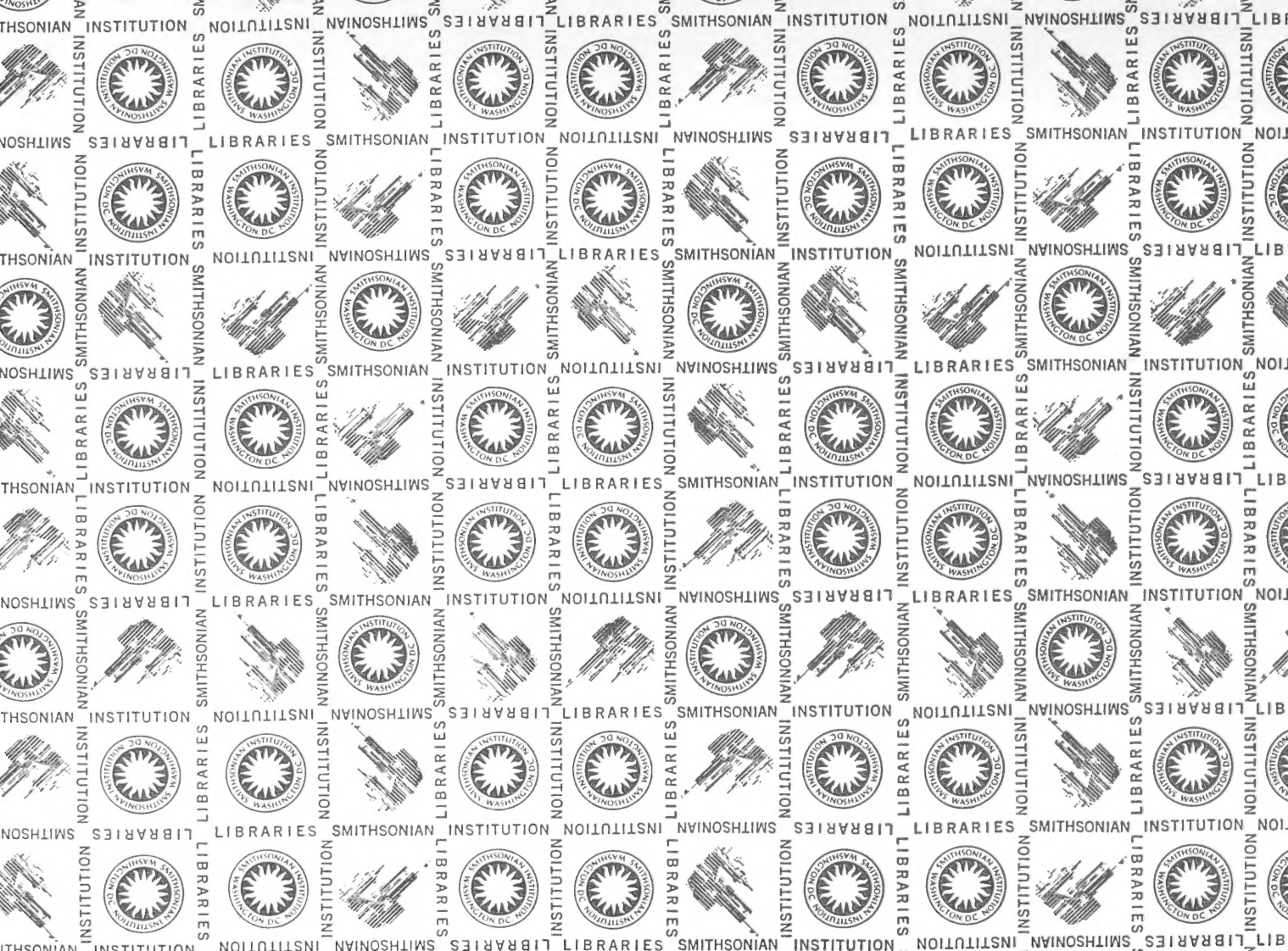
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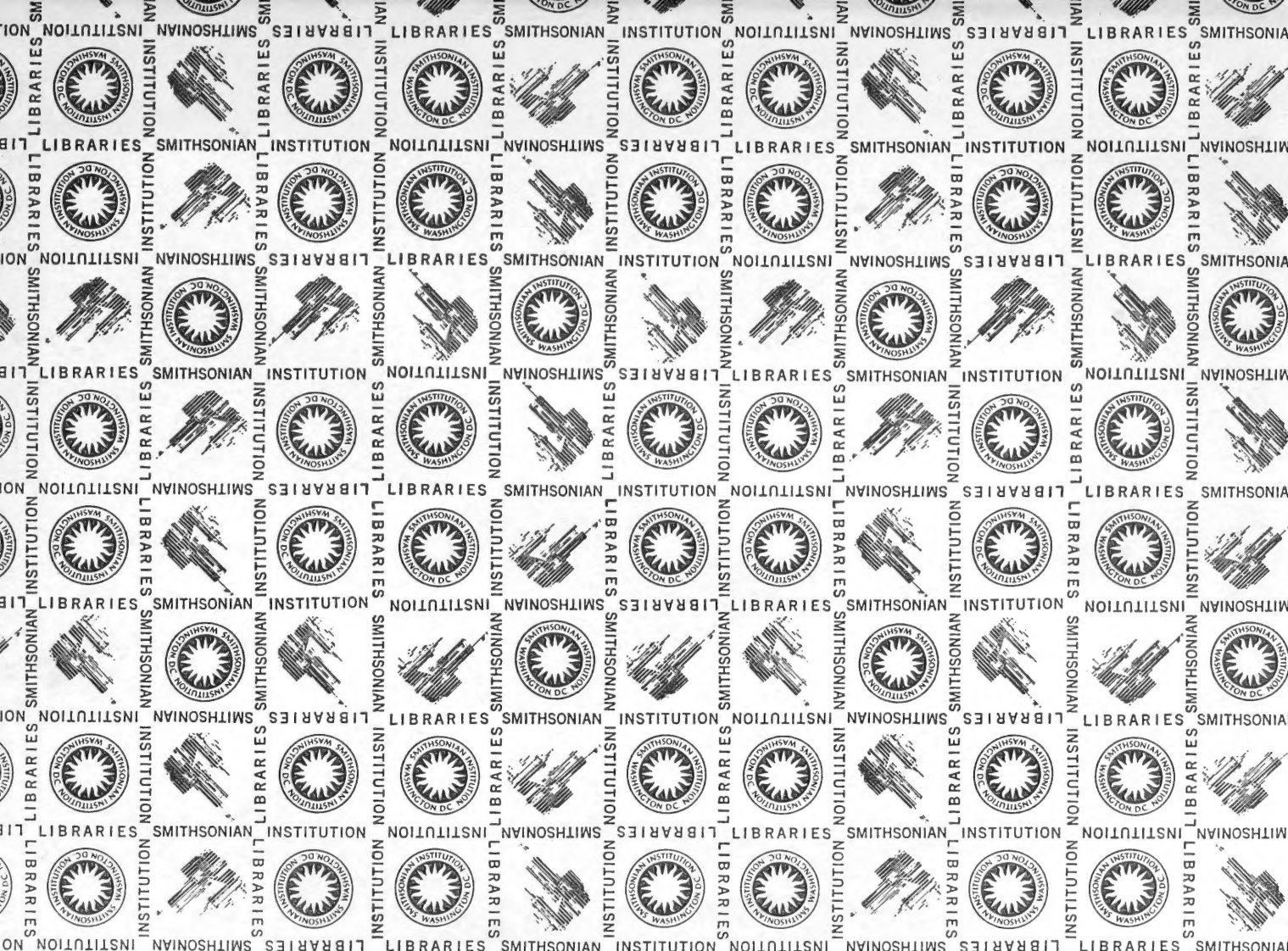
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DETERMINATION OF THE SIZE AT SEXUAL MATURITY OF THE WAVED WHELK *BUCCINUM UNDATUM* LINNAEUS, 1758, IN THE GULF OF ST. LAWRENCE, AS A BASIS FOR THE ESTABLISHMENT OF A MINIMUM CATCHABLE SIZE

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ABSTRACT The reproductive biology of the whelk *Buccinum undatum* L. was investigated to determine the size at sexual maturity, as a basis for the establishment of a minimum catchable size. Samples were obtained in spring of 1988 and 1989 from 8 sites in the Gulf of St. Lawrence (Québec). Size and age at sexual maturity varied between sites and between sexes. Males reach sexual maturity at sizes ranging from 49 mm to 76 mm in shell length, around 5 or 6 yr. Females reach maturity at larger sizes (60–81 mm) and later (7 yr). Important differences occur between Magdalen Islands (in the southern part of the Gulf) and stations from the northern Gulf, which were interpreted as a result of long-term selective pressures, namely from predation. Differences were also observed at a smaller geographical scale, which were likely linked to local environmental conditions. At most sites, a decrease in the percentage of animals with undeveloped gonads at larger sizes was observed, possibly as a consequence of parasitic castration. Implications of the results for the management of the whelk resource are examined.

KEY WORDS: whelk, *Buccinum*, sexual maturity, fishery, management

INTRODUCTION

The northern whelk, *Buccinum undatum* L. (Neogastropoda, Buccinidae) is a common subtidal species along the western Atlantic coast from New Jersey to Labrador, and it supports small local fisheries throughout the Gulf of St. Lawrence. Landings in the province of Québec have fluctuated between 5 and 1300 t (fresh weight) per year since 1949, in response to local and international market demands (Villemure and Lamoureux 1975, McQuinn et al. 1988). Presently, management of this resource in the Québec region is done mainly through restriction of the fishing effort (limited entry and limited number of traps per permit). Despite this limitation, there is some evidence that local stocks have been depleted and have not yet recovered (Gendron 1991).

B. undatum exhibits life history traits which make local stocks particularly vulnerable to overfishing. Its life history is characterized by the absence of a planktonic dispersal stage, and in the Gulf of St. Lawrence it is a slow-growing species (Boivin et al. 1985, Jalbert et al. 1989). Eggs are contained in capsules laid on rocky substrates, and larval development (trocophore and veliger) occurs inside the capsules. In the northern Gulf of St. Lawrence, juveniles (3 mm shell length) emerge from the capsules after a 5 to 8 mo period (Martel et al. 1986a). Moreover, the adults are relatively sedentary. They spend most of their time immobile and half-buried in the sediment (Himmelman 1988, Hamel 1989, Jalbert et al. 1989), although they are capable of significant movements when offered a prey (Himmelman 1988, McQuinn et al. 1988), or confronted by a predator (Harvey et al. 1987).

Overfished stocks may therefore remain depleted for a long time, until adult whelks gradually recolonize the depleted areas and breed successfully. To prevent overfishing, a management strategy based on the protection of the local brood stock could be implemented. Although no clear stock-recruitment relationship has been defined for whelks, it can nevertheless be assumed that the establishment of a minimum catchable size above the size of sexual maturity could be an effective tactic to prevent local recruitment overfishing. It would ensure that a portion of the population can reproduce before being fished.

Life history traits of *B. undatum* (i.e. direct development and limited adult movement) tend to reduce mixing between adjacent populations. Individuals may therefore become locally very well adapted. Great morphological and genetic differentiation over very short distances have been documented for a number of gastropods lacking a planctonic dispersal phase (Behrens Yamada 1989). Implementation of management tactics must therefore take into account the possible occurrence of several distinct stocks within the area concerned.

The present study determines the size and age at sexual maturity of *B. undatum* from different Québec localities in the Gulf of St. Lawrence. Whelks were also described morphologically, in the light of the morphological differences recently observed for *B. undatum* from different sites (Thomas and Himmelman 1988).

MATERIAL AND METHODS

1. Sampling

Sampling was done in each of the major geographic areas inside the Québec jurisdiction limits. In the northern part of the Gulf of St. Lawrence, sampling was done along the Gaspé Peninsula (Saint-Joachim and Mont-Louis), the Lower North Shore (La Tabatière), the Middle North Shore (Mingan and Magpie), the Upper North Shore (Sept-Iles and Moisie), and in the southern part of the Gulf, along Magdalen Islands (Fig. 1). Sampling was done on known fishing grounds readily accessible in early spring. Sampling was conducted in April and May 1988 and 1989, before the beginning of reproductive activities (copulation and egg-laying) as observed by Martel et al. (1986a).

At 6 sites, approximately 100 whelks of each sex ranging from 40 to 120 mm in shell length (SL) were collected haphazardly by SCUBA divers at depths varying between 15 and 20 m, on mixed substrates (sand-mud, pebbles). At the two other sites (Magdalen Islands and La Tabatière), whelks were obtained from baited traps

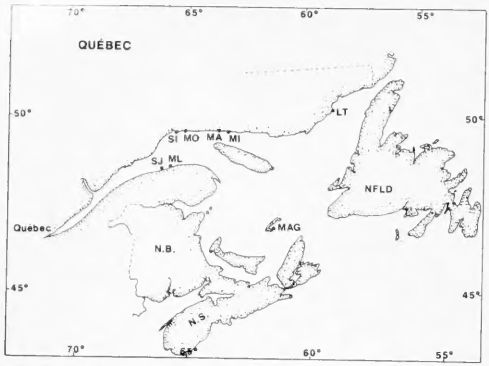

Figure 1. Map of Eastern Canada showing the location of the 8 sampling sites. St-Joachim SJ; Mont-Louis ML; Mingan MI; Magpie MA; Sept-Iles SI; Moisie MO; Magdalen Islands MAG; La Tabatière LT.

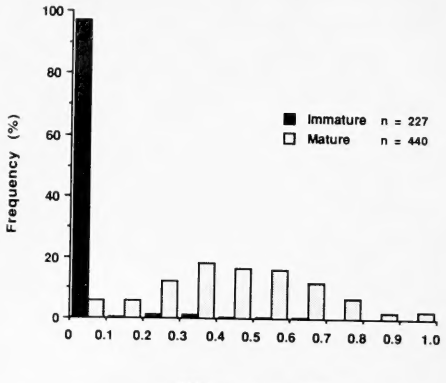

Figure 2. Frequency distribution of the seminal vesicle index of *B. undatum* (seminal vesicle weight/somatic tissue weight) × 100, for mature and immature males, according to the relative penis size criteria (penis length/shell length < 0.5 = immature; ≥0.5 = mature).

set at the same depths. At each site, whelks were gathered from an area of approximately 1 km of shoreline. Whelks were preserved in 10% formalin in seawater.

2. Sexual Maturity

All whelks collected were examined for determination of sexual maturity. Sexual maturity of males was determined from examination of the penis. Santarelli-Chaurand (1985) classified as mature, males having a penis at least as long as half the total shell length (SL). This was based on observations made by Køie (1969) that the ratio of penis length to total length increased when sexual maturity was attained. Males with a penis length ≥0.50 SL were therefore classified as mature. The relationship between relative penis size and gonadal development was nevertheless examined, to confirm the validity of the use of the penis size as a criteria for sexual maturity. The frequency distribution of the seminal vesicle index (ratio seminal vesicle weight to somatic tissue weight), for mature and immature males (according to the relative penis size) is presented in Figure 2, confirming the association between a large penis and gonad development. Only the seminal vesicle was considered here since at the period of sampling, the seminal vesicle is fully developed while the testis is decreasing in size (Martel et al. 1986a).

Females maturity was determined from a gonado-somatic index (GSI), defined as the ratio of gonadal weight (ovary + albumin and capsule glands + seminal receptacle + bursa) to the total eviscerated weight (total body weight − (gonad + digestive gland)). At the period of sampling, females had fully-developed ovaries (Martel et al. 1986a). The eviscerated weight was used instead of total weight mainly to eliminate the effect of the highly variable weight of the digestive gland (see Martel et al. 1986b). All weights were determined after allowing specimens to drain on a paper towel for 2 min. Females having a GSI ≥0.06 were considered mature, following observations of Martel et al. (1986b). Histological examination of females indeed revealed the absence of large oocytes in the ovaries of females with a GSI < 0.06, thus suggesting they were not capable of reproducing in that year.

The percentage of mature animals in each 5 mm size class (SL) was computed and a logistic curve was fitted to the data. Size at sexual maturity, defined here as the size at onset of maturity was derived from the inflexion point of the curves. The logistic equation was reparametrized in order to obtain from the calculation procedures, a direct estimation of the variance (fitting error) of the size at sexual maturity. Therefore,

$$y = \frac{y_{max}}{1 + e^{b(a+x)}}$$

where y is the proportion of mature animals, a is the inflexion point, b is a constant of the curve and x is the shell length. The parameters a and b were approximated using the Marquardt method outlined in SAS/STAT/NLIN program, and y_{max} was ad-

TABLE 1.

Parameters of the logistic equation (see text) used to determine the size at sexual maturity (a) for *B. undatum* (mm SL) for each site and sex.

Site	Sex	y max	a (size at sexual maturity)	sd	b
St-Joachim	M	0.75	76.38	2.06	−0.166
St-Joachim	F	0.70	75.62	1.10	−0.268
Mont-Louis	M	1.00	71.78	1.39	−0.821
Mont-Louis	F	1.00	73.76	2.70	−0.153
Mingan	M	1.00	75.55	1.25	−0.259
Mingan	F	1.00	78.33	1.87	−0.285
Magpie	M	0.85	67.06	0.62	−0.855
Magpie	F	1.00	73.61	1.09	−0.236
Sept-Iles	M	1.00	69.22	2.15	−0.124
Sept-Iles	F	1.00	80.76	1.05	−0.342
Moisie	M	0.95	58.94	3.91	−0.315
Moisie	F	1.00	79.54	2.65	−0.165
Magd Is.	M	0.75	49.09	2.26	−0.290
Magd Is.	F	0.70	60.29	0.87	−4.425
La Tabat	M	1.00	61.87	4.29	−0.095
La Tabat	F	1.00	73.67	1.63	−0.250

sd represents the standard deviation of the size at sexual maturity.

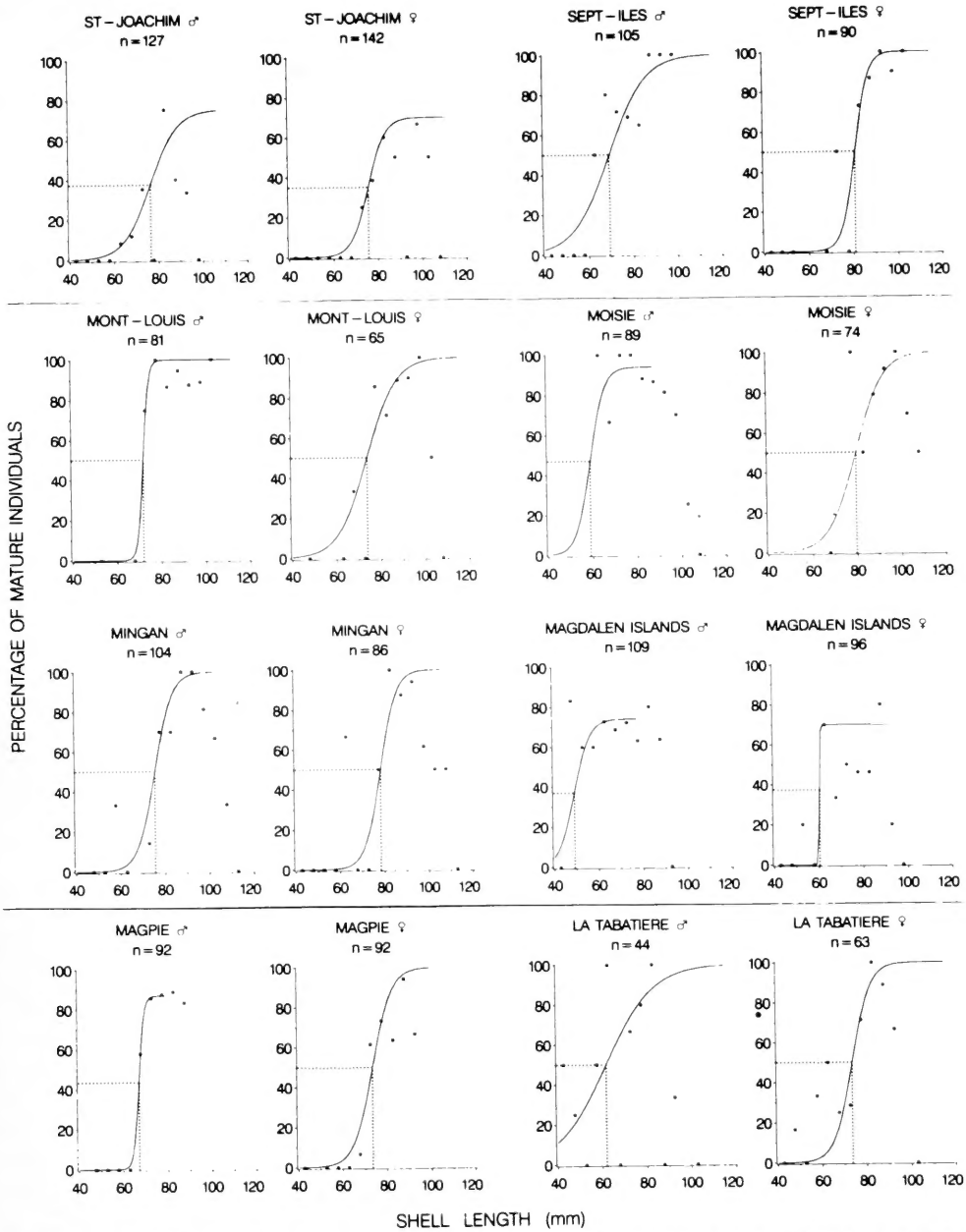

Figure 3. Percentage of mature male (♂) and female (♀) whelks *B. undatum* as a function of shell length, for the different sites in the Gulf of St. Lawrence. Size at onset of maturity was estimated from a logistic curve fitted to the data (ascending portion). n refers to the total number of animals examined.

justed to values ≤ 1, depending on sites (Table 1). The logistic equation was fitted on the ascending portion of the data as explained below.

3. Shell Morphometry and Age Determination

Five morphometric characteristics (Table 2) were examined on the shell of each whelk to assess possible morphological differences between sites, as reported by Thomas and Himmelman (1988). Characteristics examined were the general form of the shell, the form of the aperture, and shell robustness. Variability was examined using a canonical discriminant analysis (Legendre and Legendre 1984), based on the correlation matrix of the 5 morphometric variables.

Age of whelks was estimated by counting annuli on the operculum, after coloration with methylene blue. The annual periodicity of annulus deposition was demonstrated by Santarelli and Gros (1985). Plots of mean shell length at age were constructed for each sex and each sampling site. Size at sexual maturity was reported on these plots and a rough estimate of age at maturity was then derived visually.

RESULTS

1. Sexual Maturity

Percentage of mature animals as a function of size followed a logistic curve up to approximately 90–100 mm SL. Thereafter, at certain sites, the percentage of mature animals decreased as size increased (Fig. 3). In these cases, the logistic curve was fitted to the ascending portion of the data only. Moreover, at some of these sites, the percentage of maturity did not reach 100% (Table 1).

Males reach sexual maturity at sizes ranging from 49 to 76 mm, and mainly at 5–6 yr (Fig. 4). Females reach sexual maturity at larger sizes (60–81 mm) and at a later age (roughly 7 yr) (Fig. 4). As explained above, mean age at maturity was derived visually from the mean shell length at age curves presented in Figure 5.

The sample from Magdalen Islands (southern part of the Gulf of St. Lawrence) differed markedly from those from the northern Gulf; sexual maturity occurring at a smaller size and also at an earlier age in the former. In the northern Gulf, samples tend to form a more homogeneous group, at least as far as females are concerned. In this area, females reach sexual maturity roughly at 76 mm (Fig. 4). Besides differences observed between the southern and northern parts of the Gulf, intersite differences are also obvious in the northern Gulf. Whelks from St-Joachim (site 1) reach sexual maturity later than those located only 60 km downstream, at Mont-Louis (site 2) and differences in size are obvious for males, between neighbouring sites along the North Shore (Fig. 4).

2. Shell Morphometry

Canonical discriminant analysis revealed that whelks from Magdalen Islands particularly, had a thicker and heavier shell than those from the other sites (Fig. 6; Table 2). The first canonical variable accounts for 49% of total variance and is associated with

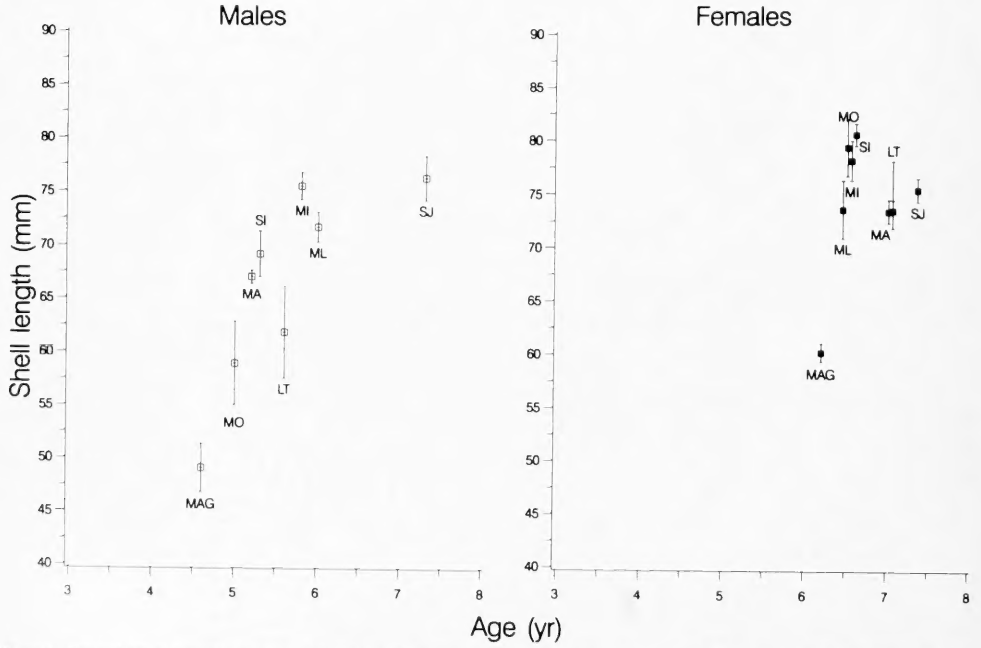

Figure 4. Size and age at maturity of male and female whelks *B. undatum* from the different sampling sites in the Gulf of St. Lawrence. (Vertical bars indicate one standard deviation). St-Joachim SJ; Mont-Louis ML; Mingan MI; Magpie MA; Sept-Iles SI; Moisie MO; Magdalen Islands MAG; La Tabatière LT.

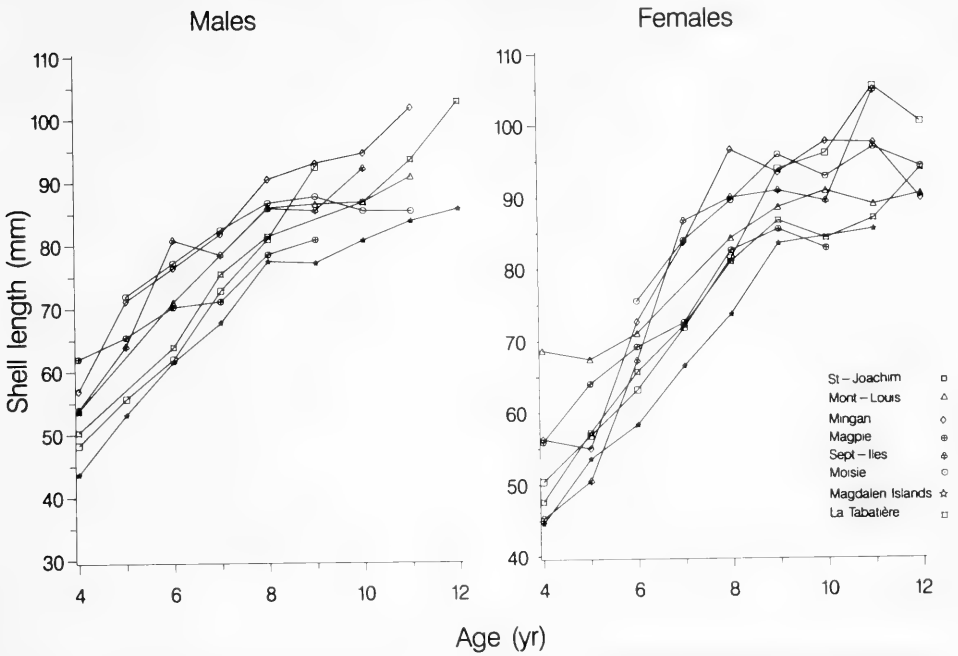

Figure 5. Mean shell length at age for male and female whelks *B. undatum* from the different sampling sites in the Gulf of St. Lawrence.

shell robustness (shell thickness and relative shell weight). In this respect, shells from Magdalen Islands tend to isolate on this first axis. The second axis accounts for 30% of total variance and is associated with the size of shell aperture. Whelks from St-Joachim tend to show a relatively greater aperture than whelks from the other sites. This characteristic appears also to distinguish males from females, the latter having a relatively smaller aperture. This type of sexual dimorphism in shell aperture was already recognized in *B. undatum* by ten Hallers-Tjabbes (1979). Unfortunately, this sexual dimorphism is not obvious enough to be used in the field to distinguish sexes.

DISCUSSION

Size at sexual maturity varies throughout the region studied and important differences were observed between samples from Magdalen Islands (in the southern part of the Gulf) and those from the northern part of the Gulf of St. Lawrence. Further investigations should be undertaken in the southern part of the Gulf in order to determine to what extent the observations made in Magdalen Islands in 1989 are representative of the whole region.

Sexual maturity occurs at a smaller size and apparently at an earlier age in Magdalen Islands compared to what was observed in the northern Gulf. Moreover whelks from Magdalen Islands, unlike those from the other sites have a more robust shell. Shell robustness in *B. undatum* was interpreted as a response of whelks to crustacean predation (Thomas and Himmelman 1989). Magdalen Islands supports a major lobster (*Homarus americanus*) population, unlike the other sites studied. In the northern Gulf, the main predators are *Cancer irroratus, Hyas* sp., *Leptasterias* and *Asterias* (Thomas and Himmelman 1989). Whelks from Magdalen Islands are similar, with respect to shell robustness to those from Maces Bay in the Bay of Fundy, as described by Thomas and Himmelman (1989), and where lobsters are abundant. Shell weight of a 40 mm whelk (SL) is 3.8 and 6.9 g for whelks from Mingan and Magdalen Islands, respectively. These values are comparable to those given by Thomas and Himmelman (1989), i.e. 4.5 g for the Gulf (near Mingan) and 6.1 g for Maces Bay.

Mean shell length at age for samples from Magdalen Islands are generally smaller, which can be accounted for by the fact that shell length measurements do not take into account growth in thickness. This may explain to a certain extent why size at sexual maturity appears to be smaller at this site.

Although age at maturity was only roughly approximated, sexual maturity appeared to be attained at a younger age in Magdalen Islands. This could be the result of long-term selective pressure. It is generally accepted that intense predation pressure can select for smaller sizes at maturity and reduced maximum size, compared to populations subjected to reduced predation (Stearns and Crandall, 1981). Differences in size and age at maturity between the Magdalen Islands and sites located in the northern part of the Gulf may therefore be linked to differences in predation pressure characterizing the two different benthic assemblages.

Within the northern part of the Gulf, no clear pattern of variability in size and age at sexual maturity can be observed. Martel et al. (1986b) noticed that the size at sexual maturity was smaller along the Gaspé coast (6–6.5 cm), than on the North Shore (7–8 cm) and suggested that this reduction could be a consequence of a

heavy fishing. Information on landings and relative fishing effort for the major geographic areas show that on the whole, the fishing pressure has been heavier on the Gaspé coast (Gendron 1991). However, it was not possible to quantify the level of fishing mortality in each of the sites sampled and further, it is not certain whether fishing pressure on the sampling grounds is identical to the pattern of fishing observed at the larger spatial scale. Our observations show that variability in the size and age at sexual maturity appears to exist at a smaller scale than the one defined by the geographical areas. Whelks from sites distant of only 60 km showed striking differences in their age at sexual maturity and in their morphology (Gaspé Peninsula), and in their size at maturity (North Shore). These observations suggest the existence of distinct morphological stocks at very short distances from one another. Small-scale factors such as on-site whelk density (related to fishing intensity) and food availability, may also be important in regulating size and age at maturity.

In most of the sites, a number of large whelks showed no sign of sexual maturity. For females, this can be explained by the fact that any given year, 20% to 40% of the females do not reproduce (Martel et al. 1986b). Nevertheless, an ovary is always present (Martel et al. 1986a). In our samples, a number of large males and females exhibited complete resorbtion of the gonads (GSI = 0), which in males, was associated to an atrophy of the penis. This gonad resorbtion can be linked to the presence of trematode parasites (Køie 1969), causing castration and affecting secondary sexual characters (Fretter and Graham 1962). This has already been reported by Hamel (1989) for whelks from the Mingan area. Although parasites were observed at certain sites, we did not systematically examine all whelks to assess the presence of parasites and therefore cannot confirm that gonadal atrophy is strictly linked to parasitic castration.

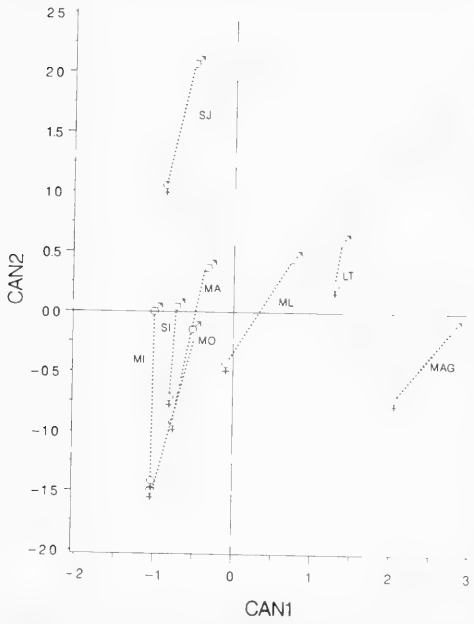

Figure 6. Results of the Canonical Discriminant Analysis showing the positions of the centroids (group means) along the first two canonical axes (CAN 1 and CAN 2) for male (♂) and female (♀) whelks *B. undatum* from the different sampling sites in the Gulf of St. Lawrence. St-Joachim SJ; Mont-Louis ML; Mingan MI; Magpie MA; Sept-Iles SI; Moisie MO; Magdalen Islands MAG; La Tabatière LT.

TABLE 2.

Standardized coefficients of the two first canonical variables (discriminant functions, CAN1 and CAN2) based on five morphometric variables for the whelk *B. undatum*.

Morphometric variable	CAN1	CAN2
shell width / shell height	0.232	−0.068
aperture height / shell height	0.013	1.363
aperture width / aperture height	0.152	−0.129
shell thickness / shell height	0.942	0.440
shell weight / total weight	0.698	−0.145
% variance explained	48.5%	29.6%

Percentage of total variance accounted for by each canonical variable is also presented.

Alternatively, gonad atrophy could be explained by the occurrence of reproductive senility affecting older animals. Cursory examination of whelks showing complete gonad atrophy indicated that the phenomenon could be related to age. Whelks under 8 yr did not show gonad atrophy. The occurrence of reproductive senility could eventually be investigated quantitatively, in a way similar to what has been done by Peterson (1983) for *Mercenaria mercenaria*.

To be effective, a minimum catchable size regulation would have to take into account the variability (sex and site) observed in the size at sexual maturity. In practical terms however, it may be difficult to establish and enforce. First of all, sexes cannot be separated in the fishery, and since the size at sexual maturity differs between sexes (females > males), the establishment of a minimum catchable size would have to adjust on the size at sexual maturity of the females. A management tactic providing protection of reproductive females would also be effective in protecting reproductive males while the reverse is not true. Sexual maturity of females is late and therefore implies that the minimum catchable size will itself be high, to a point where in some areas fishing may be compromised (Gendron 1991). In such instances, conflicts between resource protection objectives and short-term socio-economic priorities are likely to arise in the process of enforcement of the management tactic. Secondly, because of the geographical variability in the size at sexual maturity, different minimum catchable sizes should be established by partitioning the whole region into different sub-regions which are homogeneous in respect to the size at sexual maturity. A single minimum catchable size established for the whole area may, at certain sites, offer insufficient protection of the brood stock while in others, provide unnecessary protection for the large non-reproductive animals. In

practical terms, the definition of homogeneous sub-regions may present certain difficulties since the exact spatial scale of differentiation is unknown although it seems to be small.

Notwithstanding these difficulties, enforcement of a (or more) minimum catchable size in such a way that it reflects variability of the size at sexual maturity will become most important in the context of heavy exploitation. The stock-recruitment relationship is not known for this species, but it can be assumed that heavy exploitation in the absence of any catch size regulation, thus allowing the landing of whelks of size below the size at sexual maturity, will easily lead to severe recruitment overfishing and ultimately to the collapse of the local fishery.

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GROWTH AND DEVELOPMENT IN LARVAL AND POST-METAMORPHIC *RANGIA CUNEATA* (SOWERBY, 1831)

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ABSTRACT Larval and juvenile *Rangia cuneata* were reared at 8–10 ppt and 23–26°C for 7 weeks. Egg diameter averaged 59.2 ± 3.1 μm (mean ± 1 SE). Cell division began before the end of the first hour after fertilization (hour 1). By hour 8, 95% of the larvae had developed into rotating blastulas. A few trochophores had developed by hour 12, the first D-hinge larvae by hour 14, and umbonate larvae by day 4. Pediveligers settled by day 6 or 7 at lengths between 160 and 200 μm. After 7 weeks in the laboratory, juveniles reached an average length of 1348 ± 326 μm and an average height of 1191 ± 308 μm. An exhalent siphon developed 4–5 d after metamorphosis when juveniles were about 250 μm long, but did not extend beyond the valves until 10–14 d after metamorphosis. The tentacles of the future inhalant siphon had begun to develop in 400–900 μm long juveniles (by 21 d after metamorphosis). Development of the inhalant siphon was complete and the siphon was functional when juveniles measured about 1,000 μm in length (about 42 d after metamorphosis).

KEY WORDS: *Rangia cuneata*, larvae, development, settlement, growth, siphons

INTRODUCTION

Rangia cuneata is a large (up to 7 cm) suspension-feeding mactrid bivalve found at salinities below 15 ppt (Cain 1975) in the Gulf of Mexico and in southeastern coastal waters as far north as upper Chesapeake Bay (Hopkins et al. 1973). Published studies of development and growth of the planktonic young or benthic juveniles did not continue past metamorphosis (Fairbanks 1963, Chanley 1965) or provided incomplete information on rearing conditions (Hopkins et al. 1973). No study has described development of the siphons. Thus we measured developmental times and growth of larvae and juveniles and monitored siphonal development for 7 weeks after fertilization. Our source of spawning adults lies near the northern extreme of the species' reported range (Gallagher and Wells 1969, Hopkins et al. 1973).

MATERIALS AND METHODS

Adult *Rangia cuneata* were collected in early June, 1990 from the tidally-influenced upper Choptank River (0–3 ppt salinity), a tributary of Chesapeake Bay (approx. 38°48′N, 75°53′W). They were held in ambient flowing seawater (6–8 ppt, 23°C) at our hatchery about 30 km downstream from the collection site. Animals were batch fed for about 4 h each day at a starting concentration of 1–5 × 10^5 cells/ml of *Isochrysis galbana* (clone T-Iso) per 80 animals to condition them for spawning.

Clams were spawned on June 19 by increasing water temperature from ambient to 30°C over a short time period and by adding a suspension of sperm for further stimulus (Loosanoff and Davis 1963). More than two males and two females spawned (actual numbers were not recorded). When spawning began, males and females were placed in separate containers where they continued to spawn. When spawning ended, egg and sperm suspensions were combined and frequent observations were made under a microscope to ensure that the sperm concentrations did not induce polyspermy. Fertilized eggs were placed in 150-L plastic containers with filtered (5 μm), aerated Choptank River water. Larvae were fed daily with *I. galbana* and water was changed every 48 h. Larvae were reared in the containers until they metamorphosed. The juveniles were then transferred to 50-L aquaria with river sediment and maintained until siphon development was complete (about 7 weeks after fertilization). Water temperature and salinity in both the larval and juvenile cultures ranged from 23–26°C and 8–10 ppt, respectively.

To estimate the time course of development, we observed larvae during the first hour, then sampled larvae 1, 2, 3, 4, 8, 12, 14, 16 and 24 h after fertilization, and at 24 h intervals for 1 week. Samples of settled juveniles were examined periodically over the next 6 weeks. Larvae or juveniles were placed in a Sedgewick-Rafter cell and examined microscopically. An ocular micrometer was used to measure either the diameter of pre-D-hinge larvae, or the length (the largest anterior-posterior distance), height (the largest distance from the umbo or the center of the hinge to the ventral margin), and hinge length (before the umbo formed) of the shells of at least 20 larvae or juveniles per sample. Values are reported as $\overline{X} \pm 1$ SE. We also observed particle intake into the shell cavity and the timing and sequence of siphonal development in juveniles. Some juveniles were anaesthetized with a few drops of a saturated solution of $MgCl_2$ in seawater to allow us to observe siphonal morphology.

RESULTS

While spawning, male and female clams extended their siphons just beyond the edge of the shell and released a thin stream of gametes through the exhalent siphon. The eggs measured 59.2 ± 3.1 μm in diameter.

By the end of the first hour after fertilization (hour 1), cell division had begun, and larvae with 2, 4, and 8 or more (8+) cells were present (Table 1). Larvae with 8+ cells were not yet spherical. Duration of the 2- and 4-cell stages was 15–30 min each. Most larvae were multicellular, spherical blastulas by hour 3, with all larvae at that developmental stage by hour 4. About 5% were rotating and rolling on the Sedgewick-Rafter cell bottom by hour 3, 14% by hour 4, and 95% by hour 8 (20% of these were swim-

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TABLE 1.

Growth and development of *Rangia cuneata* larvae raised at 23–26°C and 8–10 ppt.

Time From Fertilization	Developmental Stage	Dimension	Size (μm) Range	Size (μm) Mean ± SE
1 h	2 to 8+ cells		—	—
2 h	8+ cells		—	—
3 h	Spherical blastula		—	—
4 h	Spherical blastula	D	57.6–72.0	65.5 ± 4.6
8 h	Rotating blastula	D	57.6–76.8	70.3 ± 5.5
12 h	Rotating blastula	D	62.4–86.4	74.2 ± 6.0
	Trochophore (<1%)		—	—
14 h	Rotating blastula (<1%)		—	—
	Trochophore (50%)	H	67.2–76.8	73.2 ± 4.0
		W	48.0–62.4	57.6 ± 5.9[a]
	D-hinge (50%)		—	—
16 h	Trochophore (10%)		—	—
	D-hinge (90%)	L	72.4–86.9	81.5 ± 4.0
		H	67.6–77.2	68.6 ± 2.5
24 h	D-hinge	L	82.0–91.7	88.8 ± 3.2
		H	37.6–82.0	75.0 ± 3.6
2 d	D-hinge	L	86.9–101.3	97.9 ± 4.0
		H	82.0–91.7	85.7 ± 3.4
3 d	D-hinge	L	96.5–130.3	110.5 ± 8.2
		H	82.0–115.8	97.0 ± 9.0
4 d	Umbonate	L	115.8–173.7	139.9 ± 14.5
		H	106.2–154.4	123.5 ± 10.4
5 d	Umbonate	L	135.1–173.7	162.0 ± 11.0
		H	115.8–159.2	147.9 ± 11.5
6 d	Pediveliger	L	164.1–197.8	179.0 ± 8.2
		H	159.2–173.7	165.5 ± 4.0
7 d	Pediveliger	L	164.1–202.7	187.7 ± 11.8
		H	159.2–183.4	175.4 ± 7.2

[a] At the ciliated girdle, width was 72.5 ± 4.1 μm (n = 4).
Mean height (H) and width (W) measurements for trochopores are for four individuals. For other larval stages, mean diameter (D), length (L), and height (H) measurements are for 20 individuals.
— = not measured.

ming in the cell's water column). All the larvae still in the rotating blastula stage were in the water column by hour 12. Over the 8-h period from hour 4 to hour 12, average blastula size increased from about 66 μm to 74 μm (Table 1).

A few (<1%) of the larvae had reached the trochophore stage by hour 12. Trochophores were somewhat acorn shaped, with a long apical flagellum, and swam with the velum upward. Their height was greater than their width (Table 1). About 50% of the larvae were D-hinge at hour 14, and 90% at hour 16, with hinge length ranging from 38.6 to 57.9 μm by hour 16 (n = 20). At days 2 and 3, the hinge on some animals had a slight saddle-shape, but most remained straight. By day 3, hinge length ranged from 48.3 to 57.9 μm (n = 20). Throughout the D-hinge stage, average larval length was 12–14 μm greater than average larval height (Table 1). As the larvae aged, they assumed a yellow color.

All larvae were umbonate by day 4. The early umbo was rounded and not very distinct, but became more angular and noticeable with time. Pediveligers developed by day 6, using their long slender foot to crawl on the cell bottom or keeping it extended as they propelled themselves with their velum along the bottom. Pediveligers were only rarely observed swimming vertically in the cell's water column with the foot extended. The presence of the foot and swimming behavior along the cell bottom was the first and only indication that the larvae were competent to settle; we saw no eyespot. On days 6 and 7, most of the larvae settled and metamorphosed on the bottom of the rearing container in the absence of sediment. No byssus thread was observed. The velum was completely resorbed as the clams commenced a benthic existence. Average length of the pre-metamorphic clams was about 179 μm on day 6 and 188 μm on day 7 (Table 1). From the first shell measurements of the D-hinge stage at hour 16 until settlement, average shell length increased about 230% and shell height increased about 250% (Table 1).

After 7 weeks in the laboratory, juveniles reached an average length of 1348 ± 326 μm (n = 27) and an average height of 1191 ± 308 μm (Fig. 1). As the clams aged, the variation in size increased (Fig. 1). The linear regression of height (μm) on length (μm) of larvae and juveniles from day 1 to 49 was:

$$\text{height} = 1.08\ \text{length} + 5.27$$
$$r^2 = 0.99;\ n = 254$$

Unlike adult *Rangia cuneata* that have two siphons fused along

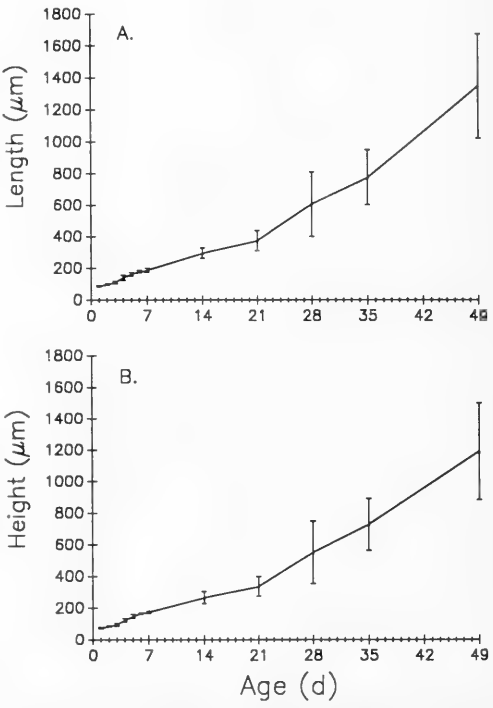

Figure 1. A. Mean length (±1 SE) and B. mean height (±1 SE) of *Rangia cuneata* larvae and juveniles reared in the laboratory at 23–26°C and 8–10 ppt, measured periodically up to 49 d after fertilization. N = 20, except on days 28 (N = 23), 35 (N = 24), and 49 (N = 27).

their length, recently-metamorphosed juveniles had no siphons. Particles were drawn into the mantle cavity through the pedal gape, presumably by pedal cilia. Pseudofeces and feces were expelled through the pedal gape at the anterior end of the gills. An exhalent siphon developed and was used by spat to expel feces 4–5 d after metamorphosis, when the spat were about 250 μm long. The exhalent siphon did not extend beyond the valves until 10–14 d after metamorphosis.

Several small siphonal tentacles developed, one pair at a time, with one tentacle of each pair growing on the edge of each mantle lobe. The first pair developed by 21 d after metamorphosis when juveniles were 400 to 900 μm long (Fig. 2). This pair appeared just ventral to the exhalent siphon, with subsequent pairs developing ventral to earlier pairs. After three or four pairs had formed, two sections of the mantle fused to form an intact inhalant siphon. The upper section that fused was immediately below the exhalent siphon and just above the first tentacle pair. The second point of fusion was a few μm ventral to the most recently formed pair of tentacles, so that the newly formed siphon had six or eight siphonal tentacles, all of which were on the rim of the siphon adjacent to the exhalent siphon. Tentacles continued to develop on the siphon rim, two at a time, ventral to the most recently developed pair; we counted a maximum of six pairs by day 49. The inhalant siphon was functional in half the juveniles with three tentacle pairs and in all juveniles with four or more pairs; juvenile size was about 1000 μm and age was 42 d.

DISCUSSION

The earliest published account of the development of *R. cuneata* larvae is that of Fairbanks (1963) who described development to the D-hinge stage. He artificially spawned clams from Lake Pontchartrain, Louisiana, by stripping eggs and sperm from the adults. In his study, key developmental stages occurred when the larvae were older than in our study, even though the larvae were reared at about the same temperature (23–28°C in Louisiana; 23–26°C in Maryland). Fairbanks (1963) reported that ciliated blastulas rolling along the bottom of a watch glass first appeared 8.5 h after fertilization, much later than in our study. Other comparisons include the first appearance of trochophores by about hour 26 and the first appearance of veliger larvae by about hour 34 in Louisiana clams compared with 12 h and 14 h, respectively, in our study.

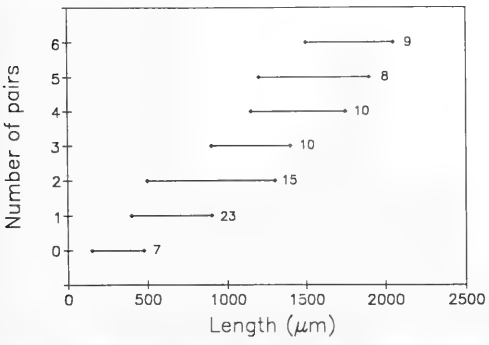

Figure 2. Relationship between number of pairs of siphon tentacles and length of juvenile *Rangia cuneata*. Numbers beside each line are sample size.

The differences in timing between the two studies may be due to the fact that Fairbanks (1963) used stripped eggs and sperm. This may have resulted in a greater proportion of ova that were not completely mature, as suggested by Fairbanks' (1963) report of a number of abnormal embryos. Fairbanks (1963) did not report on salinity, embryo density, and rearing chamber volume, nor if the larvae were fed, so meaningful comparisons with our study are difficult to make.

Chanley (1965) described larval development (but not timing) from the D-hinge stage until metamorphosis in *Rangia cuneata* from the James River, lower Chesapeake Bay (collection salinity, 5 ppt; spawning conditions, 15 ppt and 22–24°C). Length of the different larval stages measured by Chanley (1965) generally overlapped those we measured; our metamorphosing clams (160–203 μm) were somewhat larger than his (160–175 μm). He did not observe eyespots, and found metamorphosis to begin by day 7; our findings corroborate his. Hopkins et al. (1973) reported larval growth rates of *R. cuneata* from Galveston Bay, Texas (22°C, 3–5 ppt). Larvae also settled by day 7, but at a smaller size than ours. Growth slowed after day 8, so that 14-d old *R. cuneata* were only 140–150 μm long, compared to 220–350 μm in our study. The Texas larvae had been fed only once during the week before metamorphosis and it is not clear what and when post-larval clams were fed. Finally, Mann et al. (1990), while rearing larval *R. cuneata* produced by James River adults (25°C, 10 ppt) for a study of their swimming behavior, reported that the larvae metamorphosed after 12 d, i.e., 5 to 6 d later than in any other study. We know of no explanation for this difference.

Rangia cuneata is different from most other western Atlantic bivalves studied in terms of size at and timing of settlement. Of 15 bivalves in Chesapeake Bay studied by Chanley and Andrews (1971), nine produce larvae that are as small or smaller than *R. cuneata* when they reach the D-hinge stage. However, all but one (*Petricola pholadiformis*) are larger at metamorphosis, including another mactrid bivalve, *Mulinia lateralis*. By contrast, Kennedy et al. (1989) found that an additional species, *Macoma mitchelli*, both produces D-hinge larvae that are similar in size to *R. cuneata* larvae and metamorphoses at a similar size (160–205 μm). *Rangia cuneata* larvae also metamorphose at a younger age than most other larvae studied to date, which require at least 10 d or longer at 20–24°C (Loosanoff and Davis 1963). However, *Macoma mitchelli* (Kennedy et al. 1989), *Macoma balthica* (Kennedy, personal observations), and *Mulinia lateralis* (Calabrese and Rhoades 1974) also metamorphose in 7 d or less.

Siphonal development has not been described in detail for many species, but for most that have been described, the exhalent siphon develops and functions earlier than the inhalant (e.g., Ansell 1962, Lutz et al. 1982, Aabel 1983, Kennedy et al. 1989), suggesting the importance of removing waste from the vicinity of the individual. Development of the exhalent siphon in *R. cuneata* (first appearance and use 4–5 d after metamorphosis) was slightly slower than that described for *Abra alba* (functioning siphon at metamorphosis; Aabel 1983), but faster than described for *Macoma mitchelli* (first use at 8–9 d after metamorphosis; Kennedy et al. 1989). Although *M. mitchelli* is older than *R. cuneata* when it starts to use the exhalent siphon, it is about the same size (about 250 μm), whereas *A. alba* is larger (about 350 μm).

Development of *R. cuneata*'s inhalant siphon was similar to

that described for *Venerupis pullastra* (Quayle 1950/51), *Venus striatula* (Ansell 1962), and *Arctica islandica* (Lutz et al. 1982). The inhalant siphon took less time to develop in *R. cuneata* juveniles (six weeks after metamorphosis, about 1 mm long) than in *A. alba* (3 months after metamorphosis, about 1 mm long; Aabel 1983) and about the same time but at a larger size than *M. mitchelli* (45 d after metamorphosis, 390–910 μm; Kennedy et al. 1989). In addition, in both *R. cuneata* and *A. abra* the inhalant siphon was being used to draw in food particles soon after it had fully developed. However, the inhalant siphon in *M. mitchelli* was not seen to be used by juveniles up to 3.4 mm in shell length (the largest animals examined by Kennedy et al. 1989).

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PARASITE AND SYMBIONT FAUNA OF JAPANESE LITTLENECKS, *TAPES PHILIPPINARUM* (ADAMS AND REEVE, 1850), IN BRITISH COLUMBIA

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ABSTRACT No infectious diseases were detected in accidentally introduced Manila clams (*Tapes philippinarum*) from 21 localities in British Columbia. However, parasites or symbionts consisting of intracellular bacteria, protozoa, and metazoa were observed. Some of these organisms were thought to be enzootic to the Manila clams. These were: intracellular bacteria (Rickettsia or Chlamydia) in the cytoplasm of the epithelial cells of the gills (at 10 localities, mean prevalence of 11.8%) and the digestive gland tubules (at 20 localities, mean prevalence 27.7%); *Trichodina* sp. along the surface of the siphons and mantle (at 18 localities, mean prevalence 46.3%); Rhynchodida ciliates attached to the gills (at 14 localities, mean prevalence 9.6%); Rhabdocoela turbellaria in the gut lumen (at 19 localities, mean prevalence 24.7%); and parasitic copepods, *Mytilicola orientalis* in the gut lumen (at 9 localities, mean prevalence 3.9%) and *Pseudomyicola ostreae* attached to the gills (at 4 localities, mean prevalence 2.5%). The other organisms found in the Manila clams probably strayed from other molluscan hosts native to British Columbia. These were: *Nematopsis*-like apicomplexan spores usually in the connective tissue of the gills (at all localities, mean prevalence 40.7%); two different apicomplexans associated with the gut, 1) a gregarine-like Apicomplexa (at one locality, 33% infected) and 2) a coccidia-like Apicomplexa (at one locality, 15% infected); trematode metacercaria of several species including members of the family Echinostomatidae and *Derogenes varicus* (family Hemiuridae), and parasitic cysts apparently consisting of a successful host response surrounding a degenerating metacercaria, all in the connective tissue of the body (at 13 localities, mean prevalence 8.6%); and pea crabs (*Pinnixa faba, Pinnixa littoralis,* and *Fabia subquadrata*) in the mantle cavity (at 10 localities, mean prevalence 13.7%). Possibly some of the unidentified species of *Trichodina* and Rhynchodida (both mentioned above) may also be native to North America and may therefore represent new invaders of the Manila clam.

KEY WORDS: *Tapes philippinarum*, Manila clam, parasites, symbionts, protozoa, metazoa, intracellular bacteria, Pinnotheridae

INTRODUCTION

The Japanese littleneck or Manila clam, *Tapes philippinarum* (=*Tapes japonica*, =*Tapes semidecussata*, =*Venerupis japonica* =*Venerupis philippinarum*) was accidentally introduced into British Columbia, presumably with seed of the Pacific Oyster (*Crassostrea gigas*) that was being imported from Japan between about 1912 and 1961 (Quayle 1964, 1988). First observed in Ladysmith Harbour, Vancouver Island in 1939, the Manila clam has since spread throughout the Strait of Georgia, along the entire western coast of Vancouver Island, and north to the central coast region of British Columbia to become one of the major intertidal bivalves (Bourne 1982). In 1988, 87% of the intertidal clam landings were Manila clams (3908 tonnes) with a landed value exceeding $7 million (Fisheries and Oceans Canada, Annual B.C. Catch Statistics, Fisheries Management Pacific Region). Recently, the possibility of culturing Manila clams in British Columbia has come under investigation (Broadley *et al.* 1988).

To date, little is known about the parasite fauna of Manila clams despite the fact that this clam is fast becoming the major species cultured worldwide. The following initiates the documentation of parasites and symbionts in Manila clams and provides reference information for the developing Manila clam culture industry in British Columbia. The parasites and symbionts encountered during the survey of Manila clams in British Columbia, their distribution, prevalence, and potential for causing disease is presented below.

MATERIALS AND METHODS

Between October 1985 and June 1990, 994 Manila clams consisting of 30 samples from 21 localities on the coast of British Columbia were examined for parasites and symbionts (Table 1, Fig. 1). Between 10 and 40 Manila clams from each location were examined histologically. These clams were fixed in Davidson's solution with acetic acid within 24 hr after collection, except for clams from Campbell Island which were held moist at 4°C for 3 days before being fixed. Prior to fixation, clams were shucked, the shell length measured, and the soft tissues and shell examined superficially. Clams over 5 grams in wet weight were cut in half. After at least 24 hr in the fixative two cross-sections, one through the region of the stomach and digestive gland and the other through the region of the heart and kidney were made. Additional sections through the adductor muscle and siphon were taken from samples collected in 1987 to 1990. All tissues were prepared using routine histological techniques. Sections (5 μm thick) were stained with Harris modified haematoxylin and 0.5% alcoholic eosin. Stained sections were examined under a compound microscope (100 to 1000× magnification).

In addition, about 30 Manila clams from 16 localities (Table 1) were examined fresh within three weeks of collection. These clams were collected at the same time as those for histological examination but were held in 50 L fiberglass tanks supplied with flow through ambient sea water (29‰ salinity with seasonal temperature variation from 8°C to 18°C) for up to 3 weeks during the examination process. Each clam was shucked, the shell length measured, and the soft tissues and shell given a visual superficial examination. The specimen was kept cold (<10°C) throughout the examination process. The entire volume of mantle fluid collected from shucked clams was examined under a dissecting microscope (25× magnification) and all metazoans and large protozoans were identified and counted. A subsample of mantle, gill, digestive gland, gonad, gut content, and kidney of each clam was excised, separately pressed between a glass slide and coverslip, and examined for protozoans under a compound microscope (400× magni-

TABLE 1.

Localities in British Columbia, Canada from which Manila clams (*Tapes philippinarum*) were examined for parasites and symbionts.

Locality*			Length (mm) of Clams Examined	Number Examined
Identity Code	Place Name	Date of Examination		Fresh; Histology
A-1	Nanoose Bay	Oct. 1985	35.4 ± 5.9	30 ; 10
A-2	Nanoose Bay	June 1986	43.6 ± 4.5	30 ; 10
A-3	Nanoose Bay	April 1988	34.2 ± 5.3	0 ; 40
B	Redonda Island	Nov. 1985	41.4 ± 1.9	30 ; 10
C-1	Cortes Island	Nov. 1985	42.3 ± 2.5	30 ; 10
C-2	Cortes Island	Nov. 1987	41.4 ± 2.8	30 ; 10
D	Kendrick Arm	Jan. 1986	44.1 ± 4.5	30 ; 10
E-1	Lasqueti Island	Jan. 1986	38.0 ± 2.7	30 ; 10
E-2	Lasqueti Island	Oct. 1986	45.6 ± 3.3	30 ; 10
F-1	Pipestem Inlet	March 1986	41.3 ± 3.6	0 ; 10
F-2	Pipestem Inlet	Aug. 1986	40.2 ± 2.7	30 ; 10
G	Davis Lagoon	April 1986	44.8 ± 8.6	24 ; 10
H-1	Denman Island	April 1986	48.7 ± 6.8	30 ; 10
H-2	Denman Island	Jan. 1987	51.2 ± 7.5	0 ; 10
H-3	Denman Island	March 1989	39.5 ± 5.7	0 ; 20
I	Okeover Arm	May 1986	54.3 ± 6.4	30 ; 10
J	Read Island	July 1986	49.6 ± 18.4	30 ; 10
K-1	Sibell Bay	April 1987	42.1 ± 7.0	30 ; 10
K-2	Sibell Bay	April 1989	39.2 ± 5.1	0 ; 20
L	Mud Bay	May 1987	39.6 ± 4.3	30 ; 10
M	Savary Island	July 1987	49.7 ± 4.3	30 ; 10
N	Hornby Island	July 1987	48.0 ± 5.1	30 ; 10
O-1	Piper's Lagoon	Oct. 1987	45.8 ± 5.3	30 ; 10
O-2	Piper's Lagoon	March 1989	38.3 ± 2.7	0 ; 20
P	Boulder Point	March 1989	37.3 ± 5.0	0 ; 20
Q	Baynes Sound	March 1989	36.7 ± 6.3	0 ; 40
R	Qualicum Beach	April 1989	33.6 ± 2.9	0 ; 20
S	Parksville Beach	April 1989	32.8 ± 2.8	0 ; 20
T	Yellow Point	April 1989	30.5 ± 3.3	0 ; 20
U	Campbell Island	June 1990	46.1 ± 4.6	30 ; 10

* Localities are indicated on Figure 1 by the identity code.

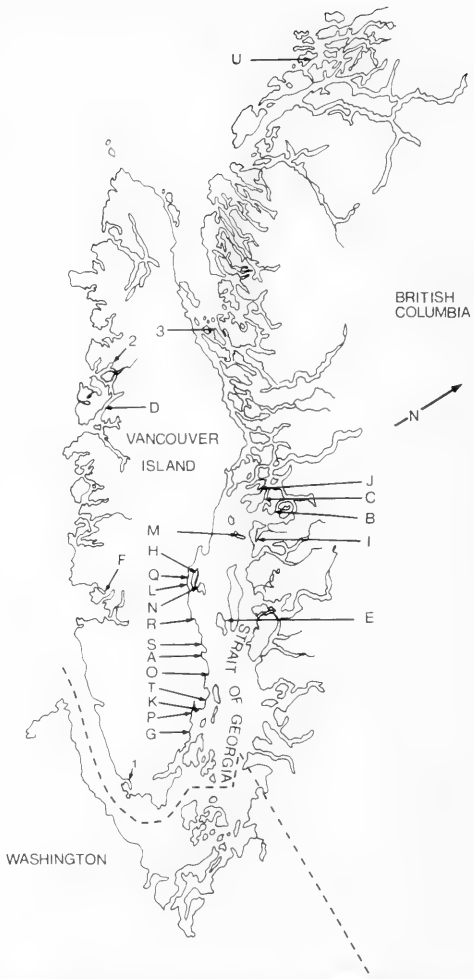


Figure 1. Map of coastal British Columbia indicating the localities by a letter code from which Manila clams (*Tapes philippinarum*) were sampled. The locality name, date, size, and number of clams examined is given in Table 1. Native littleneck clams (*Protothaca staminea*) were from localities indicated by a numeral code where: 1 = Sooke Basin where clams were collected from Roche Cove (head end) and Coppers Cove (at mouth) in December 1986; 2 = mouth of Little Espinosa Inlet, sampled in July 1986; and 3 = Canoe Pass on Turnour Island, sampled in May 1990.

fication) for 5 min. The remaining mantle, gills, adductor muscles, and visceral mass were separated, cut into thin slices if necessary, pressed between 2 glass plates (5.0 × 7.5 cm), and examined under a dissecting microscope (25× magnification) for large protozoans and metazoans. All organisms that were found were counted and removed for identification. Whenever possible, the organisms were photographed and preserved in a permanent collection (housed at the Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C., Canada, V9R 5K6, c/o Dr. Susan Bower).

An infection of *Nematopsis*-like spores (Apicomplexa) in the gills of one clam collected from Lasqueti Island in January 1986 was sufficiently heavy to allow for examination under a transmission electron microscope. Pieces of infected gill were fixed in 3% gluteraldehyde in 0.1 M phosphate buffer with 0.35 M sucrose (pH 7.2) for 2 hr followed by washing and 1 hr post fixation in 1% osmium tetroxide in the same buffer. After dehydration in ethanol and embedding in Epon, sections were stained with uranyl acetate followed by lead citrate and examined with a Philips electron microscope 300.

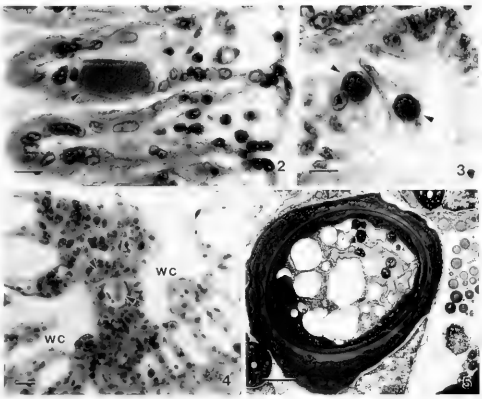
RESULTS

The majority of samples were obtained from the Strait of Georgia (18 of 21 localities) during the months of March through June (17 of 30 samples) (Table 1). All clams from all localities contained numerous unidentified free-living ciliates within the mantle

fluid or adjacent to the tissue lining the mantle cavity. In addition, various species of nematodes, rotifers, polychaetes, mites, chironomid larvae, ostracods, free-living copepods, and amoeba with tests (suborder Difflugina) were often observed in the mantle fluid. These organisms were not considered to be parasites or symbionts. Thus, they are not given further consideration in this paper. However, parasites or symbionts consisting of intracellular bacteria, protozoa, and metazoa were found (Table 2) and these form the basis of the present paper.

Colonies of intracellular bacteria (Rickettsiae or Chlamydiae) were observed histologically within the epithelial cells of the gills (Fig. 2) and the digestive gland tubules (Fig. 3). The infection in the gills was about half as prevalent as that of the infection in the digestive gland, the latter being virtually ubiquitous (Table 2). Also the intensity of infection in the gills was low (11 or fewer colonies per histological section) in all 22 infected clams. Intensity of infection in the digestive gland was heavy (greater than 25 colonies per histological section) in 6 of the 115 infected Manila clams. Identity of the intracellular bacteria from the gills and digestive gland is unknown and the bacteria may represent more than one species. There was no inflammatory response to any of the infections and only individual infected cells showed pathological changes. Even in the heaviest infections, only a low percentage of the epithelial cell population was infected. Overall pathology was therefore minimal and the infection had no apparent adverse affects on the clam.

Apicomplexan spores similar in morphology to *Nematopsis* sp. were observed at all localities (Table 2). The spores (about 20 μm in length and 8 μm in diameter) were usually found in the connective tissue of the gills and were frequently surrounded by a focal inflammatory response (Fig. 4). These spores were also occasionally observed in the connective tissues of the kidney and digestive gland but early developmental stages of this parasite were not found. An increase in prevalence at any given locality



Figures 2 to 4. Bacteria and protozoa observed in histological sections of Manila clams (*Tapes philippinarum*) from various beaches in British Columbia (haematoxylin and eosin stain, scale bars are 20 μm).

Figure 2. One colony of a Rickettsia-like intracellular bacterium (arrow) resembling a basophilic inclusion in the cytoplasm of an epithelial cell of a gill filament.

Figure 3. Two colonies of a Rickettsia-like intracellular bacterium (arrows) resembling basophilic inclusions in the cytoplasm of secretory epithelial cells of the digestive gland tubules.

Figure 4. Two *Nematopsis*-like apicomplexan spores (arrows) in the connective tissue between the water channels (WC) of the gill and surrounded by a focal inflammatory response.

Figure 5. Electron micrograph of a dead *Nematopsis*-like apicomplexan spore surrounded by several layers of tightly bound haemocytes from a heavily infected *T. philippinarum* (scale bar is 2 μm).

TABLE 2.

Prevalence of parasites and symbionts in Manila clams (*Tapes philippinarum*) from 21 localities in British Columbia, Canada.

Parasite/Symbiont	Localities*	Prevalence**
Rickettsia or Chlamydia in epithelium of gill	10: A-2,O-2,K-2,Q,C-1,E-1,H-1,H-3,M,P,S	11.8 (20–10)
Rickettsia or Chlamydia in epithelium of digestive gland	20: H-3,H-2,K-1,H-1,O-1,M,J,A-3,P,T,Q,U,A-2,I,O-2,S,C-1,E-2,L,N, K-2,G,B,E-1,F-1,F-2,R	27.7 (70–5)
Apicomplexan spores (*Nematopsis*-like)	21: R,E-1,E-2,N,A-2,H-3,O-1,L,J,H-1,O-2,A-3,S,T,C-2,Q,B,C-1,D, K-1,H-2,K-2,G,I,P,M,U,A-1,F-2	40.7 (85–2.5)
Apicomplexa, Gregarine-like	1: B	33
Apicomplexa, Coccidia-like	1: U	15
Trichodina spp.	18: C-2,N,E-2,O-1,M,B,C-1,I,F-2,H-1,L,K-1,J,A-2,E-1,K-2,G,A-1, Q,R,A-3,D,F-1,H-2,H-3,P	46.3 (100–5)
Order Rynchodida (attached to gills)	14: F-1,H-1,L,I,P,J,Q,K-2,A-2,K-1,H-3,F-2,E-2,S,G,M,C-2	9.6 (30–2.5)
Rhabdocoela turbellaria	19: O-1,B,K-1,C-2,A-1,N,U,E-2,E-1,C-1,P,S,G,M,K-2,A-2, O-2,L,J,A-3,Q,H-1,I,H-3,T,F-2	24.7 (60–5)
Trematode metacercaria	12: E-1,T,Q,H-1,L,C-2,U,A-3,A-1,A-2,G,I,N,O-1	6.3 (22.5–2.5)
Parasitic cysts	6: H-3,S,T,Q,E-2,O-2	14 (30–10)
Mytilicola orientalis	9: H-1,L,N,I,A-3,G,E-1,M,O-1	3.9 (7.5–2.5)
Pseudomyicola ostreae	4: H-1,I,A-2,E-2	2.5 (2.5–2.5)
Pinnotheridae	10: H-2,H-1,U,I,K-1,O-1,G,A-2,J,L,N	13.7 (60–2.5)

* Recorded as the number of localities where the organism was found in Manila clams: followed by the identity code of the localities (see Table 1 and Fig. 1) listed in descending order of prevalence of each organism.

** Recorded as the mean prevalence of infected Manila clams from all positive localities (followed by the range of prevalences in parenthesis).

was usually associated with an increase in the intensity of the infection. The majority of the spores (from a heavily infected Manila clam collected from Lasqueti Island in January 1986), as judged by electron microscopy, were dead (Fig. 5). The dead spores were encased in several layers of tightly bound haemocytes. Apart from the focal inflammatory reaction, there was no indication that this parasite was pathogenic.

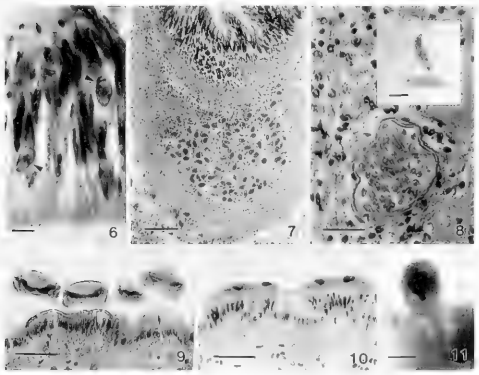
The pear-shaped Gregarine-like Apicomplexa (Fig. 6), found in Manila clams from Redonda Island in November 1985, were observed in 8 of 10 clams examined histologically and 5 of the 30 examined fresh. Most infections were light (fewer than 10 organisms per clam) but up to 150 organisms were observed in 4 Manila clams. Different morphological stages, including dividing forms, were not found. All of the organisms were associated with the gut and were located extracellularly in the gut epithelium, in the intestinal muscles, or in the surrounding connective tissue. There was no host response to the infection and the only pathology appeared to be a distention of the intestinal circular muscle bands of the most heavily infected clam to accommodate the parasite (Fig. 7). This parasite was not observed in Manila clams at other localities.

Sporocysts containing elongate sporozoites (28 μm by 4 μm, Fig. 8) of a Coccidia-like Apicomplexa were observed in the connective tissue around the gut in 6 of 40 Manila clams from Campbell Island. Similar organisms have been observed in 37% to 100% of the native littleneck clams (*Protothaca staminea*) from all four localities in British Columbia (Fig. 1) from which this species of clam was examined. Early developmental stages of this parasite were not observed in the Manila clams and the life cycle of the parasite remains unknown. Manila clams had little or no inflammatory response to this coccidian.

The only other parasitic protozoa observed were two types of ciliates. One type, *Trichodina* sp., was located along the inner surface of the siphons and occasionally adjacent to the foot or mantle (Fig. 9) of up to 100% of the Manila clams from 18 of the 21 localities (Table 2). High prevalences (>70%) occurred at all localities sampled during periods of elevated water temperatures (between July and November). In some Manila clams, the *Trichodina* sp. were closely attached to the outer surface of the epithelial cells (Fig. 10). However, there was never any indication of pathology, and the number of ciliates was insufficient for species identification (using silver impregnation techniques). The other ciliate, of the order Rhynchodida, was found attached between the epithelial cells of the gills (Fig. 11) of 30% or less of the Manila clams from 14 localities (Table 2). As with the *Trichodina* sp. the intensity of all infections was too low to obtain sufficient specimens for taxonomic investigations and there was no evidence of pathology.

Turbellaria (Graffillidae-like, of the order Rhabdocoela) (Fig. 12) were observed in the lumen of the gut (Fig. 13) and trematode metacercaria were found encysted in the connective tissues of the gills or adjacent to the digestive tract (Fig. 14). Although the turbellaria were more prevalent than the trematodes, the intensity of both metazoa was low (Table 2). The metacercaria consisted of: 1) unidentified species (could not be excysted intact by mechanical manipulation or digestion with pepsin and/or trypsin under various conditions (pH and temperature)); 2) representatives of the family Echinostomatidae as observed in littleneck clams; and 3) *Derogenes varicus* (family Hemiuridae) in Manila clams from Campbell Island. In addition, 16 Manila clams from 6 localities had a few cysts within the connective tissue adjacent to the digestive tract (Table 2). These cysts appeared to consist of a successful host response surrounding a degenerating metazoan, perhaps remnants of trematode metacercariae.

Two species of parasitic crustacea, *Mytilicola orientalis* and



Figures 6 to 11. Protozoa observed in histological sections of Manila clams (*Tapes philippinarum*) from various beaches in British Columbia (haematoxylin and eosin stain).

Figure 6. Pear shaped Gregarine-like Apicomplexa (arrows) between the epithelial cells of the gut (scale bar is 10 μm).

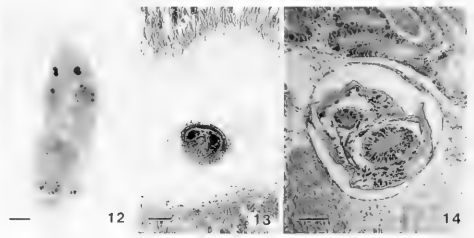
Figure 7. Large group of pear shaped Gregarine-like Apicomplexa between the circular muscle cells of the gut showing considerable muscle distortion but minimal inflammatory response (scale bar is 50 μm).

Figure 8. Sporocyst with elongate sporozoites in the connective tissue below the intestinal circular muscles (scale bar is 25 μm). Inset shows whole mounts of two sporozoites stained with haematoxylin and eosin (scale bar is 10 μm).

Figure 9. *Trichodina* sp. adjacent to the epithelium lining the inner surface of the siphon (scale bar is 40 μm).

Figure 10. *Trichodina* sp. attached to epithelial cells lining the inner surface of the siphon (scale bar is 40 μm).

Figure 11. Rhynchodida-like ciliate attached between ciliated epithelial cells of a gill filament (scale bar is 10 μm).



Figures 12 to 14. Metazoa collected from Manila clams (*Tapes philippinarum*) from various beaches in British Columbia (scale bars are 50 μm).

Figure 12. Whole mount of a gravid Graffillidae-like turbellaria from the intestinal tract (Semichon's acetic acid-carmine stain (Noble and Noble 1962)).

Figure 13. Histological section through the eye spots of a turbellaria in the lumen of the gut (haematoxylin and eosin stain).

Figure 14. Section through the anterior and posterior suckers of an encysted trematode metacercaria in the connective tissue adjacent to the digestive gland (haematoxylin and eosin stain).

Pseudomyicola ostreae, were rare in Manila clams (Table 2). Except for three *M. orientalis* detected in histological sections, crustacea were found only in clams that were examined fresh. No evidence of pathology was observed in association with the crustacean infections.

Forty five pea crabs (family Pinnotheridae) were observed in 42 of 404 Manila clams from 10 localities (Table 2). The majority of the crabs were immature or male *Pinnixa faba* and *Pinnixa littoralis*. However, one gravid female *P. faba*, one female *P. littoralis*, and two male and one immature female *Fabia subquadrata* were also recovered.

The sample of Manila clams from Campbell Island were collected near the northern limit of distribution for this species in British Columbia. Manila clams from this locality had an increased diversity of pea crab species and infections of a coccidia-like apicomplexan (Fig. 8) previously not found in Manila clams. In addition, what appeared to be ectopic clam gametocytes (mainly oocytes) in various stages of development and degeneration were occasionally observed throughout the tissues but mainly at the periphery of the mantle.

DISCUSSION

Most of the parasites and symbionts appeared to be randomly distributed throughout the Strait of Georgia. Disparities in observing certain organisms probably occurred—a consequence of the method of examination used and the small number of clams sampled in some instances. For example, the prevalence of intracellular bacteria in clams (only observed histologically) from localities in which only 10 clams were examined histologically may have influenced the accuracy of the results. Also, the lack of *Pseudomyicola ostreae* (only observed in fresh specimens) in localities from which clams were only examined histologically probably does not indicate the absence of these organisms at those particular localities. Most sampling sites were located within the Strait of Georgia and the lack of samples from the west coast of Vancouver Island and central coast of British Columbia prohibited a distribution analysis of any of the parasites outside the Strait of Georgia. Similarly, the collection of just over half of the samples between March and June prevented the seasonality of most parasites from being examined. Despite these inherent weaknesses in the data, interesting conclusions can be made.

In British Columbia, the accidentally introduced Manila clam is apparently free of infectious diseases that adversely impact on populations. Nevertheless, various bacteria, protozoa, and metazoa inhabit the tissues of these Manila clams either as parasites or as symbionts (in the latter case, the relationship between the Manila clam and associated organisms is unknown). Increases in prevalence of any particular organisms usually corresponded with an increase in its intensity. However, the high prevalence and intensity of one organism at any locality did not correspond with high prevalence or high intensity of other organisms. Also, parasites that have been associated with abnormal behaviour (e.g. a failure of clams to bury themselves in the substrate) in other clams of the Family Veneridae on the Pacific Coast of North America were not observed. For example, the coccidian in the kidney of *P. staminea* from Washington (Morado *et al.* 1984) and larval cestodes (*Echeneibothrium* sp.) in connective tissues of *P. staminea* and *Protothaca laciniata* from California (Sparks and Chew 1966, Katkansky and Warner 1969, Warner and Katkansky 1969) were not found in the Manila clams we sampled.

The relationship between the Manila clam and the observed organism varied from symbiotic (in this case defined as an association between two organisms in which the nature of relationship is unknown) to parasitic. An example of symbiosis was illustrated by the Graffillidae-like turbellaria. Turbellaria have been reported from the mantle cavity and alimentary tract of many species of bivalves worldwide but the exact status of the relationship has not been defined (Lauckner 1983). For species of *Paravortex* (family Graffillidae) from the intestinal tract of various lamellibranchs, Jennings (1974) and Jennings and Phillips (1978) considered the host-symbiote relationship to be midway between being "entocommensal" and parasitic. The acoelour turbellarian *Convoluta japonica* pathogenic to Manila clams in Japan (Kato 1951) was not observed in British Columbia.

The relationship between the *Trichodina* sp. and Manila clam also bordered on symbiosis. Although no pathology was observed in *Trichodina* infected Manila clams in British Columbia, the close association between some specimens and the mantle epithelium (Fig. 10) suggests that the relationship may be parasitic. Also, mortalities attributed to *Trichodina* sp. have been reported in cockles (*Cardium edule*) and oysters (*Crassostrea angulata*) in Europe (Lauckner 1983). Heavy intensities of *Trichodina* sp. as reported in European bivalves were not observed in British Columbia. Thus, the potential for *Trichodina* sp. becoming pathogenic to Manila clams in British Columbia is probably dependent on conditions that will allow considerable increases in *Trichodina* numbers. To date these conditions have not been encountered in British Columbia.

The wide distribution and benign host relationships of *Trichodina* and turbellaria suggests that these organisms may be enzootic to Manila clams and that they were introduced into British Columbia with their hosts. Other parasites observed that may also be enzootic to the Manila clam were the intracellular bacteria, the Rhynchodida ciliates, and both parasitic copepods (*M. orientalis* and *P. ostreae*). All these parasites have been reported from various bivalves in other parts of the world (Lauckner 1983). Without knowing the specific identity of the intracellular bacteria and Rhynchodida ciliates it is impossible to track the origin of these parasites. *Mytilicola orientalis* was thought to originate in mussels (*Mytilus crassitesta*) from the Inland Sea of Japan. This parasite now occurs in a wide variety of bivalves and has spread along the Pacific coast of North America and into coastal Europe through the movement of bivalves for culture purposes (Bernard 1969, Lauckner 1983). *Pseudomyicola ostreae* originally described from oysters (*Ostrea denselamellosa*) in Japan (Yamaguti 1936) is now known to occur in other bivalves from the Pacific coast of Asia (Nakamura and Kajhara 1979) as well as in at least *Crassostrea* oysters (*C. gigas* and *C. virginica*) and Manila clams in British Columbia. It is likely that both parasitic copepods also occur in Manila clams in enzootic areas of Asia. The long association between Manila clams and these parasites without indication of disease suggests that these parasites are not likely to be a problem in the future.

The remaining parasites, observed in the Manila clams, probably strayed from other hosts native to British Columbia. They can be grouped into one of three categories based on their potential for causing disease. The first category consists of parasites, such as *Nematopsis* sp. and the trematode metacercaria, that appear to have difficulty surviving in Manila clams. Many of the *Nematopsis* sp. that were examined with the electron microscope were dead. A morphologically similar and healthier looking *Nematopsis*

sp. is abundant in local mussels (*Mytilus* sp.) with little indication of pathology (Bower 1991). Known species of *Nematopsis* require development in a crab in order to complete their life cycles. In British Columbia, the normal cycle may occur in mussels and crabs with Manila clams representing aberrant hosts.

Manila clams were shown to be susceptible to infection by metacercaria (*Himasthla quissetensis*) usually found in marine molluscs on the east coast of North America (Cheng et al. 1966). Hence, the observation of various species of metacercaria in Manila clams from British Columbia was not unusual. Metacercaria of *Derogenes varicus* are normally found in free-living copepods, arrow worms, and hermit crabs (where progenesis may occur) and adult stages inhabit the gut of many species of fish (Koie 1979, Rohde 1982). Thus, the Manila clam is probably a "dead end" host for *D. varicus*. In addition, some of the metacercaria in Manila clams were being destroyed as indicated by the presence of the "parasitic cysts". This host response was similar to that described by Cheng and Rifkin (1968) in Manila clams from Hawaii against larval cestodes. Consequently, it was concluded that in British Columbia, Manila clams represent aberrant and hostile hosts for metacercaria of local species of trematodes. The various species of metacercaria, including the gymnophallid reported from Manila clams in Japan (Endo and Hoshina 1974) and species that were pathogenic for a closely related clam (*Tapes decussatus*) in Europe (Palombi 1934, Bartoli 1973) were not found in British Columbia.

The second category consists of parasites, such as the gregarine-like apicomplexan and possibly some Rhynchodida ciliates, that seem capable of surviving but are not pathogenic for Manila clams. A gregarine-like apicomplexan similar to that in Manila clams was observed in Pacific oysters from two different locations in British Columbia (Bower, unpublished data). In both hosts, there was no evidence of parasite multiplication. Although the identity and life cycle of this parasite is unknown, its low prevalence, low intensity, lack of associated pathology, and limited distribution suggests it is of no consequence for Manila clams in British Columbia.

The final category consists of parasites, such as the coccidia-like apicomplexan, pea crabs, and possibly some *Trichodina* sp., that currently occur in low prevalences but may be a cause for concern if they become more abundant. The identity and life cycle of the coccidia-like apicomplexan is not known. However, it appears to be a common parasite of native littleneck clams throughout British Columbia. The reason for the occurrence of this parasite only in the most northern stocks of Manila clams is uncertain.

The pea crab (*Pinnotheres sinensis*) reported as a pest of Manila clams in Japan (Sugiura et al. 1960) was not found in British Columbia. The three species that were observed are all enzootic to the Pacific coast of North America (Hart 1982). The presence of a gravid female *Pinnixa faba* in a Manila clam is unusual since adults of this species are typically found in the flat gaper (or hores clam, *Tresus capax*) (Hart 1982). Although no pathology (as reported in some bivalves infected with pea crabs (Pearce 1966)) was observed, high prevalence (about 50%) at one locality suggests that pea crabs may cause a problem in marketing Manila clams from some areas.

The unusual parasites and ectopic gametocytes observed in Manila clams from the host northerly population sampled (i.e. Campbell Island near the northern limit of Manila clam distribution) may be attributed to natural environmental conditions that are slightly stressful for Manila clams at this location. In order to substantiate this speculation, it would be necessary to compare parasite populations in Manila clams with those of other species of bivalves from this and more southerly locations using samples that were all collected at about the same time. The results of this comparison could further support the hypothesis that stress in bivalves reduces resistance to disease (Sindermann 1990).

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EMBRYONIC AND LARVAL RESPONSES TO SELECTION FOR INCREASED RATE OF GROWTH IN ADULT BAY SCALLOPS, *ARGOPECTEN IRRADIANS CONCENTRICUS* (SAY, 1822)

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ABSTRACT 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 the three selected lines tested when compared to the control (unselected) lines. 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 (control) line brood stock had significantly higher shell length values at 10 and 14 days when compared with those from two selected line stocks. Progeny of the control line stock 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 adult *Mercenaria mercenaria* L..

KEY WORDS: embryos, genetic selection, growth, larvae, scallop, survival

INTRODUCTION

While rearing several generations of the southern bay scallop *Argopecten irradians concentricus* as part of a quantitative genetic selection program (see Crenshaw et al. 1991) a pattern of lower survival rates among select line progeny was suggested at the mass culture level (pers. obs.). A similar trend was also detected earlier in mass culture work with selected lines of *Mercenaria mercenaria* and has been examined experimentally (Heffernan et al. 1991a). In the *Mercenaria* study, lower embryonic viability levels and slower larval growth rates were detected among the offspring of selected parents when compared to those from unselected control parents (Heffernan et al. 1991a, b). The purpose of this study was to examine experimentally the growth and viability levels of offspring from selected and unselected (control) lines of the southern bay scallop.

METHODS

The original brood stock employed in our genetic selection work with the southern bay scallop were collected in St. Joseph Bay, west of Apalachicola, Florida in the fall of 1987. Detailed descriptions of the methodology used in the propagation of successive scallop generations have been presented previously (Walker et al. 1991a, Walker et al. 1991b and Crenshaw et al. 1991). Briefly, a parental generation same-age cohort for the selection effort was established from the offspring of the 1987 wild-stock spawnings. In October 1988, this parental generation was subjected to truncation selection (upper 15.9% = *ca.* shell length mean of 1.5 standard deviations above the mean) with the offspring of the resultant select and control lines grown to maturity through November 1989 (facilitating the calculation of a heritability estimate, see Crenshaw et al. 1991). On November 18, 1989 another truncation selection process was initiated (upper 23.3% = *ca.* average shell length of 0.69 standard deviations above the mean) as outlined in Table 1, to produce F_2 generation select (SS) and control (S) line stocks, both from the F_1 line that had been subjected to one selection event. An F_2 generation stock (C) was also produced by mass spawning the F_1 unselected control line stock. Thus, we had three F_2 genetic cohorts available for experimental evaluation of offspring dynamics, namely; Control (unselected line = C), Select (line subjected to one selection event = S), and Select-Select (line subjected to two selection events = S-S). Following the calculation of a cut-off point for selection in the F_2 generation, S-S, S and C, brood stocks (N = 40/treatment) were established. These scallops were held in pearl nets (20 scallops/net) within three separate conditioning tanks (500 L). Temperature was held between 18–20°C, with each tank receiving equal daily amounts of a mixed monocultured algal diet (*Isochrysis galbana* strain T.Iso and *Chaetoceros muelleri* strain Chaet 10).

Embryonic Trials

Previous experience with the southern bay scallop suggested a tendency to spawn spontaneously in November within the laboratory holding tanks. All tanks were checked hourly during normal working hours (7am–6pm) to ensure rapid detection of spawning events. Spawnings took place on November 19, 1990 (S; egg total = 2.41×10^6), November 21 (C; egg total = 8.38×10^6), November 23 ($S\text{-}S_1$; egg total = 4.37×10^6), and November 27, a second spawning by the same cohort ($S\text{-}S_2$; egg total = 2.53×10^6). Following spawning, fertilized egg material was strained (20 μm mesh) from the holding tank *ca.* two hours after spawning was noticed, and a fertilized egg suspension (10 L) was established by adding filtered sea water. Five 1 ml subsamples were taken from each egg suspension and after enumeration under a compound microscope, a mean fertilized egg count was calculated for each event. A similar experimental design was subsequently employed to evaluate survival and growth rates for the offspring of each spawn. One liter flasks (N = 15 per treatment) were stocked with appropriate volumes from each egg suspension to create an initial estimated stocking density of 20 embryos/ml (i.e., 20,000/flask). Seawater in all replicates was filtered (1 μm), while temperature

TABLE 1.

Background information on the various selection events performed on bay scallop lines used in this study.

Parental generation shell length at selection (Oct. 1988).

Cohort Mean Size	Std Dev	Cut-off point	Select Mean
42.8 mm	4.37	46.90	49.12

F1 generation shell length at selection (Nov. 1989).

Control Line (C)			
Cohort Mean Size	Std Dev	Cut-off point	Select Mean
49.96 mm	3.79	—	—
Select Line (S)			
Cohort Mean Size	Std Dev	Cut-off point	Select Mean
50.99 mm	3.34	53.20	55.17

(25.4°C ± 0.4 SE) and salinity (26‰) were maintained relatively constant in temperature control rooms for the duration of the test (*ca.* 48 hours). In order to validate the initial stocking density levels, five 1 L replicates from each treatment were sacrificed immediately. Each of these replicate flasks was filtered through a 20 μm mesh and the filtrate concentrated in a small volume of seawater (20–30 ml) and fixed (v:v) with 10% neutral buffered formalin. Fixed samples were stained with Rose Bengal.

After 48 hours, the remaining replicates in each treatment were similarly processed, fixed and stained. Preserved samples were washed through a 20 μm mesh with freshwater prior to microscopic analyses. A 50 ml sample suspension in freshwater was prepared and three homogeneous sub-samples (1 ml) were obtained from each replicate suspension. These larval stages were counted on a compound microscope, and shell length data at 48 hours were gathered for all replicates, with 30 specimens per replicate measured randomly (100×).

Larval Trials

Prior to the establishment of each larval culture, one control, C, and two treatments, S and S-S fertilized-egg stages were mass cultured in 500L tanks at densities of *ca.* 20/ml. Forty eight hours after spawning, these tanks were drained through a 20 μm mesh sieve, and the filtrate resuspended in 10L. Enumeration of larval density in the 10L suspensions was carried out as for embryos. Twenty five replicate 1L flasks/treatment were then stocked to approximate an initial density of 1 larva/ml (i.e., 1000/flask, see above). Due to constraints of space and time, only the C, S, and S-S_1 treatments were examined for larval dynamics. Initial stocking densities were evaluated by the immediate sacrificing of five replicates per treatment. Further samples (n = 5) were taken randomly from each treatment on 6, 10, and 14 days following the establishment and the culture. All sample flasks were examined for evidence of set stages (spat), prior to draindown, using a stereo microscope (20–40×) at the time samples were taken. Replicates (i.e., larval flasks) were qualitatively classified as follows: fully set (none to very few swimming, >90% of larvae set); partially set (majority swimming, some set); or swimmers (none to very few set stages, >90% of larvae swimming). Sample processing, fixing, and staining were as for embryonic stages. Enumeration of all larval samples involved total counts. Shell length was randomly measured for 30 specimens/replicate in all cases. Water changes were carried out every other day and each flask received a daily food ration of 4×10^7 cells of T. Iso (i.e., 40,000 cells/ml concentration in each flask, see Heffernan et al. 1991).

Embryonic and larval data sets were analyzed by ANOVA using Statview 512$^+$ and SAS software. One way ANOVA and Nested ANOVA were used in the analyses of survival and shell length data, respectively. All percentage survival data were arcsine transformed prior to statistical analyses. The Nested ANOVA design (SL data) used an error term based upon the mean square for replicates.

RESULTS

Embryonic Trials

ANOVA revealed that differences among cohort initial densities (Table 2) were highly significant ($p = 0.0024$), with $C < S\text{-}S_1 = S\text{-}S_2$, according to a Scheffe F-Test ($\alpha = 0.05$). However, there were no significant differences (Scheffe F-Test $\alpha = 0.05$) detected between C and S nor among S, S-S_1 and S-S_2 cohorts. The mean density estimates for each cohort provide the best estimates of true densities and were employed in the estimation of percentage survival after 48 hours for each cohort (Table 2) (see Discussion). For example, the final density of each replicate (N = 10) within a cohort was divided by the mean validated estimate of initial stocking density for that cohort to produce a percentage survival figure. ANOVA showed 2d survival rate differences among groups to be highly significant ($p < 0.0001$) with $S < S\text{-}S_1 = S\text{-}S_2 < C$ as determined by a Scheffe F-Test ($\alpha = 0.05$) (Table 2).

Mean treatment shell length data after 48 hours ranged from 49.4 μm (S) to 63.5 μm (S-S_2) (Table 2). Highly significant differences among cohorts were detected by ANOVA (Table 2) and a Scheffe F-Test ($\alpha = 0.01$) revealed treatments to be significantly different from one another in the following order; $S < S\text{-}S_1 < C = S\text{-}S_2$.

Larval Trials

Analysis (ANOVA) of the replicates sacrificed at the start of the larval trials revealed mean initial stocking density differences among treatment to be highly significantly (Table 3). A Scheffe F-Test ($\alpha = 0.05$) showed all treatments to be significantly different from one another in the following order; $S\text{-}S_1 < C < S$. These validated mean treatment initial stocking densities have

TABLE 2.

Summary of data from two-day embryonic trials evaluating survival and growth rates among three cohorts of bay scallops in a selection program; Control (C), Select (S) and Select-Select (S-S; with two spawns, S-S_1 and S-S_2).

Cohort	Initial Density $\bar{X} \pm SE$	2d % Survival $\bar{X} \pm SE$	2d SL (μm) $\bar{X} \pm SE$
C	13,140 ± 896	48.5 ± 0.1	60.7 ± 0.6
S	17,080 ± 970	19.9 ± 0.7	49.4 ± 0.6
S-S_1	17,320 ± 1,071	27.3 ± 1.0	55.0 ± 0.7
S-S_2	19,240 ± 778	28.7 ± 0.3	63.5 ± 0.7
p value	.0024	<.0001	<.0001

P values represent the probability of differences among groups as determined by ANOVA.

TABLE 3.

Summary data from larval (2d–14d) trials evaluating survival and growth rates among three cohorts of bay scallops in a selection program, Control (C), Select (S) and Select-Select ($S\text{-}S_1$).

Cohort	Initial Density $\bar{X} \pm SE$	6d % Survival $\bar{X} \pm SE$	10d % Survival $\bar{X} \pm SE$	14d % Survival $\bar{X} \pm SE$	6d SL (μm) $\bar{X} \pm SE$	10d SL (μm) $\bar{X} \pm SE$	14d SL (μm) $\bar{X} \pm SE$
C	1,062 ± 21.7	74.7 ± 4.9	69.6 ± 13.8	29.0 ± 3.1	99.4 ± 1.0	151.4 ± 1.9	229.0 ± 5.9
S	1,286 ± 34.6	87.0 ± 2.0	74.1 ± 1.5	50.9 ± 4.8	90.9 ± 0.7	140.7 ± 1.9	173.3 ± 2.4
$S\text{-}S_1$	623 ± 29.7	55.3 ± 6.5	45.1 ± 3.8	29.8 ± 4.8	102.4 ± 1.1	131.0 ± 1.8	191.8 ± 3.6
p value	<.0001	.0056	.0772	.0053	.0002	.0116	.0025

P values represent the probability of differences among groups as determined by ANOVA.

been utilized in the calculation of survival rates for each subsequent sampling date (see Discussion).

Day six survival rates (ranged from 55.3% in $S\text{-}S_1$ to 87.0% in S), were revealed by ANOVA to have highly significant differences among treatments (Table 3). A Scheffe F-Test ($\alpha = 0.05$) showed that only treatments S and $S\text{-}S_1$ were significantly different from one another. Day 10 mean survival rates ranged from 45.1% in $S\text{-}S_1$, to 74.1% in S (Table 3). However, ANOVA revealed the differences among treatments to be non-significant ($p \sim 0.08$) (Table 3). Highly significant differences in 14d survival rates were detected among treatments by ANOVA (Table 3). A Scheffe F-Test ($\alpha = 0.05$) revealed that survival in treatments C (29.0%) and $S\text{-}S_1$ (29.8%) were not significantly different, while both were significantly lower in survival than treatment S (50.9%). Day 14 data must be interpreted in the light of setting activity, while no evidence of setting was detected on days six and ten. However, qualitative assessment of settlement behavior on day 14 revealed all of C replicates were classified as "fully set"; while 40% of S replicates were "fully set" with 60% still at the "swimming stage"; none of $S\text{-}S_1$ replicates were "fully set," while 40% displayed evidence of being "partially set," and 60% were at the "swimming stage."

Mean treatment shell length values ranged from 90.9 μm (S) to 102.4 μm ($S\text{-}S_1$) on day six (Table 3, Fig. 1). ANOVA (Table 3) revealed highly significant differences among treatments, while a Scheffe F-Test ($\alpha = 0.05$) showed S to be significantly lower than C and $S\text{-}S_1$, which were not significantly different from one another. By day 10, treatment shell length values displayed significant differences among groups ($p \sim 0.012$) (ANOVA, Table 3, Fig. 1). A Scheffe F-Test ($\alpha = 0.05$) showed that C was significantly greater than $S\text{-}S_1$, but S was not significantly different from C or $S\text{-}S_1$. Employing the same test, but using $\alpha = 0.1$, revealed that C was significantly different from both S and $S\text{-}S_1$, which were not significantly different from one another. Similarly, on day 14 highly significant differences among groups were found (ANOVA, $p = 0.0025$) and the control cohort C was significantly greater (Scheffe F-Test, $\alpha = 0.05$) than both select groups, $S\text{-}S_1$ and S, which were not significantly different from one another (Table 3, Fig. 1).

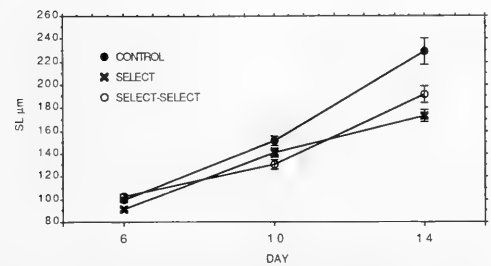

Figure 1. Mean shell length (SL) of 6, 10 and 14d larval stages from Control (C), Select (S) and Select-Select (S-S) cohorts of southern bay scallops. Error bars represent the 95% confidence intervals.

DISCUSSION

Strictly speaking, any significant results of these experiments apply only to descendents of that wild population from which the experimental selected and control populations were derived, namely populations of St. Joseph Bay on the northern Gulf coast of Florida. There are no factors of which we are aware that would suggest that this population is other than a single interbreeding deme. Future experiments will determine the extent to which our findings are applicable to other populations of the same species or to other species. Similarly, it would have been desirable to have replicated all treatments in this program within the same time frame. However, that would have required space and resources considerably greater than those available to us. Rather we have concentrated on basing our experiments upon the largest gene pools, i.e. numbers of spawning adults, practicable and the maintenance of the most carefully controlled experimental conditions possible. Growth rate heritability estimates already obtained for these populations (Crenshaw et al. 1991) will be refined in future generations of selection, and results reported here will be tested in future, more highly selected generations.

Our interpretation of the results of this study are contingent on the assumption of the validity of using verified initial stocking densities. It is clear that our attempts to establish intended densities, employing generally accepted techniques, were not as accurate as desirable, and that our verified densities are a great improvement. By using density validation procedures, we have reduced considerably the error variance that resulted from the procedure employed to establish initial densities. Initial cultures were established in such a way that density distributions among cultures within a cohort or treatment should be random, and it is reasonable to assume that such randomness would persist among validated culture densities within a cohort. Complete counts of initial densities of individual embryonic cultures would have been unacceptably time consuming, and, of greater importance, procedures involved in such counts could inflict serious and unpredictable damage on the delicate zygotes. We are fully aware that error variance is introduced by the assumption of a single initial cohort density for the calculation of survival rates. However, such vari-

ance would be considerably less than that eliminated by employing validated initial densities, and if significant differences emerge from ANOVA comparisons, it will be in spite of modestly inflated error variance, which, again, is not nearly so inflated as it would have been had we employed initial intended but unverified densities.

Inspection of the embryonic survival data show that the low validated initial density of the control, relative to densities of the selected lines, was associated with a significantly higher survival figure than that of the selected lines. The same association was not evident among the cohorts of the three selected lines. Initial densities were established intentionally at levels below which densities appear to have effects upon survival, and it is our contention that the observed differences detected among experimental treatment survival and shell length data are, by and large, independent of initial density. We offer the following points in support of this assumption: 1) In both the embryonic and larval trials, our verified mean initial stocking densities, which ranged from 13.1/ml–19.3/ml for embryos and 0.6/ml–1.3/ml for larvae, were well below density levels recommended for the hatchery production of this and other commercially important bivalve molluscs. Typical density levels recommended in the literature are as follows: for embryos; *Argopecten irradians* Lamarck—17 to 40/ml (Castagna and Duggan 1971, Castagna 1975), *Amusium pleuronectes* (Linne)—30/ml (Belda and Del Norte 1988), *Mercenaria mercenaria*—30 to 60/ml (Castagna and Kraeuter 1981), and *Crassostrea gigas* (Thunberg)—up to 100/ml (Wilson 1981), for larvae; *A. irradians*—5/ml (Castagna 1975), *Pecten maximus* (L.)—5 to 10/ml (Gruffydd and Beaumont 1972), *M. mercenaria*—15/ml (Castagna and Kraeuter 1981) and *Patinopecten yessoensis* (Jay)—1.5 to 1.75/ml (Bourne et al. 1989) and <2/ml (MacDonald 1988). Given that our density levels were below all of these reported critical levels, we assume the initial densities employed in our studies would not have caused significant effects on embryonic or larval survival or growth rates. 2) While there was a significant correlation between mean embryonic initial stocking density (X) and survival rates at 48 hours (Y) ($r = 0.71$, $p < 0.01$), there was little consistency to such "density effects." Group C had the lowest initial density and the highest survival rate, but S-S_2 survival was significantly higher than that of S, while S-S_2 initial density was also significantly higher than that of S. Furthermore, 3) size data at 48 hours (Y) in the embryonic trial were positively and significantly correlated ($r = 0.474$, $p < 0.01$) with initial stocking densities (X), which is the opposite to what one would expect in the case of a density dependent growth factor. Points 2) and 3) suggest that factors other than density were responsible for detected growth and survival differences among treatments. 4) A similar trend was evident in correlations between mean larval initial densities (X) and survival rate(s) (Y) at 6 ($r = 0.776$, $p < 0.01$), 10 ($r = 0.583$, $0.01 < p < 0.05$), and 14 days ($r = 0.426$, NS), where once again the observed trend in survival rates was the reverse of what one would expect in the case of a density dependent factor. 5) Day six data were the only data set which shows a significant correlation between initial density (X) and size (Y) ($R = -0.781$, $p < 0.01$). In spite of published reports to the contrary (Castagna 1975), these data suggest that there may have been negative effects on growth as a result of increased density in the first six days of larval development. However, it was only the greatest density (S) (initial level ~ 1.3/ml, density at day 6 ~ 1.1/ml) that was associated with significantly reduced size at day six. Due to the possibility of a complicating density factor influencing our day six data set, we will limit our interpretation of the effect of genetic selection on larval growth and survival dynamics to day 10 and 14 data sets. No significant correlations were detected between larval initial density levels (X) and shell length data (Y) at 10 or 14 days, nor could replicate density and replicate mean size be shown to be correlated on days 10 and 14. Both of these points suggest the densities employed in these tests were unlikely to have produced the observed differences among treatments.

Based on the embryonic trials, the simplest hypothesis is that genetic selection for increased growth rate in adult bay scallops had a significant negative impact on embryo survival rates. The unselected control group (C) had survival rates significantly higher than the three groups which had been subjected to selection pressure. It remains to be explained why the S group (59% lower survival than controls) faired worse than the two S-S groups (41–44% lower survival than controls) when it had been subjected to less selection pressure. These results are in agreement with experimental data reported for *Mercenaria mercenaria* (Heffernan et al. 1991a, b), and with mass culture experiences with this species (pers. obs.).

Size data at 48 hours showed the groups with the highest survival rates (C and S-S_2) to also have the largest shell length (with the two top groups in terms of survival having a reversed order in the shell length ratings). It is possible that initial egg size, for which we do not have data, is positively associated with embryo size at 48 hours. A positive correlation between egg size and survival at 48 hours would suggest that differences in egg size may have contributed to the patterns we have observed. Egg size has been demonstrated to significantly affect larval survival in *Argopecten irradians* and *M. mercenaria* (Kraeuter et al. 1982). Egg size may be related to the quantity and quality of nutrient reserves stored in eggs of bivalves, and the effects of selection pressure on such nutrient reserves, warrants further investigation (see Gallager and Mann 1986 and George et al. 1990 for examples of the impact of egg quality on subsequent development in bivalves and echinoids, respectively).

In general, there was no significant impact on larval survival rates associated with selection pressure during the 2–10 day period. Interpretation of day 14 data is complicated by the onset of settlement in groups C and S, where one would expect metamorphosis associated deaths. However, the relatively low survival rate of group S-S_1, which had not undergone settlement by day 14 and thus not yet suffered metamorphosis associated mortalities, would suggest a negative impact of selection on survival in at least one selected test group. The remarkably high survival of group S tends to contradict this finding. It is apparent that a post-settlement (nursery phase) study will be required to elucidate further details of the impact of selection on offspring performance (see O'Foighil et al. 1990 for a detailed analysis of mortality patterns in scallop nursery culture).

This study clearly demonstrates a negative impact of selection for increased growth rate in adult bay scallops on the growth rates of larval progeny. Shell length data for days 10 and 14 show the offspring of the unselected control to be larger at face value than those of both selected groups tested. Day 10 data, not complicated by settlement mortality factors, at face value, suggest a linear relationship between this negative larval growth effect and selection pressure (i.e., S-S_1 < S < C). However, the only significant difference ($p < 0.05$) is that between C and S-S_1, with that between C and S only approaching significance ($0.05 < p < 0.1$). Selected groups also displayed a delayed time to settlement and metamorphosis. Delay in the time of metamorphosis and a reduc-

tion in size at metamorphosis would both contribute to higher predation pressures in nature, effectively selecting against the progeny of "select" parents. This increased susceptibility to predation pressure coupled with reduced embryonic viability levels would constitute reductions in fitness traits, which are to be expected in a population subjected to appreciable selection pressure, according to the principle of genetic homeostasis (Lerner 1954). The reduced growth rate in the larvae of selected parents reported here is in agreement with that reported earlier for *Mercenaria mercenaria* (Heffernan et al. 1991a). As suggested in the same report (Heffernan et al. 1991a) these results bring into question the merits of hatchery culling practices, whereby smaller egg-larval stages are routinely discarded (Castagna and Kraeuter 1981). Commercial scale evaluations of culling practices from hatchery, selected, unselected (control) and wild stocks of bivalves would help in increasing our understanding of how best to handle these life stages (for maximum efficiency) in the commercial production of various species.

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DIFFERENCES IN INDIVIDUAL GROWTH RATE AMONG SCALLOP (*CHLAMYS TEHUELCHA* [(D'ORB.]) POPULATIONS FROM THE SAN JOSÉ GULF (ARGENTINA): EXPERIMENTS WITH TRANSPLANTED INDIVIDUALS

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ABSTRACT The basis for differences in individual growth rate among populations of *Chlamys tehuelcha* from the San José Gulf (Argentina, Patagonia) was examined. Scallops from 2 populations with different individual growth rate were (1) transplanted to a third site and (2) reciprocally cross-transplanted. In experiment 1, no growth differences were detected between the scallops originated from the two San José populations. In experiment 2, no growth differences were detected between indigenous and transferred scallops. The *C. tehuelcha* stock from San José Gulf has been characterized as a megapopulation with strong genetic flow among the component populations. My results strengthen this depiction and indicate that interpopulational differences are not due to genetic divergence among the San José populations.

KEY WORDS: scallop, *Chlamys tehuelcha*, growth, transplant experiments, megapopulation

INTRODUCTION

The *Chlamys tehuelcha* stock from the San José Gulf has been described as a megapopulation (Orensanz, 1986, Orensanz et al. 1991), that is, a number of local populations (grounds in this case) interconnected by some kind of dispersion (larval dispersal in this case; Roughgarden et al. 1985, Roughgarden and Iwasa 1986).

Orensanz (1986) documented differences in individual growth rate among populations of *C. tehuelcha* from San José Gulf along a NW–SE oriented environmental gradient (NW: higher growth; SE: lower growth). These interpopulational differences were attributed to the availability of phytoplankton and resuspended microphytobenthos (Orensanz 1986). Ciocco (1991) confirmed the pattern: a) mean productivity did not differ between a northern ground and a southwestern one, and was greater in both these localities than in the SE; b) scallop growth rate estimated for a strong year class settled in the 3 grounds showed a similar pattern: (N $\simeq$ SW) > SE.

There are, however, exceptions not explained by the environmental gradient in food availability. Ciocco (1985, 1990), for example, reported a ground with low growth rate in a zone with high to moderate food availability level, and suggested that these situation should be produced by the spionid polychaete *Polydora websteri* Hartman infestation. An alternative explanation might be genetic divergence among populations, as has been shown in other scallop species (Huelvan, 1985; Buestel et al. 1986). The objective of this study is to test this hypothesis.

STUDY AREA

The San José Gulf (Fig. 1) is a 814 km^2 basin with a mean depth of 30 m. Sublittoral bottoms include sandy and muddy sediments with different proportions of boulders and shells, and zones of compacted bare clay. The tide regime is semidiurnal (range = 2.9 to 8.7 m, mean neap and spring tides). Surface water temperature varies seasonally from 9 to 17°C. Mean salinity is 33.9‰ and mean concentration of dissolved oxygen 5.81 ml $\cdot$ l^{-1} (Pizarro 1976). Average gross and net annual production were estimated at 223 and 161 g C $\cdot$ m^{-2} $\cdot$ yr^{-1}, respectively (Charpy and Charpy 1977). Primary production is limited by the availability of nitrates (Charpy and Charpy 1977, Charpy-Roubaud et al. 1982). Abundance of phytoplankton has two peaks: the highest in spring (generally in early October) and the second, smaller, in late summer/early fall (February–March).

The Nuevo Gulf (Fig. 1a, b) is a semi-circular basin, about 2470 Km^2 in surface, with a mean depth of 104 m. The tide regime is semidiurnal (mean range = 3.78 m) and the mean salinity is 33.8‰ (Rivas, 1985). In Nueva Bay (Fig. 1c) surface water temperature varies seasonally from 9 to 18°C, mean concentration of dissolved oxygen is 4.45 ml $\cdot$ l^{-1} and mean chlorophyl a concentration is 0.71 mg $\cdot$ m^{-3} (De Vido de Mattio & Estevez 1978).

MATERIAL AND METHODS

Scallops from populations with different individual growth rates were (1) transplanted to a third site (Experiment 1), and (2) reciprocally cross-transplanted (Experiment 2). San Román and Tehuelche populations (Fig. 1d) were chosen since previous studies (Orensanz 1986, Ciocco 1991) reported that both the asymptotic maximum size (L_∞) and the growth constant (k; von Bertalanffy model) are greater in scallops from San Román than in those from Tehuelche (Fig. 2).

Experiment 1. One hundred scallops from the San Román population and 100 scallops from the Tehuelche population were collected by scuba diving and transferred to Nueva Bay, Nuevo Gulf (Fig. 1c). The experimental site was located 1.5 km off Cuevas Point (Puerto Madryn, Fig. 1c, sandy bottom, 8 m mean depth).

The scallops were mostly in age class 1+ (by growth ring reading). Scallops were labelled by etching the shell, measured (height, ±0.1 mm) and perforated in the anterior auricula. Steel hooks with 0.30 m plastic threads were passed through the perforation and tied at 0.25 m intervals to ropes fastened to the bottom. A total of 4 ropes with 50 animals each were deployed: 2 with San Román scallops and 2 with Tehuelche scallops.

Possible predators, especially volutid snails (*Odontocymbiola magellanica* (Gmelin), and *Zidona dufresnei* (Donovan)) and the crab *Ovalipes trimaculatus* Stelling (Decapoda, Portunidae) were removed from the study area before starting the experiment. The experiment started in June 2, 1988, and ran for 76 days. Four surveys (June 14, June 30, August 1 and August 16) were conducted to monitor scallop condition, to examine the state of ropes

Figure 1. The study area. a) general situation, b) South portion of the North-Patagonic Gulfs, c) Nueva Bay (Nuevo Gulf): study site (black circle) (depth in meters), d) San José Gulf: scallop grounds (shaded areas) and study sites (black circles) (depth in meters).

and hooks and to measure bottom water temperature. During the second inspection the ropes were raised 0.50 m off the sea bed to minimize snail attacks. The experiment was interrupted after the last survey to prevent imminent scallop losses due to hook deterioration.

Initial height (IH) and height increment (ΔH = final height − initial height) were compared by means of single classification ANOVA; homogeneity of variances was confirmed previously with Bartlett's and Hartley's F max tests (Sokal and Rohlf 1981). Covariance analysis was not applied due to linearity between IH and ΔH was not demonstrable, although logarithmic and square root transformations were attempted. Statistical tests were applied used the BIOM (Rohlf, 1986) statistical package.

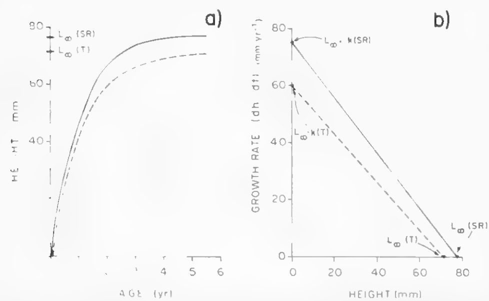

Figure 2. Growth curves (a) and growth rates: size relationships (b) from San Román (SR, solid line) and Tehuelche (T, dashed line) populations (von Bertalanffy model; figures fitted from Ciocco, 1991, data).

Experiment 2. Almost 400 scallops were collected by scuba diving at San Román and Tehuelche grounds (almost 200 at each site) between December 27 and 28, 1988.

Each individual was measured (height, ±0.1 mm) and tagged by etching the shell. Scallops were distributed as follows:

SAN ROMAN	TEHUELCHE
96 San Román scallops	96 Tehuelche scallops
96 Tehuelche scallops	96 San Román scallops

Scallops were kept suspended to prevent difficulties experienced in Experiment 1. The scallops were placed in "japanese" lantern nets (20 mm mesh, 4 compartments), held at approximately 2 m above the sea bed, in 6 m depth (mean low water). Indigenous and transplanted animals were held in alternate compartments at a density of 12 per compartment. In all, 4 nets were placed at each site. The experiment started in December 28, 1988, and ran for 119 days at San Román and 164 days at Tehuelche, due to difficulties in recovering lantern nets at the latter site. Growth (Δheight = final height − initial height) was compared between indigenous and transplanted scallops at each site through covariance analysis (x = ln initial height; y = ln Δheight) (Sokal & Rohlf 1981).

RESULTS

Experiment 1. Scallop transplant from 2 populations differing in growth rate to a third site.

Bottom water temperature varied between 8.8°C (August 1) and 11.5°C (Jun 2) (Jun 14 y Jun 30: 11°C; August 16: 9.2°C). Scallop losses (unfastened individuals) at the end of the trial were similar for scallops originating from the two sites (San Román: 36%; Tehuelche: 40%). Mortality (attached animals found dead at the end of the trial) was greater in Tehuelche scallops (63.3%) than in San Román scallops (32.8%) (significant at $p < 0.001$; chi-square test).

Height increment (ΔH = final height − initial height) at the end of the experiment (Table 1) did not differ significantly between the 2 populations (mean initial height of scallops from both populations were statistically similars, too).

Experiment 2. Reciprocal cross-transplants between 2 populations with different growth rate.

Bottom water temperature varied between 13°C (December 28) and 16°C (April 25) in San Román and between 13.8°C (December 28) and 16°C (April 26) in Tehuelche (June 9: 14°C). Scallop losses and mortality at the end of the experiment were similar among groups. Overall mean scallop loss was 7.28% and overall mean scallop mortality was 22.2%. Mean growth ($\overline{\Delta H}$) at the end of the experiment varied between 4.09 mm and 5.88 mm (Table 2). Growth did not differ significantly between indigenous and

TABLE 1.

Transplant from San José Gulf to Nuevo Gulf.

Original Population	$\overline{IH}$ (mm)	s($\overline{IH}$)	$\overline{\Delta H}$ (mm)	s($\overline{\Delta H}$)	n
San Román	56.8	3.35	1.53	1.09	43
Tehuelche	57.2	6.53	1.32	1.32	22

Mean initial height ($\overline{IH}$) and growth ($\overline{\Delta H}$) of the scallops surviving at the end of the experiment. (s = standard deviation; n = number of scallops). Experiment duration: 76 days.

TABLE 2.

Reciprocal transplants in San José Gulf.

Original Population	Placement Population	Experiment Duration (days)	$\overline{IH}$ mm	s($\overline{IH}$)	$\overline{\Delta H}$ (mm)	s($\overline{\Delta H}$)	n	Growth Rate ($\overline{\Delta H}$(mm)/119 days)
San Román	San Román	119	60.60	9.47	5.88	4.70	82	5.88
San Román	Tehuelche	164	61.02	10.28	4.95	3.93	63	3.59
Tehuelche	Tehuelche	164	62.72	6.53	4.39	2.64	77	3.19
Tehuelche	San Román	119	63.06	6.05	4.09	2.40	56	4.09

Mean initial height ($\overline{IH}$) and growth ($\overline{\Delta H}$) of the indigenous and transferred scallops (s = standard deviation; n = number of scallops surviving at the end of the experiment).

transplanted scallops at each site (covariance analysis; differences among adjusted $\overline{\Delta H}$: significant at $p < 0.75$; differences among slopes significant at $p < 0.25$).

DISCUSSION

Genetically determined intraspecific growth or reproductive schedule differences among geographically related scallop populations have been documented in *Argopecten irradians* (Lamarck) (Sastry 1966a, 1968) and *Pecten maximus* (L.) (Gibson 1956, Huelvan 1985, Buestel et al. 1986). However, in most other cases, the differences have been attributed to environmental factors, especially temperature, depth and food availability (Sastry 1966b, 1970, 1979, Caddy 1970, Posgay 1979, MacDonald & Thompson 1985, Bricelj et al. 1987, Eckman 1987, Schick et al. 1987).

If differences in growth between populations of *C. tehuelcha* from San José Gulf were due to a NW-SE environmental gradient in food availability (Orensanz's hypothesis), the expected results should be as follows: Experiment 1), similar growth in scallops from both original populations, and Experiment 2), similar growth in both indigenous and transplanted scallops at each experimental site, but different growth between grounds [greater in the N (San Román) and lesser in the S-SE (Tehuelche)].

Similar growth rates were detected in scallops from San Román and Tehuelche populations in the first experiment, although the trial ran only for 76 days between late fall and mid-winter [$\overline{\Delta H}$ (San Román) = 1.53 mm; $\overline{\Delta H}$ (Tehuelche) = 1.32 mm].

No differences were detected between transplanted and indigenous scallops at each site in the second experiment. This trial ran between early summer and fall for 119 days at San Román and 164 days at Tehuelche. Assuming linear growth (which is reasonable because of the experiment duration), it is possible to standardize and compare the growth between sites (grounds) starting from growth rate ($\Delta H/t$) estimations. Calculated growth for the period when the scallops were in the water at both sites ($\overline{\Delta H}$/119 days) tended to be greater for transplanted and indigenous scallops located in the N (San Román = SR) than for the transplanted and indigenous scallops located in the S-SE (Tehuelche = TE) (SR in SR = 5.88 mm; TE in SR = 4.09 mm; SR in TE = 3.59 mm; TE in TE = 3.19 mm).

Results of both experiments suggest that growth differences between the 2 populations compared are not due to genetic divergence. The *C. tehuelcha* stock from San José Gulf was described as a megapopulation (Orensanz 1986, Orensanz et al. 1991). Orensanz (1986) and Ciocco and Aloia (1991) reported inferential evidences supporting the concept of connectedness by larval dispersal among the component populations of this megapopulation. Genetic divergence among component populations of a megapopulation are expected when the connectedness among populations is weak. Orensanz et al. (1991) included these cases in the boundary between megapopulation and Rassenkreis (Endler 1977, Mayr 1982) concepts. Pectinid megapopulations comprise examples of weak interrelation (Sinclair et al. 1985) and cases of strong genetic flow among the component populations (Fairbridge 1953, Orensanz et al. 1991). The *C. tehuelcha* megapopulation from San José Gulf was included in the last class by Orensanz et al. (1991). My results strengthen this characterization and suggest that genetic divergence is not responsible for the interpopulational differences in growth rate that have been observed.

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IMMUNOTOXICITY OF CADMIUM FOR THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA* [GMELIN, 1791]): EFFECTS ON HEMOCYTE CHEMILUMINESCENCE

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ABSTRACT Reactive oxygen metabolites produced by phagocytically stimulated oyster hemocytes were quantified by a luminol-dependent chemiluminescence (CL) assay. In vitro exposure of hemocytes to sublethal cadmium concentrations resulted in dose-dependent suppression of CL, suggesting an impairment of the cells' ability to kill microorganisms via reactive oxygen intermediates (ROIs). The presence of autologous serum in the medium protected the cells from the CL-suppressive effects of cadmium. In serum-free medium, hemocyte-mediated CL was abolished by 3 h exposure to ~77 ppm cadmium; a 2.5-fold greater cadmium concentration was required to produce this effect in the presence of 66% serum. The in vitro exposure studies showed that a critical intracellular cadmium concentration was necessary for CL inhibition. In vivo attempts to modulate hemocytic CL activity by exposing oysters to ≤0.25 ppm cadmium for ≤2 weeks were unsuccessful, probably because the critical intracellular cadmium concentration was not achieved.

KEY WORDS: *Crassostrea virginica*, chemiluminescence, hemocytes, immunotoxicity, cadmium

INTRODUCTION

Environmental xenobiotics may exert a variety of sublethal physiological effects on the eastern oyster and other estuarine organisms. Immunotoxic effects can be particularly insidious because of their potential for delayed expression of decreased resistance to infectious disease. Bivalves rely heavily on phagocytic blood cells to defend against infectious agents; therefore, toxicants that interfere with normal hemocyte functions may increase disease susceptibility.

Molluscan phagocytes, like their mammalian counterparts, typically undergo a respiratory burst of metabolic activity upon appropriate membrane stimulation. This activity provides cytotoxic reactive oxygen intermediates (ROIs) that are involved in cell-mediated antimicrobial reactions (Adema et al. 1991). The respiratory burst is characterized by increased oxygen utilization, activation of the hexose monophosphate shunt, and production of superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$) and singlet oxygen ($^1O_2$). Aside from the direct cytotoxicity of ROIs, their activities can be amplified in powerful antibacterial systems of blood cells, such as that involving myeloperoxidase, H_2O_2, and halide (Klebanoff 1975). Nakamura et al. (1985) were the first to measure ROIs in mollusks by quantifying H_2O_2 production by scallop hemocytes. Since that investigation, the presence of ROI activity in many species of snails and bivalves has been confirmed. For example, Dikkeboom et al. (1987) reported that phagocytic stimulation of snail hemocytes resulted in O_2^- production and H_2O_2 generation. Anderson et al. (1992) quantified O_2^- generation via nitroblue tetrazolium reduction by *C. virginica* hemocytes.

The emission of photons or chemiluminescence (CL) during phagocytosis was first described by Allen et al. (1972) in mammalian polymorphonuclear leukocytes. The addition of luminol, a cyclic hydrazide that emits light upon oxidation, has been shown to enhance the CL signal strength (Allen and Loose 1976) and has proven useful in studies of low level ROI production by monocytes and macrophages. Previous studies have described luminol-enhanced CL by *L. stagnalis* hemocytes (Dikkeboom et al. 1987, 1988) and by *C. virginica* hemocytes (Larson et al. 1989, Fisher et al. 1990). It is widely accepted that the CL response of phagocytes is correlated with bactericidal activity associated with the respiratory burst (Welsh 1980, Horan et al. 1982).

The aim of this study was to measure the effect of exposure to sublethal cadmium concentrations on the chemiluminescent response of oyster hemocytes. Inhibition of hemocytic CL is an indication of immunosuppression and can result in reduced resistance to infectious disease. Cadmium is a priority pollutant found in water and bound to sediments, it is persistent in the environment and is bioaccumulated in the tissues of aquatic organisms (Eisler 1985, U.S. EPA 1991). Attempts were made to measure modulation of hemocyte chemiluminescence after in vitro exposure of the cells to cadmium and in blood cells withdrawn from oysters exposed to cadmium in vivo. An effort was also made to relate chemiluminescence modulation to intracellular cadmium concentration.

MATERIALS AND METHODS

Hemolymph samples were withdrawn from the adductor muscle hemolymph sinuses of 4–7 oysters and pooled in a conical plastic centrifuge tube held on ice to minimize cell aggregation. Large cell clumps and other debris were removed from the bottom of the tube and the cell suspension gently mixed. One-ml aliquots were immediately placed in pony scintillation vials for CL studies; other 0.5 ml aliquots were saved for subsequent cell viability determinations. This method allowed simultaneous evaluation of the effects of a range of Cd concentrations on the CL response and viability of cells from the same pool. The percent inhibition of control CL produced by various Cd concentrations was calculated

for individual aliquots from each cell pool, and these data were compared between pools.

The cells were exposed to Cd in the presence of *C. virginica* serum (+serum) or in serum-free medium (−serum). In order to minimize osmotic shock to the hemocytes, the media contained filtered ambient water collected from the oysters' tanks on the day of the experiment; the salinity ranged from 12–17 ppt. In the +serum series, $CdCl_2$ in 300 µl dextrose-augmented, filtered (0.45 µm) ambient estuarine water (DAFA) was added to 1 ml of whole hemolymph (as prepared above), and 20 µl of antibiotic-antimycotic solution added (200 U penicillin G, 0.2 mg streptomycin, 0.5 µg amphotericin B). In the −serum series, 1 ml whole hemolymph samples were centrifuged (525 xg, 10 min, 21°C), supernatant removed, cells resuspended in 1 ml filtered ambient water (FA), and centrifuged out of suspension as above. The hemocytes were then resuspended in 1.3 ml DAFA containing $CdCl_2$ and 20 µl of the antibiotic-antimycotic solution added. The final dextrose concentration in all vials (+ or −serum) was 5.55 mM. The CL assays on both + and −serum samples were carried out in an identical fashion from this point on.

Cadmium exposure was carried out in the dark for 3 h at 21°C. Glassware and reagents were dark-adapted >24 h and all CL procedures were carried out under dim red illumination. After exposure, the cells were washed free of Cd-containing medium, using the centrifugation steps outlined previously, and resuspended in 1 ml DAFA. Unexposed hemocytes in DAFA were also held for 3 h at 21°C and were subjected to the same washing and resuspension steps as the Cd-exposed cells. One ml of 250 µM luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) in FA was added to all cell aliquots and resting (unstimulated) CL levels were determined for 10 min with a Packard Tri-Carb liquid scintillation analyzer, programmed for CL quantitation. Then 0.3 ml FA was added, and the cells were stimulated by the addition of 0.2 ml of heat-killed yeast suspension (10 mg Sigma Chemical Co. type II baker's yeast/ml FA). The resultant CL was measured at 0.33 min intervals for about 1 h. Parameters calculated from control and Cd-exposed hemocytes included: 1. background CL: the level of CL emitted by the unstimulated cells immediately prior to the addition of heat-killed yeast; 2. peak CL: the maximal CL value elicited by the addition of yeast (corrected for background CL level); 3. total elicited CL: the area under the CL curve stimulated by the addition of yeast (corrected for background).

RESULTS AND DISCUSSION

The effects of Cd exposure on hemocyte CL activity are shown in serum-free medium (Fig. 1) and in the presence of autologous serum (Fig. 2). These data were generated from aliquots of the same cell pool exposed to various Cd concentrations for 3 h prior to CL determinations; the results are representative of those from the cell pools examined (6 pools −serum, 5 pools +serum). In all cases, both control and Cd-exposed hemocytes produced resting luminol-augmented CL in the absence of phagocytic stimuli. The addition of heat-killed yeast particles to untreated hemocytes induced a marked increase in CL activity that peaked after 5–7 min. This activity gradually decreased with time but remained above background level for more than 60 min. The presence of Cd in the medium consistently produced a dose-dependent suppression of hemocyte-mediated CL; however, higher concentrations were required to produce this effect when oyster serum was included in the medium (Figs. 1 and 2).

The data from the Cd-exposed hemocyte aliquots were expressed as percent inhibition of control CL of an untreated aliquot from the same pool; a summary of these data from six individual pools is presented in Table 1. Arc-sine transformation (Gomez and Gomez 1984) was carried out to normalize the raw percent inhibition data generated from the study. The resultant mean percent inhibition of the various CL parameters induced by 3 h exposure to sublethal cadmium levels are given in the table, along with the upper and lower 99% confidence intervals. These confidence intervals were selected to keep the experiment-wide error rate at ~0.05, using the method of Bonferroni (Tarone 1990). Cadmium concentrations that produced inhibitions with lower 99% confidence intervals <5% were considered to have no effect on

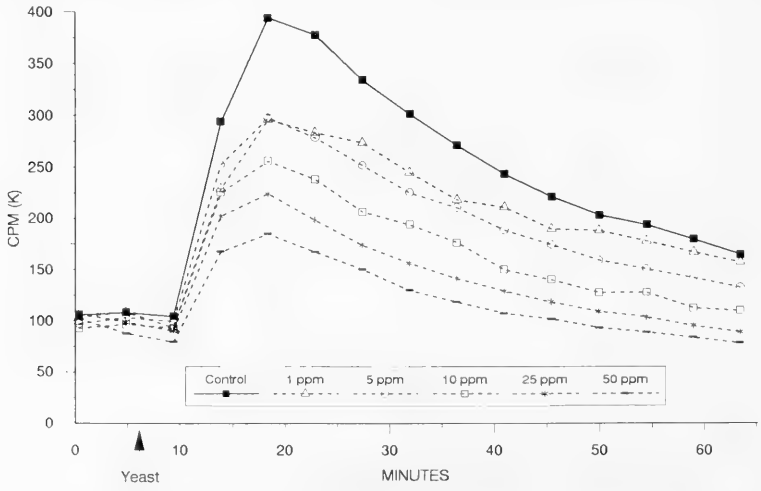

Figure 1. Effects of cadmium exposure on hemocyte chemiluminescence in serum-free medium.

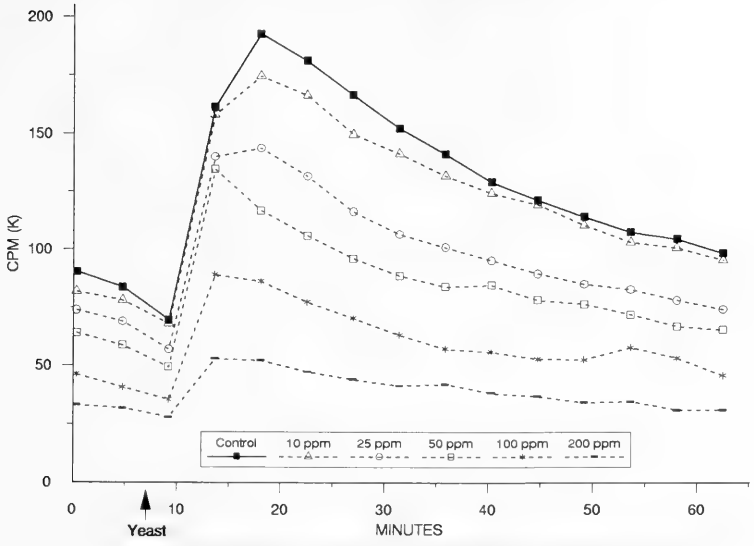

Figure 2. Effects of cadmium exposure on hemocyte chemiluminescence in the presence of serum.

chemiluminescence. The highest cadmium exposure levels used in the serum+ and serum− groups were selected based on their lack of 3 h lethality, by the trypan blue exclusion assay. Concentrations of 10, 25, and 50 ppm were run on both groups to enable direct evaluation of serum effects on CL.

Interpretation of the data in Table 1 leads to the following conclusions. Although a trend of increasing inhibition of basal CL with increasing Cd concentrations is seen, examination of the lower 99% confidence interval values suggests that there is no meaningful inhibition at any Cd level tested, with or without serum in the medium. However, Cd does suppress peak CL in the absence of serum ($\geq$10 ppm) and in the presence of serum ($\geq$100 ppm). A similar effect was seen in the case of total CL, where dose-dependent suppression was induced at $\geq$2 ppm (in serum-free media) and $\geq$25 ppm (in serum-containing medium). These data indicate that more cadmium was required to produce levels of inhibition of peak or total chemiluminescence comparable to those seen in the absence of serum. Other pieces of supporting evidence included the 3 h EC_{50} (effective concentration of Cd that produced 50% inhibition of peak CL after 3 h exposure, calculated by linear regression) data; these were 73 ppm in the presence of serum, and 21 ppm in the absence of serum. The cadmium effects on CL could not be attributed to lethality because, even at the highest concentrations tested, the average reductions in viability were minimal (3.2% at 50 ppm Cd, −serum; 1.5% at 200 ppm Cd, +serum).

The data were analyzed by ANOVA to determine the statistical significance of the changes in CL parameters observed following exposure to cadmium and the apparent protective effect of serum in the medium (Table 2). If significance is set at $p \leq 0.05$, it can be concluded that cadmium produced marked inhibition of phagocytically induced peak and total chemiluminescence, but caused no significant reduction in unstimulated, resting CL. Using the same criterion, serum in the medium protected against the CL-suppression by cadmium, regardless of which CL parameter was considered. As indicated in Table 2, there were no interactive effects (synergy, etc.) of the CL responses to cadmium and serum. The protective effect of oyster serum in these in vitro assays was predictable. No doubt extracellular Cd was bound to serum proteins and possibly to other ligands such as metallothioneins. The data reemphasized the fact that caution is needed in interpreting in vitro toxicity determinations. In this case, inclusion of autologous serum to mimic physiological conditions caused apparent reduced sensitivity, probably by reducing the actual dose of the toxicant available to the hemocytes.

Some preliminary attempts were made to determine the Cd concentration in the hemocytes under the conditions of the experiment. Exposure to Cd was carried out following the protocols previously reported, the cells washed thoroughly to remove unincorporated Cd and atomic absorption analyses of the cells was performed by the Riverside Clinical Laboratories (Newport News, VA). Cells were exposed to 200 ppm Cd (+serum) or 50 ppm Cd (−serum) for 3 h, treatments already shown to strongly inhibit the CL response. In this study, both treatments produced ~50% reduction in peak CL and total CL, somewhat less inhibition than would be predicted based on Table 1, but substantial. Hemocytes exposed to 200 ppm Cd (+serum) contained an average of 15.0 μg Cd/mg protein, those exposed to 50 ppm Cd (−serum) contained an average of 15.8 μg Cd/mg protein. Based on these limited data, it appeared that modulation of CL was primarily a function of cellular Cd level.

In this regard, we had previously attempted to study the effects on subsequent hemocyte CL after two weeks of in vivo exposure of oysters to water borne Cd (Oliver and Anderson unpublished). Several Cd levels were tried, including 0.25 ppm (nominal) which was selected as the highest experimental dose. Cadmium toxicity, as manifested by decreased condition index (Roesijadi and Klerks 1989) and reduced shell growth (Shuster and Pringle 1969), starts to become evident at ~0.20 ppm. The mean Cd concentration in

TABLE 1.

Percent inhibition of hemocyte chemiluminescence (CL) by cadmium.

		Serum-Free Media			Serum-Containing Media		
	Cd ppm	Mean Percent Inhibition	Lower 99% CI	Upper 99% CI	Mean Percent Inhibition	Lower 99% CI	Upper 99% CI
Basal CL	1	2.6	−2.3	20.8			
	2	5.9	0.7	16.1			
	10	5.7	−0.7	28.4	1.9	−2.3	17.0
	25	13.1	1.0	35.8	4.8	−1.3	27.8
	50	13.7	1.2	36.3	7.8	−0.4	34.7
	100				21.6	−5.8	87.5
	200				38.3	1.0	89.3
Peak CL	1	16.0	0.3	48.1			
	2	23.0	2.3	56.1			
	10	36.3	8.5	70.5	17.0	−1.9	69.7
	25	60.4	31.8	85.9	39.8	24.2	56.5
	50	66.3	43.9	85.4	34.4	0.9	84.1
	100				78.3	23.6	99.1
	200				92.5	62.8	99.0
Total CL	1	15.4	0.0	52.8			
	2	26.2	5.9	54.4			
	10	43.0	5.3	86.7	21.9	2.7	52.4
	25	69.1	38.5	92.5	34.8	24.5	45.8
	50	75.8	52.6	92.9	40.2	14.3	69.4
	100				74.4	51.9	91.5
	200				90.9	67.9	100.0

Basal CL = chemiluminescence produced by hemocytes prior to phagocytic stimulation.
Peak CL = maximal chemiluminescence recorded after phagocytic stimulation of the cells.
Total CL = the area under the curve of chemiluminecent activity induced by phagocytosis.

hemocytes from the 0.25 ppm group was 2.2 μg Cd/mg protein; Cd concentrations in hemocytes from unexposed oysters were several orders of magnitude lower. The effects on CL responses of hemocytes collected from oysters after in vivo exposure to a range of Cd concentration ≤0.25 ppm were variable and showed no significant dose dependency.

However, as reported in this paper, in vitro exposure of oyster hemocytes to sublethal concentrations of Cd can suppress CL, suggesting impairment of cell-mediated cytotoxic mechanisms, such as bactericidal activity. Such evidence implicates Cd as a potential immunotoxicant, by extension of the criteria developed for mammals (Tam and Hinsdill 1990). Our inability to show CL inhibition in the whole-animal exposure studies possibility may be explained by the fact that the requisite hemocyte Cd levels (indicated by the in vitro results) were not attained.

TABLE 2.

Percent inhibition of hemocyte chemiluminescence (CL) by cadmium: Direct comparison of 10, 25, and 50 ppm and effect of autologous serum.

	p Values, Data Analyzed by ANOVA		
	Cd Effect	Serum Effect	CD * Serum Interaction
Basal CL	0.1387	0.0205	0.9205
Peak CL	0.0078	0.0202	0.7635
Total CL	0.0052	0.0193	0.6235

Few reports exist concerning the effects of metals, or other environmental contaminants, on the CL response of bivalve hemocytes. Copper (≥4 ppm) was shown to inhibit phorbol myristate acetate-induced oyster hemocyte CL, whereas cadmium, aluminum and zinc were reported to slightly enhance CL at ≤160 ppm, but suppressed CL at a higher concentration of 320 ppm (Larson et al. 1989). It appears that our current method can more sensitively monitor CL modulation by Cd; this could result from differences in the composition of the media, length of exposure, and nature of the respiratory burst stimulus. As part of a study of tributyltin (TBT) on defense-related activities of oyster hemocytes, Fisher et al. (1990) showed that 0.4–400 ppb TBT produced a dose-dependent inhibition of zymosan-induced CL activity. Our data confirm and extend these observations on the utility of hemocyte CL modulation as a method to identify environmental xenobiotics with potential immunotoxicity for bivalve mollusks. Furthermore, CL provides a sensitive means of more fully understanding the details of protective mechanisms available to invertebrate blood cells.

ACKNOWLEDGMENTS

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TISSUE DISSEMINATION AND RETENTION OF MICROBE-SIZE ABIOTIC PARTICLES ADMINISTERED TO OYSTERS BY GASTRIC INTUBATION

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ABSTRACT We describe a technique which can be used to investigate the uptake of known numbers of particles while avoiding the selective processes associated with gill filter feeding in oysters. Particles the size of bacteria can be localized in the tissues, and total retention experiments allow investigations of "self cleansing" or depuration. Such experiments could simulate the retention of microorganisms that are resistant to digestion and slowly depurated. One can infer from our data that particles avoiding destruction by hemocytes or tissue phagocytes can persist for extended periods in oysters.

KEY WORDS: oyster, *Crassostrea*, particles, intubation

INTRODUCTION

Bivalve molluscs such as oysters and clams are gill filter feeders exposed to many kinds of abiotic and biotic particles in their environment. They possess elaborate selection systems, externally and internally, to handle these materials and guide them to regions of digestion or endocytosis. Although favorable for the bivalve, these systems complicate investigations on bioaccumulation, intracellular digestion, and depuration. Experimental particles are frequently administered suspended in the surrounding water and must passively await activity by the animal. Additionally, various degrees of size selection and rejection then take place when pumping and filtration ensue.

In order to administer a known quantity of particles, and bypass external rejection, a method has been developed whereby materials are deposited directly into the stomachs of oysters by intubation. Using fluorescent microspheres followed by sectioning or dissolution, these particles may be followed into the tissues of the animal and their distribution observed or uptake quantified.

Abiotic particles the size of single bacteria can be easily visualized in such preparations, and are not degraded or digested. The method thus provides a valuable comparative model for similar experiments designed to evaluate the persistence of microbes in bivalve mollusc tissues.

MATERIALS AND METHODS

Experimental Animals

Crassostrea virginica Gmelin were collected from Old Tampa Bay, Florida (27°, 53′, 20″ north latitude, 82°, 38′, 13″ west longitude) and maintained in the laboratory at 20°C in aerated, biologically filtered, recirculated artificial seawater of 27 ppt. salinity. Salinity and nitrate concentration were measured weekly, with water replaced regularly to control nitrate accumulation. Animals were kept unfed, not used immediately after collection, and routinely replaced. They were inspected for firm valve closure at the initiation of an experiment and for firm valve closure and body condition at its termination.

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Gastric Intubation Procedure

Oysters are prepared for intubation by cutting a window on the anterior ventrolateral aspect of the left valve just caudal to the beak with a Dremel #409 cutting disc in the chuck of an osteological drill (Fig. 1A). Four cuts are made at right angles to form a rectangle, about 1 cm across its narrow dimension, just under, but not damaging, the lip of the shell. The cuts initially penetrate the prismatic layer of the shell but should not go through the deeper nacreous (calcitostracum) layer. The prismatic layer can then be pried free, frequently intact, without perforating the shell. Next, a second shallow cut is made to perforate the shell window, allowing it to be broken away with a blunt instrument. This exposes the mantle of the anterior ventral region of the animal (Fig. 1B). When the mantle margins are repositioned and retracted, the labial palps and the mouth can easily be located (Fig. 1C).

The injection apparatus, consisting of an 18 gauge stainless steel feeding tube with the syringe fitting cut off is connected by polyethylene tubing to a Manostat 2ml microburette. It is loaded with an ultrasonically dispersed suspension of 2 micrometer diameter fluorescent polystyrene latex beads (Fluoresbrite, Polysciences, Inc.) in artificial seawater. The feeding tube is carefully inserted between the labial palps up and into the mouth which is easily probed and entered. Once within the esophagus, the tube is advanced up to a stop (a larger piece of tubing fit around the needle) serving to position its blunt tip within the stomach (Fig. 1D). After 0.05 ml of microsphere suspension has been injected, the tube is carefully withdrawn.

Following intubation, the window is closed using, when possible, the plate of prismatic shell removed when the opening was made (or alternatively with a plate cut from a donor shell). Strips of Parafilm "M" Laboratory Film rolled to a cylindrical diameter of 1–2 mm are made to line the rectangular opening, and the plate is pressed into place. The windowed surface of the shell is then coated with Trim Dental Plastic (a proprietary dental product of Harry J. Bosworth Co., Skokie, IL). This external layer of plastic is allowed to become firm, and loose sealing material is cut off. After the plastic has hardened and residual solvent dissipated in air, the animal is rinsed and placed in a 40 l artificial seawater environment.

Localization of Particles

To locate particles in tissues, oyster soft parts are removed by breaking the valve hinge with an oyster knife, and cutting the

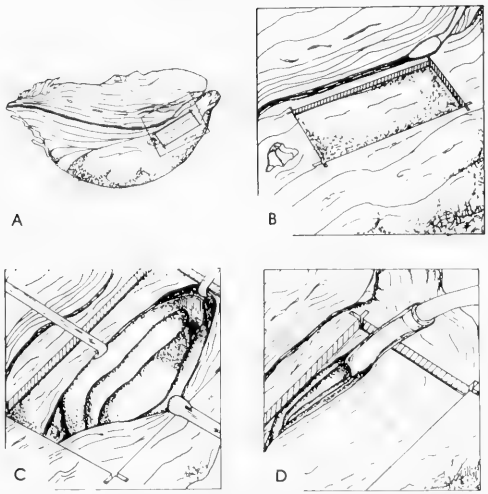

Figure 1. Descriptive views of windowing and intubation procedures: (A) *Crassostrea virginica*, external anteroventral aspect identifying window region on left valve, (B) Window from inset region of A with prismatic and nacreous layers removed and showing mantle, (C) Mantle margins repositioned and retracted to reveal underlying labial palps and mouth (dark area between anterior regions of inner and outer palps), (D) Pictorial showing feeding tube inserted into mouth up to "stop" tubing which has been positioned to place the terminal blunt tip within the stomach of the oyster.

adductor muscle attachments. Prior to sectioning with a Vibratome (E.M. Corp, Micro-Cut H1200), tissues are hardened in 15% formaldehyde in seawater overnight. A body region containing the stomach, digestive diverticula, and intestinal segments is mounted on a stub and 200 micrometer thick sections are made through designated regions using a Vibratome. These sections, affixed to a 1″ × 3″ slide, are then projected onto photographic printing paper using a photographic enlarger. A Zeiss epi-illumination fluorescence photomicroscope equipped with a Bioquant (R & M Biometrics, Inc.) image analyzing system is used to visually locate microspheres and place them on tracings of the photographic image.

Quantification of Particles

In order to estimate the retention of beads in the whole oyster, a tissue solubilization method is used. To do this the hinge is broken and the soft parts, excluding the adductor muscle, are removed. These are rinsed in distilled water and placed in a weighed, double, 80 ml capacity plastic bag to be inserted in a Stomacher paddle blender. The bag and contents are weighed again and wet tissue mass is determined by weight difference. Next, 30 ml of 5.25% Sodium Hypochlorite solution (commercial bleach) is added, and the Stomacher blender operated at half speed using a powerstat for two spaced 30 second intervals (these blenders "knead" the bag contents and avoid shearing forces generated by rotating blades, however, bag perforation can be a problem with this kind of animal tissue). The homogenate is then washed and rinsed into a 50 ml centrifuge tube with 45 ml of distilled water.

After centrifugation for 30 min at 1600 × g, the supernate is decanted into a second tube and made to 45 ml with distilled water. Twenty ml of distilled water are used to suspend the pellet. Both are re-centrifuged for another 30 min at 1600 × g, and the 45 ml supernate in the second tube is again decanted, discarded, and replaced by the 20 ml supernate from the first pellet. This supernate is re-centrifuged for another 20 min at 1600 × g, decanted, and discarded. The second pellet is re-suspended in distilled water and added to the first, the total, a suspension of the whole animal less adductor, brought to 10 ml with water.

Particles are suspended by sonication and five 10µl samples are applied to alcohol-cleansed slides and dried as spots about 1 cm in diameter. Using a fluorescence microscope, microspheres are counted in these volumes, averaged, and utilizing dilution factors, expressed as beads per 100 g wet weight of animal soft parts. Adductor muscles are not included because of fibers they leave in the suspension.

Measurements of Animal Activity

In order to compare activities of intact and intubated oysters, valve movements are monitored in experimental environments. To do this, oysters are mounted left valve down on Plexiglas bases with dental plastic. A stainless steel hook is affixed to the top right valve with the plastic, this in turn attached to a thread leading to the lever of a Harvard Apparatus rotary motion transducer. The shell motions are then recorded over intervals sufficiently long to establish meaningful activity patterns for intact, intubated, and sham-operated oysters. Experimental environments are typically stirred, air-saturated artificial seawater at 27 ppt salinity and 20°C.

RESULTS

Localization of Fluorescent Microspheres after Gastric Intubation

Uptake of microspheres from the gut of oysters occurs rapidly after intubation. Figure 2A shows a typical Vibratome section taken through the stomach region at 48 h after intubation. Accompanying this section is a tracing, Figure 2B, showing the distribution of fluorescent microspheres. It can be seen that the particles are widely disseminated, even to the mantle edge. Beads also remain surrounding gastric and intestinal structures and in the digestive gland region. On the side of the animal away from the gill region are three clumps of beads too concentrated to count (numbers estimated). The outer two clumps appear to represent beads in the lumina of kidney tubules, the inner clump has no recognizable space closely associated with it and may have been displaced from a hemolymph sinus.

Activity Records of Intubated Oysters

Figure 3 shows a valve activity record of an intubated oyster. Such records show that the valve activity behaviors of intubated and intact oysters do not appear to differ substantially over our typical experimental periods (72 hours maximum).

Total Retention of Microspheres after Intubation

Since measured amounts of suspended particles can be administered, the number recovered in solubilized oysters can be used to estimate the retention of particles at designated times after intubation. Anatomical studies have shown that the gastric region of oysters 10 cm in length can easily accommodate 0.05 ml of intubate. This is our standard volume, and typically contains an elec-

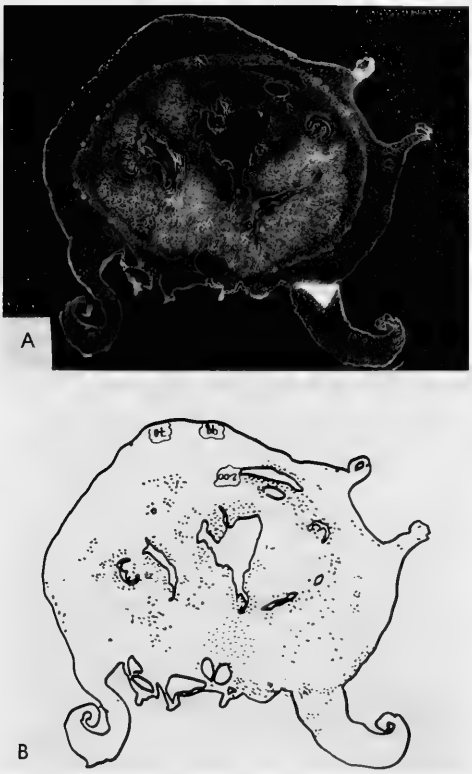

Figure 2. (A) Vibratome section of oyster used to identify internal anatomical structures. Posterior aspect of section with left mantle margins at lower left and upper right. Right mantle margins at upper right and lower right. Alimentary structures and major hemolymph sinuses are easily identified. Animal processed 48 h after intubation. (B) Tracing of "A" showing the dissemination of fluorescent microspheres (black dots) throughout the animal. Three dense aggregations are seen at top with numerical estimates of beads labeled.

tronically-counted 450,000 beads. Oysters examined within one hour of intubation retain about one third of the administered microspheres (time zero). After 24 hours under standard conditions (circulating air-saturated artificial seawater, 27 ppt, 20°C), the numbers dropped an order of magnitude and remained about the same until the experiment was terminated at 72 h. Figure 4 shows these data. After 24 h particles are lost, but on a whole animal basis, per gram loss over the next 48 h did not represent a log change in numbers.

The total retention investigations have indicated that uptake of microspheres from gastric intubation is not complete using the inoculum indicated. Less than half of the particles are retained initially, and although retention is variable, the particles apparently persist in the tissues for days afterwards. In localization experiments we have followed them for up to 10 days (Alvarez et al., 1992).

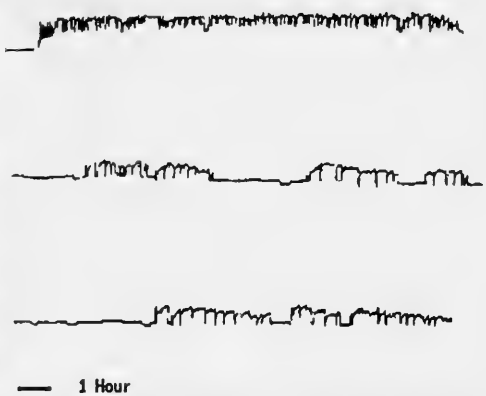

Figure 3. Computer scanned and edited partial record of valve movement of windowed oyster. Continuous record beginning at top left with open valves deflecting upward. Activity was indicated over approximately 60% of a 38 h time period.

DISCUSSION

These investigations were undertaken to study the uptake of bacteria-size particles introduced into the gastric region of the alimentary canal of oysters. The data shown are representative of many such experiments we have performed. Control experiments have shown that when dead (formaldehyde fixed) animals are intubated, uptake does not occur and microspheres are not distributed within the body tissues as in living animals. This control preparation also demonstrates that microsphere "smearing" does not happen as a result of sectioning.

Since the intubated particles are indigestible, once in the tissues, those retained may be identified days after administration. This allows accumulation as well as self-cleansing (depuration) to be investigated. However, there appear to be both differences and similarities in the way these materials are handled by the animals when compared with results of investigations employing other routes of administration.

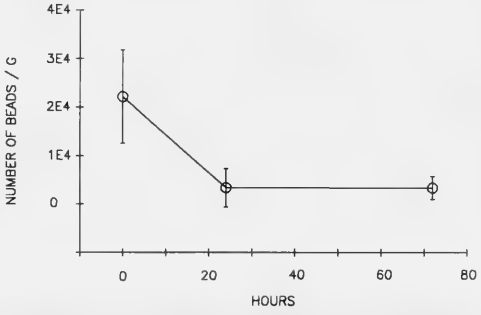

Figure 4. Microspheres retained by *Crassostrea virginica* after gastric intubation. Each circle represents a mean for 5 oysters with standard deviations indicated. After time zero (1 h), they were kept in air-saturated artificial seawater at 27 ppt salinity and 20°C. These data represent one of six similar datasets including over 90 intubated animals.

The seminal investigation by Stauber (1950) wherein he inoculated India ink particles into the circulatory system of eastern oysters indicated that arterial vessels were occluded by the material followed by its removal by phagocytes. Subsequently, they were distributed throughout the body, and eventually were transported through epithelia to the outside of the animal. The arterial occlusion was cleared in 17 days. Tripp (1958, 1960), inoculating bacteria, yeast, and erythrocytes intravascularly made similar observations. Phagocytosis and diapedesis ensued and the particles were eventually cleared from the animal. Living organisms that were susceptible to intracellular digestion were soon killed and degraded, whereas resistant stages were shed over a longer period of time (Tripp 1960).

Cheng (1984) and Feng et al. (1977) have presented evidence to indicate that in bivalves, two-way trafficking of hemocytes takes place, the gut to tissue pathway being related to nutrition. They suggest that the phagocytes in some way distinguish food particles.

Our research indicates that inward movement of abiotic particles does indeed occur, and that subsequent distribution throughout the body of the animal follows. Based on observations of phagocyte interactions with microspheres and examination of sectioned material, we feel these cells are the carriers of these particles. It is also clear that the columnar epithelium of the intestine is a barrier and that transport through this layer is a biological event. It is reasonable to assume that in lamellibranchs luminal phagocytes internalize materials and traverse the lining of the alimentary canal, and considering ours and other investigations (e.g., Yonge 1926), one might infer that these phagocytes then become or behave as hemocytes and distribute materials throughout the body of the animal. Assuming phagocyte intervention, the uptake of abiotic particles that we have demonstrated indicates a lack of discrimination for nutritive particles, and suggests that luminal phagocytes are recognizing "non-self" in the same way hemocytes and tissue phagocytes do. Particle internalization and transport would then depend largely on surface characteristics of the particle, and specific or non-specific recognition abilities of the phagocytes. Finally, Galtsoff (1964) cites evidence that in lamellibranchs bacteria may serve as food. This is reasonable for a filter-feeding organism, and if so, bacteria-size particles in the gut may be expected to be ingested by phagocytes and distributed in the same way we have shown abiotic particles to be disseminated. Those bacteria digested would be destroyed, but if a bacterium escaped digestion, it might become a non-pathogenic tissue resident that could be retained for extended periods. In fact, Feng (1966) has shown that patterns of removal of intracardially injected bacteria differ depending on the species, some remaining relatively constant in numbers, or even increasing before elimination. It has been suggested that for susceptible particles (Feng 1988, Tripp 1970) destruction by digestion is more important than loss in migrating cells. In any case, particle-cell interactions and phagocyte behavior have important implications for cleansing shellfish of human pathogens by various depuration processes.

Our research strongly suggests that pathways of accumulation of shellfish with exogenous microorganisms are not entirely contaminative accidents but at least partially biologically mediated events. As such, enhancing effective tissue depuration of potential human pathogens that initially evade phagocytosis or phagosomal degradation by these animals undoubtedly requires a more intimate knowledge of their cell biology, physiology, and behavior than what is presently available.

ACKNOWLEDGMENT AND DISCLAIMER

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EFFECTS OF MARINA PROXIMITY ON THE PHYSIOLOGICAL CONDITION, REPRODUCTION, AND SETTLEMENT OF OYSTER POPULATIONS

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ABSTRACT Concerns over the possible effects of marinas on nearby shellfish beds prompted a study to evaluate physiological condition, gametogenesis, and settlement of oyster populations, *Crassostrea virginica*, in relation to marina proximity. Results indicated no major differences between a marina and control site with respect to oyster condition or gametogenic state. However, significantly lower spat settlement was noted on artificial substrates at the marina versus the control site throughout much of the spring and summer. In order to clarify whether this pattern was typical, oyster settlement patterns were studied around three other marina complexes. Two of the marinas were located in well-flushed waters and the third was situated in a poorly flushed embayment. Replicate fouling tubes were placed intertidally at several locations within each marina, and at pre-determined distances away from the marinas in both directions. Spat settlement within each of the two well-flushed marina complexes was not significantly lower than settlement outside the marinas, which suggested that the reduced number of spat observed at the marina studied previously was probably due to some other factor. Very little spat settlement occurred in or around the poorly flushed marina.

INTRODUCTION

Studies recently completed in several southeastern states have documented elevated levels of metal and hydrocarbon contaminants in sediments collected from numerous marina complexes compared to reference sites (Marcus and Swearingen 1983, Voudrais and Smith 1986, Wendt et al. 1990, NCDEH 1991). There is also documented evidence of reduced water quality at some marina locations, particularly those having poor flushing characteristics (Nixon et al. 1973, Marcus and Swearingen 1983, Marcus et al. 1988, NCDEH 1990). However, relatively few studies have evaluated the ecological effects of marinas on the estuarine biota within and adjacent to marinas (Reish 1961, 1963, Nixon et al. 1973, Soule and Oguri 1977, Holmes et al 1985, Marcus and Swearingen 1983, Marcus et al 1989, Wendt et al. 1990).

In South Carolina, oyster populations represent one of the primary resources of concern to the state's regulatory agencies and the public. Although current regulations prohibit the construction of new marinas in waters open to shellfish harvesting ("SFH" waters), many existing marinas were built in waters now classified as SFH prior to these regulations being implemented. Marinas also occur in waters having lower water quality standards and extensive shellfish resources adjacent to these facilities. Oyster tissue samples collected at a few of these marinas frequently had relatively high concentrations of some metals and polyaromatic hydrocarbons [PAHs] relative to nearby control areas (Marcus and Stokes 1985, Marcus and Thompson 1986, Marcus et al. 1989, Wendt et al. 1990). However, relatively little is known about the effects of marinas on shellfish settlement, physiological condition, gametogenesis, or other aspects of oyster biology.

This paper describes results obtained from studies designed to (1) compare the physiological condition and gametogenic state of oysters collected at a marina facility in South Carolina and a nearby reference site, and (2) evaluate settlement patterns of oyster spat relative to marina proximity at several marinas in different hydrologic regimes.

METHODS

Study Sites

During 1986, oyster physiological condition, gametogenic state, and spat settlement patterns were measured at two locations near Hilton Head, South Carolina: the Skull Creek Marina and a nearby reference area (Fig. 1). The Skull Creek Marina is a moderate-size facility (100 slips) located in a broad tidal creek with no other obvious sources of pollution nearby. It has fuel and sewage pump-out facilities and had been in operation for approximately 8 years prior to the initiation of this study. The reference area was located in an undeveloped area of Mackay Creek about 3 km northwest of the marina (Fig. 1). Within this area, one site (A) was used to collect oysters for gametogenic and condition index analyses. This site had an extensive oyster bed that was comparable in tidal height to the oyster bed sampled on the shoreline behind the Skull Creek marina. Another site (B) was selected on the opposite shore of Mackay Creek for the spat settlement studies. This site was similar to the marina with respect to distance from Port Royal Sound and shoreline orientation (Fig. 1). Both Skull Creek and Mackay Creek are well-flushed tidal systems (mean tidal range of 2.1 m) that drain into the lower portion of Port Royal Sound in approximately the same location. In the vicinity of the study sites, each creek was approximately 400 m wide.

A second series of studies was conducted in 1989 at three additional marinas, all located near Charleston, South Carolina. The Stono River/Buzzards Roost marina complex consisted of two commercial facilities located adjacent to each other along a portion

Contribution 308 from the South Carolina Marine Resources Center.

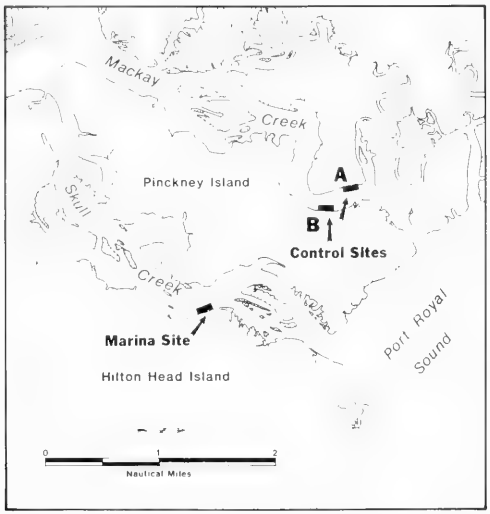

Figure 1. Location of marina and control sites sampled during the first study phase. Control site A was used for condition index and gametogenic studies. Control site B was used for spat settlement studies.

of the Stono River that was well-flushed tidally (Fig. 2). Together, these two marinas have approximately 400 slips, forming one of the larger marina complexes in the state. The Bohicket Creek marina was also located in a well-flushed section of a large tidal creek (Fig. 2) and was moderate in size with approximately 140 slips. The Toler's Cove marina was a moderate-sized marina (138 slips) located in a dredged basin that was connected to the Atlantic Intracoastal Waterway by a small tidal creek (Fig. 2). Tidal current velocities in this marina basin were not as great as those observed at the other marina sites. All of the marina complexes sampled in 1989 had been in operation for at least five years prior to the beginning of this study and all had fuel and sewage pump-out facilities. The shoreline adjacent to all three marina facilities supported extensive salt marsh habitat with abundant intertidal oyster banks along the banks. There were no obvious sources of pollution near any of the marinas. Reference sites for each marina were located along the adjacent shoreline at varying distances from the marinas (see settlement methods section).

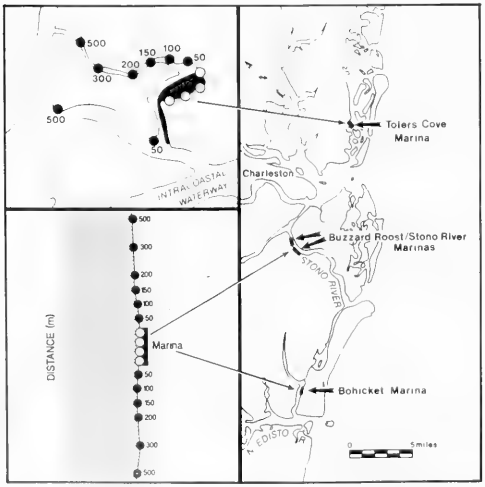

Figure 2. Location of marinas sampled for oyster settlement patterns during the second study phase, and approximate location of sampling sites in and near each marina. Four stations were located within the boundaries of the Bohicket Creek marina whereas there were six stations located within the Buzzards Roost/Stono River marina.

Condition Index of Oysters

Biweekly samples of oysters were collected from the Skull Creek Marina and the Mackay Creek site from February to July, 1986 in order to determine physiological condition before and during the reproductive season. Approximately 25 oysters (>50 mm shell height) were gathered from various locations within the intertidal beds at each site and brought to the laboratory for analysis. Water temperature and salinity were recorded on each sampling date.

After a thorough cleaning, the oyster meat was removed from the shell and wet weight measurements of the shells and meat were taken separately. The shell and meat were then dried for 24 h at 100°C and weighed again. A volumetric condition index (CI) was calculated using the formula described by Lawrence and Scott (1982):

$$CI = \frac{\text{dry meat weight } (g)}{\textit{internal shell cavity capacity } (g)} \times 100$$

Shell cavity capacity was determined by subtracting dry shell weight (g) in air from total live weight (g) in air of the cleaned oyster. This index is a more sensitive indicator of stress than other indices which have been used for evaluating physiological condition of bivalves (Crosby and Gale 1990), and has been frequently used as an index in previous studies of *C. virginica* (Lawrence and Scott 1982, Abbe and Sanders 1988, Marcus et al. 1989, Crosby and Gale 1990). Values obtained at the marina and at control sites using the condition index were statistically compared for each sampling date using a t-test on data transformed with the arcsin $\sqrt{CI/100}$.

Oyster Gametogenesis

Additional oyster samples were collected from February to July at the Skull Creek Marina and the reference area to compare gametogenic condition. Collected oysters were transported to the laboratory in cool, dry containers to avoid premature aborting of gametes. Shell heights of 25 oysters from each study site were measured to the nearest millimeter using a vernier caliper. Physiological condition (Quick and Mackin 1971) and macroscopic gonadal condition (Burrell et al. 1984) were determined before shell removal. The visceral mass (gonad, digestive diverticulum) was then dissected and fixed in formalin-acetic acid-alcohol (FAA) for three to four weeks. Following dissection, three cross-sections were excised, washed in running tap water for approximately one hour, and stored in 50% ethyl alcohol. The tissues were then prepared histologically for microscopic examination, by dehydration in alcohol, clearing in xylene, and infiltration in paraplast at 57°C (Preece 1972). After embedding in paraplast, three 7

μm sections were taken from each sample at approximately 20 μm intervals.

After examination of gonad preparations, a gametogenic index was devised, incorporating staging concepts from other studies of *Crassostrea virginica* (Kennedy and Battle 1964, Berg 1969, Loosanoff 1969, Kennedy and Krantz 1982) and other bivalves (Durve 1965, for *Crassostrea gryphoides;* Keck et al. 1975, Eversole et al. 1980, Manzi et al. 1985, for *Mercenaria mercenaria*). Table 1 lists the criteria used for the various reproductive stages considered in this study. The frequency of oysters in each reproductive stage was statistically compared between the control site and marina site samples using a chi-square analysis for each sampling date.

Spat Settlement Studies

During 1986, two arrays of artificial substrata were placed at the Skull Creek marina and Mackay Creek control site to compare oyster spat settlement over the course of one reproductive season, and to evaluate the suitability of different test surfaces. Both arrays were placed at identical elevations within the lower intertidal zone of each site, with all arrays oriented parallel to the shoreline to minimize effects of shading and desiccation. Artificial substrata placed at the marina were located adjacent to the dock facilities, approximately midway along an unshaded section of shoreline spanned by the marina.

One array of test substrata consisted of 225-cm^2 formica panels having the texture and color of dark gray slate. Four replicate panels were suspended vertically from a PVC frame so that the bottoms of the panels were approximately 3 cm above the sediment surface. The second array of artificial substrata, commonly called "french collectors," consisted of 0.75-m lengths of gray, longitudinally corrugated PVC tubing (21 mm OD). Four replicate tubes were inserted in the sediment adjacent to the panel frames so that the top 20 cm section of each tube was exposed approximately 3 cm above the sediment surface. This length of tubing also provided a 225-cm^2 test surface for spat settlement due to the surface corrugation.

The panels and tubes were placed in the intertidal zone on April 8, 1986, and subsequently collected and replaced with new surfaces at two-week intervals through October 14, 1986. This generated 13 replicate sets of data for comparison of spat settlement between the two types of surfaces. Water temperatures and salinities were recorded on all sampling dates in both areas. After

TABLE 1.

Criteria used to identify various phases of gonadal development in *Crassostrea virginica*.

Reproductive Phase	Gonadal Condition	
	Males	**Females**
Inactive	The gonads are in a state of quiescence and the follicles are either absent or few in number and small in size. When present, follicles are found in the area between the body wall and the digestive gland or, more rarely, scattered in the form of small islands throughout the mass of vesicular connective tissue. Sex was not distinguishable.	
Early Development	The follicles begin to enlarge; however, the vesicular connective tissue still occupies considerable space between the follicles. Primary spermatocytes appear at the basal membrane of these follicles.	Small primary oocytes are present around the periphery of small follicles. Some are elongated on stalks and accompanied by a decrease in the number of follicle cells and inclusions.
Late Development	As development proceeds, primary spermatocytes increase in number and there is the appearance of some spermatids beginning to migrate toward the center of the follicle where they arrange themselves in radial columns.	The follicles begin to enlarge, anastomose, and proliferate; however, the connective tissue still occupies a considerable amount of space. A central lumen is present in each follicle, into which protrude the stalked oocytes.
Early Ripe	Proliferation of spermatids and differentiation into spermatozoa begin to occur rapidly, with a general reduction in the earlier stages of gametogenesis.	Oocytes increase in size and number and begin to fill the lumen of the follicles. There is a gradual appearance of a nucleolus.
Ripe	The follicle is filled with dense radiating bands of spermatozoa, the tails of which project into the central lumen.	Many well-defined, mature ova, averaging 70–75 μm in diameter, appear to be free within the follicular lumen. There are a few traces of earlier stages of gametogenesis. The voluminous vesicular connective tissue has disappeared and the enlarged follicles seem to come in contact with each other.
Spawning	The lumen of the shrunken follicle is often empty because of the recent discharge of mature spermatozoa. A few spermatozoa remain in the radiating banks, but the rows of follicle cells gradually increase to replace the spawned spermatozoa.	There is a slight shrinkage of the follicles from which mature oocytes are gradually discharged. Very small oocytes are embedded in the follicle cells at the periphery of the empty alveoli. Usually, a large number of follicles still retain spawn, with the lumina almost entirely filled with mature ova.
Spent	The follicles are almost completely filled with follicle cells and the reduced lumen contains a few sex cells. The vesicular connective tissue proliferates.	Unspent oocytes, some in early phases of cytolysis, are present. Shrinking follicles are invaded with phagocytic cells, both inside the lumina and around the outside walls. Simultaneously, the vesicular connective tissue proliferates.

collection, the substrata were returned to the laboratory and preserved in a buffered 10% formalin solution.

In the laboratory, three of the four panels and french collectors from each treatment group were randomly selected and examined under a dissecting microscope to identify and count all oyster spat that had settled within the 225-cm^2 sample area. Scars of oyster spat were also observed and counted. Panels or french collectors which showed signs of damage were discarded and replaced with the fourth replicate from that treatment group.

A second series of studies was completed in 1989 using a different sampling design. The general design used in 1989 involved placing a large number of french collectors at a number of sites located along the shoreline both within and away from (up to 500 m) the three marinas described previously. The rationale for this design was to determine whether spat densities were significantly reduced in the immediate vicinity of the marinas compared to more distant locations and, if so, to quantify how far these effects extended. Figure 2 shows the approximate location of where french collectors were placed at each marina. At all locations, 10 collectors were placed at identical tidal heights approximately 1–2 m apart, with the top 25 cm of each collector exposed above the sediment surface. When the collectors were established at these locations, the water temperature at all three marinas was 23°, and salinities varied from 23–29 ppt. All of the collectors were left in place for approximately 6 weeks (mid-May to July) to allow for spat settlement. The collectors were then retrieved, and preserved in a buffered 10% seawater–formalin solution. In the laboratory, spat which had settled on each collector (225 cm^2 surface area) were counted and recorded.

Statistical comparisons for the settlement studies were limited to non-parametric tests due to large and heterogeneous variances among treatment groups, even after data transformations. Mann Whitney U tests were used to compare mean densities of spat on the panels and tubes collected from Skull Creek and Mackay Creek on each sampling date. Kruskal Wallis tests were used to compare spat settlement densities on the tubes at varying distances away from the other marinas studied.

RESULTS AND DISCUSSION

Condition Index

The volumetric condition index of oysters collected at the study sites ranged from 5.29 to 9.49 (Fig. 3). Values were highest in late April and early May, which corresponded to the period when the highest percentage of ripe individuals were noted (Fig. 4). The index values were greater at the marina site than at the control site on 11 of the 13 sampling dates, but the differences were only statistically significant on two of those dates (Fig. 3). During this study period, salinities ranged from 26–33 ppt and temperatures ranged from 11–31°C at the two sites, with comparable conditions noted among the sites on each sampling date.

The physiological condition of oysters is known to undergo seasonal variations which reflect reproductive and physiological state (Walne 1970, Trevallion 1971, de Wilde 1975). These variations are considered normal and have been correlated with seasonal changes in nutrient storage and utilization (Walne 1970, Trevallion 1971). Galtsoff (1964) indicated that seasonal fluctuations are related to reproductive activity in spring and summer, and glycogen storage in fall. In our study, seasonal fluctuations in condition indices were clearly related to the storage and release of gonadal material, but there appeared to be no substantial difference in the condition of oysters between the marina and control sites. Marcus et al. (1989) also found no significant differences in the physiological condition of *C. virginica* at three marinas compared with non-marina sites. Additionally, the condition index values observed at both our study sites were similar to Crosby and Gale's (1990) study of South Carolina *C. virginica* populations that were not close to marinas, and they were consistently higher than the CI values noted by Lawrence and Scott (1982) in two other South Carolina estuaries.

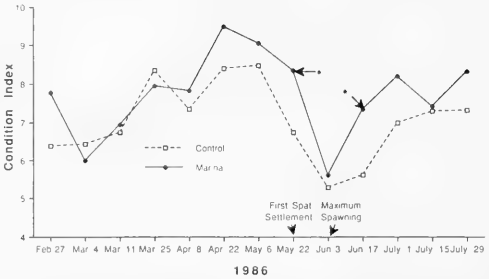

Figure 3. Average condition index of oysters sampled at the Skull Creek marina site and Mackay Creek control site during 1986. Asterisks represent sampling dates where a significant difference was observed in the CI of oysters collected at the marina and control site ($P < 0.05$).

Gametogenic Comparisons

Histologic examination of the oyster gonads collected from the Skull Creek marina and the Mackay Creek control site failed to reveal any consistent differences among the sites with respect to the frequency of oysters in various stages of gonadal development (Table 2, Fig. 4). Gonads in early and late phases of development were observed in over 80% of the population from February through early March, when water temperatures were 11–15°C. By late March (water temperatures of 17°C), at least 50% of the oysters sampled from both locations were in an early ripe phase. As the water temperature increased in April to 20–24°C, over 80% of the oysters became completely ripe and began spawning. Ripe and spawning conditions were evident in most of the oysters sampled throughout the remainder of the study period at both sites (Fig. 4).

Statistical comparisons of the proportion of oysters in various stages of gonadal development at each site indicated significant differences among the two locations on only 4 of the 11 sampling dates. On two of those dates, a greater proportion of the oysters at the control site were in more advanced stages of gonadal development than those at the marina site; however, the opposite pattern was observed on the other two dates. Thus, while there were significant differences among locations on a few dates, the patterns observed did not suggest that marina proximity had a detrimental effect on gonadal development of the oysters sampled.

Although our data indicate no consistent differences between marina and control site populations in the progression of gametogenesis, other sublethal effects may be occurring that were not tested in this study. Among the sexually ripe oysters examined in this study, less than 1 percent of the control site oysters were hermaphrodites compared to 3 percent of the marina site oysters. The latter percentage is higher than the normal proportion indi-

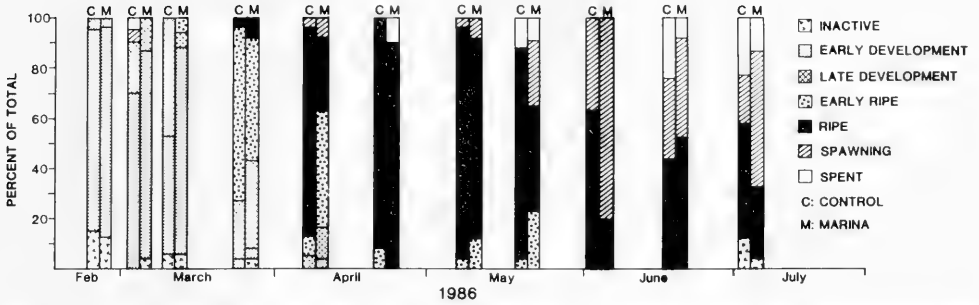

Figure 4. Percentage of oysters in each reproductive phase collected at the Skull Creek marina and Mackay Creek control site during 1986.

cated by Galtsoff (1964). Joseph and Madhyastha (1984) suggest that hermaphroditism can be a result of environmental stress. The slightly higher percentage of hermaphrodites observed at the marina may indicate stressful conditions, but further sampling would be needed to conclusively demonstrate this.

Settlement Patterns

During the study phase completed in 1986–87, oyster spat were first observed settling on the test surfaces exposed from May 6 to May 20, when water temperatures were 24–28°C. Settlement continued to occur on both types of test substrata until October 1 at the marina site and October 14 at the control site (Fig. 5). Water temperatures at that time were 29–30°C. Periods of peak spat settlement differed between the two types of substrata, however, and there were also differences between the marina and control sites with respect to spat densities on both the formica panels and french collectors.

The mean density of spat settling on the formica panels was

TABLE 2.

Number of oysters in each stage of development at Skull Creek Marina (M) and MacKay Creek Control (C) site.

Date	Area	N	Developmental Stages						X^2	Development State
			ED	LD	ER	R	SP	ST		
2-27-86	M	22	21	1					0.36	M = C
	C	18	17	1						
3-04-86	M	21	18	3					2.08	M = C
	C	23	17	4			1	1		
3-11-86	M	16	14	1	1				9.55**	M < C
	C	20	9	11						
3-25-86	M	20	1	8	10	1			2.14	M = C
	C	24	1	5	17	1				
4-08-86	M	25	1	3	11	8	2		12.98*	M < C
	C	23		1	2	19	1			
4-22-86	M	25				23		2	4.00	M = C
	C	25			2	23				
5-06-86	M	24			3	19	2		1.42	M = C
	C	23			1	21	1			
5-22-86	M	21			5	9	5	2	12.39**	M > C
	C	23			1	21		3		
6-03-86	M	24				5	19		9.31**	M > C
	C	25				16	9			
6-17-86	M	15				8	6	1	0.93	M = C
	C	25				11	8	6		
7-01-86	M	22				7	12	3	4.39	M = C
	C	21				10	5	6		

* $p < 0.5$.
** $p < 0.1$.
ED = early development; LD = late development; ER = early ripe; R = ripe; SP = spawning; ST = spent.
Chi-square values represent comparison of frequencies of different gonadal stages at the marina versus control site.

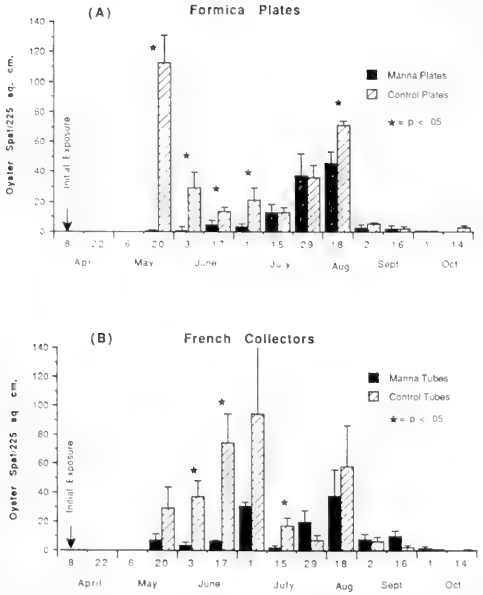

Figure 5. Mean number of oyster spat on (A) formica plates and (B) PVC "french collectors" deployed for two-week periods at the Skull Creek marina and Mackay Creek control site during 1986. Stars indicate significant differences between sites ($P < 0.05$, Mann-Whitney U test) in the mean number of spat collected on a particular sampling date. Dates are the pickup date and error bars represent 1 standard error of the mean.

generally greater at the control site than at the marina site, with significant differences noted between sites on five of the sampling dates ($P < 0.05$, Mann Whitney U test; Fig. 5). Temporal differences in the settlement patterns also occurred between sites during spring and summer. In the control area, spat settlement to the panels was highest during the May 6–20 exposure period (24–28°C), with a second smaller peak of settlement occurring in August. In contrast, spat at the marina site remained low during the spring and early summer, with a single peak in settlement occurring in late July and August (30–31°C). Even during August, the density of oyster spat on the panels was significantly lower at the marina than at the control site.

Settlement of oyster spat on the french collectors was also generally greater in the control area than at the marina, with significant differences observed between sites on three of the sampling dates ($P < 0.05$, Mann Whitney U test; Fig. 5). As noted for the formica panels, spat settlement in the control area showed two peaks of abundance separated by a period of lower settlement during July. A similar pattern was observed on collectors at the marina, although spat densities were generally lower. In contrast to the formica panels, the initial peak period on the french collectors was noted in late June–early July (29–30°C) rather than late-May (25–28°C). The second peak of settlement occurred in August, which corresponded to the same period of peak settlement observed on the formica panels.

The bimodal peak in spat densities observed on all but one of the arrays of artificial substrata (marina formica panels) during the first year of this study represents a settlement pattern which has been commonly noted for *C. virginica* (McNulty 1953, Shaw 1967, Hayes and Menzel 1981, Kenny et al. 1990). It is not clear why we observed a disparity between the panels and french collectors in the timing of the first peak in spat densities. However, our gametogenic analyses indicate that peak spawning of the oysters sampled at both sites occurred in June. If this spawning pattern was typical of oyster beds in the Port Royal Sound area, then greatest settlement should have occurred on the test surfaces sometime after this period, which corresponds with the settlement patterns observed on the french collectors. These collectors have the added advantage of being simple to construct and deploy, and their cylindrical shape minimizes differences among treatments related to surface orientation effects. As a result, they were the only type of test substrata used in the second phase of our settlement studies.

The lower spat settlement on the panels and tubes deployed at the Skull Creek marina site versus the control site suggested that the presence of this marina may have had an adverse effect on oyster spat settlement within a localized area. This could be due to a number of factors, such as proximity to boats which release toxic antifouling compounds from hull paints, and small but frequent oil and fuel spills which could reduce larval viability, spat settlement, or spat survival. Smith and Hackney (1989) observed a significant reduction in oyster spat settlement on panels treated with oil; however, they observed no effects on panels treated with a 40:1 gas:oil mixture. Thus, the effects of petroleum hydrocarbons on spat settlement may be quite variable, depending on the compounds involved.

An alternative explanation for the reduced spat in the Skull Creek marina area may be related to natural differences between Skull Creek and Mackay Creek with regard to larval density. The banks of Mackay Creek were much more densely populated with oysters than were the banks of Skull Creek. In fact, a large portion of the oyster bed adjacent to the marina was composed of dead oysters (empty shells), and field teams had difficulty finding sufficient live specimens for the other study components. While the cause of this mortality is unknown and may be independent of the marina, it is clear that this area did not support a productive oyster bed in comparison to the control area. Although such a discrepancy could have affected the pool of available spat, oyster veligers have an extended free-swimming period before settlement occurs (Loosanoff and Davis 1963), which should lead to wide dispersal and adequate mixing of the pool of oyster larvae generally available for settlement in the two areas. Differences observed between areas were probably not related to salinity or water temperature since values of those parameters were equivalent at both sites (Wendt et al. 1990).

In order to better determine whether oyster settlement patterns are influenced by marina proximity, the second series of experiments were conducted at three other marinas as described in the methods section. Similar sampling was not feasible at the Skull Creek marina due to a change in shoreline and hydrographic characteristics away from that marina. The results from these experiments suggest that proximity to well-flushed marina sites does not have a significant detrimental effect on oyster spat settlement (Fig. 6). Spat settlement patterns at both the Stono River/Buzzards Roost marina complex and the Bohicket marina were significantly different among the stations sampled (Kruskal Wallis test, $P < 0.001$). At both sites, spat densities on the collectors within the marina were greater than they were on the collectors located at various distances away from the marina. One possible explanation for this pattern may be related to the presence of pilings, docks and

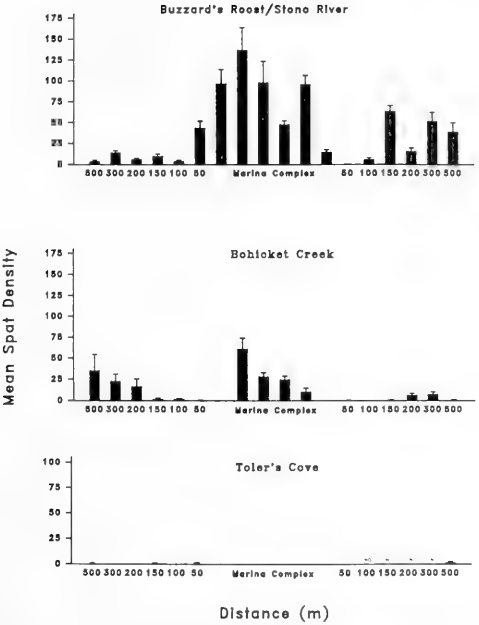

Figure 6. Mean number of oyster spat collected on french collectors deployed for a six-week period at the three marinas in 1989. ND indicates no data collected at that site.

other structures at the marinas, which probably affected water currents and wave energy along the shoreline where the tubes were placed. Bushek (1988) evaluated *C. virginica* settlement patterns relative to the degree of water motion and found that highest settlement occurred where water motion was lowest. Other factors, such as the relative abundance of adult oysters may have also influenced settlement patterns. Although live oyster beds and oyster shell were prevalent along the shoreline at both marina complexes, many of the sampling sites outside the marina did not have oyster beds immediately adjacent to the collectors. Hidu and Haskin (1971), Hidu et al. (1978) and others have documented gregarious settling patterns for *C. virginica* related to the presence of adult conspecifics. Therefore, the presence of oyster beds on the shoreline, both within and away from the marinas, may have prompted spat settlement in those areas. The density of adult oysters at each site was not measured in this study, so we cannot confirm whether this factor influenced spat settlement patterns. Regardless of the cause of the spatial variability in spat settlement, it appears that any toxicants associated with marina operations (e.g., antifoulant compounds, heavy metals, or petroleum compounds) were not present in sufficient concentrations to lower spat settlement.

In contrast to the well-flushed marinas, little or no spat settlement was observed within or adjacent to the Toler's Cove marina, even though the collectors were exposed during the same time period as those deployed at the other two marina complexes. Although these results may indicate a marina effect, it should be noted that spat settlement was also absent or extremely low even at sites most distant from the marina. Most of the sites in this study area were in sheltered habitats that received relatively little wave action and low tidal current velocities, which should have enhanced oyster settlement (Bushek, 1988). Additionally, there were dense beds of living oysters at many of the sites close to the marina, and living oysters were observed on the pilings within the marina. Therefore, it is not clear why we observed little or no set on the tubes deployed in this area. In a separate study of benthic patterns at the Toler's Cove marina, Wendt (unpublished data) measured lower dissolved oxygen levels at this marina compared to a nearby reference site, and she found elevated concentrations of lead (~30 mg/kg), copper (~15 mg/kg), and tributyltin (49–56 ppb) in the marina sediments compared to sediments at the reference site, where no contaminants were detected. The high contaminant levels observed at the Toler's Cove marina may be due to the poor flushing of this marina and the levels may have been sufficiently high enough to have affected spat settlement and survival.

In summary, our analyses of oyster condition, gametogenic state, and spat settlement patterns did not reveal any consistent patterns related to marina proximity, with the possible exception of reduced spat settlement at a poorly flushed site. It should be noted, however, that our analyses were designed to assess gross effects only. Further research is warranted to study these effects and to confirm whether the reduced spat settlement observed at the poorly flushed marina occurs at other marinas having similar flushing characteristics.

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TEMPORAL AND SPATIAL CHANGES IN FECUNDITY OF EASTERN OYSTERS, *CRASSOSTREA VIRGINICA* (GMELIN, 1791) IN THE JAMES RIVER, VIRGINIA

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ABSTRACT Adult *Crassostrea virginica* (Gmelin) were examined during the reproductive season of 1986 to determine temporal and spatial variation in fecundity among individual female oysters from four reefs in the James River, Virginia. Sex ratio and oyster abundance were also determined to facilitate estimation of total reproductive output of oyster assemblages. Fecundity was highly variable, both within and among locations. Variation was attributed to differences in oyster size, asynchrony and variation in time since prior spawning, prevalence of parasites (especially *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus*) and differing salinity regimes.

KEY WORDS: oysters, *Crassostrea*, fecundity, reproduction, James River

INTRODUCTION

The James River, third largest and southernmost tributary of the Chesapeake Bay, has traditionally been a seed oyster harvesting area (Haven & Fritz 1985). Between 1930 and 1980, an estimated 75% or more of the seed oysters planted in Virginia originated from the James River (Haven et al. 1981). Approximately 5,658 hectares of public oyster reefs extend from near the James River Bridge upriver to Deepwater Shoal Light (Fig. 1) (Andrews 1951, 1954, 1983, Haven and Whitcomb 1983).

Despite its importance, little is known about the broodstock that produces the larvae that recruit into the James River seed area. Andrews (1983) states "The importance of large broodstock populations was shown after 1960, when setting rates declined to one-tenth the 1950's level; this followed cessation of private oyster planting in the lower river." This statement suggests the importance of broodstock oysters in downstream areas of Hampton Roads from which larvae were thought to be transported upstream to the seed area by gravitational circulation. Results of dye studies in the James River Hydraulic Scale Model emphasize the importance of broodstock oysters in the low salinity seed area upstream of the James River bridge (see Fig. 1), suggesting that larvae originating here would provide maximum coverage of reefs in the seed area (Ruzecki and Hargis 1989). The downstream populations have essentially been eliminated by losses to two parasites, *Haplosporidium nelsoni* and *Perkinsus marinus*, leaving upstream oyster reefs as the only remaining broodstock source.

The contribution of oysters in the seed area to local recruitment is dependent upon their fecundity; however, quantitative information describing fecundity in James River oysters has not been examined. This study examined temporal and spatial variation in oyster fecundity within and among four oyster reefs in the James River seed area during the 1986 reproductive season. Several biotic and abiotic factors potentially affecting fecundity, including temperature, salinity, dissolved oxygen and oyster size, were examined. Estimates of the number of eggs spawned by the four oyster assemblages in 1986 were calculated from average female fecundity, sex ratio, and oyster abundances on each reef.

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MATERIALS AND METHODS

Oysters were collected from four reefs, Horsehead, Wreck Shoal, Thomas Rock, and Dog Shoal in the James River seed area (Fig. 1). The spawning season for oysters in the James usually extends from June through October (Haven and Fritz 1985). Ten collections of live oysters were made at approximate 2 week intervals from June 2 through October 16, 1986 using a 60 cm oyster dredge with teeth of 7.5 cm length. Tows were not replicated. Collections were also made by divers on June 2, August 25, and October 16. On those days, four quantitative samples, used to estimate size frequency distribution and areal density at each location, were obtained by haphazardly placing a 0.25 m^2 quadrat on the bottom and collecting all live oysters within the quadrat. These oysters were frozen for subsequent analysis.

Samples of bottom water were obtained from all stations on every collection date using a modified Van Dorn bottle. Temperature was measured in the field to the nearest 0.5°C using a stem thermometer. Water was placed in glass bottles and returned to the laboratory for salinity and dissolved oxygen analyses. Salinity was determined using a Beckman Induction Salinometer Model RS-10. Dissolved oxygen was determined using the azide modification of the Winkler titration method (APHA 1980). Percent oxygen saturation was calculated for every water sample.

Quantitative quadrat samples were treated as follows. All oysters with the exclusion of young-of-the-year, which were considered to be the only reproductively inactive size class, were counted and measured. Natural log transformed mean oyster abundance was calculated and abundance was compared among stations and over time.

Sex ratio (male:female) was calculated for oysters from the 4 reefs using 40 randomly chosen individuals from every dredge collection. Shell height (defined here as the maximum distance between the umbo and the ventral shell margin) was measured to the nearest millimeter using dial calipers. Gonadal material was collected with a Pasteur pipette and examined microscopically. Sex was determined by the presence of eggs or sperm. Calculated ratios were compared to the expected 1:1 ratio using chi-square analysis for both individual collections and pooled data for the entire study period. Gonadal smears were also examined for the presence of cercaria of the digenean trematode *Bucephalus cuculus*

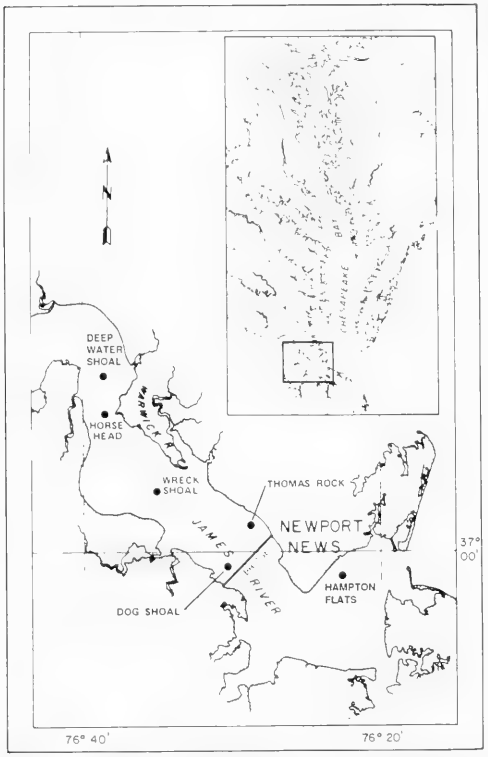

Figure 1. Locations of sample collection sites in the James River, Virginia. The seed bed area is generally considered to be upstream of the James River Bridge and below Deepwater Shoal. Significant oyster beds were located on Hampton Flats prior to disease losses after 1960.

(McCrady), which occurs in the gonads and digestive gland (Tennent 1906, Menzel and Hopkins 1955). No examinations were made for prevalence and intensity of either *H. nelsoni* or *P. marinus*.

Fecundity values were obtained as direct estimates of the number of eggs in the gonad, not the number of eggs released at spawning. The two values will only be equal in instances where the gonad is completely evacuated on spawning. The present method differs from previously reported approaches using histological or direct spawning methods. The practical limitation of processing the number of animals collected in this study by either of the latter methods precluded their use. Ten females were randomly selected from each collection for fecundity determination. Individual oyster meats were placed in a commercial kitchen blender containing 150 ml of filtered York River water adjusted to 20‰ with distilled water, and blended on medium setting for 30 seconds. The resultant homogenate was sequentially filtered through 90 um and 53 um mesh Nitex nylon sieves; the liquid containing the eggs was retained in a 1 l graduated cylinder. Blender, sieves and funnel were rinsed with 750 ml of estuarine water; additional water was added to bring the volume in the cylinder to 1 l. Total number of eggs was estimated following mixing of the cylinder contents, removing a 10 to 1000 ul aliquot (depending upon egg concentration) to a Sedgwick-Rafter Cell, counting on a compound microscope, and correcting for proportional volume of aliquot and cylinder contents. The mean of three aliquots counted for each oyster was used as the estimated fecundity.

Boundaries of Dog Shoal, Thomas Rock, and Horsehead were estimated and area was obtained by digitizing NOAA Chart 12248. Boundaries of Wreck Shoal were unclear, so area was estimated as that reported by Moore (1910). The product of reef area, number of females per m^2, and mean fecundity was used to estimate spawning potential of the four broodstock assemblages.

Statistical analyses were performed using SPSS-X (Release 2.1) on a PRIME 9955 computer. Unless otherwise indicated, data were analyzed using one-way or multivariate Analysis of Variance (ANOVA) and subsequent Student-Neuman-Keuls (SNK) multiple range tests when appropriate. When nonparametric statistics were necessary, the Kruskal-Wallis (KW) nonparametric one-way ANOVA was employed; multiple comparisons were made using Dunn's Approximation (Dunn 1964). In all statistical tests, significance was tested at an alpha level of 0.05.

RESULTS

Bottom water temperature and salinity values recorded at the study sites are depicted in Figure 2. Horsehead had the largest range of temperature and salinity, 19–31°C and 6.6–18.4‰, respectively. Thomas Rock experienced the smallest temperature and salinity ranges, 22–29°C and 14.9–20.8‰. Temperature varied significantly over the study period (ANOVA: $F = 63.98$, df = 9, $P < 0.00005$), but there was no temperature difference among the stations (ANOVA: $F = 1.36$, df = 3, $P = 0.277$). Salinity generally increased over the course of the summer (ANOVA: $F = 13.68$, df = 9, $P < 0.00005$) at all sites. Variation in salinity among stations was significant (ANOVA: $F = 65.51$, df = 3, $P < 0.00005$) with that at Horsehead significantly lower than at the other locations (SNK: $P < 0.05$). Oxygen saturation did not vary significantly among stations (ANOVA: $F = 2.96$, df = 3, $P = 0.052$) and was never below 75% saturation.

Oyster distribution was irregular on all reefs, ranging from 0–107 oysters per 0.25 m^2. Oysters were more abundant at Horse-

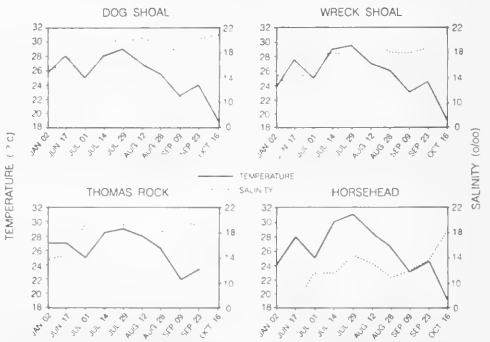

Figure 2. Temperature and salinity records for sample locations during the summer of 1986.

head than at other stations (SNK: $P < 0.05$). Thomas Rock and Wreck Shoal had significantly more oysters than Dog Shoal (SNK: $P < 0.05$), but abundance was not different between the two (SNK: $P > 0.05$). Mean abundance of oysters remained fairly constant at Dog Shoal and Wreck Shoal through the study (Table 1). Abundance decreased at Thomas Rock and Horsehead; the decline at Thomas Rock from June 2 to October 16 was significant (SNK: $P < 0.05$).

Individual oysters varied in height from 22 to 122 mm, with the majority between 30 and 105 mm. Mean size of Horsehead oysters was significantly smaller than that of oysters from all other locations (Dunn's Approx: $P < 0.05$).

In most collections male oysters were more abundant than females, the male to female ratio ranging from approximately 1 to 4 (Table 2). Since sex ratio was not found to differ significantly over time (ANOVA: $F = 0.76$, $df = 1$, $P = 0.607$), a seasonal mean sex ratio, pooling data from all dates was calculated for every station. Seasonal mean sex ratio of oysters was significantly greater than the expected 1.0 for each reef.

Fecundity differed significantly among the stations (KW: $X^2 = 11.97$, $n = 330$, $P = 0.0075$). Oysters from Thomas Rock were the most fecund, followed by those from Wreck Shoal, Dog Shoal, and finally Horsehead (Table 3). The most fecund Thomas Rock individual contained approximately 45.95×10^6 eggs, while the most fecund Horsehead individual contained approximately 22.03×10^6 eggs. Unequal sample size and large variation among individual females limited statistical comparisons; however, oysters from Thomas Rock were more fecund than oysters from Dog Shoal (Dunn's Approx: $P < 0.05$). A regression between fecundity and oyster shell height was not attempted because values of the former were not normally distributed at each value of the latter. The prerequisite of homogeneity of variance was not fulfilled.

Synchronous mass spawning was marked by a statistically significant decrease in mean fecundity between two dates with small variation about the mean values. Large variation about the means suggests that spawning was not synchronous at any station except Horsehead (Table 3). Asynchronous spawning events may be characterized by a reduction in mean fecundity but an associated high variability about the mean. Unlike the synchronous events, the high variability generally precludes identification of asynchronous spawnings by statistically significant decreases in mean values. Four asynchronous periods of spawning are apparent at Dog Shoal: these being represented by high mean fecundity and large variation about the mean on June 17, July 14, August 12, and September 9, with subsequent samples characterized by low mean fecundity. Characterizing the decrease in mean egg number from June 17 to July 1 as a spawning was potentially compromised by the lack of demonstrably ripe gametes at this site (unpublished observations made during sex ratio studies); however, gametes may have ripened subsequent to the June 17 sampling and been released before the July 1 sampling.

Large scale spawning at Thomas Rock appears to have begun between July 14 and July 29. Mean fecundity on July 14 differed significantly from other dates (Dunn's Approx: $P < 0.05$). Large variation in fecundity within the June 17 and July 1 samples can probably be attributed to asynchronous gonad maturation of the oysters. As in the case of Dog Shoal, Thomas Rock oysters did not contain fully mature eggs in June. There was no indication of a second spawning event at Thomas Rock.

Wreck Shoal and Horsehead oysters began producing mature eggs before oysters at Thomas Rock and Dog Shoal. Although there was no significant date effect on fecundity at Wreck Shoal, the increase in mean fecundity and variation around the mean indicated that spawning began between July 1 and July 29. Further comment is limited by the omission of a July 14 sample, damaged in processing. Fecundity data suggest a second spawning event in Wreck Shoal oysters at the beginning of September; however, the increase is not statistically significant (Dunn's Approx: $P > 0.05$).

Spawning patterns at Horsehead were different than those seen at Dog Shoal and Thomas Rock. There was a mass spawning at Horsehead at the beginning of July. A second, significant (Dunn's Approx: $P < 0.05$) increase in fecundity in late July was followed by spawning which persisted into September. Horsehead oysters ceased spawning activity before those at other stations; no oysters contained eggs in the October collection.

The mean fecundity per female was calculated for each location from the mean values obtained in biweekly sampling to avoid weighting against limited samples of less than 10 individuals (see Table 3). Estimated spawning potential, as calculated from mean fecundity, density of female oysters and area of oyster reef (see Table 4) indicate there were more eggs produced at Thomas Rock than at the other three sites; however, large variations in individual fecundity measurements around mean values for any chosen date (see Table 3) suggest a large range estimate for spawning potential at all sites. The estimate of spawning potential for Dog Shoal was an order of magnitude lower than at Wreck Shoal and Horsehead.

Bucephalus cuculus cercaria were present in oysters from all collection locations; however, no difference in prevalence was evident between locations. Oysters of both sexes, as well as those of indeterminate sex, were observed to contain cercaria. No evidence of hermaphroditism or "sexual aberration" as described for *Bucephalus*-infected oysters by Tripp (1973), was observed. Percentage of oysters parasitized could not be reliably estimated since examination was focussed on presence of cercaria in a single gonad smear, and certain cercaria may reside in the digestive gland and/or gonad of oysters.

TABLE 1.

Oyster abundance at four stations in the James River, Virginia.

	Dog Shoal		Thomas Rock		Wreck Shoal		Horsehead	
Date	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
6/2	10.3	11.0	45.5	38.0	18.0	7.8	71.7	40.7
8/25	6.3	5.0	21.5	5.6	20.8	6.1	65.0	17.4
10/16	9.8	7.4	9.8	7.4	16.3	9.6	44.5	22.2

Abundance is mean ± standard deviation (s.d.) of four quadrat collections of 0.25 m^2 each.

DISCUSSION

Fecundity of female oysters from four major reefs in the James River was highly variable, both within and among locations, during the 1986 spawning season. The large variation may be influenced by several biotic and abiotic factors. Major abiotic factors potentially influencing fecundity include water temperature (which affects gonad maturation rate), and salinity regime. Biotic influences include time since last spawning, food supply and resultant nutritional status of the individuals, size of females, and prevalence of parasites.

TABLE 2.

Sex ratio of oysters collected from four stations in the James River, Virginia between June 17 and September 19, 1986.

	Dog Shoal		Thomas Rock		Wreck Shoal		Horsehead	
Date	Ratio	P	Ratio	P	Ratio	P	Ratio	P
6/17	3.0	0.002*	3.0	0.002*	1.9	0.058	0.7	0.206
7/1	4.0	0.000*	1.7	0.114	3.4	0.001*	0.9	0.752
7/14	2.1	0.027*	1.7	0.114	2.1	0.027*	2.1	0.027*
7/29	1.7	0.114	1.2	0.527	0.9	0.752	1.2	0.631
8/12	3.4	0.001*	1.2	0.527	1.5	0.206	3.0	0.002*
8/26	1.7	0.144	2.1	0.027*	2.5	0.009*	1.6	0.170
9/9	1.3	0.637	3.4	0.011*	2.0	0.046*	0.8	0.655
All	2.4	<0.001*	1.8	<0.001*	1.9	<0.001*	1.3	<0.05*

* Denotes a ratio significantly different from 1.0 (Chi-square analysis).

Variation in fecundity among females on the same reef occurred in part because of asynchrony in gonadal development and spawning activity (see also Nelson 1928, Loosanoff and Davis 1952, Kennedy and Krantz 1983). An oyster which spawned immediately prior to collection presumably contained fewer eggs than a similar oyster in which spawning was imminent. Methods used in this study do not distinguish between oysters which recently spawned and those which had not but were truly less fecund. Fecundity estimations were also influenced by the time of sampling relative to spawning events. Seasonal mean values which include a preponderance of immediate post-spawning animals will not reflect the high fecundities of immediate pre-spawning animals. Large oysters usually produce more eggs than do small oysters (Davis and Chanley 1956). Consequently, it would appear reasonable to attribute some of the recorded variation in fecundity within and among reefs to body size. The observed lack of normal distribution of fecundity values at each shell height in the present study was probably related to asynchrony in spawning activity at each location, and limited the use of regression analysis to examine size versus fecundity relationships.

Egg maturation and peak spawning occurred earlier at the upriver stations Horsehead and Wreck Shoal, than it did downriver at Thomas Rock and Dog Shoal. Even though bottom water temperature did not vary significantly among sites during the study period, earlier differences in temperature may account for the timing of spawning in that gametogenic development is time and temperature dependent (Price and Maurer 1970). Whitcomb (1986) recorded a bottom water temperature of 19.5°C on May 1 at Horsehead. On May 6, temperature at Wreck Shoal was 19.0°C, while downriver at two shoals close to Thomas Rock and Dog Shoal, temperatures were 17.1°C and 17.0°C, respectively. Egg maturation and subsequent spawning were probably delayed in 1986 at the downriver locations by the extended period of cooler temperatures (see Loosanoff and Davis 1952). The consistently high observed values for percentage dissolved oxygen suggest that this was not a source of stress to oysters examined in this study.

Egg production is dependent upon the nutritional health of the oyster since accumulation of egg reserves takes place at the expense of stored glycogen (Gunter 1941, Medcof and Needler 1941, Engle 1950, Galtsoff 1964, Gabbott 1975, Mann 1979). Horsehead oysters are notably slower growing than from higher salinity locations in the James River (unpublished personal observations) and their occurrence in dense assemblages on the reef suggests possible intraspecific competition and food limitation, with subsequent reduction in physiological status and energy that may have influenced the amount of energy available for reproduction. Quantitative data to support this hypothesis are, however, lacking.

TABLE 3.

Fecundity of oysters (in millions of eggs) collected from four stations in the James River, Virginia between June 2 and October 16, 1986.

	Dog Shoal			Thomas Rock			Wreck Shoal			Horsehead		
Date	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
6/02	8.58	—	1	3.29	3.39	9	2.70	—	1	1.34	1.48	10
6/17	8.34	6.85	10	14.52	9.30	10	5.04	3.22	10	5.47	2.08	10
7/01	2.78	3.69	10	15.55	13.42	10	13.86	13.18	10	**11.86***	6.16	10
7/14	8.81	11.85	6	**26.93***	15.41	10	sample damaged			3.15	3.34	10
7/29	1.90	2.06	10	14.11	12.19	10	6.13	7.22	10	**6.09***	3.30	10
8/12	6.06	4.01	10	4.22	4.97	10	5.62	3.28	10	4.66	3.27	10
8/26	1.11	1.38	10	3.52	2.47	10	2.01	1.55	10	2.34	1.51	10
9/09	8.48	14.23	9	3.75	5.66	10	4.61	5.38	10	1.59	1.75	10
9/23	1.96	3.30	10	2.50	3.15	10	1.88	3.43	7	0.39	0.80	7
10/16	1.09	0.53	4	0.91	—	1	0.68	0.71	5	—	—	—

Values given are mean, standard deviation (SD), and number of individual oysters (N).

* Denotes a significant difference ($P < 0.05$, Dunn's Approximation) from preceding and following collections at the same station.

TABLE 4.

Oyster spawning potential at four stations in the James River, Virginia.

	Dog Shoal	Thomas Rock	Wreck Shoal	Horsehead
Reef area (m^2)	449,912	1,559,700	2,047,709	603,062
Female oysters m^{-2}	10.4	33.8	27.7	105.5
Mean fecundity	4.91×10^6	8.93×10^6	4.73×10^6	4.10×10^6
Spawning potential	2.30×10^{13}	4.71×10^{14}	2.68×10^{14}	2.61×10^{14}

Reef area is estimated from NOAA chart 12248 or data of Moore (1910), see text.
Abundance (Table 1) and mean sex ratio (Table 2) data for all dates are used to estimate mean number of female oysters m^{-2}.
Mean fecundity is estimated from biweekly fecundity data (Table 3) as mean of biweekly mean values to avoid weighting against samples containing <10 individuals.
Spawning potential = (reef area × female oysters m^{-2} × mean fecundity).

Parasitism probably contributed to the observed variation in fecundity. Barber et al. (1988) demonstrated the deleterious effects of increasing infection intensity of *H. nelsoni* on *C. virginica:* a progression from uninfected to epithelial to systemic infection was accompanied by 31 and 81% decreases in fecundity. Record high salinities, caused by consecutive droughts in 1985 and 1986, allowed oyster diseases to invade areas of the James River where they normally would not occur (Burreson 1986). Oysters from Wreck Shoal, Dog Shoal, and Horsehead were infected by *H. nelsoni* with Wreck Shoal showing the highest infection rate in August, 1986 (Burreson, personal communication). Although Thomas Rock oysters were not heavily infected by *H. nelsoni,* at least 20% and perhaps as many as 96% were infected by the protozoan parasite *P. marinus* (Burreson 1986). Finally, one to several pea crabs (*Pinnotheres ostreum*) were found in a large percentage of oysters from all sites, potentially reducing energy available for egg production (see Haven 1959).

Male oysters generally were present in greater numbers than females at all sites. This contrasts with findings of Morales-Alamo and Mann (1989) who found a sex ratio of approximately 1.0 for Wreck Shoal oysters of greater than 60 mm height. Two scenarios may be invoked to explain these differences. The observations of Burkenroad (1931) and Needler (1932) suggest that a greater proportion of small (<60 mm) oysters are males. Therefore, the inclusion of small oysters in the present study may account for the observed preponderance of males. Alternatively, Kennedy (1983) suggests that an increased proportion of males in an oyster population is indicative of environmental stress such as the occurrence of pea crabs (from Awati and Rai 1931, in Coe 1938). If stress related to infection by *H. nelsoni* and *P. marinus* is similarly reflected in sex ratio data then the current observations may be indicative of increased infections since the collections of Morales-Alamo and Mann (1989). Unfortunately, estimates of the prevalence and intensity of these parasites were not made in either Morales-Alamo and Mann (1989) or the present study. Although the contributions of the parasite *Bucephalus cuculus* to stress on the host organism also remains unquantified, all other cumulative effects of parasitism, acting either directly on individual fecundity or indirectly sex ratio change, are consistent in reducing population egg production.

Spawning events were more predominant in periods of increasing temperature and stable salinity; however, exceptions were observed. Of the four projected spawnings at Dog Shoal two, in late June and mid August, coincided with decreasing temperature whereas the July and September events coincided with increasing temperature. All occurred in periods of increasing or relatively stable salinity conditions. The July spawnings at Thomas Rock, Wreck Shoal and Horsehead occurred during or after a period of considerable rise in temperature accompanied by stable salinity. The projected minor spawning at Wreck Shoal coincided with a brief temperature and salinity increase after a consistent decrease in both. The late July spawning at Horsehead occurred directly after summer maxima for both temperature and salinity, as both began a period of consistent decline. With the exception of Horsehead, all spawnings occurred at or above 17‰ salinity. Only the September spawnings at Dog Shoal and Wreck Shoal occurred at less than 25°C.

Although no data are available to allow comparison of the relative contributions of oysters in the Hampton Roads and seed bed regions of the James River to the spawning potential and maintenance of the James River oyster stocks prior to losses to *H. nelsoni* (see Haven and Fritz 1985), the present data allow some statements concerning relative contributions from spatially distinct reef populations within the seed bed area in 1986. Warmer temperatures in the early summer in the Horsehead and Wreck Shoal area suggest that spawning commenced earlier in these upriver locations but the contribution of the higher salinity population at Thomas Rock was comparable over the spawning season. This relationship underscores the vulnerability of the James River population to both *H. nelsoni* and *P. marinus* in that the distribution of both disease organisms is strongly determined by salinity. Continuing losses of oysters in the higher salinity sections of the seed bed area (Barber, unpublished data), where salinities may be more conducive to larval development and metamorphosis, may have disproportionately large impacts on spawning potential and subsequent recruitment events, with detrimental effects on both the ecology of the James and the commercial fishery supported by the oyster resource.

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A COMPARISON OF METHODS FOR CALCULATING CONDITION INDEX IN EASTERN OYSTERS, *CRASSOSTREA VIRGINICA* (GMELIN, 1791)

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ABSTRACT A number of techniques have been reported to estimate condition index in oysters and other bivalve molluscs. We report and compare condition index, estimated by three different methods, for oysters collected from a single reef in the James River, Virginia over a four week period in the summer of 1987. Two indices express condition as a ratio of dry meat weight to shell cavity volume, but differ in methods of estimating shell cavity volume. A third method expresses condition as a ratio of dry meat weight to dry shell weight. Within the size range 36–96 mm length there is no effect of size on index values. We suggest that indices based on both shell cavity volume and shell weight have utility in reflecting biochemical or nutritive status; however, intercalibration is difficult and comparisons of data from different authors and locations limited in scope.

KEY WORDS: bivalve, condition index, oyster, clam, mussel

INTRODUCTION

A number of techniques have been reported to estimate condition index in oysters and other bivalve molluscs. Recent reviews include Mann (1978), Lucas and Beninger (1985), Bodoy, Prou and Berthome (1986), Davenport and Chen (1987), and Crosby and Gale (1990). Collectively, these contributions illustrate the abundance of indices used, the lack of consistent methods, and the difficulty in comparing published material. Mann (1978) included discussion of volumetric and gravimetric meat-to-shell ratios, biochemical and physiological indices, and a comparison of biochemical and gravimetric indices. Lucas and Beninger (1985) offered a comprehensive review of "static", physiological, biochemical, and "dynamic" indices. All of the above discuss indices based on measurement of dry tissue weight, shell cavity volume and shell dry weight. In this contribution we compared three methods based on dry meat : shell cavity volume ratios and dry meat : dry shell weight ratios, and discuss their utility in comparison with gross biochemical indices.

METHODS

Oysters, *Crassostrea virginica* Gmelin, were collected from Horsehead reef in the James River, Virginia. Sampling locations were randomly selected from a uniform grid overlaying the reef. Samples were collected at weekly intervals from 1 July to 29 July, 1988, using a 60 cm oyster dredge with 7.5 cm teeth. Tows were not replicated. A one half bushel subsample was haphazardly taken from the collected material, sorted and all whole oysters were retained. Twenty five oysters, selected randomly and without regard to size, were removed for estimation of condition index.

All animals were measured for length (defined as the longest dimension measured from the hinge) to facilitate subsequent examination of size versus index relationships. The overall size range for the entire study was 36–96 mm length. The following relationships were used to estimate condition index (Ci):

$$Ci = (\text{dry meat weight} \times 100/\text{shell cavity volume}) \quad (1)$$

$$Ci = (\text{dry meat weight} \times 100/\text{dry shell weight.}) \quad (2)$$

Equation 1 is that of Hopkins as described in Higgins (1938). When the resultant value is multiplied by ten it is the volumetric index (Ci-vol) of Crosby and Gale (1990). Equation 2 is the relationship used by Walne and Mann (1975) and is similar to the shell weight index (Ci-shell) of Crosby and Gale (1990) with the exception that the meat:shell ratio here is multiplied by 100 rather than 1000.

Shell cavity volume was estimated from the difference between the volume of water displaced by the live animal, after removal of attached epifauna and debris, and the volume displaced by the clean, separate valves after removal of the meat. Displacement was estimated using two different methods. Individual oysters were placed in a water filled container equipped with an overflow pipe. Surface tension around the exposed surface of the water moderates the flow of water and is a potential source of error. It is this moderating force which has led us to label this a passive method. Passive methods were also used by Hopkins (1938) and Crosby and Gale (1990). Condition index calculated by equation 1 using these data will be referred to as volumetric and passive, abbreviated to Ci-vol-p, to conform with Crosby and Gale (1990). Individual oysters were then transferred to a second, cylindrical chamber fitted with a piston inserted from above after addition of the oyster, which came to rest against a stop. Displaced water moved through a small bore hole in the piston into a graduated glass buret attached to the piston. The displaced water volume was the calculated difference of the calibrated buret measurement before and after addition of the oyster. Errors due to surface tension by this method are markedly reduced by comparison with the former method. Condition index calculated by equation 1 using this data will be referred to as volumetric and active, abbreviated to Ci-vol-a. All measurements were replicated three times for each individual animal.

Dry meat weight and dry shell weights were estimated after drying to constant weight at 100°C in tared pans. Condition index calculated by equation 2 using this data will be referred to as shell weight indices, abbreviated to Ci-shell.

RESULTS

The descriptors of oyster size (whole animal volume, dry meat weight, shell cavity volume, and shell length) of the animals examined are shown in Table 1. The regression relationships between condition indices (Ci-vol-p, Ci-vol-a, and Ci-shell) and both

TABLE 1.

Size range of animals examined.

Whole animal displacement volume	(V) 6–73 ml
Shell length	(L) 39–96 mm.
Shell displacement volume	4–44 ml
Shell cavity volume (V − D)	2–26 ml
Meat dry weight	0.3–2.0 g
Shell dry weight	10–110 g

Letters in parentheses indicate descriptor variable name in Table 2.

whole animal volume and length, that is size descriptors not used in condition index calculation, are given in Table 2. In all comparisons very low r^2 values indicate a large scatter of points about the line and slopes that are not significantly different from zero. No relationship between size and condition is observed. Consequently, all data obtained by one method at one date are pooled (n = 25 per week) and plotted as Figure 1, a bar histogram, to examine variation in index over time by all methods. The similarity in temporal trend is evident regardless of the index in use.

Figures 2A and 2B illustrate, respectively, comparisons of Ci-vol-p versus Ci-vol-a, and both Ci-vol-p and Ci-vol-a versus Ci-shell using all 125 individual values collected during the study period. The lack of correlation between Ci-vol-p and Ci-vol-a is unsettling given that they differ only in the method of volume estimation and suggest measuring error in one or both methods. Only the plot of Ci-vol-p versus Ci-shell exhibits a slope significantly different than zero ($p < 0.001$). The accompanying r^2 value of 0.209 suggests a modest predictive capability for this relationship.

DISCUSSION

Condition indices based on both shell weight and shell cavity volumes have limitations. Shell weight indices do not account for possible changes in shell volume caused by changes in shell shape or thickness. Shell cavity volume indices for oysters are only valid if specimens of the same age are used because oysters from overcrowded natural reefs and young oysters are usually flat, with little space between the valves (Galtsoff 1964). Oysters are notably ecomorphic, volume condition index values from these animals are comparatively high because the soft tissues occupy almost the

TABLE 2.

Linear regressions of relationships between Ci-vol-p, Ci-vol-a, and Ci-shell when plotted respectively against volume, V, and length, L, as listed in Table 1.

y	x	m	c	p	r^2
Ci-vol-p	V	0.014	7.715	0.765	0.001
Ci-vol-p	L	−0.031	9.44	0.629	0.002
Ci-vol-a	V	−0.052	9.792	0.076	0.003
Ci-vol-a	L	0.040	4.936	0.927	0.003
Ci-shell	V	−0.005	2.173	0.300	0.009
Ci-shell	L	−0.005	2.351	0.305	0.008

All relationships as y = mx + c where y is Ci value and x is V or L. N = 125 oysters in all cases; 25 each on July 1, July 8, July 15, July 22, and July 29, 1987

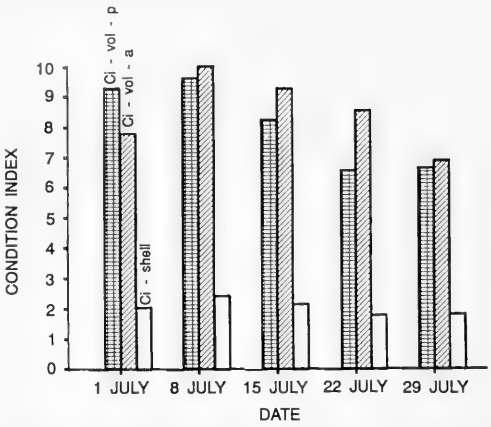

Figure 1. A comparison of mean condition index (of 25 animals) assessed as Ci-vol-p, Ci-vol-a and Ci-shell at weekly intervals during the period July 1–29, 1988.

entire shell cavity. The lack of relationship between Ci-vol-p and Ci-vol-a in the present study suggests that error in measurement of volume, especially so in passive systems where surface tension problems in large bore measuring containers may result in errors that are a significant fraction of the reading, may be a more widespread and significant problem than previously appreciated. Finally, uncoupled growth of tissue and shell may be quite typical for healthy oysters, resulting in reduced condition values that do not accurately reflect nutritional or physiological status of the oyster (see discussion in Hilbish 1986). Indeed, all condition indices will vary due to seasonally related changes in growth of the animals under study and have been used as descriptors of such change.

In their recent review Crosby and Gale (1990) examine the following indices:

$$\text{Ci-vol} = \text{dry soft tissue weight (g)} \times 1000/\text{internal shell cavity vol (ml)}$$

$$\text{Ci-grav} = \text{dry soft tissue weight (g)} \times 1000/\text{internal shell cavity capacity (g)}$$

$$\text{Ci-shell} = \text{dry soft tissue weight (g)} \times 1000/\text{dry shell weight (g)}$$

In their discussion Crosby and Gale (1990) state that Ci-shell "is an "absolute" index (as opposed to a relative index such as Ci-vol and Ci-grav) comparing metabolism directed towards calcification processes and metabolism focused towards somatic and gametic processes of glycogen storage, protein synthesis, and vitellogenesis. Ci-shell is not, then, an index of nutritive status and should not be used as an indicator of recent catabolic or anabolic activity within a bivalve." We disagree with this conclusion and suggest that all three have utility as indices of nutritive stress. The processes of glycogen storage and catabolism, protein synthesis and possible utilization in respiratory pathways with resultant ammonia excretion, and balance between somatic and gametic processes are all affected by short term stress and continually adjusted by anabolic and catabolic pathways (see Gabbott 1975). If condi-

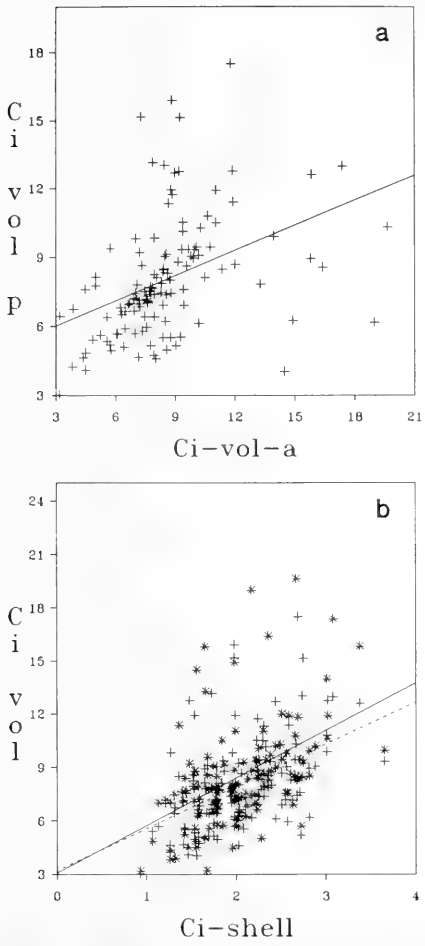

Figure 2. A comparison of (a) Ci-vol-p versus Ci-vol-a ($r^2 = 0.032$, $p = 0.967$), and (b), both Ci-vol-p versus Ci-shell (+, ——; $r^2 = 0.209$, $p < 0.001$) and Ci-vol-a versus Ci-shell (*, - - -; $r^2 = 0.000$, $p = 0.850$) as individual values obtained from 125 animals collected in equal groups of 25 at weekly intervals during the period July 1–29, 1988.

tion is defined as "the ability of an animal to withstand an adverse environmental stress, be this physical, chemical or biological" (Mann 1978), and stress as "a measurable alteration of a physiological, or behavioural, or biochemical, or cytological, steady-state which is induced by environmental change, and which renders the individual (or the population, or the community) more vulnerable to further environmental change" (Bayne 1975), then the obvious requirement of any "static" [sensu Lucas and Beninger (1985)] condition index ratio is to provide a stable denominator to compare with a sensitive numerator. In this instance shell weight is as useful as cavity volume. Both are considered to increase over time as the animals grows but are essentially immune from decreases in value, with the exception of possible minor weight loss due to abrasion or boring organisms.

If, in presenting a quantitative condition index, the intent is to examine short term stress effects or nutritive status then it would arguably be more appropriate to ignore indices based on tissue weight : shell cavity volume or tissue weight : shell weight ratios and use one of the biochemical indices reviewed in Mann (1978) or the one of the "dynamic" indices offered by Lucas and Beninger (1985). Mann (1978) discusses the use of percentage carbohydrate content [equivalent to the glycogen content as discussed by Ingle (1949), Walne (1970) and Gabbott and Stevenson (1974)], carbohydrate : nitrogen ratio (as an index of stored respiratory substrate compared to somatic tissue), carbon : nitrogen ratio (total organic content compared to somatic tissue) or percentage organic content. All of these biochemical indices have been used and compared to one or both of Ci-vol (dry meat weight : shell cavity volume) and Ci-shell (dry meat : dry shell) indices. Walne (1970) and Gabbott and Stevenson (1974) both report a good correlation in *Ostrea edulis* between the dry weight : shell cavity ratio (Ci-vol-p of this study, Ci-vol of Crosby and Gale, 1990) and a glycogen condition index calculated as [glycogen (g) / internal shell volume (ml)] ($P < 0.01$ and $P < 0.001$, respectively). Mann (1978, Table 1) compared dry meat : dry shell condition indices (Ci-shell of Crosby and Gale, 1990) with percentage carbohydrate, carbohydrate : N ratios and percentage organic content values for field populations of *Crassostrea gigas* (data of Matsumoto et al. 1934), *Ostrea edulis* (data of Walne and Mann 1975) and *Mytilus edulis* (data of Dare and Edwards 1975). In all but two instances highly significant ($P < 0.001$) positive relationships were observed, and significance values for the remaining plots were $P < 0.01$ and $P < 0.02$. Further, Table 2 of the same study compares four biochemical indices; percentage carbohydrate, carbohydrate : N ratio, C : N ratio, and percentage organic content to the same shell condition index for laboratory maintained populations of *Crassostrea gigas* and *Ostrea edulis* (data subsequently published in Mann 1979a) and *Tapes philippinarum* (Adams and Reeve) = *Tapes japonica* (data subsequently published in Mann 1979b) and in all instances found highly significant ($P < 0.001$) positive relationships. Lucas and Beninger (1985) expand this offering to include net growth efficiency, scope for growth, O:N ratio (oxygen consumption relative to nitrogen excretion) and relative maintenance cost).

Both Ci-vol and Ci-shell condition indices reflect biochemical or nutritive status, and generate a quantitative measure by comparing a sensitive numerator, dry meat weight, against a relatively stable demoninator, shell weight or volume measured in absolute units. Efforts to generate intercalibration factors between indices, especially cavity volume and shell weight based indices, within a single group of animals have been limited. The relationships illustrated in Figure 2 suggest that simple linear algorithms cannot be generated to intercalibrate shell and volume condition indices, although this may be a function of possible volumetric measuring error as mentioned earlier. Further, the aforementioned comments of Galtsoff (1964) underscore the problem of attempting to compare data collected by different investigators at different times and locations and strongly suggests possible age (and presumably size) dependency. Size, measured as length or volume, dependency was not observed in the present study for any of the three indices measured; however, size dependency in condition (Ci-vol-p of this

study, Ci-vol of Crosby and Gale) was observed in oysters collected from Virginia estuaries by Austin, Haven and Mustafa (in review). Condition indices clearly have value for comparisons within data sets that have been consistently collected; however, comparisons with quantitative data of other authors and/or historical data sets collected by other investigators or methods may be limited to discussion of temporal trends rather than absolute values.

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THE INFLUENCE OF INTERTIDAL HEIGHT ON GROWTH, MORTALITY AND *HAPLOSPORIDIUM NELSONI* INFECTION IN MSX MORTALITY RESISTANT EASTERN OYSTERS, *CRASSOSTREA VIRGINICA* (GMELIN, 1791)

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ABSTRACT Oysters, *Crassostrea virginica* (Gmelin), selectively bred for resistance to mortality associated with *Haplosporidium nelsoni* (Haskin, Stauber and Mackin 1966), were held at five different intertidal levels in the Delaware Bay during their first growing season. Survival was directly proportional to aerial exposure (AE) with the most exposed oysters (28.2% AE) suffering the highest mortality (30%). Growth, condition, fouling, *Polydora* infestation and a measure of marketability were all inversely proportional to aerial exposure. There was no detectable relationship between *H. nelsoni* incidence or intensity of infection with aerial exposure at the end of the experiment. Due to the heavy infestation of *Polydora* blisters at low levels, we suggest that more aerially exposed ("hardened") oysters grown at lower intertidal levels for a second growing season would lead to a higher overall yield.

KEY WORDS: intertidal, *Crassostrea virginica*, *Haplosporidium nelsoni*, MSX, hardening

INTRODUCTION

The oyster parasite *Haplosporidium nelsoni* (Haskin, Stauber and Mackin 1966), commonly known as MSX, has been responsible for extensive mass mortalities of the native American oyster *Crassostrea virginica* (Gmelin), leading to the steady decline of oyster populations along the eastern seaboard of the United States since the early 1950s (Andrews 1968, Farley 1968, Ford and Haskin 1982, Andrews 1984). Despite its recognition as a major pathogen, the full details of the life cycle of this ascetosporidan protozoan remains unclear. The MSX infection cycle is seasonal (Andrews 1982, 1984) yet our knowledge of the life cycle is incomplete largely because neither *C. virginica* tissue nor *H. nelsoni* can be cultured *in vitro* and there is no direct evidence of there being an intermediate host or host-to-host transfer (Haskin and Ford 1979). The presence of *H. nelsoni* in *C. virginica* poses an energetic burden on the oyster host (Barber et al. 1988a, Newell and Barber 1988, Barber et al. 1991), debilitating its feeding capacity (Newell 1985), reducing the biochemical energy store (Barber et al. 1988b) and lessening its reproductive capacity (Ford and Figueras 1988). The combined effects of parasitism and fluctuations in natural and anthropogenic environmental factors are also considered to increase the energetic burden imposed by MSX disease (Littlewood and Ford 1990).

Ever since MSX epizootic mortalities have occurred in the Delaware Bay (Haskin et al. 1965), selective breeding programs have been implemented to develop stocks of *C. virginica* resistant to MSX induced mortality (Ford and Haskin 1987). There is also evidence that similar disease tolerance has evolved in native oyster populations (Haskin and Ford 1979, Ford 1988) although these populations remain too devastated to support the ailing oyster industry. Aquaculture of *C. virginica*, as an alternative to fishing wild stocks, is in its relative infancy in the Delaware Bay (Aprill and Maurer 1976). Furthermore, aquaculture of disease resistant strains has been largely untested. Resistant strains are limited in quantity and are not presently commercially available.

Of the wide variety of oyster culture techniques available, intertidal cultivation may be the most pertinent method in the Delaware Bay region for the following reasons. Extensive tidal flats enable relatively easy access to intertidally cultivated stocks, and oysters which are regularly exposed to air are comparatively free from fouling and predation pressure (Arakawa 1980, Gibbons and Chu 1989). Thus, oysters held in the intertidal zone may be expected to experience greater survival than oysters held subtidally unless fouling and predation is controlled (e.g. see Ogasawara et al. 1962, Wisely et al. 1979, Littlewood 1988, Gibbons and Chu 1989).

The proportion of time an intertidally held animal is exposed to air, is dependent on its position (vertical height) relative to the tide and the amplitude of the tide. These parameters may be integrated into a proportion of aerial exposure (AE). Oysters held in the subtidal zone experience 0% AE and have the maximum time available to feed. Consequently, with increased aerial exposure, growth and the total yield of oysters is expected to be reduced as feeding time becomes limiting.

MSX is thought to be a water-born parasite (Ford and Haskin 1982) and we were interested in whether infection prevalence and intensity were affected by intertidal height, and how growth, mortality and yield of cultivated *C. virginica* might be affected at different exposure levels in a bay experiencing an MSX epizootic. Our overall goal was to investigate the viability of an aquaculture technique in the context of MSX disease. Although we also aim to shed light on the effects of aerial exposure on the parasitism of *C. virginica* by *H. nelsoni* it must be recognised that we used only MSX mortality resistant stock.

MATERIALS AND METHODS

Three multi-level racks constructed from tubular steel were anchored firmly on the tidal flats at the Rutgers Shellfish Research

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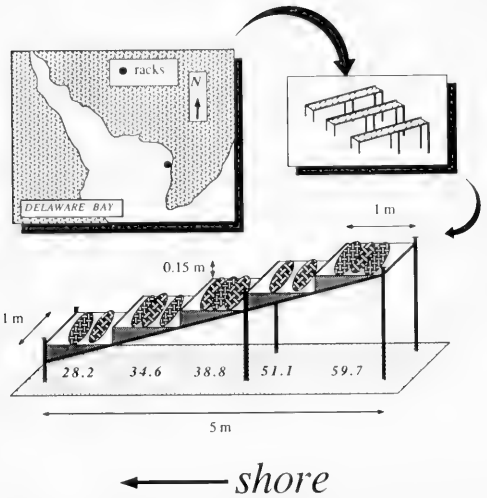

Figure 1. Location, position of racks and their dimensions. Mesh bags were held at five levels; italicized numbers represent estimates of per cent aerial exposure (see Fig. 2).

Laboratory's Cape Shore field station in the Delaware Bay. Each rack consisted of a step-wise series of horizontal PVC coated metal mesh tiers which in turn supported the experimental animals. The location, orientation of each rack relative to the shore and approximate dimensions of each rack are illustrated in Figure 1.

Only hatchery reared oysters, bred in this laboratory for resistance to MSX induced mortality, were used in the experiment although comparisons are later made with unselected stocks grown by this laboratory at the same site during the same months. Two to three nylon mesh (25 × 25 mm) bags, each with 100 cultchless oysters (9 months old) were placed at each level on each rack on 2 May 1988 and were sampled periodically until the experiment was terminated on 6 December 1988. Bags were weighed, cleaned and reweighed to determine fouling and siltation load between sampling periods. Dead oysters were counted to calculate mortality and the shell height of 25 oysters from each bag was measured (±1 mm) to estimate growth.

At the end of the experiment 50 oysters were collected from each level, equally distributed between the bags on each rack. Shell height, shell length, and total weight was determined for each animal. Oysters were shucked, dried shells were weighed and oyster tissue was fixed in Davidson's fixative and later stored in 70% ethanol. Whole fixed meats were weighed and sections of tissue were removed and processed for histology according to standard methods (Haskin et al. 1966, Douglass and Haskin 1976) to determine MSX presence and intensity. MSX disease in oyster body and gills was scored to give a single discrete scale of infection intensity using a standard, repeatable method described by Barber et al. (1988a), where 0 represents no infection, 1 represents light epithelial gill infections, 2 represents light systemic infections, 3 represents a heavy gill infection, and 4 represents an advanced systemic infection. The remainder fixed meat was dried to constant weight and reweighed to estimate total dry meat weight. Condition index was calculated both as the ratio of total dry meat weight to shell weight multiplied by 100 (CWT) and total dry meat weight to shell cavity volume multiplied by 100 (CVOL). For our purposes shell cavity volume was calculated as the difference between whole animal and shell weight and then converting this weight to a volume assuming the density of oyster meat is equal to that of sea water (see Lawrence and Scott 1982). The total number of *Polydora* mudworm blisters on the inside of each upper valve was recorded. A previous study has shown that there is no significant difference in *Polydora* infestation between upper and lower valves of an oyster, either in terms of number or area of shell affected (Wargo and Ford unpublished data).

The vertical height of each rack level above datum was recorded for the field site. The proportion of time each rack level was exposed to air during the experiment, i.e. % aerial exposure (AE), was determined from tide tables (NOAA 1987) using the method described by Littlewood (1988). Condition index ratios and mortality data were arcsine transformed prior to statistical analyses. Data were analyzed with the SAS (SAS Institute Inc. 1987) and SuperANOVA (Abacus Concepts Inc. 1989) statistical packages.

RESULTS

Tide and Aerial Exposure

The height above datum, the tidal cycle and the proportion each rack level was aerially exposed are shown in Figure 2. All subsequent results are presented as a function of aerial exposure. This should allow comparison of data between sites where, for example, beach profiles, tidal cyclicity and tidal amplitude may differ.

Survival, Growth, Fouling and Condition

Figure 3 illustrates survival, growth and the rate of fouling as a function of aerial exposure during the course of the growing season. 95% confidence intervals about the mean values in Figures 3a, b are so small they are occluded by the symbols. Solely for illustrative purposes and in order to clarify the effects of aerial exposure we chose to pool the data within each rack level for these figures.

Oysters held at the 28.2% AE (lowest) level suffered highest mortalities, and the differences in survival between levels were

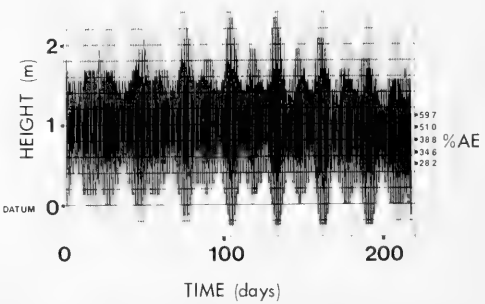

Figure 2. Tidal cycle based on highs and lows relative to datum; data were taken from published tide tables (NOAA, 1987). Estimate of the proportion of time each rack level was exposed to air during the experiment (% AE) calculated according to Littlewood (1988).

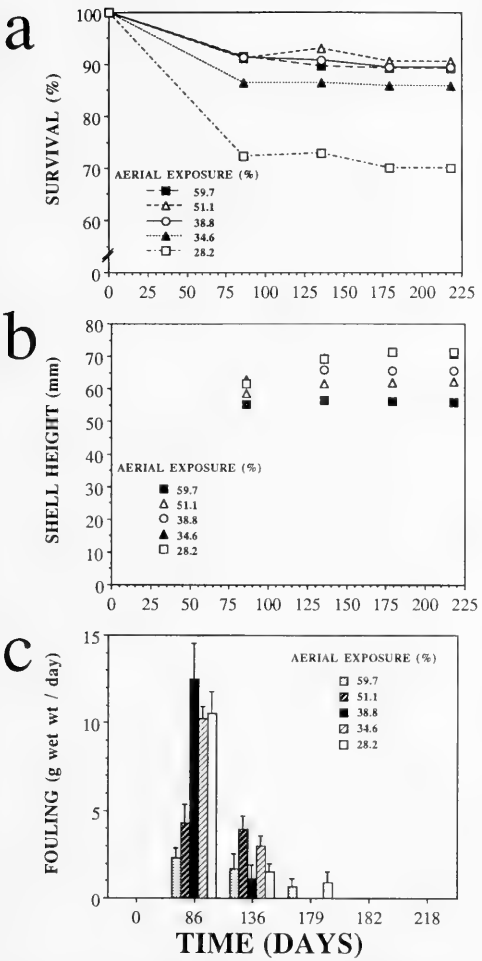

Figure 3. a. Survival, b. shell height and c. rate of fouling accumulation at each of five intertidal levels during one growing season. All data are presented as means ± 95% confidence intervals; confidence intervals are too narrow in a. or b. to be seen.

established by day 86 (August 10, 1988; see Fig. 3a). There were significantly fewer oysters at 28.2% AE level from this date until the end of the experiment (χ^2-test; $P < 0.01$). Mean shell height was significantly different between levels at each sampling date (ANOVA, $P < 0.001$) with shell height inversely proportional to aerial exposure (Fig. 3b).

The major component of fouling was mudworm infestation, probably *Polydora ligni*, and the large volume of silt associated with the species (Stauber and Nelson 1940). In general, fouling accumulated at a greater rate at levels experiencing less aerial exposure (Fig. 3c). The rate of fouling was not consistent during the growing period. The greatest weight of fouling was recorded during the first three months of the growth period, irrespective of exposure level.

Table 1 illustrates the results of a nested analysis of variance conducted on data accumulated on the last day of the experiment. Although there were strong bag and rack effects, indicated by low P values, we were particularly interested in the effects of aerial exposure (among levels component). The strong influence of intertidal exposure is demonstrated in the ANOVA table, where all growth and condition variables were significantly different among levels, and also in Figures 4a, b where mean values ± confidence intervals do not overlap. The trend towards decreased growth and condition with increased aerial exposure is clear.

Oysters grown on the lowest intertidal exposure level (28.2% aerial exposure) had the greatest number of *Polydora* blisters on the upper valve. The mean number of blisters diminished with greater aerial exposure (Fig. 4b). ANOVA indicated a statistically significant difference in numbers of blisters between levels (Table 1) with oysters grown on the lowest level experiencing the highest levels of infestation (mean = 1.88 blisters per valve).

MSX Infection

Fewer than 20% of the oysters sampled at each level (from 2 to 8 individuals) were infected with *Haplosporidium nelsoni* (Fig. 4c). Again, for clarity, only the overall means in Figures 4a, b are illustrated. Aerial exposure had no statistically significant effect on MSX scores (Table 1). A χ^2-test indicated that there was no significant difference in the proportion of MSX infected and uninfected oysters between exposure levels ($\chi^2 = 6.816$, 4 df, $P = 0.146$). Although neither MSX incidence nor mean MSX intensity amongst infected oysters follow regular or similar trends with aerial exposure (Fig. 4c) each tended to be higher with greater aerial exposure.

Marketability

Figure 5 illustrates the proportion of the initial number of oysters attaining marketable quality as a function of intertidal height. We have chosen two criteria for marketability to discuss. Those

TABLE 1.

Results of nested analysis of variance determining the effects of bag, rack and level on oyster growth variables, oyster condition, MSX infection, and *Polydora* infestation; probabilities (italicized) and F ratios [df].

	Source of Variation					
	Among Levels		Among Racks Within Levels		Among Bags Within Racks Within Levels	
Dependent Variable	$F_{[4,206]}$	*(P)*	$F_{[9,206]}$	*(P)*	$F_{[25,206]}$	*(P)*
shell height	46.18	*(<0.01)*	3.40	*(<0.01)*	1.60	*(0.04)*
shell length	33.79	*(<0.01)*	1.06	*(0.39)*	1.80	*(0.15)*
whole wt.	20.56	*(<0.01)*	1.67	*(0.10)*	1.53	*(0.06)*
shell wt.	14.31	*(<0.01)*	1.64	*(0.10)*	1.43	*(0.09)*
dry meat wt.	31.14	*(<0.01)*	1.74	*(0.08)*	2.06	*(<0.01)*
condition	7.89	*(<0.01)*	1.53	*(0.14)*	1.22	*(0.22)*
MSX rating	1.26	*(0.29)*	1.67	*(0.10)*	1.29	*(0.17)*
Polydora	67.95	*(<0.01)*	4.18	*(<0.01)*	0.94	*(0.55)*

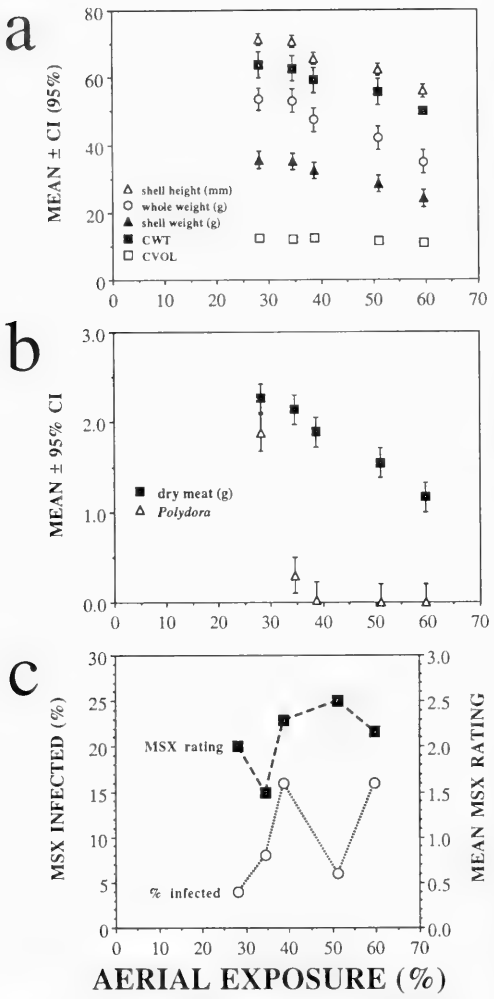

Figure 4. a. Final oyster dimensions and condition index, b. MSX intensity, dry meat weight and *Polydora* infection; values are means (n = 50) ± 95% confidence intervals, c. proportion of oysters parasitized with MSX, and intensity of infection of oysters with MSX. All variables are presented as a function of aerial exposure.

oysters with a shell height of 70 mm or more are considered to be of marketable size, and of those, oysters without mudworm blisters are considered to be preferable for the half-shell trade (Fig. 5). Approximately 50% of the oysters grown at each intertidal height below 35% exposure reached marketable size, whereas fewer than 25% were marketable at each level above 35% exposure. Indeed, based on size alone there is a direct relationship between aerial exposure and marketability. However, when those oysters (≥70 mm) with mudworm blisters are eliminated, the marketability of

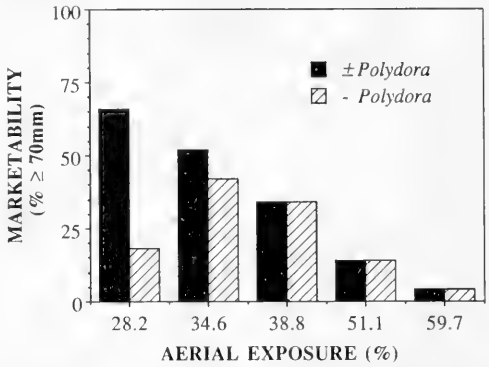

Figure 5. Marketability of oysters as a function of aerial exposure. Marketability is defined as the proportion of the initial number of oysters attaining a shell height of ≥70 mm; i. regardless of mudworm blisters (± *Polydora*), and ii. without blisters (− *Polydora*).

oysters grown at the lowest level (28.2% AE) is severely reduced, with those oysters grown at 34.6% and 38.8% AE yielding the highest proportion of oysters suitable for the half-shell trade.

DISCUSSION

Condition Index

Based on shell weight, condition index (CWT) is recognised as a useful index of physiological condition as it is essentially a ratio of tissue to shell growth (Lucas and Beninger 1985). In retrospect however, it is inappropriate to compare CWT between levels, as shell weight itself changes as a function of aerial exposure. Although both shell and dry meat weight change in a similar fashion with aerial exposure, they do so at a different rate so that increased aerial exposure results in a reduction in CWT. This is probably due to the considerable increase in shell thickness with greater exposure, observed in this experiment and others (e.g. Littlewood 1988).

Condition, based on shell cavity volume (CVOL), is a more appropriate index when comparing levels as it deals more specifically with meat, rather than whole oyster, condition (Mann 1978). Although statistically significant, the reduction in CVOL with increased aerial exposure is less dramatic than with CWT. Meats were of high quality at all levels and we consider any differences in CWT between levels to be negligible. Therefore, we consider aerial exposure to have little effect on meat quality, although it is recognised that increased aerial exposure considerably reduced meat growth.

Fouling and Polydora

The difference in the rate of fouling during the experiment corresponds with the settlement of the mudworm *Polydora ligni* which occurred predominantly between June and July of 1988 (personal observations, RNW). The large volumes of silt which also accumulated up to August 10 (day 86) were attributable to the presence and characteristic burrow forming behaviour of *P. ligni*

(Stauber and Nelson 1940). After day 86, when bags were cleaned, relatively less fouling collected; from this time few *P. ligni,* or indeed any other epibiont, settled. We conclude that the fouling (recorded mainly as silt) was seasonally coincident with the settlement of *P. ligni* and that increased aerial exposure may have limited the recruitment of mudworms as well as having limited the time available for siltation. The temporal and spatial incidence of *P. ligni* in the present study coincides with previously recorded settlement patterns of *P. ligni* (Green and Hobson 1970, Orth 1971).

Mudworm blisters in the experimental oysters are believed to have been caused exclusively by *Polydora websteri* (Blake 1969, Zottoli and Carriker 1974). Both *P. ligni* and *P. websteri* infection intensity may be limited by controlling aerial exposure, and blisters may be avoided by holding oysters at levels experiencing greater than 40% aerial exposure, at least during times of mudworm settlement.

MSX Parasitism

The incidence and intensity of *Haplosporidium nelsoni* parasitism of *Crassostrea virginica* at the termination of this experiment had not been significantly affected by aerial exposure (between 28 and 60%), although there were indications that greater exposure may increase both infection and intensity of infection (Fig. 4c). As MSX infection was determined only at the end of the experiment it is difficult to determine its role in growth and mortality directly from this experiment, where aerial exposure *per se* incorporates so many variables. Nonetheless, a continuing monitoring program at this laboratory enables us to discuss the progress of the MSX infection cycle during the course of the present experiment. In 1988 Delaware Bay experienced a major MSX epizootic. During the first 86 days of the growth period in our experiment (from May to August) each of two stocks of 1988 year class oysters imported from Virginia and Maryland (unselected MSX-susceptible), and held adjacent to oysters in this experiment, experienced MSX mediated mortalities greater than 90% (Ford et al. 1989 unpublished report). Mortality of the resistant stock used in the present study was less than 30% at any aerial exposure, with less mortality at higher levels.

Despite the high infectivity and transmissibility of *Perkinsus marinus* (Ray 1954, Andrews 1984), another water-born parasite pathogenic to *C. virginica,* it does not seem to be affected by tidal exposure either (Burrell et al. 1984, Gibbons and Chu 1989). However, there still exists the possibility that the infectivity and intensity of infection of some water-born protozoan parasites are directly affected by aerial exposure. For instance, *Mikrocytos roughleyi* which has been implicated as the causal agent of winter mortality in *Saccostrea cucullata* (Farley et al. 1988, R. J. G. Lester pers. comm. 1990) has been shown to be limited when oysters are held high in the intertidal zone (P. H. Wolf and A. J. Collins pers. comm. in Wisely et al. 1979). This suggests that disease may be controlled by limiting the time oysters are exposed to infective particles or agents in the water column. In the present study the lack of a relationship between exposure and disease may be explained by a number of possibilities. One such scenario may be that there are too few infective particles or intermediate hosts in the water column, so that the probability of the pathogen, or its vector, coming into contact with and infecting the host is very small. This is unlikely in the present study considering the high mortalities experienced by the adjacent, unselected stocks. Alternatively, infection may be temporally or spatially constrained within a specific tidal cycle(s) such that oysters at all intertidal levels are equally as likely to be infected.

Marketability

The yield of marketable *Crassostrea virginica* grown in mesh bags within the intertidal zone of a bay with MSX was clearly dependent on the degree of aerial exposure. Unlike other oysters, *C. virginica* was capable of growing at intertidal levels exceeding 50% AE (c.f. *Ostrea edulis* which reportedly fails to grow above 30% AE, Walne, 1958), indicating further its remarkable adaptation to an intertidal existence (Galtsoff 1964). Gillmor (1982) showed that intertidally grown *C. virginica* grew better at certain intertidal levels than subtidally, even in the absence of fouling organisms. This suggested that the oyster has the ability to supplement energy input within the intertidal zone (see also Littlewood 1988). Our experiment did not include subtidally held oysters, but we do show an additional advantage in holding oysters intertidally, at least during their first growing season.

The occurrence of mudworms, which reduce oyster condition (Wargo and Ford unpublished data), and mudworm blisters, which weaken oyster shells and thereby interfere with shucking, reduce the market value of oysters (Haigler 1969, Kennedy and Breisch 1981). As a consequence, those oysters grown at the lowest exposure level were not the most suitable for the half shell trade, despite their larger shell size and whole weight.

This experiment followed oysters through to the end of their first full growing season. For commercial purposes oysters would probably best be grown for all or part of a second growing season to maximize the number of marketable, blister-free oysters. However, despite the size advantage of oysters grown in the low intertidal range, the high survival and mudworm free condition of oysters grown in the high intertidal range justifies their use as suitable stock to be grown for a second season. Oysters with mudworm blisters, i.e. those grown at low aerial exposure, are best sold at the end of the first growing season.

Keeping oysters high in the intertidal zone during the first growing season is known to enhance survival and increase shell thickness in *Crassostrea gigas* (Ogasawara et al. 1962). In Japan, *C. gigas* are cultured for these reasons because they become "hardened". A "hardened" oyster is one held intertidally for its first year; it is small and thick shelled. In their second growing season growth rate is so high that they attain the size of oysters of the same age held subtidally (Ventilla 1984). The advantage of this technique with *C. gigas,* and with *C. virginica* in the present study, is that there are a greater number of "hardened" oysters than those originally less exposed or subtidal. In the light of our results and considering the economic advantages of "hardening" *C. gigas* (Ogasawara et al. 1962, Ventilla 1984) we recommend a thorough investigation of the technique as applied to *C. virginica.*

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DESIGN AND MANAGEMENT OF A CLOSED, RECIRCULATING "CLEARWATER" HATCHERY SYSTEM FOR FRESHWATER PRAWNS, *MACROBRACHIUM ROSENBERGII* DE MAN, 1879

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ABSTRACT Management techniques, including hatchery design, start-up, maintenance, and harvesting, found to be most effective and efficient for successful larval culture of the freshwater prawn *Macrobrachium rosenbergii* in a closed, recirculating "clearwater" system are described. This detailed information is based upon several years of practical experience that has included the evaluation of both newly developed techniques and published recommendations that have been either followed or modified. Heretofore undetailed descriptions of biofilter start-up and maintenance as a separate unit and efficient feeding practices to minimize organic build-up and to maximize food utilization are presented. The quality of the procedures have been judged by the achievement of reliability and consistency in production yields.

INTRODUCTION

There are many published descriptions of larval culture of freshwater shrimp. These descriptions either do not provide sufficient detail or specifically concentrate on one particular aspect of culture. During the past five years of study at the Aquaculture Unit of the Mississippi Agricultural and Forestry Experiment Station (MAFES), a management system for larval culture has evolved and is based upon the evaluation of newly developed techniques combined with modifications of the closed recirculating "clearwater" system described for the intensive larval culture of *Macrobrachium rosenbergii* (De Man) by Aquacop (1977, 1983).

The procedures described are based upon the overall goal of maintaining not only a "clearwater" but also a "clean" system free of excessive levels of heterotrophic bacteria and nitrogenous waste. Efforts have been directed toward minimizing the organic load within the culture tank by providing sufficient water exchange and filtration and by maximizing feeding efficiency (i.e., the ability of the larvae to effectively locate and crop off the introduced feed). Efficiency of nitrogenous waste removal can ultimately depend upon the type of substrate composing the biological filter and the substrate conditioning procedure. Feeding efficiency is affected by intensity and angle of lighting, water quality, and feeding regimes. Many of the management procedures that are presented have applicability to the larval culture of other organisms.

HATCHERY DESIGN AND OPERATION

Hatchery Design

The design of the hatchery should be based upon the desired rate of production of postlarvae (pls). Past experience suggests that the density of newly-hatched larvae that can be successfully stocked and reared is between 40 and 100/L of water. At the end of the culture period survival ranging from 40 to 80% can be expected and is principally dependent upon hatchery design and management. For example, a tank containing 10,000 liters (10 tonnes) of water and stocked at 50 larvae/L (i.e., 500,000 larvae) and with a survival of 80% at the completion of the larval cycle would yield 400,000 pls. Source of the larvae to stock the system must also be carefully planned. A healthy 45 g female will produce approximately 450 larvae per gram of her weight; larger females will produce more larvae per gram of weight because fecundity is a linear function of body weight and an exponential function of body length (Malecha 1983). To produce 500,000 larvae, approximately 25 egg-carrying female prawn averaging 45 grams each would be needed. A smaller number of larger prawns would also be effective.

Assuming a desired production of 2 million pls (2.5 million larvae at 80% survival), 5 larval culture tanks containing 10 tonnes of water each and totaling 50 tonnes (50,000 L) would be required. Volume of the biological filter (biofilter) is approximately 6% of the volume of the total culture system (Griessinger et al. 1989) or 2.5 tonnes (2500 L). As the stocking rate is increased, the turnover rate must be accordingly increased. The highest stocking rates may require a maximum turnover rate of 70 to 100%/h.

A 50,000 L larval culture system requiring a 70% turnover rate would require a flow rate of 35,000 L/h. Therefore, a pump, sand filter and ultraviolet light should be purchased to perform efficiently in meeting these requirements. The proper sand particle size (850 μm) should be used in the sand filter to remove particulate matter before water is exposed to the ultraviolet light and enters the biofilter to maximize their efficiencies. Also, the sand filter must be flushed on a regular basis (once to several times daily) to prevent any accumulation of organic material which causes clogging, channeling, and growth of potentially pathogenic bacteria. Other types of particulate removal systems, such as upflow bead filters, may be utilized. Although an ultraviolet light system is an expensive item, the purchase is well worth the expense, especially to the novice. The ultraviolet light used at the Aquaculture Unit of MAFES typically reduced the number of bacteria from a high concentration of 10^6 to 10^9 bacteria/ml (inlet) to

a low concentration of 1 to 10/ml (outlet). The size of the ultraviolet light system used is dependent upon the flow rate. Other types of disinfectant systems may be applicable to this type of larval system. All equipment should be suitable for use in saltwater systems and be free of potential contamination from leaching of metal such as copper, brass, or zinc.

Lighting, especially indirect sunlight, is extremely important for freshwater prawn larval culture. Moller (1978) reported that larvae capture food by chance encounter. Contrary to this belief, prawn larvae do feed by sight as evidenced on cloudy days when feed consumption by larvae is significantly reduced. Therefore, sufficient lighting is necessary to insure maximum food consumption (Aquacop 1977). Aquacop (1977) further states that direct sunlight may cause mortalities by apparent sunburning of the exoskeleton. Natural lighting should be used whenever possible because of the difficulty in achieving a sufficient light intensity with artificial lighting. The intensity of lighting should be 30,000 to 700,000 lux (i.e., typical of partly cloudy to clear days).

The system for hatching *Artemia*, the principle food for prawn larvae, should also be designed to meet the maximum requirements of the desired level of postlarval production. For example, if 2.5 million larvae have an upper feeding rate of 100 *Artemia*/larva/day and assuming an average hatching rate of approximately 150,000 *Artemia* nauplii per gram of cysts, then a daily *Artemia* hatch of 1667 g of cysts is required. *Artemia* are generally hatched at 1 to 10 g of cysts/L of water (Sorgeloos, 1980). Therefore, following the use of 1 g of cysts/L of water, the *Artemia* hatchery system would require an 1667 L working volume or about 3–550 to 600 L tanks. Hatching tanks should be constructed of fiberglass and have a gently sloping, conical bottom equipped with a bottom drain for harvesting and transparent or opaque sides. A holding tank for mixing and storing artificial saltwater for *Artemia* hatches and pumps for transferring water from the storage tank to the *Artemia* hatching tank are also required. Additional tanks will be required if fortification of *Artemia* with presumed essential fatty acids or other nutrients is then desired.

The hatchery should be enclosed in a building with a concrete floor wherein sterilization procedures can be effectively performed and cleanliness can be easily maintained. The building should be designed to have temperature control and to maximize use of indirect sunlight. Provisions for the availability of intense artificial lighting as backup should be included in the design. The building should be serviced with a backup generator to operate the pump, air blower, and heaters during an emergency involving the loss of electricity.

Preparation of the Larval Culture System

The larval culture system should be cleaned and flushed with filtered, fresh water prior to filling. Filtered fresh water or filtered natural salt water can be used to fill the system. If fresh water is used then a commercially manufactured, artificial marine salt mixture should be added until the desired salinity is achieved. After the system is filled and operating, it should be sterilized by adding 5 mg/L of available chlorine a few days prior to stocking. Use of a powdered chlorine product (calcium hypochlorite) is preferred over a liquid product (sodium hypochlorite) because the liquid product is more persistent and not as easily removed by aeration. The availability of chlorine in a commercial product must be taken into account in calculating the amount to add (e.g., a product with 65% available chlorine requires 5 mg/l ÷ 0.65 = 7.7 mg/L). If the system is equipped with adequate aeration and filtration most chlorine will dissipate in a few days. However, a chlorine test should be performed to verify that none remains. If chlorine persists, addition of sodium thiosulfate at a concentration of 3 mg/L for each 1 mg/L of chlorine will effectively remove the chlorine. Caution should be exercised in the use of sodium thiosulfate because it is toxic to penaeid (marine) shrimp larvae (Johnson and Cichra 1985) at low to medium levels (4 to 50 mg/L) and to stage I freshwater prawn larvae (Daniels, unpublished data) at levels of 100 ppm but possibly at lower levels, too.

Water in the culture system should be thoroughly filtered after addition of the artificial salt mixture because most of these products use a protein-based carrier molecule that leaves a film or color. Salinity and temperature should be adjusted to, or near, optimal larval culture conditions and allowed to stabilize prior to stocking. Systems should have adequate heating and buildings should have proper ventilation to provide a controlled water temperature (28–32°C) independent of air temperature. Salinity should be kept within 12 to 16 ppt (Sandifer et al. 1977) and abrupt changes should be avoided. An artificial seasalt mixture or natural salt water can be used, but selection of the brand of artificial salt mixture or the source of natural seawater should be considered carefully. While some artificial salt mixtures are adequate for finfish culture, some may not provide proper growth and good survival of freshwater prawn larvae (Daniels, unpublished data). Artificial seasalt mixtures have been successfully used, but whenever practical a good source of natural seawater should be selected.

To reduce costs associated with the use of an artificial salt mixture, salinity in the hatching system and culture tank can be initially lower (5 to 7 ppt) than that of eventual grow out conditions. The salinity in the culture tank can then be increased after stocking by introducing the salt mixture through the sand filter and biofilter tank prior to the addition of any biofilter substrate. Addition of either salts or fresh water to adjust salinity during the larval culture phase should not be performed via the biofilter tank once the biofilter substrate has been added. Bacteria in the biofilter are sensitive to sudden changes in temperature and salinity. New water should be sterilized and filtered prior to introduction into the system.

Biological Filtration

The biofilter should hold approximately 6% of the volume of the larval culture tanks (Griessinger et al. 1989). Many biofilter designs are acceptable as long as they effectively and efficiently remove all ammonia and nitrite produced during the cycle. Any calcareous material can be used as filter substrate but smaller particles such as crushed oyster shell or coral are recommended. Non-calcareous substances lack the buffering capacity to assist in regulating the pH (8.0 to 8.5) of the water in the system. Substrate material can be enclosed in bags fashioned from plastic or nylon netting of the appropriate mesh to facilitate handling. Adequate aeration is necessary to maintain proper dissolved oxygen levels for nitrifying bacteria.

"Activation" of the biofilter substrate (Griessinger *et al.* 1989) is extremely important in promoting growth of beneficial bacteria and in efficient removal of nitrogenous waste products. Biofilter substrate should be "activated" in a separate preconditioning tank and added to the biofilter as needed to compensate for increases in nitrogenous wastes as feeding and larval biomass increase. Temperature and salinity in the preconditioning tank should be constant

and equal to conditions in the larval culture tank. The amount of "activated" substrate in the biofilter at any time should be able to nitrify 100% of the maximum expected ammonia produced by the larvae in 24 h. The following procedure for the "activation" or preconditioning of filter substrate is suggested.

1. Determine the daily maximum expected ammonia nitrogen load in the larval culture system based on the desired level of postlarval production. The maximum ammonia nitrogen rate of production of a closed, recirculating system for *M. rosenbergii* larviculture based on empirical data is about 30 µg/larvae/day (L. de Parseval, unpublished data). If 60 g of ammonia nitrogen is the maximum expected amount produced within the system in a 24 h-period (i.e, 2 million larvae), then 226.8 g of ammonium chloride (i.e., 1.0 g of ammonium nitrogen per 3.78 g of ammonium chloride) should be completely oxidized by the biofilter substrate being "activated" in the preconditioning tank. A bag of well-maintained crushed coral weighing 2.26 kg usually contains a good population of nitrifying bacteria that will nitrify (oxidize) 1.0 g of ammonium chloride in 24 h. Therefore, 227 bags of crushed coral would be used to nitrify 60 g of ammonia nitrogen. Maximum coral volume, representing less than 4% of the total rearing volume, is reached by the 17th day of rearing and larval stage index = 8.5 (Griessinger et al. 1989).
2. Initially, add 10% of the required total ammonium chloride (NH_4Cl) to the water containing the substrate material. Other inorganic sources of ammonia can also be used.
3. After a few days check the levels of total ammonia (NH_3-N) and nitrite (NO_2-N). Low range ammonia (0.0–0.8 mg/L ammonia-N) and nitrite (0.0–0.2 mg/L nitrite-N) test kits for salt water are satisfactory for such determinations. If both levels are below detection, then add the same amount of NH_4Cl as in #2. If either total ammonia or nitrite is still present, do not add any additional NH_4Cl and check after another 24 h.
4. Continue to add this predetermined amount of ammonium chloride (see #2) and to check the levels of NH_3-N and NO_2-N until none is detected within 24 h of its addition. When this amount of ammonium chloride is completely nitrified within 24 h double the amount and follow the same procedure.
5. As each level of NH_4Cl is consumed within the desired 24 h period, double the amount of NH_4Cl until the maximum required load is consumed daily (i.e., within 24 h).
6. Once the maximum load is achieved, the production cycle can begin. But, the coral remaining in the preconditioning tank must be maintained at maximum level of ammonia and nitrite consumption.

Addition of substrate to the biofilter should coincide with the increase of ammonia-nitrogen produced by an increase in the larval biomass (Griessinger et al. 1989). Typically, beginning 3 days post-stocking, increasing amounts of "activated" media must be added daily to the biofilter tank. The bacterial population provided through daily addition of substrate should always be sufficient to remove all ammonia and nitrite. Extra substrate should be added if nitrite or ammonia levels begin to rise. Upon completion of the larval cycle the substrate of the biofilter can be thoroughly rinsed with brackish water to remove excess organic buildup and either stored dry or returned to the substrate conditioning tank to reestablish the bacterial colony. Alternatively, the substrate can be chlorinated to kill all bacteria, dechlorinated, and then reseeded with stock bacteria from another conditioning tank.

Broodstock Care

Broodstock can either be collected from ponds or an indoor holding facility depending upon location and season of hatch. Hatcheries operating in temperate climates will have to either import broodstock or maintain an indoor broodstock holding facility. Broodstock held indoors for extended periods of time will require a nutritionally complete diet and excellent water quality to promote superior egg production and quality. Harrison (1990) has summarized current nutritional practices and commercial diet formulations for broodstock of decapod crustaceans. The diet should ideally be in a pelleted form. Two principal considerations in the formulation of this diet are that the level of protein (having a balanced amino acid profile) be at least 30% (dry weight) and a total of approximately 0.5% $\geq$ 20 C n-3 and n-6 polyunsaturated fatty acids be available. Commercially available pelleted diets may not be sufficient. Therefore, pieces of beef liver or squid, cut to the appropriate size, should be fed as a supplement to some diets at least twice per week. On the day of supplemental feeding, 50% of the amount of the pelleted diet should be substituted with a dry weight equivalent of beef liver or squid. As beef liver and squid have moisture contents of approximately 80%, one kg of either one would be only 200 g by dry weight. The amount of feed fed per day should be divided equally into two feedings (early morning and late afternoon). Broodstock should be fed at a daily rate of 1–3% of total biomass. The feeding rate should be adjusted to match consumption.

Broodstock may be held in fresh or brackish (<15 ppt) water at 27–32°C. Colder temperatures will reduce the number of eggs, increase the time for egg development, and appear to promote fungal growth on eggs. Indoor broodstock facilities should be kept clean by siphoning excess food and waste several times weekly. Habitat (e.g., netting or shelters) should be provided in the water column of the holding tank to reduce the potential for cannibalism and to increase fecundity by providing shelter and increased surface area. Hanging small mesh nylon netting vertically or horizontally in the water column is one method of providing habitat.

Density of brooders in the holding tanks appears to influence the egg-carrying capacity of females such that the total number of eggs that eventually hatch is reduced at higher densities. A stocking density of 1 individual per 20–60 L and a ratio of one or two blue-clawed (BC) males per 20 females is recommended. If broodstock are collected in early to late October and larval production is planned for December or January, then orange-clawed (OC) males should be stocked at a ratio of 2 or 3 OC per 20 females in addition to the BC males. Ra'anan and Cohen (1985) define the different male morphotypes (i.e., BC and OC). The OC males should weigh >35 grams when stocked. If newly hatched larvae are needed after March then OC males should be stocked at 3 to 4 per 20 females to offset increased mortalities of males over a longer holding period.

Egg Collection and Hatching

Gravid females should be collected in adequate numbers to provide the number of larvae necessary to stock the culture system. Usually an additional 10 to 20% are collected to insure that a sufficient number of larvae are collected within a 3-day period. Eggs that are in an advanced state of development (grey color)

should hatch within 1 to 4 days. Females may be chemically treated to remove any bacteria, parasites, etc. prior to placement into the hatching system or treated within the system. New (1990) states that berried females are disinfected for 30 min in aerated water containing 0.2–0.5 mg/L copper or 15–20 mg/L formalin. Other antibiotics or chemicals might be employed but caution should be exercised with all methods and pretested on a few females to evaluate the effects on broodstock and larval hatching. Only FDA approved chemicals should be used for commercial purposes. Failure to prevent introduction of potentially pathogenic organisms from the hatching system into the larval culture system can result in extensive mortality.

A typical hatching system consists of a hatching tank, larval collector, and a mixing tank or biofilter. Females are placed into the hatching tank supplied with adequate habitat (e.g., a piece of pvc pipe for each individual). The interior of a hatching tank is black except around the area where the overflow pipe is located. This area is painted with a lighter color. Black painted grating (e.g., egg crating or louver material) divides the tank into two chambers with the chamber for holding the females occupying about 80% of the tank volume. Water overflows from the hatching tank into the collection tank, passes through an 180 μm mesh screen located around a center standpipe, and then flows into the mixing tank. Water is returned to the hatching tank from the mixing tank by airlifts. Larvae can be hatched in fresh or brackish water (0 to 12 ppt), but hatching success is higher in brackish water (New 1990). Gravid females can be placed into these salinities without a need for acclimatization. A total of 600,000 larvae can be hatched in a system consisting of a 300 L rectangular hatching tank with two 120 L circular tanks used as collection and mixing tanks.

During the evening the hatching tank is completely covered with black plastic except directly over the overflow pipe where an incandescent light (25 to 60 watts) is placed. Newly hatched larvae are attracted to the light source. As water flows from the hatching tank, larvae are transported into the collection tank. Collected larvae are usually healthy and free of debris.

On the following day, the number of collected larvae is determined by enumerating the number of larvae in a known volume of water following a procedure similar to that described by Aquacop (1983). This procedure consists of thoroughly mixing the water by hand and taking 8 to 16 samples of a known volume of water (e.g., 30 ml beaker) and counting the number of larvae in each sample. The average number of larvae/ml in the samples is then multiplied by the total volume of the collection tank to yield the total number of collected larvae. All larvae are then removed from the collection tank by siphoning, separated from any debris, and then placed into the larval culture system. This procedure is repeated for up to 3 days until the desired number of larvae are collected and stocked. If more than one day of collection is needed, a partial water exchange in the hatching system is advised and feeding should be reduced.

Daily Maintenance of the Culture System

Water quality should be monitored regularly. Temperature should be measured and recorded 3 times daily (e.g., early morning, noon, and late afternoon). Low levels of dissolved oxygen are usually not encountered except in the biofilter where bacterial respiration exerts a high oxygen demand. However, new systems require periodic monitoring to identify potential problems. Salinity should be checked daily and adjusted as needed. At pH levels ranging from 7.60 to 8.34, total ammonia ($NH_3 + NH_4^+$) should be maintained below 32 and 14 mg/L, respectively (Armstrong et al. 1978). Nitrite should be kept below 1.8 mg/L (Armstrong et al. 1976). Sublethal levels of total ammonia and nitrite can reduce the growth rate of larvae and can increase their susceptibility to parasites and disease. Ammonia and nitrite need to be monitored at least several times a week.

Bacterial concentrations should be monitored several times a week by a dilution method or other method to identify unwanted increases in the concentration of all types of bacteria. However, this procedure is not obligatory as a reduction in the consumption of feed by larvae is an excellent, indirect indicator of bacterial or water quality problems. An experienced manager can use this indicator to identify potential problems and act before a major loss of larvae occurs.

Numerous bacterial flora have been identified in association with prawn and shrimp larviculture. Anderson et al. (1989) identified numerous aerobic heterotrophic bacterial flora found from various *M. rosenbergii* hatcheries in Malaysia. Bacteria in the larval culture water was dominated by gram-negative organisms. They concluded that the high incidence of *Vibrio* sp. isolated from larvae reinforces the opinion that while members of this genus are pathogenic they are not the primary pathogens. Baticados et al. (1990) evaluated numerous chemicals as controls of *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larval culture. They, however, concluded that prevention of disease through rigorous water management and sanitation was the best methodology for control of pathogens. Many of the chemicals used to treat the larvae caused deformities in larvae or their use was cost prohibitive.

Various chemical treatments have been used in the past to try to control disease problems in freshwater prawn hatcheries. However, the use of these chemicals with *Macrobrachium rosenbergii* is not approved by FDA for commercial use in the United States. Aquacop (1977) used streptomycin and bipenicillin at 1.25 to 2.5 mg/L every two or three days as a preventative or at 5 mg/L when necrosis or filamentous bacteria increase. Our experience suggests that oxolinic acid is effective in alleviating pathogenic bacterial problems in research culture tanks while having no adverse effects on those species involved in nitrification. Use of oxolinic acid has been effective in the successful treatment of burn spot lesions in juvenile *M. rosenbergii* by killing *Aeromonas hydrophila* around the lesions (El-Gamal et al. 1986). The effect of oxolinic acid on prawn larvae has not been documented and it should be used with caution. Schnick (1988) describes oxolinic acid as a broad-spectrum antibacterial agent registered for use in fisheries in Europe and Japan with possible future registration in the United States. She cited a personal communication with G. L. Bullock of the U.S. Fish and Wildlife Service that oxolinic acid yielded favorable results against many gram-negative pathogenic bacteria; however, Bullock also found resistant organisms after only 10 transfers in studies to determine resistance.

Daily cleaning of the larval tank is imperative for maintenance of a "clean" environment. The bottom and sides of the larval culture tank must be thoroughly scrubbed daily to remove all algae, organic accumulations, etc. Cleaning should begin by day 3 and end after the appearance of postlarvae. Cleaning can also be performed after each harvest of postlarvae. An ordinary nonmetallic household sponge mop and a large sponge mop can be used to clean the sides and bottom, respectively. The large sponge mop

can be constructed by attaching a piece of foam around a 7.6cm diameter PVC pipe with contact cement and attaching a handle. Aeration must be maintained while cleaning the tank to prevent larvae from being trapped between the mop and sides or bottom of the tank.

After the larval tank has been cleaned, all air and water flow to the larval tank is stopped and the stand-pipe is capped. In circular tanks, a circular current of water can be created by stirring with paddles to concentrate all excess food, dead larvae, etc. in the center of the tank. This material is then siphoned into a plastic bucket where the quantity (number of ml) of excess food is noted and the number of dead larvae are enumerated in a known volume of water (see Egg Collection and Hatching for procedures). The number of dead larvae is then subtracted from the number of estimated larvae in the culture tank to yield a new estimate of larvae. Cannibalism on dead or weak larvae may reduce the accuracy of this estimation.

Before water is again recirculated through the biofilter, the sand filter should be backwashed. After supplemental feeding begins, the sand filter must be backwashed at least twice daily, usually in the early morning and after the tank is cleaned. In a closed, recirculating system using an artificial salt mixture, sterile, filtered fresh water prepared in a storage tank can be used to backwash the filter. However, care needs to be exercised to avoid introduction of this fresh water directly into the biofilter.

Larval Feeds and Feeding Practices

Larvae should be neither overfed nor underfed. Overfeeding results in a buildup of organic matter which causes a proliferation of bacteria and fouling of the water and filtration material. Underfeeding results in poor growth, weak animals, cannibalism, and an extended duration of the larval stages leading to metamorphosis to postlarva.

The establishment of feeding regimes, including feeding schedules and types of feed fed, is extremely important in maximizing feeding efficiency. Food should be available to larvae from daybreak until sunset at which time guts should be completely full. Initially, larvae are fed only live or frozen *Artemia* (22 to 24 h post-hatch) at regular intervals each day during the production cycle. *Artemia* nauplii may later be fortified by feeding them commercial products which contain highly unsaturated fatty acids or other nutrients presumed to be essential to freshwater prawn larval growth. *Artemia* constitute the sole food source until day 10 when a prepared diet partially replaces *Artemia* as the food source (Aquacop 1983). These supplemental feeds must be carefully prepared to provide a highly water stable diet of proper particle size. Initiation of supplemental feeding is based on the discretion of the culturist. Use of supplemental feed compensates for the presumed nutritional deficiencies of an exclusive *Artemia* diet. Additionally, overall feed costs are reduced by feeding these less expensive prepared feeds.

Artemia

While *Artemia* nauplii are an excellent food source for larvae, they are also potentially a principal source of disease (Léger et al. 1986). Therefore, *Artemia* cysts should be sterilized and hatched under clean conditions. The nutritional value of the *Artemia* nauplii is enhanced when culture occurs under optimal conditions (Sorgeloos 1980). Larvae are fed newly hatched *Artemia* nauplii which have an undigested yolk sac that presumably constitutes a major portion of their value as a source of nutrition. The nutritional value of *Artemia* as a feed for larval fish and crustacean species varies according to geographical source (Léger et al. 1987a); therefore, caution should be used in selecting brands.

Sorgeloos et al. (1983) list several advantages for decapsulation of *Artemia* including: (1) disinfection of cysts, (2) improved hatching, (3) lower threshold for light stimulation at the onset of the hatching metabolism, and (4) direct feeding of decapsulated cysts to larvae including *M. rosenbergii* (Laviña and Figueroa; cited in Sorgeloos et al. 1983). The following procedure may be used.

1. Cyst hydration: Cyst hydration is accomplished after a 2 h exposure in fresh or sea water (≤35 ppt) at 25°C. Cysts are then washed and filtered over a 120 μm screen.
2. Decapsulation: Sorgeloos et al. (1983) give examples for calculating the amounts of chemicals and the procedures to use in decapsulation. A choice of liquid bleach (NaOCl) and bleaching powder ($Ca(OCl)_2$) are given. For 100 g of cysts and using bleaching powder with 70% (by weight) active ingredients, a decapsulation solution of 1400 ml of seawater (35 ppt), 71 g of bleaching powder and 67 g Na_2CO_3 (or 40 g of CaO) is needed. The amount of bleaching powder is based on 0.5 g of active ingredient/g of cyst and a 70% activity (i.e., $100 \times 0.5 \times 100/70$). The amount of Na_2CO_3 or CaO is based on 0.67 or 0.40 g/g of cysts. The decapsulation solution should be cooled to 15° to 20°C and maintained below 40°C by addition of ice or ice packs. The decapsulation treatment requires only 10 to 15 min and is finished after a complete color change in the cysts (i.e., dark brown to grey when using calcium hypochlorite or grey to orange with sodium hypochlorite).
3. Washing and deactivation: Cysts are thoroughly washed on a 120 μm screen with fresh water or seawater until the odor of chlorine is no longer detected. Toxic chlorine residues that may be adsorbed to the decapsulated cysts can be deactivated by dipping them two times into a 0.1 N HCl (hydrochloric acid) or HOAc (acetic acid) solution as recommended by Bruggeman et al. (1980). The deactivation should be performed for less than one-half minute and then the cysts are washed again. The cysts should be kept away from direct sunlight during the decapsulation process.

In lieu of decapsulation, cysts can be simply hydrated in fresh or salt water for 1 h followed by thorough washing on a 120-μm screen (Sorgeloos et al. 1983) prior to stocking in the hatching tank.

Sorgeloos et al. (1983) describes the environmental conditions for maximal hatchability in 75-L, funnel-shaped containers made of transparent PVC and stocked at 5 g of cysts/L. Natural seawater or artificial salt solutions diluted to 5 ppt can be enriched with 2 g $NaHCO_3$/L of hatching medium. The pH should not drop below 8.0 and temperature should be kept constant within the range of 25° to 30°C. Oxygen levels should remain above 2 mg/L. Illumination at 20 cm above the hatching tank with four 60-watt fluorescent light bulbs (1000 lux) should be provided for the duration of the hatch.

Artemia (22 to 24 h post-stocking of cysts) are harvested as follows:

1. Turn off air, remove standpipe (if one is used), heater, and airstones and cover with a dark lid or black plastic for 5 to 10 min (Sorgeloos et al. 1983).
2. Drain off any unhatched cysts and egg shells which will be

dark brown. *Artemia* nauplii will be bright orange. Decapsulated cysts will have little or no debris that needs to be separated from the newly hatched nauplii.

3. Slowly drain the water containing the newly hatched nauplii into a 120 μm mesh screen until the brown *Artemia* egg shells begin to reappear.
4. Thoroughly rinse nauplii with fresh (or brackish) water.

Léger et al. (1983) described a high density storage of *Artemia* nauplii at 4°C with viability remaining over 90% after 48 h. *Artemia* can be transferred directly to culture tank conditions (25°C) without affecting survival. They also demonstrated that cold-stored nauplii remained in instar I stage. Léger et al. (1986) cite minimal loss in nutritional value of cold-stored *Artemia* nauplii and an advantage in feeding slower moving prey. The following procedure has been followed at the Aquaculture Unit of MAFES for *Artemia* harvested from 454 g of cysts and held for a 6–9 h period.

1. Fill an insulated cooler with 10 L of water having a salinity of 10 to 12 ppt.
2. Remove about 1 L of water from the cooler to use in rinsing harvested *Artemia* into the cooler.
3. Rinse harvested *Artemia* into cooler and refill to original 10 L volume.
4. Add 5 artificial ice packs to reduce the water temperature to 4°C. Maintain the temperature at approximately 4°C (*not* less than 2°C). Usually, 5 ice packs are adequate for 5 to 7 hours, but an extended period of storage will require replacement with new ice packs.
5. Provide suitable aeration and cover with lid.

Feeding larval freshwater shrimp with *Artemia* nauplii which have been enriched with essential, highly unsaturated fatty acids (HUFA) has been shown to reduce the time required for *M. rosenbergii* to undergo metamorphosis to postlarva (Deru et al. 1989, abstract; Devresse et al. 1990). Léger et al. (1986, 1987b) describe various methodologies for *Artemia* enrichment and the relative uptake of these enrichment diets by *Artemia*. One of these, the Belgian technique, involves feeding *Artemia* with either a product of coated microparticles or a self-emulsifying enrichment concentrate which is added to the culture medium. The emulsion can be added to the hatching tank water with the cysts or fed to the newly-hatched nauplii before or after separation of nauplii from the hatching debris. The latter procedure involves separation of nauplii from the debris after 24 h incubation time (28–30°C) and stocking into another tank at a density of up to 3×10^5 nauplii/L for 12, 24, or 48 h enrichment. Devresse et al. (1990) fed 24 h enriched nauplii to *M. rosenbergii* larvae.

Assuming that 10 L of *Artemia* are stored in a cooler for feeding larvae during a 24 h period, 5 L of cold-stored *Artemia* will be fed during the morning hours and 5 L during afternoon hours. Extra *Artemia* may be frozen and stored as a reserve and can be fed at any time to compensate for an insufficient amount of live *Artemia*. While feeding *Artemia* to larvae the center standpipe should have a 90 to 120 μm mesh screen to maintain water circulation between the filters and larval tank while preventing the escape of *Artemia*. The amount of aeration in the larval tank should be just enough to keep *Artemia* suspended in the water column. Frozen *Artemia* require slightly more aeration to remain suspended.

Aquacop (1983) gives recommendations for the number of *Artemia* nauplii to feed per larvae per day during the cycle. The amount ranges from 5 per larvae per day at the beginning of the cycle to 50 per larvae per day by day 15. Rates may vary widely with culture conditions; however, successful production cycles completed at the Aquaculture Unit of MAFES during the past two years typically use much higher rates, reaching twice the recommended amounts by day 15. Supplemental frozen *Artemia* nauplii should always be held in reserve.

Feeding should be spread over time to maximize the nutritional value of the *Artemia* nauplii and to minimize bacterial growth on uneaten food. If all *Artemia* for the morning feeding are placed into the larval culture tank at the same time then uneaten *Artemia* will digest some of their nutrient-laden yolk sac before being consumed by the larvae later in the morning. A typical feeding schedule might involve feeding 40% of the estimated number of *Artemia* needed for the morning feeding (sunrise to noon) and then begin checking after 1 to 2 h to see if any remain. Once this initial amount of *Artemia* has been consumed then feed another 40% and check for *Artemia* remaining as before. If by late morning the guts of larvae are still not full (see Checking and Staging Larvae) and all *Artemia* in the larval culture tank are gone, then the remaining 20% should be fed. By noon all *Artemia* in the larval culture tank should have been consumed. Additional *Artemia* may be fed if larval guts are observed to be less than 100% full. After the noontime cleaning, larvae are again fed *Artemia* following a procedure similar to the morning schedule and larval guts should be completely full by sunset. This procedure is designed to avoid introducing excess food while providing enough food to fill all larval guts.

Supplemental Feeds

Commencement of supplemental feeding may begin on day 10 (Aquacop, 1983). No specific time of day for supplemental feeding has been recommended; however, larval guts should be as full of *Artemia* as possible, as early as possible, to allow maximal flushing time (i.e., using a larger mesh screen to remove uneaten *Artemia* and feces) during supplemental feeding. For example, in the afternoon, larval guts must be 100% full at least 1 to 2 h before sunset and before supplemental feeding begins.

A typical supplemental diet formulation consists mainly of fish or squid, chicken eggs, beef liver powder, and a marine fish oil high in highly unsaturated fatty acids, HUFA, (Appendix 1). Alternative ingredients can be used but care should be exercised to provide a similar nutrient composition. Diet preparation is really an art that is developed with experience and the following procedures are based upon those reported by Aquacop (1983).

1. Defrost squid or fish in microwave for at least 10 min. Clean squid by removing pen, ink sac, skin, eyes and beak or clean fish by removing scales, skin, and bones. Sterilize 7 min/kg on high setting in a microwave oven. Homogenize fish or squid in a commercial-type food processor (e.g., Robot Coupe R172) until well blended (i.e., smooth texture with no chunks).
2. Blend in the chicken eggs, marine fish oil, and beef liver powder until well mixed.
3. Continue mixing and add a binder ingredient (e.g., alginate) slowly until the paste begins to detach from the walls of the food processor.
4. The paste is then placed in a plastic bucket containing about 4 g/L of calcium chloride ($CaCl_2$). The outer layer of paste will begin to harden quickly. Slowly break the paste into smaller particles until it has gone from a sticky texture to a cardboard-like consistency. Twenty minutes of mixing should be adequate. Binding may occur slowly if an insuffi-

cient amount of binder was used. Extra $CaCl_2$ can be added to the water to increase the rate of binding. If binding is insufficient, much of the diet will be lost as fine particulate.

5. Manually push the material through brass sieves to obtain proper particle sizes. Suggested mesh sizes are 250, 425, 600, 850, and 1000 μm. Rinse the sieved diet thoroughly to remove fine particulates which can foul the water and contribute to bacterial growth within the culture tank. Feed should be well drained before storing and may be refrigerated for several days or frozen. The size of particle fed normally ranges from 300 to 1000 μm depending on the size of larvae (Aquacop 1977).

An alternative to this prepared diet would be an appropriate larval feed manufactured by a commercial supplier. Most of these diets have been formulated for larval culture of penaeid shrimp. The proper size of particle to feed should be based on the size of the particle after hydration.

Aquacop (1983) recommended feeding rates ranging from 0.07 to 0.20 g (dry weight) per larvae per day. Feeding rates should be adjusted to match a consumption rate specific to each culture tank. The size of the mesh used on the standpipe should always be smaller than the food particle size to allow flushing without loss of feed. The amount of food to be fed should be weighed and then measured volumetrically to compare to that amount of excess feed siphoned during tank cleaning. A portion of the food is added to the culture tank and larvae are observed to determine if most of them have grasped a piece. When this food begins to disappear then more food can be added to the culture tank. Little or no food from the morning feeding should remain when the culture tank is siphoned. If food remains then the amount fed should be adjusted. In the afternoon, the supplemental diet should be fed while at least a few hours of daylight remain. Little or no food should remain at daybreak.

Checking and Staging Larvae

Larval guts are checked twice daily. Larvae are staged once daily until the first postlarvae are observed. A stereoscope with a range of magnification from 60 to 100 × is recommended for staging and checking guts. A microtitre plate may be used to help in examining larvae individually. Staging can be performed any time of day. Stages are determined according to the criteria developed by Uno and Kwon (1969) and abridged by Malecha (1983). A larval stage index (LSI) as defined by Manzi et al. (1977) and used by Aquacop (1983) is a calculated weighted average of stage determinations. For example, if 30% (.3) of the larvae are identified as stage 2, 40% (.4) are identified as stage 3, and 30% (.3) are identified as stage 4, then the average stage index is $(.3 \times 2) + (.4 \times 3) + (.3 \times 4) = 3$. Metamorphosis from one larval stage to the next generally takes 2 days until LSI 7 or 8, and larvae are generally distributed within 2 or 3 stages with postlarvae appearing on day 19 (Aquacop 1983). Malecha (1983) presents graphically the typical number of larval stages represented on a daily basis.

Aquacop (1983) states "that food consumption, measured by the percentage of larvae with full gut, is a good index to estimate the health of larvae". Larval guts are checked after most of the morning ration of *Artemia* has been fed and can be rechecked if they are not yet 100% full. If full, guts will appear bright orange and will be pressed against the heart and upper carapace. The final daily check of guts is performed after the entire afternoon ration of *Artemia* has been fed. If guts are not 100% full, then additional *Artemia* should be fed until the guts become full. Completely overcast days can increase the time required for larvae to feed and can result in larval guts being only partially full at the onset of darkness. If guts remain less than 100% full for several consecutive days then a problem is assumed.

Harvesting Postlarvae (pls)

Smith and Hopkins (1977) describe an apparatus for separating postlarval prawns which involves removal of the population to a separation tank. The following is a description of techniques employed commercially and at the Aquaculture Unit of MAFES.

Several harvests of pls may be performed before completion of the production cycle. If pls are observed by day 19, then the first harvest of pls will generally occur between day 23 and 28 (usually when about 25–30% of the larvae have undergone metamorphosis). Aquacop (1983) recommends cropping of postlarvae when postlarval density on the bottom of the tank is above $2/cm^2$ or 100,000 larvae/ $2\text{-}m^3$ tank. Despite the potential loss of pls due to cannibalism by larvae, economics of the operation may dictate a single harvest after most larvae have metamorphosed. Otherwise, subsequent harvests will generally occur at 3–4 day intervals until the final harvest. Each harvest should contain about 25–30% of the original number of larvae that were stocked. A slower rate of metamorphosis will increase the time interval between each successive harvest, and under these conditions the harvest should be based on the estimated number of new pls.

Harvesting should begin early in the morning prior to feeding. Aeration is discontinued and the water is stirred by hand (Aquacop 1983). Larvae are dipped from the tank and either held temporarily in a smaller tank (at least 800 to 1000 L) or transferred directly into an empty larval culture tank where growout continues. Very few pls will be removed using this procedure because most of the pls cling to the side of the tank. The holding tank for the larvae should be equipped with air to circulate food as in the larval tank. No heating of the water in the holding tank is necessary if the harvest is done quickly and in a warm environment. Larvae in the holding tank should be fed *Artemia* nauplii.

After the larvae have been removed, the majority of the water must then be pumped out through a small mesh screen into a holding tank for temporary storage. Extra brackish water must be available to replace water lost during the harvesting process. The pls can then be concentrated by draining the remaining water, removed with a dip net, and then placed into a nursery tank or a holding tank. Water in the holding or nursery tank should have a salinity similar to that of the larval culture tank (10–15 ppt) but can be lower (5–7 ppt). The pls are then acclimated to fresh water over at least a 12-h period (Aquacop 1983) by allowing fresh water to slowly enter and dilute the salt water. The total number of harvested pls can be estimated by enumerating in a known volume of water (Aquacop 1983) or gravimetrically (Aquacop 1977). The gravimetric procedure involves weighing the total biomass and dividing that amount by the average weight from a sample of 2000 individuals to yield the total number of pls (e.g., 1200 g ÷ 0.006 g = 200,000).

When all pls are harvested the larval culture tank should be thoroughly scrubbed and rinsed with fresh water and then refilled with the old culture water and the new make-up water. Be careful to insure that the salinity and temperature of the new water, the water in the biofilter, and the water in which the larvae are being

held are similar. The larvae are then returned to the larval tank and reared as previously described. An estimate of the number of the larvae to be returned should be made by enumerating the number in a known volume. The amount of feed can then be reduced accordingly. The final harvest is performed when most of the larvae have metamorphosed and is accomplished by drain-down.

SUMMARY

The time table for daily duties will vary with each hatchery and cycle; however, the general pattern of maintenance will be similar. A possible schedule for daily hatchery maintenance would be similar to that presented in Appendix 2. As the manager becomes better acquainted with the operation of the system and the handling of the larvae, the amount of time spent in caring for the larvae will decrease to a minimum level. However, additional time spent checking larval guts and feed consumption will be beneficial in achieving higher rates of metamorphosis and survival at higher densities.

Culture methods and equipment will vary with location and management goals. Therefore, the basic concepts and procedures presented in this text should be used as guidelines for design and management of a hatchery. Careful management and meticulous observation will enable the manager to make adjustments for each particular system and to develop different larval rearing procedures as warranted.

APPENDIX 1.

Supplemental Diet Formulation

Ingredients	Percentage (by wet weight) in Diet
Squid, cleaned	85
Cod liver oil	2
Eggs	10
Beef liver powder	3

APPENDIX 2.

Daily Duties

Time	Duties
0600	—Change to feeding screen (120μm)
	—Feed frozen *Artemia* nauplii.
	—Harvest *Artemia* nauplii.
	—Hydrate *Artemia* cysts
	—Check temperature (T°) and salinity.
	—Backwash sand filter (when feeding supplemental feed).
0700	—Feed live *Artemia* nauplii.
	—Rinse and stock *Artemia* cysts or decapsulate cysts.
0800	—Stage larvae
	—Check remaining *Artemia* in larval tank (If all gone, feed more; if not, check later).
0930–1000	—Once supplemental feeding begins, check guts; if full, put on flushing screen and feed.
1030–1100	—When *Artemia* is the sole food source, check guts and begin flushing tank, if guts are full.
1130	—Clean larval tank.
1200	—Siphon larval tank and estimate food consumption and mortality.
	—Backwash sand filter.
	—Add additional substrate to biofilter.
1230	—Feed live *Artemia*.
	—Check T°.
1230–1600	—Continue feeding *Artemia* in allotments until larvae are 100% full.
1600	—Check guts (if <100% full, then continue to feed *Artemia* until full).
	—If 100% full, then feed supplemental feed and change to flushing screen.
1700	—Check T°.
	—Final system check.

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ECONOMIC ANALYSIS OF COMMERCIAL PRODUCTION OF FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII* DEMAN, 1879 POSTLARVAE USING A RECIRCULATING "CLEARWATER" CULTURE SYSTEM

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ABSTRACT An economic analysis of constructing and operating a closed, recirculating "clearwater" hatchery for the commercial production of postlarvae of the freshwater prawn *Macrobrachium rosenbergii* is presented. The study is based upon proven practical techniques and published recommendations for larval culture. Investment requirements for a prawn hatchery, producing 6.3 million postlarvae per year, located in Mississippi are estimated to be $83,523. Annual cost to operate this hatchery is estimated to be $7.87 per 1,000 prawns produced. Price/survival sensitivity revealed that minor variations in survival result in substantial variability in net returns.

An accurate economic assessment of culturing freshwater prawn in temperate climates is required before producers can make informed decisions regarding the potential of this enterprise. Aquacop (1979) projected the cost of producing *Macrobrachium rosenbergii* postlarvae at $12 per 1,000 annually. It was pointed out however, that this figure would undoubtedly be lower in many other regions, because of the high cost of living in Tahiti. Cost of producing freshwater prawn postlarvae in temperate climates was estimated by Leventos (1986) to be U.S. $7.74 per thousand. Leventos' study however, was based upon untested management procedures and additional research (Daniels et al. 1991) suggest a need to change the management procedures described in 1986. Therefore, it is necessary to assess hatching costs for the management regime outlined by Daniels et al. (1991).

This study presents estimates of the cost of producing postlarvae of freshwater prawns under a successful management scheme. The specific objectives were (1) identification of a practical size hatchery and estimation of its investment requirements, (2) estimation of annual ownership and annual operating costs associated with the hatchery, and, (3) estimation of net returns and returns on investment according to selected survival and prices of postlarvae.

MATERIALS AND METHODS

To meet the objectives, a commercial size firm was synthesized with the capacity to produce 6.3 million postlarvae for transfer to a nursery phase of culture. The design and operation of the firm was based upon the management techniques of Daniels et al. (1991). Data necessary to estimate investment requirements for the firm were gathered from research personnel, suppliers, and contractors. Data regarding the type and use of labor were provided by research personnel. The economic-engineering approach was used to estimate the costs associated with hatching and larval rearing. The economic-engineering technique has been used extensively in aquaculture economic analysis and allows for the development of budgets from technical input-output coefficients (D'Abramo et al. 1991; Keenum and Waldrop 1988; Foster and Waldrop 1972).

Production Scenario

The duration of a hatchery cycle is approximately 30 days. The hatchery in this study is designed to produce a total of 6.3 million postlarvae in two separate 30-day production cycles. Only two cycles are evaluated because of the temperate climate of Mississippi, which limits the "marketing window" for postlarvae. The initial stocking rate was specified at 60 larvae $\cdot$ L^{-1} of water. Survival was estimated to be 80% after the completion of each cycle. Each 30-day cycle consists of two hatchings which eases management of the hatch as well as the harvest of postlarvae. Commencement of the first cycle was the first week of January, dividing the stocking into two discrete three-day periods within each of two consecutive weeks. Allowing one week for wash down and disinfection, the second staggered cycle would begin during the second and third weeks of February. The labor and physical resources assumed necessary for successful operation of the hatchery are derived from the management practices described by Daniels et al. (1991).

Physical Requirements

Building requirements include a 9.1 m $\times$ 27.4 m galvanized metal building with windows on the east and west sides for the entrance of indirect, natural lighting. A large center concrete drain for ease of cleansing and sterilizing is an assumed requirement. A smaller building (3.1 m $\times$ 3.1 m) is also included to house blowers for the aeration system. The hatchery design consists of six round, conical-bottomed tanks with slopes not exceeding 10°. Each tank is specified as having a 3.1 m diameter, with an effective working volume of water of approximately 11,000 L. The tanks are mounted on a fiberglass skirt and have a 25.4 cm clearance from the ground. The bottom and the sides, up to approximately 30 cm from the bottom, are painted a lighter color to provide a distinct contrast. Fittings for each tank include a 7.6 cm top lip and a 10.2 cm bottom drain.

Five additional tanks are required for biological filters, conditioning of filter media, and saltwater storage. These fiberglass tanks are circular and, flat bottomed, with diameter and depth being 3.05 and 1.52 m, respectively. The tanks used to condition filter media are also used to acclimate postlarvae to freshwater after all of the filter media has been transferred to the biofilter. A schematic of the hatchery is provided in Figure 1. While circular tanks were assumed for use in this study, Daniels et al. (1991) recommends rectangular tanks equipped with baffles and coral as

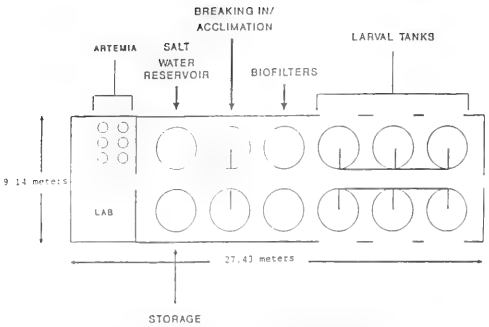

Figure 1. Schematic of the synthesized hatchery.

media for biological filtration. Substitution of rectangular tanks should have little, if any, effect on the cost estimates provided. Other calcareous materials such as limestone gravel, oyster shells, and clam shells can be used as biological filtration media.

Pressure sand filters are required for removing particulate material from the water. Two sand filters rated at 227 L per minute are specified for filtering water within the larval culture system; and two smaller sand filters rated at 151 L per minute are specified for filtering water prior to its initial use in the hatchery. Two 2-horsepower pumps rated for saltwater use are necessary to transport water through the system.

Mechanical aeration is designed to provide high levels of dissolved oxygen necessary for larval survival and to keep food suspended in the water column after it has been introduced into the larval culture tank. The 3-horsepower blower is designed to give high volume, low pressure, uncontaminated air. Two blowers are specified, one to be used as a backup if the primary unit fails to operate.

TABLE 1.

Estimated investment requirements for a freshwater prawn hatchery, Mississippi, 1990.

Item	Investment ($)	Percent of Total Cost
Land	600	0.72
Building	28,800	34.48
Fiberglass tanks		
Conical-bottomed	15,300	18.32
Flat-bottomed	6,550	7.84
Artemia tanks	2,370	2.84
Storage tank	1,850	2.21
Mechanical Aeration system	3,673	4.40
PVC	3,092	3.70
Heaters (immersion)	2,154	2.58
Microwave oven	400	0.48
House for aeration system	1,000	1.20
Filters	1,663	1.99
Pumps	1,260	1.51
Accessories	762	0.91
U.V. disinfection system	7,200	8.62
Binocular microscope	795	0.95
Refrigerator	260	0.31
Freezer	315	0.38
8500 watt generator	1,429	1.71
Food processor	350	0.42
Heating system (air)	2,500	2.99
Miscellaneous	1,200	1.44
Total	83,523	100.00

TABLE 2.

Estimated annual ownership cost for a freshwater prawn hatchery, Mississippi, 1990.

Item	Cost	Percent of Total
Depreciation		
Building	1,440	11.63
Fiberglass tanks		
Conical-bottomed	1,020	8.24
Flat-bottomed	437	3.53
Filters	238	1.92
Pumps	180	1.45
Accessories	109	0.88
House for aeration	33	0.27
Aeration system	367	2.97
Heaters	308	2.48
Microwave oven	80	0.65
Artemia tanks	158	1.28
PVC	155	1.25
Binocular microscope	53	0.43
Refrigerator	26	0.21
Freezer	32	0.25
Miscellaneous	240	1.94
8500 watt generator	143	1.15
Food processor	70	0.57
Storage tank	123	0.99
U.V. disinfection system	720	5.82
Heating system	167	1.35
Total, depreciation	6,099	
Interest on investment		
Land	66	0.53
Building	1,584	12.79
Fiberglass tanks		
Conical bottom	842	6.80
Flat bottom	360	2.91
Filters	91	0.74
Pumps	69	0.56
Accessories	42	0.34
House of aeration	55	0.44
Aeration system	202	1.63
Heaters	118	0.96
Microwave oven	22	0.18
Artemia tanks	130	1.05
PVC	170	1.37
Binocular microscope	44	0.35
Refrigerator	14	0.12
Freezer	17	0.14
Miscellaneous	66	0.53
8500 watt generator	79	0.63
Food processor	19	0.16
Storage tank	102	0.82
U.V. disinfection system	396	3.20
Heating system	138	1.11
Total, interest on investment	4,626	
Taxes	442	3.57
Insurance	1,214	9.81
Total, ownership cost	12,381	100.00

Low winter temperatures in temperate climates necessitate a heating system to insure optimal culture conditions. Heaters with the capacity to maintain water temperature at 28°C are needed. Liquid petroleum (LP) gas heaters were selected as the most economical and available means of heating the hatchery. Based on calculations using the total air volume of the building, a heater producing 144,000 BTU per hour is required to keep the hatchery at the desired temperature. Additionally, maintenance of proper water temperature requires supplemental heat (4kw) derived from immersion heaters positioned in the biofilters.

A disinfection system is specified for each larval culture system to minimize potentially harmful pathogens. Two disinfection systems would be included in this production scenario.

Artemia, hatched from dehydrated cysts, are the major food source for larval prawns. Six fiberglass, conical-bottomed tanks of 492 L capacity are specified for *Artemia* production.

Operation of a hatchery requires regular monitoring of water quality variables such that pH, ammonia, nitrate, nitrite, and salinity levels are maintained at satisfactory levels for optimum growth. A refractometer is specified for salinity measurements and a saltwater test kit is specified for the determination of important water quality parameters.

A microwave oven and a commercial-grade food processor are specified for preparation of supplemental feed. Other designated equipment include a refrigerator and a chest-type deep freezer for storage of supplemental feed ingredients. A binocular microscope (60–100x range) is needed for daily inspection of the quantity of food in the guts of the larvae. A 8500 watt backup generator is specified as a source of electricity in the event of a power failure. Polyvinyl chloride pipes and fittings are used to transport water and air from their sources to the culture system. Other pipes and fittings are specified for return of water in the recirculating system. Additional miscellaneous equipment such as test tubes, racks, stands, and dip nets are specified for daily operation of the hatchery.

Costs

Costs were categorized according to initial investment (Table 1), annual ownership costs (Table 2) and annual operating costs (Table 4). Annual ownership costs consist of depreciation, interest on investment, taxes, and nonliability insurance. These costs are fixed and incurred in the short run regardless of whether the facilities are operated. Annual operating costs are incurred upon actual operation of the hatchery and include repairs and maintenance, labor, feed, utilities, liability insurance and interest on operating capital.

Annual Ownership Costs

Annual depreciation was estimated by the straight-line method and was based on the expected useful life of each piece of equipment. A zero salvage value was assumed on all items constituting the facilities. The hatchery building was assumed to have a useful life of only 20 years because of the saltwater environment. Housing for the blower was assigned a useful life of 30 years and all of the fiberglass tanks were assigned useful lives of 15 years. The life expectancies of equipment ranged from 5 to 20 years (Table 3).

Interest was charged at a rate of 11 percent on one half of the original investment for all depreciable items that compose the

TABLE 3.

Estimated depreciation, interest, and repairs and maintenance for a freshwater prawn hatchery, Mississippi, 1990.

Item	No. of Units	New Cost Each ($)	Total Cost of Items ($)	Repairs as a % of New Cost (%)	Estimated Life (yrs)	Annual Depreciation ($)	Annual Interest on Investment ($)	Annual Repairs and Maintenance ($)
Land	1	600	600				66	
Building	1	28,800	28,800	80	20	1,440	1,584	1,152
Fiberglass tanks								
Conical-bottomed	6	2,550	15,300	10	15	1,020	842	102
Flat-bottomed	5	1,310	6,550	10	15	437	360	44
Filters	1	1,663	1,663	60	7	238	91	143
Pumps	1	1,260	1,260	60	7	180	69	108
Accessories	1	762	762	50	7	109	42	54
House for aeration	1	1,000	1,000	20	30	33	55	7
Aeration system	1	3,673	3,673	70	10	367	202	257
Heaters	6	359	2,154	40	7	308	118	123
Microwave oven	1	400	400	50	5	80	22	40
Artemia tanks	6	395	2,370	100	15	158	130	158
PVC	1	3,092	3,092	20	20	155	170	31
Binocular microscope	1	795	795	50	15	53	44	27
Refrigerator	1	260	260	50	10	26	14	13
Freezer	1	315	315	50	10	32	17	16
Miscellaneous	1	1,200	1,200	100	5	240	66	240
8500 watt generator	1	1,429	1,429	75	10	143	79	107
Food processor	1	350	350	75	5	70	19	53
Storage tank	1	1,850	1,850	10	15	123	102	12
U.V. disinfection system	2	3,600	7,200	50	10	720	396	360
Heating system	1	2,500	2,500	80	15	167	138	133
Totals			83,523			6,099	4,626	3,180

hatchery. Interest, at the annual rate of 11 percent, was charged on the full value of the land.

For this study, the hatchery was assumed to be located in a rural area of Mississippi, outside the boundaries of a city, with only county taxes applicable. Tax rates and assessment procedures defined by the Mississippi State Tax Commission were used to estimate ad valorem taxes.

The assumed insurance coverage includes fire, vandalism, and extended coverage such as hail, windstorm, and smoke damage. In Mississippi, rates are set by the Mississippi State Rating Bureau, based on the construction of the facility. The rate used in this study was $14.54 per $1,000 of total investment in the hatchery.

Annual Operating Costs

Annual costs of repairs and maintenance for the hatchery was computed as a percentage of purchase price. Estimates of repairs as a percentage of purchase price were provided by manufacturers, industry representatives, and appropriate research scientists of the Mississippi Agriculture and Forestry Experiment Station. Total repairs and maintenance costs were divided by estimated life to calculate annual repairs and maintenance.

Labor requirements were estimated based on the particular needs for seasonal operation of the hatchery. A manager and four laborers working full-time were assigned to the operation of the hatchery, and this labor was assumed to be seasonal. Labor cost for each individual was calculated on an hourly basis at $5.00 per hour, including all fringe benefits. The salary of the manager was set at $35,000 per year including fringe benefits.

Feed cost is based upon the assumption that larvae are fed *Artemia* exclusively for the first 10 days of the cycle. Thereafter, feed is a mixture of *Artemia* and an artificial diet principally composed of squid, eggs, and fish flesh.

Based on data previously published, the cost of purchasing and caring for broodstock was estimated to be $13.51 per kilogram of prawns (Leventos 1986). The production scenario for the hatchery designed in the present study would require 153 kilograms of broodstock. This estimate was based on the assumptions that eggs in advanced stages of development (within 3 days of hatching) can be obtained from only 5% of the total female broodstock for any one proposed cycle and an average of 500 larvae are produced per gram of female weight. The cost of initially purchasing and holding (150 days) broodstock was estimated to be $2,067 based on this information (Table 4).

Electricity is used for operating the various pumps and lighting units in the hatchery. The efficiency of each pump and blower was obtained from engineering studies and used in calculating electrical consumption. Individual wattages were used to calculate the electrical requirements for illumination and operation of other appliances for hatchery operation. The average charge per kilowatt hour was provided by the Tennessee Valley Authority and assumed to be $0.06. LP gas is required at the rate of approximately

TABLE 4.

Estimated annual operating costs for a freshwater prawn hatchery, Mississippi, 1990.

Item	Cost ($)	Percent of Total
Repairs and maintenance		
Building	1,152	3.08
Fiberglass tanks		
Conical-bottomed	102	0.27
Flat-bottomed	44	0.12
Filters	143	0.38
Pumps	108	0.29
Accessories	54	0.15
House for aeration	7	0.02
Aeration system	257	0.69
Heaters	123	0.33
Microwave oven	40	0.11
Artemia tanks	158	0.42
PVC	31	0.08
Ultraviolet disinfection system	360	0.96
Miscellaneous	240	0.64
Storage tank	12	0.03
Binocular microscope	27	0.07
Refrigerator	13	0.04
Freezer	16	0.04
8500 watt generator	107	0.29
Food processor	53	0.14
Heating system	133	0.36
Total, repairs & maintenance	3,180	
Labor		
Management (3 months)	8,750	23.42
Hired labor (full time)	10,400	27.83
Feed	7,456	19.95
Broodstock purchase and care	2,067	5.53
Utilities		
Electricity	986	2.64
LP gas	2,336	6.25
LP gas tank rent	45	0.12
Artificial sea salt mixture	696	1.86
Liability insurance	452	1.21
Interest on operating capital	1,000	2.68
Total, opeating cost	37,366	100.00

TABLE 5.

Estimated total annual costs for freshwater prawn hatchery, Mississippi, 1990.

Item	Cost ($)	Percent of Total
Ownership cost		
Depreciation	6,097	12.26
Interest on investment	4,627	9.30
Taxes	442	0.89
Insurance	1,214	2.44
Total, ownership costs	12,380	
Operating costs		
Repairs and maintenance	3,179	6.39
Management	8,750	17.59
Hired labor	10,400	20.91
Feed	7,456	14.99
Broodstock purchase and care	2,067	4.15
Electricity	986	1.98
LP gas and tank rental	2,381	4.78
Salt	696	1.40
Liability insurance	452	0.91
Interest on operating capital	1,000	2.01
Total operating cost	37,367	
Total, annual cost	49,747	100.00

TABLE 6.

Estimated total annual cost for production of freshwater prawn at selected survivals, Mississippi, 1990.

Survival Rate	Cost/1,000 Prawns
(%)	($)
90	6.99
80	7.87
70	8.99
60	10.49
50	12.59
40	15.73

7.2 L per hour to run the 144,000 BTU/hr heaters for 90 days of hatchery operation based upon the assumption that each L of LP gas provides 19,976 BTU per hour.

The remaining annual operating costs include insurance and interest on operating capital, and the artificial sea salt mixture. An insurance company that has experience with similar operations provided the rate for the liability insurance, $2.36 for every $100 of salary paid. Interest on operating capital was based on an annual rate of 11 percent and was charged on total operating cost for one-fourth of a year. Prices for the artificial sea salt mixture were obtained from commercial sources.

Return Analysis

Net returns and returns on investment for hatchery production were computed at final survivals ranging from 50 to 90 percent and the selling price of postlarvae ranging from $6 to $20 per 1,000. Gross return was computed for each level of survival and each selling price, and net return was calculated from the gross return minus the total annual cost. Return to capital and management was computed for each level of survival and each selling price by subtracting annual operating cost from gross returns. Subsequently, return on investment was estimated by dividing returns to capital and management by initial capital investment.

RESULTS

Total estimated investment requirements for construction of the hatchery was $83,523 (Table 1). The building was the largest cost component (34.5%) of the hatchery. Fiberglass tanks and the ultraviolet light disinfection systems are the second most expensive items in equipping the hatchery, representing 26.2% and 8.6% of the total investment. The conical-bottomed tanks represent 18.3% of the total investment, while the flat-bottomed tanks used in the biofilter, filter media break-in, and saltwater storage are 7.8% of the total. The conical-bottomed tanks must be specially built and therefore are more expensive. These four components of the hatchery represent 69.3 percent of total investment requirements for production of freshwater prawn postlarvae.

Annual ownership costs were estimated to be $12,381 (Tables 2 and 3) with annual operating costs estimated at $37,368 (Table 4). Total annual cost for the postlarvae production (hatchery) phase of freshwater prawn culture was $49,747 (Table 5). Annual ownership and operating costs accounted for 24.9% and 75.1% of the total annual cost, respectively. The two major ownership cost items were depreciation and interest on investment representing 12.3% and 9.3% of total annual cost, respectively. Salaries for management and hired labor, and feed were the two highest operating cost items representing 38.5% and 15.0% of total annual cost.

The cost associated with producing post-larval prawns is expressed as a dollar value per 1,000 postlarvae. The cost of producing 6.3 million postlarvae in this hatchery design was estimated at $7.87 per 1,000. However, as the total number of postlarvae produced per cycle decreases, cost increases. For example, if 3.2 million postlarvae (approximately 40% survival) are produced, utilizing the same level of inputs, the estimated cost of production increases to $15.73 per 1,000 prawns. Hatching costs per 1,000 prawns at selected survival rates are presented in Table 6.

The $8 per 1,000 selling price at an 80 percent survival relates best to the $7.87 break even price observed in this study (Table 7). Net return at these levels is $839 and return on investment is 0.16 (Table 8).

DISCUSSION

The feasibility of producing *Macrobrachium rosenbergii* in temperature climates continues to be examined. Although returns are small, production with 80% survival and selling price of $8 per 1,000 is economically feasible under the assumptions employed.

The cost of producing *M. rosenbergii* postlarvae according to the procedures and assumptions outlined in this study, is considerably lower than currently advertised purchase prices which range from $20 to $50 per 1,000 (D'Abramo, personal communication). These costs are higher than the estimated cost generated when

TABLE 7.

Net returns for production of freshwater prawn postlarvae at selected final survival and selling prices, Mississippi, 1990.

	Selling Price of PL's ($/1,000)							
Survival (%)	6	8	10	12	14	16	18	20
90	(7,065)	7,162	21,389	35,616	49,843	64,070	78,297	92,524
85	(9,436)	4,001	17,437	30,874	44,311	57,747	71,184	84,621
80	(11,807)	839	13,485	26,132	38,778	51,424	64,070	76,717
75	(14,178)	(2,323)	9,533	21,389	33,245	45,101	56,957	68,813
70	(16,550)	(5,484)	5,581	16,647	27,712	38,778	49,843	60,909
65	(18,921)	(8,646)	1,629	11,905	22,180	32,455	42,730	53,005
60	(21,292)	(11,807)	(2,323)	7,162	16,647	26,132	35,616	45,101
55	(23,663)	(14,969)	(6,274)	2,420	11,114	19,808	28,503	37,197
50	(26,034)	(18,130)	(10,226)	(2,323)	5,581	13,485	21,389	29,293

TABLE 8.

Return on investment for production of freshwater prawn postlarvae at selected final survival and selling prices, Mississippi, 1990.

Survival (%)	Selling Price of PL's ($/1,000)							
	6	8	10	12	14	16	18	20
90	.0636	.2340	.4043	.5746	.7450	.9153	1.0857	1.2500
85	.0352	.1961	.3570	.5179	.6787	.8396	1.0005	1.1614
80	.0069	.1583	.3097	.4611	.6125	.7639	.9153	1.0667
75	(.0215)	.1204	.2624	.4043	.5463	.6882	.8302	.9721
70	(.0499)	.0826	.2150	.3475	.4800	.6125	.7450	.8775
65	(.0783)	.0447	.1677	.2908	.4138	.5368	.6598	.7828
60	(.1067)	.0069	.1204	.2340	.3475	.4611	.5746	.6882
55	(.1351)	(.0311)	.0731	.1772	.2813	.3854	.4895	.5936
50	(.1635)	(.0688)	.0258	.1204	.2150	.3097	.4043	.4989

survival is very poor. However, other potential costs for marketing, live-hauling, or any air freight charges associated with transportation after purchase were not included in this study. The magnitude of these additional costs will determine the level of savings that can be passed on to producers of market size prawns while still assuring a fair return to producers of postlarvae.

Costs presented in this study are based on limited available data. This study involved only one hypothetical firm and serves as a guideline for understanding the economics of commercial postlarvae production. Deviations from the hatchery specifications and management techniques of this study will likely result in altered costs.

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LARVAL LOBSTER (*HOMARUS AMERICANUS*, H. MILNE EDWARDS, 1837) DISTRIBUTION IN A PROTECTED SCOTIAN SHELF BAY

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ABSTRACT Although little empirical data are available on the distribution of larval lobsters (*Homarus americanus* Milne Edwards), workers have hypothesized that warmer temperatures and possibly better food availability within protected coastal embayments along the Nova Scotian Shelf enhances larval survival. Surface tows (n = 79) were collected in Jeddore Harbor, Nova Scotia on a weekly basis from July 21 to September 22, 1988. Eighteen tows yielded an average of 2.4 larvae per 1000 m^3; only stage 1 larvae were observed. Significantly more larvae were sampled during nighttime than during daytime tows. Peak abundance occurred on August 4 when 55% of the total larvae collected were captured. Failure to collect later stages and post-larvae suggests either their migration to areas not sampled within the bay, or flushing from the harbor. The harbor's flushing rate, estimated at 4.4 tide cycles (~60 h) suggests a plausible mechanism for removal of stage one larvae from the harbor. Support for this hypothesis was the occurrence of the majority of larvae near the channel contiguous to the harbor mouth. The absence of later stage lobster larvae and post-larvae was unexpected and may have important implications for larval recruitment to shallow-water, inshore embayments.

KEY WORDS: *Homarus americanus*, lobster larvae, distribution, transport

INTRODUCTION

The lobster fishery of the nearshore eastern Nova Scotian Shelf has been fully developed since 1890, when landings peaked. Subsequently, the longterm pattern has been one of multi-year cycles with secondary peaks declining with time (Miller et al. 1987). Annual yield dropped to the all time low in 1978/79. These patterns focused attention on the fishery (McCracken 1979) and resource science, resulting in a number of hypotheses developed to explain both the cycles and decline in landings. Harding et al. (1983), using Huntsman's (1923) observation that temperature and current characteristics likely dictated larval geographic distribution, reviewed the temperature, speed, direction and zooplankter prey composition of the Nova Scotia Current and postulated low larval survival for most years. They then hypothesized the warmer, near shore bays as important sites for larval development.

Little is known of lobster larval ecology in the northwest Atlantic, particularly along the inner Scotian Shelf, the location of a commercial fishery for over 100 years (Robinson 1980). Jeddore Harbor on the eastern shore of Nova Scotia (Fig. 1) has been the site of studies on lobster reproductive biology (Duggan and Pringle 1988, Jarvis 1989). The former study supports the hypothesis that these nearshore bays hold brood stock, however, the latter study gave little support to the hypothesis that egg-bearing females homed for Jeddore Harbor. Here, we detail the spatial and temporal distribution of lobster larvae in the Harbor, finding no support for the hypothesis (Harding et al. 1983) that Jeddore Harbor is important for maturation of late stage larvae or post-larvae.

METHODS AND MATERIALS

Jeddore Harbor (Fig. 1) consists of an inner bay (2.5 km by 1.3 km, average depth = 3 m), the bottom of which is interspersed with eelgrass beds and rocky outcrops. The 4 km long channel, with an average depth of 6 m, links the bay to the open ocean. Surface water temperature and salinity for May through September ranged from 5.4°C to 18.4°C and 20‰ to 31‰, respectively. The mean tidal range for the study period (July 21–September 22) was 1.05 m.

Plankton was sampled by simultaneously towing twin one-meter-square neuston-nets (1600 μm grade Nitex mesh), with a 7 m vessel, at between 0.7 and 1.0 m/sec. Nets were rigged as described by Harding et al. (1982) with a pore to mouth ratio >3. Each net carried a General Oceanic flowmeter to permit volume-filtered calculations. The portion of the bay sampled is shown in Figure 1; the remainder had insufficient water depth for the vessel's draft. Cruises were made once weekly, between 1400 h and 0200 h, when from 7–11 tows were made. Tow length averaged 24.2 min ± 4.18 min. Light intensity (Licor quantum meter), salinity (refractometer) and water surface temperature (temperature probe) were measured for most tows. Samples were sorted under a dissecting microscope in the laboratory and lobster larvae were staged according to Herrick (1911). Tow-specific light intensity readings, salinity and temperature measurements, and larval catch rates (no. per 1000 m^3) have been presented in DiBacco and Pringle (1991).

TIDAL FLUSHING

The number of tidal cycles (n) required to flush Jeddore Harbor was estimated using the following relationship:

$$1/e = (V/(V + P))^n,$$

where V represents the low-tide volume; P the intertidal volume; and e the natural base of logarithms (R. Trites, Physical Oceanographer, Dept. Fish. & Oceans, Bedford Inst. Oceanog., Dartmouth, NS, pers. comm.). Subsequently, the residence time of harbor water was estimated by multiplying n and the mean length of a tidal cycle for the period of time in question. Low tide volume was calculated by dividing the harbour into the following layers; 0

m–3.7 m, 3.7 m–7.3 m, 7.3 m–11.0 m, and 11.0 m–14.6 m. The area at each depth was digitized from a hydrographic chart and volume of each layer was obtained from the following relationship:

$$\text{Vol. of layer} = ((\text{Area of top layer} + \text{Area of bottom layer})/2)(\text{Thickness of layer})$$

where the summation of the volume of each layer yielded an estimate of the low tide volume for the bay. Intertidal volume is the intertidal area times the intertidal height; the latter was averaged daily for the entire study period due to semi-diurnal tidal height fluctuations (derived from published tide tables, Anon. 1988).

Low tide volume equalled $7.76 * 10^7$ m^3, while both the intertidal range and volume equalled 1.05 m and $1.97 * 10^7$ m^3, respectively. Flushing rate was estimated at 4.41 tides, spanning approximately 60 h.

RESULTS

Lobster larvae were captured in all cruises but two in September (Table 1). A total of 208 lobster larvae were found; all were Stage 1, the planktonic post-larval stage was not observed. Larval catch abundance estimates (Table 1) ranged from zero in 61 of 79 tows to a maximum of 37.5 per 1000 m^3, peaking in August, where catch rates were markedly greater than either July or September.

Larval catch rates were significantly higher during dark hours than during daylight (Mann-Whitney U-Test, $P < 0.05$ Fig. 2) with the majority captured during the first two hours following sunset. Larval catch rates varied amongst three regions of the harbor (Fig. 3); Region 1 (Fig. 1) represents the shallow inner bay where Regions 2 and 3 make up the channel leading to the harbor

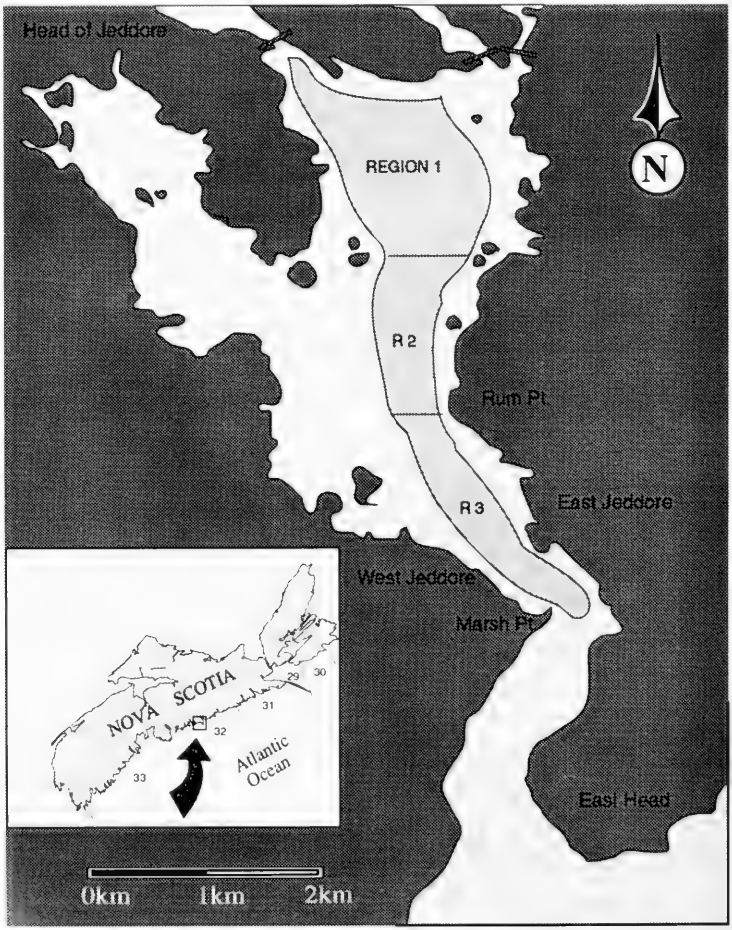

Figure 1. Jeddore Harbor consists of an inner bay linked to the open Atlantic Ocean by way of a deep narrow channel. The shaded portion of the harbor (Regions 1, 2 and 3) represents the sampled area.

TABLE 1.

Total number of lobster larvae (all stage 1) captured, both per cruise and during daylight and at night, between July 21 and September 22, 1988 in Jeddore Harbour.

Date	Number of Tows	Total Larvae	Larvae Captured During		No. Larvae per 1000 m^3	
			Day	Night	Day	Night
July						
21/22	7	4	3	1	0.91	0.97
28/29	7	1	0	1	0.00	0.85
Aug.						
4/5	11	115	1	114	0.50	20.02
11/12	8	5	2	3	0.89	2.82
18/19	7	67	0	67	0.00	14.64
25/26	7	10	2	8	0.79	3.55
Sept.						
1/2	10	5	0	5	0.00	1.85
8/9	8	0	0	0	0.00	0.00
15/16	7	0	0	0	0.00	0.00
22/23	7	1	0	1	0.00	0.81
Totals	79	208	8	200	3.09	45.51

mouth. Region 1 showed significantly fewer larvae then Regions 2 and 3; the latter two showed no significant difference (Chi-squared, $P < 0.05$). Inter-cruise and within-cruise differences in temperature, salinity, and light intensity had no significant impact on larval catch rates (Spearman rank correlations), nor did eel grass abundance in the net (Chi-squared, $P < 0.05$).

DISCUSSION

This is the first report of lobster larvae in an eastern Nova Scotia bay. The find was expected given the siting of brooding females in Jeddore Harbor during the study period (Jarvis 1989). Stage 1 larvae were in Harbor waters a minimum of ten weeks, and were likely present longer as they were observed on the first and last cruises. Similar temporal distributions were made for this species in a southern Nova Scotia bay (Pringle and Harding, unpublished data) and in the southern Gulf of St. Lawrence (Scarratt 1964, 1973, Harding et al. 1982, Hudon et al. 1986). The latter workers found stage 1 larvae in Magdalen Island waters from late June through early September, but the peak in abundance was one month earlier than Jeddore Harbor.

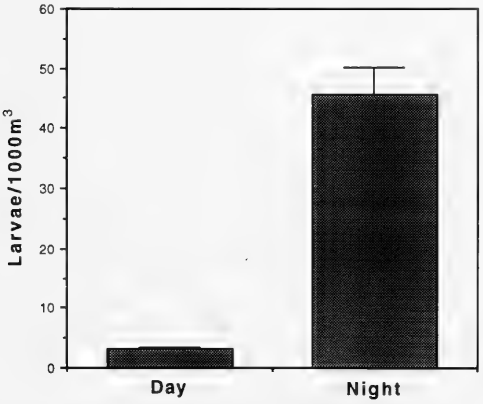

Figure 2. Total number of lobster larvae (/1000 m^3) captured in Jeddore Harbor during nighttime and daytime tows (data pooled for all regions and dates). Vertical lines represent ±1 standard error.

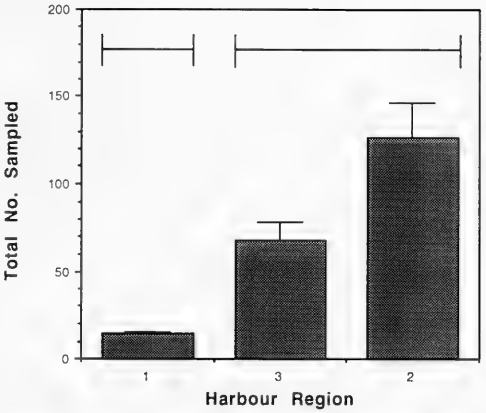

Figure 3. Number of lobster larvae sampled in each of three designated regions (data pooled for each region) of Jeddore Harbour. Vertical lines represent ±1 standard error; horizontal lines represent test of significance ($P \leq 0.05$).

The protracted spawning of stage 1 larvae suggests the absence of an external signal tightly synchronizing embryo development amongst females on this coast. This is expected since embryo development is significantly influenced by water temperature (Perkins 1972) and mature females occur in a range of water temperatures (Jarvis 1989). Nevertheless, the peak in stage 1 larval abundance in August suggests that egg hatch peaked during this month. Further, there is a strong relationship between larval intermolt length and temperature (Templeman 1936). Jeddore Harbor water temperatures for July (15.8 ± 1.2°C), August (16.0 ± 1.1°C) and early September (14.2 ± 3.5°C) were most conducive to larval maturaton (MacKenzie 1988). Moore et al. (1986) demonstrated sufficient degree days in eastern shore bays from August through early fall to permit lobster larval maturation to the post-larval stage and subsequent benthic settlement.

Larval lobster distribution in the water column was reviewed by Harding et al. (1987). Most earlier workers suggested a neustonic distribution under a variety of water and light conditions. Some hinted at a diel vertical migration, including Scarratt (1973), who found peak catch rates during the light period in southern Gulf of St. Lawrence waters. However, Hudon et al. (1986) attained peak catch rates in the neuston during dusk and at dawn, whereas Tremblay and Sharp (1989) had greater catch rates during twilight in nearshore, northern Gulf of Maine waters.

Harding et al. (1987) used a Tucker trawl to resolve the vertical diel distribution of lobster larvae in offshore, northern Gulf of Maine waters. These workers observed stage 1 larvae migrating, during darkness, from the thermocline (~25 m) into the Brown's Bank neuston. The present finding of significantly greater numbers of larvae during the dark period in Jeddore Harbor adds to the

growing support for the concept of diel, vertically-migrating lobster larvae.

Most lobster larval distributional studies (see review by Harding et al. 1987), using neuston gear, reported at least a few post-stage 1 larvae and all reported the post-larval stage. Unique to the present study is the finding of stage 1 larvae only, and no post-larvae. The boat, its rigging and tow speed were that used by Tremblay and Sharp (1989), who captured stages 1 and 2 larvae and the post-larval stage. Thus, the paucity of stage 1 larvae in our samples was not due either to equipment used, nor low water temperatures inhibiting larval maturation (MacKenzie 1988). Furthermore, it is unlikely all post-stage 1 larvae and post-larvae dispersed to that area of the bay not sampled.

This study represents the first time lobster larval distribution has been linked to brood stock distribution. Jarvis (1989) noted that the female at egg hatch moved to the Jeddore Harbor channel (Fig. 1), the site of our peak catch rates for larvae (Fig. 3). The most supportable hypothesis to explain the lack of post-stage 1 larvae, therefore, was that they were flushed from the harbor prior to maturation. The estimated stage 1 to 2 intermolt time would be from 5–7 da (MacKenzie 1988) at the water temperatures recorded during the study; longer by more than twice the Harbor's flushing rate. Stage 1 larvae are weak swimmers (Ennis 1986), thus unlikely to gain against the ebbing tidal currents of the Harbor's channel (Fig. 1) where the majority of larvae were located (Fig. 3). The well mixed channel waters (Jarvis 1989) would preclude larval retention by a two-layer circulation system. Thus it appears that not all Scotian Shelf bays retain lobster larvae sufficiently long to render a thermal advantage to those larvae hatched in them.

Evidence for a similar, rapid transport of stage 1 lobster larvae was described by Hudon et al. (1986) in open, Magdalen Island waters of the Gulf of St. Lawrence. They noted large concentrations of stage 1 larvae northeast of the islands; post-stage 1 larvae were found some distance to the southwest. The residual current was postulated as the transport mechanism. Here, the larvae were transported from open water, with less favourable temperature conditions, to a bay with enhanced thermal conditions for larval maturation. The reverse appeared to be the case for larvae hatched in Jeddore Harbor. Future research will assess the distribution of lobster larvae on the inner Scotian Shelf.

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GROWTH OF RED KING CRAB, *PARALITHODES CAMTSCHATICUS* (TILESIUS, 1815), IN ARTIFICIAL HABITAT COLLECTORS AT KODIAK, ALASKA[1]

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ABSTRACT An analysis of growth of red king crab, *Paralithodes camtschaticus* (Tilesius), from artificial habitats was conducted at Kodiak, Alaska. Modes from length frequency distributions taken from 14 samples over one year were analyzed. Nine hundred and ninety-two crabs ranging in carapace length from 1.8 to 13.0 mm were collected from artificial habitats. Nine benthic instars were identified for first year (age-0) red king crabs. Total growth in carapace length after 8 molts averaged 9.02 mm.

KEY WORDS: growth, artificial habitat, collectors, king crab, *Paralithodes camtschaticus*

INTRODUCTION

The red king crab, *Paralithodes camtschaticus* (Tilesius), is an anomuran of commercial importance in waters of Alaska. Valued at $168.7 million, the 1980 red king crab fishery was the most valuable single-species fishery in the United States (USDC 1981). During the 1989/90 fishery, 9.662 tonnes (21.3 million pounds) valued at over $105 million were harvested (ADF&G 1991). Kodiak Island, Alaska, was the focus of the commercial fishery for red king crab beginning in 1936 and extending through 1982. An average of 11,339 tonnes (25 million lb) was harvested annually between the years 1960 and 1982. Beginning in 1983, a fishery closure was announced due to low population numbers. This fishery has remained closed to the present time (ADF&G 1991).

Declines in red king crab populations and fishery closures have occurred throughout the entire Gulf of Alaska and Bering Sea and have spurred fishery scientists to investigate the reasons for the population collapses.

One aspect of investigating the collapses has been to study larval settlement, early juvenile growth, and to predict recruitment. Recruitment prediction has been successfully accomplished using artificial collectors for scallop, *Pecten alba* Tate (Sause et al. 1987), the western rock lobster, *Panulirus cygnus* George (Phillips 1986), and rock crab, *Cancer irroratus* Say (Beninger et al. 1986). At Kodiak, Alaska, an artificial habitat or collector was developed which successfully collects larval red king crabs and provides habitat for juvenile king crab instars (Blau et al. 1990).

Growth of benthic stage red king crab in Alaska has been studied by a number of authors, including Bright et al. (1960), Powell (1967), Weber and Miyahara (1962), Weber (1967), McCaughran and Powell (1977), and Stevens (1990). Only Weber (1967) at Unalaska made in situ observations of crabs during their first year (age 0) of benthic life. However, Weber's sample size was small and sampling was sporadic.

Due to their small size and cryptic behavior, first-year red king crab have not been collected in sufficient numbers throughout the year to successfully describe. Currently, there are no methods to determine age from a body structure, and the small size of juveniles and frequent molts precludes tagging. Aquarium observations may not provide useful information because of the unnatural environment.

Thus, we decided that a practical means of assessing growth was to examine modes in size-frequency distributions obtained from successive samples taken from collectors placed in the natural environment. More specifically, we determined molt increment and frequency of molting for red king crabs collected on the artificial habitats during the crabs first year of benthic life.

MATERIALS AND METHODS

Location and Description of Study Area

The study was conducted in inner Chiniak Bay, on the northeast side of Kodiak Island, Alaska (57°46′N, 152°26′W) as described in Blau et al. (1992). This area was chosen because of its known seasonal abundance of red king crab mating pairs (Powell et al. 1972). The study area consisted of shallow (<25 m) depths with dominate substrates ranging from shellhash to mud.

Artificial Habitat Collectors

Collectors were constructed of a 1.8 m long section of tubular polyethylene, 16 mm stretch mesh, and stuffed with used herring gillnet, Figure 1 (Blau et al. 1992). Twenty-five collectors were alternately attached in horizontal and vertical (float inserted) positions, two m apart, to a groundline and anchored to the substrate. Four such strings of gear were deployed in April 1990.

Sampling

Scuba divers retrieved two collectors from each site at approximately two-week intervals beginning June 1. The initial retrieval date was based on a literature review on time of egg hatch and the duration of planktonic stages (Haynes 1983; Powell et al. 1972). As the study progressed, intervals between collector retrieval were lengthened from 6 weeks to 8 weeks to correspond with an increase in the intermolt period observed. The final retrieval was made May 29, 1991.

Each collector was opened and the contents were carefully washed into a fine mesh screen and saved for microscopic examination. Red king crab glaucothoe, a form intermediate between

[1]Contribution PP-028 of the Alaska Department of Fish and Game, Division of Commercial Fisheries, Juneau.

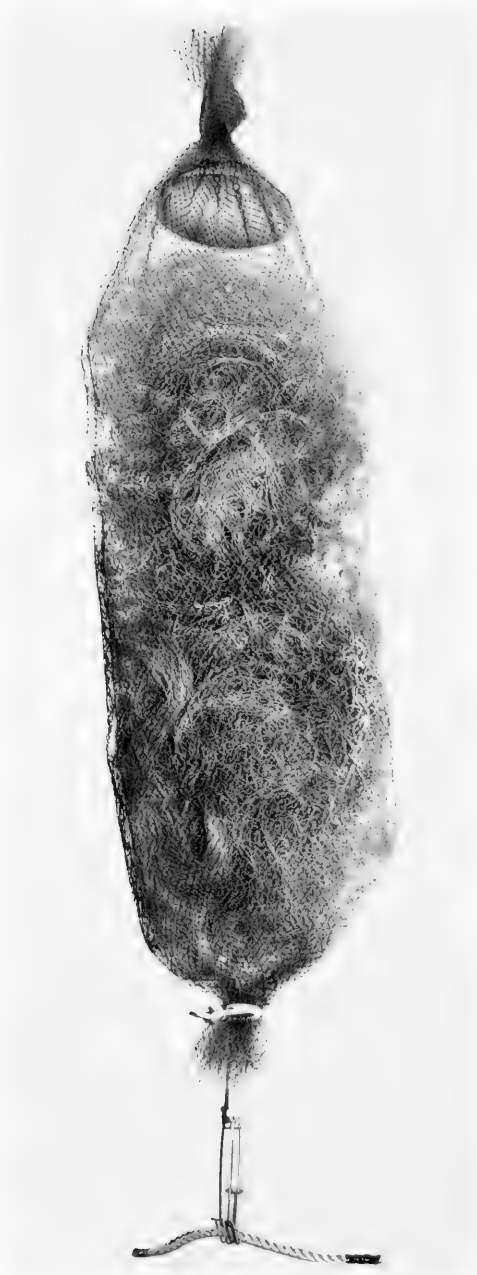

Figure 1. Artificial habitat used to collect red king crab.

larvae and benthic forms (Marukawa 1933), and benthic instars were set aside for measuring. Beginning with Instar III, the crabs were observable with the naked eye. The microscopic examination screening process was continued until no further Instar I and II's were observed. Carapace length (CL) was measured (Wallace et al. 1949) to the nearest 0.1 mm with an ocular micrometer in a dissecting microscope (120×).

Model Development

We attempted to model the carapace length data as representing growth through a succession of instars. Our goal was to estimate the mean and standard deviations of carapace length for each of the early instars present in our collections. Our development of the model required that we identify the instars present in each sample and estimate the carapace length mean and standard deviation for each identified instar. That process necessarily had a subjective component, and involved a mixture of statistical parameter estimation techniques and biological interpretations of the data. The procedure for identifying instars from the length frequency data and estimating the mean and standard deviation of carapace length for each instar is outlined in Appendix 1.

RESULTS

A total of 59 glaucothoe having a mean size of 1.71 mm CL were retrieved from the collectors over a 28-day period. Collectors were first sampled on June 1, 1990, and four glaucothoe were captured. The June 14 sample contained 54 glaucothoe and 39 Instar I crabs. The last glaucothoe appeared in the June 28 sample indicating that settlement was almost complete. Because we did not commence sampling until June 1, we did not determine when settling began; however, due to the small sample size of four glaucothoe on June 1, we inferred a late May date. On the first two sample days that contained benthic crabs their mean size was identical at 2.18 mm (Table 1). We identified this size as the first instar because we also found glaucothoe in the same samples.

A size range of 1.8 to 13.0 mm CL was noted for the 992 benthic crabs collected during the study. Box plots (Tukey 1977; Wilkinson 1990) of carapace length for each of these 14 samples plotted against week of collection (Fig. 2) help to characterize within-sample length frequency distributions, identify outliers within samples, and indicate that growth through time was approximately linear. We believe that outlier values within samples represent individuals that are a molt ahead or behind the majority in growth.

In all, nine instars were identified in our samples (Appendix 1). The estimated mean carapace length by instar ranged from 2.18 mm for Instar I, collected in June, to 11.2 mm for Instar IX, collected the following May, indicating red king crab grew 9.02 mm in carapace length in 8 molts during one year (Table 2). Using the parameter estimates in Table 2 and assuming that carapace length within instars has a normal distribution, the idealized relative frequency distributions of length for each instar was depicted graphically in Figure 3.

DISCUSSION

This study demonstrates that benthic stage red king crab in their first year of life can live and molt in the collectors. The progression of sizes through time and molt increments were ascertained by frequent sampling of the collectors, which in turn facilitated suc-

TABLE 1.

Summary statistics for carapace length measurements (to nearest 0.1 mm) by collection date for red king crabs captured in collectors near Kodiak, Alaska, June 1990 through May 1991.

Date	(N)	Min	Q1	Median	Q3	Max	Mean	Std Dev	CV
06/14	39	1.9	2.1	2.2	2.3	2.4	2.18	0.118	5.4%
06/28	77	1.8	2.1	2.1	2.3	2.6	2.18	0.171	7.8%
07/13	165	2.0	2.2	2.7	3.0	3.3	2.61	0.363	13.9%
07/26	217	2.5	2.7	2.8	3.0	3.4	2.84	0.152	5.4%
08/10	116	2.4	3.6	3.8	3.9	4.2	3.64	0.408	11.2%
08/23	98	2.6	3.6	3.7	3.9	5.4	3.78	0.362	9.6%
09/07	46	3.4	4.3	4.5	4.7	5.1	4.47	0.421	9.4%
09/21	32	4.2	4.5	4.7	5.1	6.4	4.98	0.605	12.2%
10/16	50	3.4	5.1	5.3	5.6	6.1	5.29	0.491	9.3%
12/04	51	4.6	6.4	6.7	6.9	7.9	6.58	0.703	10.7%
02/11	22	4.6	7.1	8.05	8.6	9.0	7.71	1.201	15.6%
03/27	31	6.1	7.7	7.9	8.35	10.3	7.95	0.769	9.7%
05/14	37	8.7	9.6	10.4	11.1	13.0	10.46	1.079	10.3%
05/29	11	8.6	9.7	9.4	9.7	9.9	9.33	0.474	5.1%

Σ 992

"N" is the sample size, "Q1" and "Q3" are the sample first and third quartiles, "CV" is the sample coefficient of variation; i.e. the standard deviation divided by the mean expressed as a percentage.

cessful modeling of growth of early-instar red king crabs. We were not able to determine at what larval stage red king crab enter the collectors because the initiation of sampling on June 1 was too late.

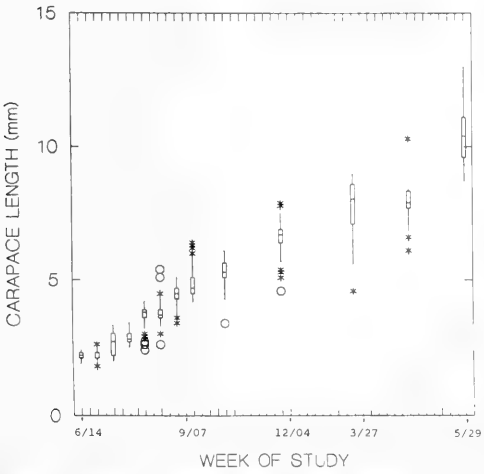

Figure 2. Box plots of carapace length by week of collection for red king crab at Kodiak, Alaska, 1991. The line inside of a "box" denotes the sample median. The lower and upper edges of a "box" denote the sample first and third quartiles, respectively. Observations in a sample that are one and one-half times the interquartile range below the first quartile or above the third quartile are considered "outliers" for a sample and are denoted by asterisks. Observations that are three times the interquartile range below the first quartile or above the third quartile are considered "far outliers" for a sample and are denoted by open circles. The range of values that fall within one and one-half times the interquartile range of the first and third quartiles are denoted by the vertical lines extending from each "box".

Instar I crabs first appeared in the June 14 sample. This is consistent with what is known about the timing of egg hatch and the length of larval stages. Egg hatch occurs during April and May in the Kodiak area (Powell et al. 1972), and the development time through larval stages 1 to 4 is reported as 60 days (Orlov and Karpevich 1965). Therefore, larvae hatching from eggs in early to mid-April, with a 60-day development period to the glaucothoe stage, would appear in the collectors in early to mid-June. Wolotira et al. (1984) in a study of decapod larvae in the Kodiak area, identified stage 4 zoea in the water column as late as June 26. In the same study, glaucothoe were observed from May 1 to June 26.

Red king crab remained in the collectors and grew throughout the year as evidenced by the progression of the length frequency modes (Fig. 2).

Analysis of the data using the fitting program allowed calculation of growth increments. Mean instar sizes derived from this study generally agree with mean instar sizes reported for red king

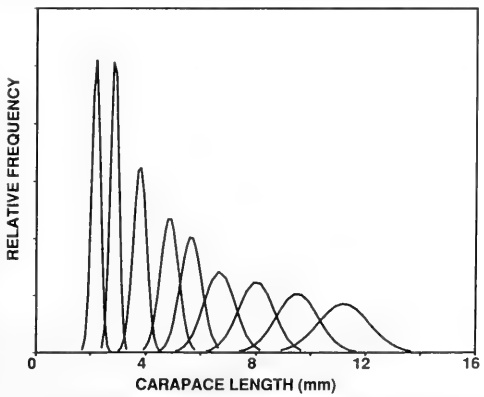

Figure 3. Modeled relative frequency distributions of lengths for first year instars of red king crab at Kodiak, Alaska, 1991.

TABLE 2.

Modeled growth of early instars of red king crab estimated means and standard deviations of carapace length by instar.

Instar	I	II	III	IV	V	VI	VII	VIII	IX
Mean	2.18	2.84	3.76	4.85	5.64	6.67	8.0	9.5	11.2
Std Dev	0.155	0.152	0.243	0.338	0.394	0.560	0.64	0.77	0.93
Increment		0.66	0.92	1.09	0.79	1.03	1.3	1.5	1.7
(% Increment)		(30%)	(32%)	(29%)	(16%)	(18%)	(20%)	(19%)	(18%)

crabs from other sources (Kurata 1959, 1961, Sato and Tanaka 1949, and Bright et al. 1960). Weber (1967) reported that in various rearing experiments the first instar was approximately 2 mm CL compared to our estimate of 2.18 mm. Based on modal analysis, Weber (1960) predicted a size of 11 mm and Bright (1967) predicted 8 mm CL for age-1 red king crab. McCaughran and Powell (1977) predicted a mean size of 12.58 mm CL for age-1 red king crab from a growth simulation model. Our estimate of size at one year of age for red king crab was 11.2 mm CL.

Additional collections of settling red king crab would provide data on interannual variations on settlement dates and growth rates.

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APPENDIX 1.

Analysis of growth and modeling of length frequency data.

Identification of and carapace length parameter estimation for each instar was performed as outlined below.

Instar I: We assumed that the first two samples from June 14 and June 28 were composed exclusively of Instar I individuals. This assumption was based largely on the timing of the first appearance of postlarval crab (versus glaucothoe) in the June 14 sample and the similarity of the length frequency distributions from the June 14 and June 28 samples. The mean and standard deviation of carapace length for Instar I were estimated by the sample statistics for the 116 length measurements in the pooled sample.

Instar II: All 217 crabs in the July 26 sample were assumed to be Instar II. The mean and standard deviation of carapace length in Instar II was estimated by all individuals collected in this sample.

Instar III: We assumed that the August 10 and August 23 samples were exclusively Instar III, with the exception of 19 individuals with carapace lengths less than 3.0 mm and three individuals with carapace lengths greater than 4.5 mm. The sample mean and standard deviation of carapace length for the remaining 192 individuals in the pooled sample were used as parameter estimates for Instar III.

Instars IV and V: With the exception of one individual with a carapace length of 3.4 mm, we pooled the September 21 and October 16 samples with the assumption that this pooled sample was composed of crabs from instars IV and V. To derive the carapace length parameter estimates for Instars IV and V, we used a commercially available computer program that fits frequency distributions as mixtures of individual component frequency distributions (MacDonald and Green 1988). We first smoothed the length frequency distribution for the 82 observations using a moving average with weights 1/9, 2/9, 3/9, 2/9, and 1/9. Then, using the computer program, we fit the smoothed frequency distribution as a mixture of two components that we believed represented Instars IV and V. To do so, we had to make the assumptions that the carapace lengths within Instars IV and V were normally distributed and that the coefficients of variation of length were equal in the two instars.

Instar VI: We pooled the September 21, October 16, and December 4 samples, again excluding the outlying value of 3.4 mm. The frequency distribution for these 132 observations was smoothed as described above. We then made the assumption that this pooled sample was a mix of Instars IV, V, and VI. Again, we assumed a normal distribution for the within-instar length frequencies and used the same mixture fitting computer program to fit the smoothed frequency distribution to a three-component mixture distribution. In the mixture fitting, the parameter estimates used for Instars IV and V were kept fixed at those derived above (see Table 1), while the fitting procedure was allowed to find the best parameter estimates for the remaining component, Instar VI.

Instars VII, VIII, and IX: In samples collected after December 4 there is greater variability and less evidence of modality in the length frequencies. Also, the sample sizes from these spring collection dates were rather small. Consequently, we were forced to rely more on subjective judgments and assumptions in estimating carapace length parameters for the instars following Instar VI.

We noted that the length frequencies from both the February 11 and March 27 samples were centered near 8 mm. Growth to 8 mm from the estimated mean for Instar VI (6.67 mm) corresponded to a 20% growth increment, which appears reasonable (Powell 1967). Therefore, we estimated the mean size of Instar VII as 8 mm.

For Instar VIII, we noted that the carapace length median and the mean (10.4 mm) of the 5/14/91 sample was higher than we had expected for the mean of Instar VIII, given our estimated mean of 8 mm for Instar VII. On the other hand, a growth from 8 mm to 10.4 mm seemed too small to be accounted for by two molts. Our examination of the length frequency distribution for the May 14 sample indicated a mode at 9 to 10 mm. Growth from 8 mm to 9.5 mm corresponded to a reasonable 19% growth increment. We also noted that the carapace length frequency for the May 29 sample had a median of 9.4 and a mean of 9.33. We believe that the May 29 sample was largely composed of Instar VIII, whereas the May 14 sample was composed largely of Instar VIII and a larger Instar IX. Guided by this interpretation of the May 14 and May 29 length frequencies, we choose 9.5 mm as a rough estimate for the mean carapace length for Instar VIII. Based on other observations of the May 14 length frequency data, we choose 11.2 mm, an 18% increment over 9.5 mm, as a rough estimate for the mean carapace length of Instar IX. To obtain estimates of the standard deviations of carapace length for Instars VII through IX, we extrapolated a linear fit of estimated instar standard deviations on estimated means of Instars I through VI to the estimated means of Instars VII, VIII, and IX.

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DEVELOPMENT OF A TECHNIQUE TO TAG ADULT RED KING CRAB, *PARALITHODES CAMTSCHATICUS* (TILESIUS, 1815), WITH PASSIVE INTEGRATED TRANSPONDER TAGS[1]

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ABSTRACT We developed a method of tagging red king crab, *Paralithodes camtschaticus* (Tilesius), with Passive Integrated Transponder (PIT) tags which were injected into the proximal segment of the fifth right leg. During the tag recovery phase of this project, the animals were interrogated for the presence or absence of PIT tags and the tags, if present, were read using a "portable electronic detector" or monitor. Initial tests using crabs held in pots indicated that PIT tags would be retained at a high percentage. We then field tested tagging and recovery methods on the Bristol Bay portion of the Bering Sea red king crab population. PIT tagging was demonstrated to be a reliable method of marking red king crab. If the recovery of tags is successfully automated, the technique could reduce overhead and increase precision in managing commercially important crab populations.

KEY WORDS: red king crab, transponder, tag

INTRODUCTION

Tagging is a basic method fishery biologists employ to gather life history and fishery exploitation related data. Red king crabs, *Paralithodes camtschaticus* (Tilesius), were first tagged in about 1927 in Japanese waters when Marukawa (1933) wired an elliptical celluloid plate, 3.5 cm by 2.4 cm in diameter, to the meropodite of the fourth leg. This tag was lost when the crab molted. In 1953 a tag was developed consisting of polyvinylchloride plastic tubing that, when inserted around or into the dorsal musculature at the connection of the carapace and abdomen, had a high degree of retention through ecdysis (Gray 1965).

This tagging technique, with some modifications in tag materials and design, has since been exclusively used by biologists working with red king crab in the North Pacific and Bering Sea for the last 27 years. However, tagging studies conducted by the Alaska Department of Fish and Game (ADF&G) in the early 1980s revealed a high degree of non-cooperation from fishermen in returning these externally marked crab (ADF&G 1982). Low return rates reduced the sample size, increased the variance of estimates of growth parameters, and confounded population estimation with tag recovery data.

Development of tags that are not externally visible and require special catch sampling for recovery could resolve difficulties associated with voluntary tag recovery programs. At least two types of internal tags are available: coded wire tags (CWT) and passive integrated transponder (PIT) tags. Bailey and Dufour (1987) and Prentice and Rensel (1977) tested the use of CWTs on snow crab *Chionoecetes opilio* (Fabricius) and the spot prawn *Pandalus platyceros* (Brandt) with some success. Prentice (1990a) in preliminary experiments using the PIT tag with two species of Crustacea, *Microbrachium rosenbergii* and *Cancer magister*, demonstrated that the tag was retained through the molt.

PIT tags have at least two advantages over CWTs used to date on crustaceans. First, they possess unique tag codes, unlike the CWT batch coding system; unique codes permit estimation of individual growth rates. Second, recovery is much improved because the electronic PIT tag monitoring equipment can distinguish tags while they remain implanted in the animal; CWTs must be removed from the animal to determine tag-batch identification. In salmonid applications a clipped adipose fin indicates the presence of a CWT to the catch sampler. The presence of external marks could bias recovery of tagged crabs.

The primary objective of this study was to determine the technical and biological feasibility of using the PIT-tag marking system with red king crab. This paper describes an initial experiment on retention by tagged crabs for a 2-month period, a large-scale field trial including recovery from commercial catch sampling, and estimation of recovery rates from a catch "seeded" with tagged crabs. Recommendations on future experimentation and applications are provided.

MATERIALS AND METHODS

Tagging Equipment

Prentice et al. (1990b), provides a complete technical description of equipment and suppliers used in this study. The tags were TX1400L 125 kHz passive integrated transponder tags available from Destron-Identification Devices, Inc. (D-IDI)[2]; each one was 11 mm in length and 2.1 mm in diameter and consisted of a microchip bonded to an antenna coil. These electronic components were encapsulated in a glass tube and uniquely programmed with a 10-digit alphanumeric code. Tags were implanted into red king crab using a Model I-300 automatic PIT tag injector available from Biomark.[2] The tag, when energized with a 125 kHz external power source provided by an HS5102L portable monitor, transmitted its unique code which was then received, processed, displayed, and stored by the monitor.

Tagging Procedure

The proximal segment of the fifth right leg was chosen as the site for tag implantation (Figs. 1 and 2). This leg was selected because it is modified as a reproductive organ and an egg clutch

[1]Professional Paper of the Alaska Department of Fish and Game, Division of Commercial Fisheries, Juneau, Alaska.

[2]Reference to trade names does not imply endorsement by the Alaska Department of Fish and Game.

grooming organ in males and females respectively, and is usually folded under the abdomen, minimizing its chances of damage during commercial fishing operations. Because this leg is not processed due to its small size, the possibility of product contamination by the tag is avoided. Also, the fifth leg normally remains attached to the abdomen of the crab and is not broken during processing.

During tagging the crab was placed in a rostrum down position. The carapace was pushed apart from the abdomen exposing the fifth leg. The tag injector needle was inserted through the articulation membrane of the leg and the tag released longitudinally into the mid portion of the leg muscle.

Preliminary Retention Experiment

In 1990 ADF&G initiated a study in the Kodiak Island area to evaluate the use of PIT tags with red king crabs (Fig. 3). Tagging methodology was tested on six male and six female adult red king crabs which were kept in pots on the ocean floor at a depth of 9 m. Observations were made on tagged crabs for a 2-month period by intermittently retrieving the pots and examining the tagged crab with a portable tag monitor.

Commercial Fishery Experiment

After the initial tag experiment was deemed successful, a large-scale tag and recovery effort was designed for the Bering Sea red king crab fishery. This experiment enabled testing all equipment under actual field conditions.

Area and Station(s) Fished

The 128-ft (39 m) crabber, Kristen Gail, was chartered to capture king crab for tagging using commercial gear in the Bristol Bay portion of the Bering Sea. Crab were tagged from August 7–26, 1990, about 90 d before the commercial fishery. Because of logistical considerations, the study area was restricted to a 5,100 nm^2 portion of Bering Sea (Fig. 3). This area produced over one-half of the harvest of red king crab between 1985 and 1989 (ADF&G 1990). Additionally, assessment surveys showed that 70% of the legal population of Bering Sea red king crab were estimated to inhabit the area studied (Stevens and MacIntosh 1990).

A total of 70 stations were fished in a stratified, systematic pattern. At each station 13 to 16 commercial king crab pots, 2.1 × 2.1 m, were set 0.2 km apart along a 2.4- to 3.0-km line. However, 11 of the 70 stations were fished with only 7 pots. Spacing between pots at these 11 stations was 0.4 km.

Figure 1. Ventral aspect of dissected red king crab abdomen with fifth legs. Tag insertion site in proximal segment depicted by an arrow. Note PIT tag (lower right).

Figure 2. Proximal segment of fifth right leg dissected longitudinally to show placement of PIT tag (arrow).

Stations were arrayed in groups of five with a north-south orientation. The distance between adjacent stations within an array was 4.8 km for a total length of 19.2 km for an array.

Pots were baited with 1.9 L frozen chopped herring, *Clupea harengus pallasi* (Valenciennes). Cod, *Gadus macrocephalus* (Tilesius), and sculpin, Cottidae, were used as hanging bait when available. Pots were fished for an average of 44.2 h ranging from 16.6 to 69.0 h.

Catch Sampling and Tagging

The contents of each pot were unloaded onto a sorting table where biologists divided the catch of king and Tanner, *Chionoecetes bairdi* (Rathbun), crabs by sex and transferred the sorted catch to a tagging and measuring table. When the catch of Tanner crab ≤139 mm carapace width (CW), male red king crab ≤146 mm (CW), and female red king crab was large, the catch was subsampled. All male red king crab ≥147 mm CW (commercial legal size) were PIT-tagged.

Every other PIT-tagged crab, or half, were "double-tagged;" i.e., in addition to a PIT tag, they were also tagged with a polyvinyl isthmus tag to test PIT-tag loss rates. After each crab was measured and tagged, its PIT tag was verified to be operational with a portable monitor. Only healthy, non-injured crabs were

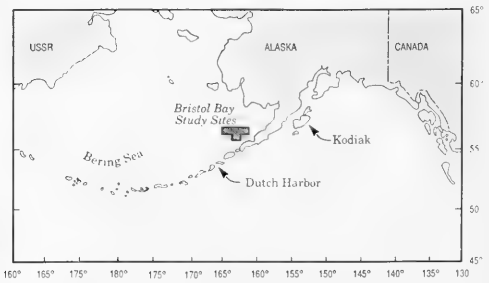

Figure 3. Locations of study sites.

tagged. Care was taken to handle crab in a gentle manner and return them to the ocean as quickly as possible. Carapace condition—new, old, and very old shell—as determined by the amount of abrasions and fouling of the exoskeleton, and size category, represented as commercially legal or sublegal, was recorded for all tagged crab.

Tag Recovery

Tagged crabs were recovered from the commercial red king crab fishery held from November 9 to 18, 1990. Fifteen ADF&G tag recovery personnel, each equipped with portable monitors were stationed at seven shore-based processors for up to 24 h each processing day. In addition, one tag recovery person was placed aboard each of three at-sea floating processors for the duration of the fishery. Tag recovery personnel, positioned on the processing lines, checked the fifth right legs after the crab had been butchered by passing the portable-monitor within 50 mm of the legs. As many legs were checked as possible; sample size was recorded along with fishing location of the delivering fishing vessel.

If a tag was present in a leg, the monitoring device would beep and automatically store the tag code. ADF&G staff and processing personnel checked the entire harvest for crabs carrying polyvinyl tags. All polyvinyl-tagged crabs recovered were tested for the presence of a PIT tag before butchering. PIT tagged legs recovered were frozen for future dissection and examination.

"Seeding" Experiment

To examine the recovery rate of PIT tags with the portable monitor at a crab processing plant and the rate of tag loss during butchering, 75 randomly chosen male red king crab were PIT-tagged and 24 were doubled-tagged. These crabs were seeded into the hold of the Kristen Gail which contained approximately 8,500 crabs that had been captured in pre-fishery tagging work by ADF&G. We assumed the tagged crab would randomly distribute themselves amongst the other crab in the hold of the vessel. Five days later the crab were delivered to a processor at Dutch Harbor. As the crab entered the processing line, two ADF&G biologists identified and monitored all the dual-tagged crabs throughout their processing, and after processing, the fifth legs from all crabs were checked for PIT tags.

RESULTS

Preliminary Retention Experiment

The initial tagging experiment was successful. All 12 red king crab retained their tags, including the six females, each of which molted once. No necrosis of the tagged leg segments was observed upon dissection indicating that the tag would probably be retained indefinitely. Based on these results, the large-scale field experiment was implemented.

Commercial Fishery Experiment

A total of 6,700 (4,700 legal and 2,000 sublegal) red king crab were PIT-tagged. Fifty percent of these crab were dual-tagged with a second polyvinyl isthmus tag. The time involved for one person to measure and PIT tag a crab was approximately 1 min. Total number of crabs tagged depended on availability of crabs, vessel running time, and incidental catch rates. Dual-tagging added substantially to total tagging time.

Out of a harvest of 3.1 million king crabs, a total of 385,695 crabs (12%) were examined for PIT tags at processing plants over a 10-d period. Thirty-four PIT tagged crabs were detected, or approximately 1 out of every 11,000 crabs examined. Of the 249 dual-tagged crabs recovered, two thirds were monitored for the presence of PIT tags, and 95% of those retained the PIT tag. Approximately 1 in every 12,400 crabs in the harvest was a dual-tagged crab.

"Seeding" Experiment

Of the 75 PIT tags seeded with 8,500 unmarked crabs, 59 were detected during processing for a 79% return rate. This test was complicated by a 40% mortality rate (deadloss) in the 8,500 crab. Because dead crabs were not processed, any dead PIT-tagged crab were not recovered. However, of the 24 dual-tagged crabs, 23 (96%) were recovered; 18 of these were recovered before butchering, and 17 (94%) still had detectable PIT tags.

DISCUSSION

The PIT tagging and monitoring equipment used in this study was reliable when waterproofed and maintained. The TX1400L 125 kHz tag withstood the strenuous field conditions often encountered in the Bering Sea. The I-300 automatic PIT tag injector was reliable as long as care was taken to occasionally clean the breach of the injector, which often fouled with coagulated crab blood. When this occurred, tags would jam and break. The HS5102L portable readers were waterproofed by inserting them in several plastic freezer bags sealed with duct tape; they performed adequately in the wet environment encountered during both tag recovery operations.

The PIT tags withstood the butchering process as demonstrated by the 79% return rate from the seeding experiment, a rate that would probably have been higher had the entire deadloss of 40% been examined for PIT tags. However, some processed 5th leg segments were not available to recovery personnel: e.g., some segments were inadvertently dropped to the processing line floor and later disposed of. This tag loss during butchering could have been quantified with the seeding experiment if it had not been for the deadloss problem.

PIT tag retention rate for dual-tagged crabs demonstrates a high rate of retention for the 5th leg. This leg is folded and tucked under the carapace in normal orientation. This provides protection for the leg and minimizes leg loss and therefore tag loss during fishing and handling. Because the commercial fishery experiment was conducted outside of the molting season, conclusions about retention through molting are not warranted. However, all six females that were tagged in the preliminary retention experiment retained their PIT tags after molting.

PIT tags were retained by the dual-tagged crabs at a fairly high rate (95%) during the 90 d between tagging and commercial fishery recapture. Though this rate is slightly less than PIT tag retention rates documented for salmonids (range 98.5–100%; Prentice et al. 1990c), it is almost identical to the retention rate (96%) of coded wire tags in crabs observed by Bailey and Dufour (1987). PIT tags were also retained at a high rate (94%) among the dual-tagged crab in the seeding experiment. The overall recovery of 34 PIT-tagged crabs from the commercial fishery was low because the recovery effort was limited. The fishery captured 3.1 million crabs that were delivered to a total of 51 processors in 10 days; we were able to cover only 10 processors and sample 12% of the catch. Furthermore, the effective range of a 125 Khz tag is no

more than 50 mm, and recovery personnel had to ensure that the wand-to-tag distance did not exceed 50 mm for the monitor to detect a tag. This required handling and orienting each leg, a very labor intensive process which substantially decreased overall sample size and limited the recovery to only 34 tags. However, the recovery rate of PIT tagged crabs among tail sections examined compared to the recovery rate of dual-tagged crabs among crabs landed was similar: roughly 1 in 10,000 crabs.

Additional design work and experimentation dealing with increasing the detection range of the tag and automated recovery techniques, similar to those in use for salmonids (Prentice et al. 1990c), is needed to increase the total number of crabs examined. Specifically, automated monitoring systems mounted on crab processing lines could reduce staffing and budget constraints related to monitoring the catch and could increase the number of crabs examined for the presence of tags because automated units could examine a greater percentage of the harvest and operate 24 hours per day.

ACKNOWLEDGMENTS

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POPULATION STRUCTURE AND HABITAT OF JONAH CRAB, *CANCER BOREALIS* STIMPSON 1859, ON THE CONTINENTAL SLOPE OFF THE SOUTHEASTERN UNITED STATES

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ABSTRACT The distribution, relative abundance, and habitat associations of the Jonah crab, *Cancer borealis*, were investigated on the continental slope off the southeastern United States. Trap catches of Jonah crab declined with increasing depth. The largest catches (9.5 crabs/trap) occurred from 274–366 m and fewer than 1 crab/trap occurred at depths greater than 458 m. Male *C. borealis* were significantly more numerous in trap catches than females (~20:1). The size of male Jonah crab differed significantly among depth strata with largest individuals occurring in the shallowest stratum (274–366 m). The presence of sperm in seminal receptacles and mature or advanced stage ovaries in most of the females collected from May—September suggested that mating had occurred. The lack of ovigerous females in collections prohibits definition of a spawning period.

The depth of maximum trap catches coincided with depths where sedimentary features were characterized by globigerina ooze, current generated ripples, or dunes. Observations from a submersible revealed densities of 8.5 crabs/1000 m^2 over soft-bioturbated sediment and 6.5 crabs/1000 m^2 over dune topography. Densities of crabs were less over the low-outcrop (1.9 crabs/1000 m^2), rippled (1.8), flat-ooze (1.7), black pebble (0.8), and coral mound (0.1) habitats than over soft-bioturbated and dune habitats. The shallow depressions, mounds, and burrows that typified the soft-bioturbated habitat may result from excavation and foraging by Jonah crab. Population estimates of 70,700–117,400 crabs varied with the proportion of habitat types in areas surveyed by submersible off Charleston, South Carolina.

KEY WORDS: Jonah Crab, *Cancer borealis*, population, continental slope, southeastern U.S.

INTRODUCTION

The Jonah crab, *Cancer borealis* Stimpson 1859, is a by-catch of the lobster fishery along the coast of New England (Marchant and Holmsen 1975, Krouse 1980), but is an unexploited resource off the southeastern United States. Limited information is available on population sizes, although harvestable resource size was estimated to be 227 million kg for the area from Georges Bank to Cape Hatteras (Anonymous 1973). The incidental nature of Jonah crab catches off the New England and Middle Atlantic states presents difficulties in estimating the amount of Jonah crab caught by commercial gear. Previous studies have provided information primarily on the crab's distribution and life history.

Occurring from Nova Scotia to south of the Tortugas, *C. borealis* inhabits the intertidal zone to a depth of 800 m (Rathbun 1930, Williams 1984). Tropical submergence has been noted in the southern portion of its range, where optimum temperatures of 6–14°C (Jeffries 1966) occur offshore (MacKay 1943, Stehlik et al. 1991). The habitat of *C. borealis* apparently changes along its geographic range, from rocky substrates in Narragansett Bay (Jeffries 1966) and off the coast of Maine (Krouse 1980) to silt and clay on the continental slope off Chesapeake Bay (Musick and McEachran 1972). Although Haefner (1977) suggested that migration of Jonah crab was limited in the Middle Atlantic Bight, Jeffries (1966) reported inshore movement from spring through fall, followed in winter by emigration to deeper, warmer waters off Rhode Island.

Information on the crab's life history is geographically limited to waters off New England and Chesapeake Bay. Size and sexual segregation with depth were reported from the Norfolk Canyon by Carpenter (1978), where smaller-sized (<30 mm) females were dominant at depths less than 150 m, and males were most abundant at depths >150 m. Ovigerous females have been reported in August and September from Maine (Smith 1879) and in mid-July from Narragansett Bay (Sastry and McCarthy 1973). Based on gonadal conditions, Carpenter (1978) proposed a spawning period of late winter to early spring in the Middle Atlantic Bight.

Recent trapping and submersible operations to evaluate the distribution of golden crab, *Chaceon fenneri* (Manning and Holthuis 1984), on the continental slope of the southeastern U.S. (Wenner et al. 1987, Wenner and Barans 1990) confirmed its co-occurrence with *Cancer borealis*. Up until that time there had been no data available describing the population structure and distribution of Jonah crabs in relation to habitat or depth for the South Atlantic Bight. This study provides information on the life history, habitat types, and densities of Jonah crab on the continental slope in that region. Estimates of population size are based on densities of Jonah crab within specific habitat types.

METHODS

Trapping studies were conducted from 5 August–16 October, 1985 and from 7 January–11 August, 1986 from the R/Vs *Oregon* and *Lady Lisa* within a geographic area bounded by latitudes 29°53.1′–32°43.1′N and longitudes 76°42.0′–79°33.6′W. Seven

depth strata were sampled: 274–366 m (stratum 1), 367–457 m (stratum 2), 458–549 m (stratum 3), 550–640 m (stratum 4), 641–732 m (stratum 5), 733–823 m (stratum 6), and >823 m (stratum 7).

Generally, three sets of traps, each set consisting of three Florida traps and three Fathoms Plus traps alternately attached at 61 m intervals to a 365.6 m groundline (Wenner et al. 1987), were made approximately 1–2 km apart within a depth stratum over a 24 hour period. Trapping locations within the strata were selected based on lack of strong surface currents and low bottom relief as determined by a fathometer. Equal distribution of effort among strata was not achieved (Table 1) due to constraints of weather and currents. These factors also influenced fishing duration, which was standardized at 20 hours, but which in fact, ranged from 10.2–25.7 hours among sets (Table 1). Because soak time (the elapsed time between setting and hauling) may influence trap catches (see Miller 1990 for a review), a least-squares regression analysis of $\log_{10}$ transformed catch per trap (y) on fishing duration (x) for sets in strata 1–3 was used to determine if a relationship existed. No statistically significant relationship was found between these variables for either the Fathoms Plus (H_0: $\beta = 0$, $F = 0.64$, $P = 0.42$, $r^2 = 0.0098$, $n = 89$) or the Florida trap (H_0: $\beta = 0$, $F = 0.34$, $P = 0.56$, $r^2 = 0.005$, $n = 89$). Therefore, trap data were not standardized with regard to soak time in analyses.

Bottom water temperature was measured with reversing thermometers at the time of trap deployment, and bottom sediments were sampled with a geological "rocket" grab for each group of three sets. Sediments were examined to aid in characterization of major habitat types. Sampling depth and location were recorded at the buoyline anchor location.

Jonah crabs from each trap were counted, individually weighed, and measured for carapace width (CW). The number of crabs per trap was calculated for each trap type per set within a stratum. Female *C. borealis* were examined for evidence of egg extrusion and mating. Presence of eggs or egg remnants on pleopods and sperm plugs in the vulvae, an indicator of recent mating, were noted. A subsample of females were sacrificed to determine gonadal condition, as described by Haefner (1977) and Carpenter (1978). Ovarian tissue and seminal receptacles of sacrificed females were examined histologically in order to validate assigned ovarian stages and determine presence of sperm or spermatophores in seminal receptacles.

Observations on density and habitat of *Cancer borealis* were made during three dives of the submersible *Johnson Sea Link* II in August 1986, 12 dives from 30 July –3 August, 1987, and 4 dives in November, 1988. An area of the upper continental slope between 31°50′N–32°05′N and 78°55′W–79°15′W, approximately 122 km southeast of Charleston, South Carolina, was divided into three equal sampling areas (Wenner and Barans 1990) that encompassed locations where trapping took place in 1985–86 (Fig. 1). During 1986 and 1987, observations and crab counts were made along 85 transects in depths of 389–567 m (Table 2). In 1988, habitat boundaries were described along 16 transects that crossed isobaths between 293–540 m (Table 2). The beginning position was selected to traverse transect paths of previous dives in 1986 and 1987. Data from 1988 transects were used only to describe habitat types and estimate their proportion across isobaths.

Prior to each transect, the submersible remained stationary near the bottom, with minimum light emission, for a 3–5 minute period while its position was determined by personnel on the R/V *Edwin Link*. Following this procedure, the submersible proceeded along the transect in the direction of the current, and counts of *C. borealis* were recorded. Survey transects were ~15 min in duration, except when there was a major change in habitat type, with distance traversed ranging from 188–522 m. Three to seven transects were completed per dive. The location of the first transect of most dives was randomly chosen in the three areas, and subsequent transects on a particular dive continued or paralleled the first. Although starting points for each transect were not randomly chosen, the pilot often directed the submersible along a different compass heading at the beginning of transects. Because of the likelihood that transects within a homogeneous habitat on a particular dive are not independent and are autocorrelated (Burnham et al. 1980), the validity of using analysis of variance is questionable (Sokal and Rohlf 1981). Therefore, we chose to use the nonparametric Kruskal-Wallis test to determine whether densities were different among habitats. During the 1988 dives, transects continued until a major change in habitat occurred, at which time the submersible's position was determined. Time, depth, bottom water temperature, current speed, habitat characteristics, biological observations, and position of the submersible were recorded at the end of each transect. Sediment was collected by a grab sampler at the beginning and end of each transect and when bottom type changed markedly and, as with previous samples, was used to characterize habitat features.

Videotape and 35-mm still photographs were used to document changes in habitat and topography along transects. Videotaping was not continuous during a transect but was initiated when crabs were sighted or habitat changed. Observations were recorded simultaneously on audiotape and videotape, and both were used to describe habitats and crab behavior. Audio documentations of crabs counted along a transect were later compared with the video coverage of habitat where crabs were encountered.

Estimates of population density were determined for the area of each transect. This area was defined as the product of the transect distance and the horizontal visibility based on estimates by a pilot experienced with previous measurements from the submersible to a point of secchi disk fade-out by Parker and Ross (1986). Submersible positions and transect lengths were determined from LORAN C coordinates, while horizontal visibility was twice the radius (~9.1 m) of the lighted arc of bottom as seen by the forward observer several meters above the bottom. Our estimate of visibility was more conservative than the measurement of 10 m to the edge of light penetration measured by Parker and Ross (1986) from the same submersible.

The population size of crabs was estimated and summarized for

TABLE 1.

Fishing duration in hours of trap sets within seven strata sampled during 1985–1986.

Stratum	Depth (m)	No. Sets	Fishing Duration (hrs)			
			x	(s)	Min.	Max.
1	274–366	12	19.0	3.78	12.4	23.8
2	367–457	53	19.4	3.30	10.2	25.0
3	458–549	22	19.7	3.99	11.2	25.7
4	550–640	8	22.0	2.04	18.6	24.7
5	641–732	4	17.9	1.23	16.4	19.8
6	733–823	20	20.4	2.46	11.7	23.3
7	824–898	9	19.9	1.29	18.2	21.9

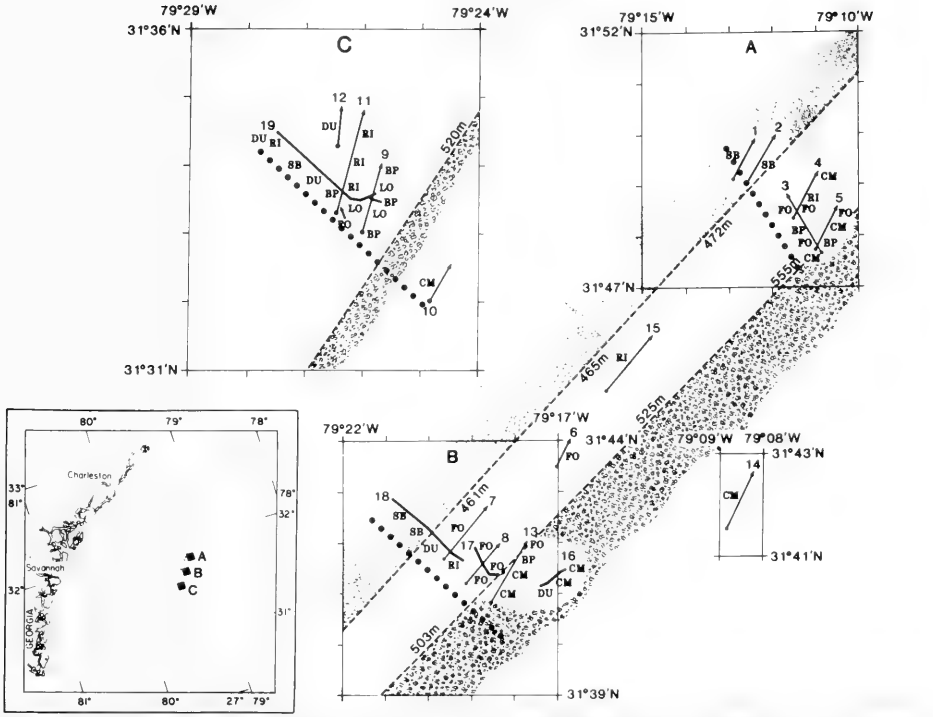

Figure 1. Location of trap sets and submersible dives (Areas A, B, and C) on the continental slope off the southeastern US (insert). Solid lines depict submersible transects, dotted lines depict length of rectangular sub-areas for population estimates, and dashed lines depict estimated depths of major habitat differences (transect numbers and habitat codes as in Table 2). Habitat generalizations depicted (stippled = soft-bioturbated ooze; rubbled = coral mounds) are based on *in situ* observations.

individual habitat areas to determine the relative total crab populations from specific habitat types within each respective fishing area (Area A, B, or C). Habitat proportions were calculated from distances along the across-isobath transects and estimated between along-isobath transects to provide proportional estimates across the range of depths observed. Then, the population size was estimated for a rectangular sub-area measuring 4 km in width and extending lengthwise the distance between shallowest and deepest depths of confirmed habitats (Fig. 1). Population size was derived by expanding mean densities from each habitat type (all areas combined) to the total area of habitats within each of the three sub-areas.

RESULTS

Description of Habitats

Bottom characteristics of the three areas surveyed were previously categorized into seven distinct habitat types (Wenner and Barans 1990). The flat-ooze habitat was the most frequently encountered habitat during crab censusing dives, occurring in 28% of the transects and accounting for 31% of the total distance surveyed in all transects. It was associated with a northerly current velocity of 1.9–2.8 km h^{-1} (Table 3). Distinct mounds of coral constituted 20% of the total distance surveyed and were associated with northerly currents of 1.4–1.9 km h^{-1}. Current-generated habitats such as ripple marks and large (4–5 m) dunes were encountered in 19% and 3% of the total distance surveyed, respectively. Currents in these habitats ranged from 1.9 to 2.8 km h^{-1} to the NNE. Black pebble (phosphorite) and low outcrop habitats often occurred in close proximity and constituted 12% and 3% of the total distance surveyed, respectively. Current velocities ranged from 1.4–1.6 km h^{-1} to the NNE. Green globigerina ooze with extensive bioturbation characterized the soft-bioturbated habitat which accounted for only 12% of the total distance surveyed on crab censusing dives. This habitat was found to represent a much larger proportion of each area's shallow topography during the 1988 habitat measurement dives. The northerly current of the soft-bioturbated habitat ranged from 0.4–0.6 km h^{-1}.

Distribution and Abundance from Traps

The 130 trap sets (770 individual trap observations) caught 2,053 *C. borealis* (566.6 kg) at depths between 293–567 m. Catch per trap was related to depth (Fig. 2). Kruskal-Wallis nonparametric analyses indicated that catches (number per trap) were significantly different among strata ($\chi^2 = 52.3$, $P < 0.01$). Catches were greatest in the 274–366 m stratum (9.5 crabs/trap,

TABLE 2.

Summary of counts of Jonah crab and habitat types encountered on submersible transects.

Dive	Transects	Year	Depth (m)	Total Area (m^2)	Number of Crabs	Habitat
1	01–05	1986	431–414	30,521	212	SB
2	06–11	1986	472–475	36,874	363	SB
3	12–18	1986	521–555	65,179	19	BP, FO, CM
4	01–06	1987	517–558	33,950	6	FO, RI, CM
5	07–11	1987	536–567	33,098	5	FO, BP, CM
6	12–15	1987	405–408	25,929	71	FO
7	16–21	1987	443–452	45,228	159	FO
8	22–26	1987	497–521	40,408	39	FO
9	27–62	1987	481–512	35,484	69	LO, BP
10	33–36	1987	531–539	21,488	0	CM
11	37–43	1987	519–461	53,458	129	FO, RI
12	44–46	1987	389–411	18,543	118	DU
13	47–54	1987	503–534	47,095	36	CM, BP, FO
14	55–62	1987	514–554	48,347	0	CM
15	63–69	1987	462–467	47,878	98	RI
16*	01–04	1988	490–517			CM, BP
17*	05–08	1988	486–540			FO, LO
18*	09–12	1988	293–481			RI, DU, SB
19*	13–16	1988	320–500			BP, RI, DU, SB

* Cross isobath habitat quantification only; dive numbers correspond to transects in Figure 1.
CM = coral mounds; BP = black pebbles; DU = dunes; FO = flat ooze; LO = low outcrop; RI = ripples; SB = soft-bioturbated.

2.5 kg/trap) and declined with depth so that <1.0 crab per trap were collected at strata depths >458 m (Fig. 2).

More individuals and greater weight of Jonah crab were collected by the Fathoms Plus trap than by the Florida trap in every stratum; however, in testing the null hypothesis of no difference in mean number or weight among trap types for each stratum, statistically significant differences (t-test of independent samples, $P < 0.05$) in these variables only occurred in the 367–457 m stratum (Fig. 2). At other depths, no significant difference in mean number or mean weight was found among trap types.

Size and Sex Composition

Male *C. borealis* were significantly more numerous in trap catches than females, outnumbering them by 19.8:1 over all strata. No ovigerous females were collected during the trapping study. Dominance of males was significant in every stratum where they occurred (Table 4). The greatest proportion (61%) of male *C. borealis* occurred at depths of 367–457 m, whereas most (74%) of the females were from depths <367 m.

Jonah crabs ranged in size from 87 mm to 155 mm CW. Male crabs which averaged 128 mm CW ($\bar{x} = 9.0$, $n = 1203$) were significantly larger than females ($\bar{x} = 104$, $s = 8.1$, $n = 97$) [$F = 667.1$, $P < 0.001$] and weighed more (Male: $\bar{x} = 268.6$ g, $s = 70.9$, $n = 1007$, Female: $\bar{x} = 106.6$, $s = 46.7$, $n = 97$) [Mann-Whitney test, $U = 2850$, $P < 0.05$]. Carapace width frequency distributions indicated that most of the males collected ranged from 120–140 mm CW. Analysis of carapace widths among strata 1–3, where most of the male crabs were collected, indicated a

TABLE 3.

Characteristics of habitats as determined from *in situ* observations in 1986–1987 (taken from Wenner and Barans 1990).

Habitat Type	Depth (m)	Current Velocity and Direction	Description
Soft-bioturbated	300–485	N, 0.4–0.6 km/hr	Green globigerina ooze, extensive bioturbation, worm tubes, low mounds, burrows
Dune	389–411	NNE, 1.9–2.8 km/hr	4–5 m height; strongly rippled slopes facing current, smooth steep scarps leeward
Flat ooze	405–567	N, 1.9–2.8 km/hr	Foraminifera-pteropod composition, with phosphorite streaks
Ripple	419–539	NNE, 1.9–2.8 km/hr	Coarse Foraminifera-pteropod fragments with phosphorite streaks; asymmetric ripple marks, current crescents and 1–2 m depressions
Black pebble	481–564	NNE, 1.4–1.6 km/hr	Globigerina ooze with phosphorite pebbles; some larger rocks (~15 cm) with live coral
Low outcrop	503–512	NNE, 1.4–1.6 km/hr	Globigerina ooze with black pebbles and low relief (10–30 cm) slabs; attached invertebrates or coral
Coral mounds	503–555	N, 1.4–1.9 km/hr	15–23 m in height; dead coral fragments, live branching *Lophelia* and *Enallopsammia*, sponges, crinoids, and pennatulids

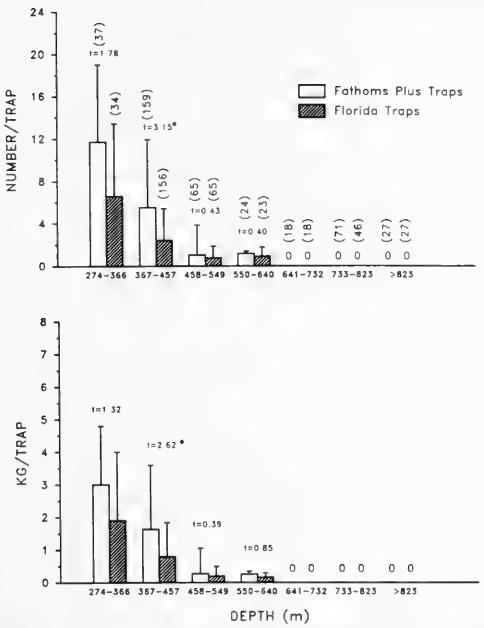

Figure 2. Catches of *Cancer borealis* in Fathoms Plus and Florida traps by depth strata. Total effort (number of traps) is shown in parenthesis. Statistically significant differences in mean number and mean weight among trap types are indicated by *($P < 0.05$) (T-test for independent samples). Error bars depict standard deviation.

significant difference (Kruskal-Wallis test, $\chi^2 = 8.4$, $P < 0.05$) (Fig. 3). Average size of males was greatest in the shallowest stratum ($\bar{x} = 129$ mm, $s = 9.5$, $n = 554$). The modal size of female crabs was 105 mm for both strata 1 and 2 (Fig. 3). No significant difference in carapace width of female *C. borealis* was noted between these strata (ANOVA log-transformed data, $F = 0.01$, $P > 0.10$).

Maturity

Ovaries were examined in 66 of the 98 females collected. Most of the individuals examined (69.7%) had mature ovaries (Table 5), and sperm was present in the seminal receptacles of 51 of the 52 individuals whose tissues were prepared for histological examination. Only three individuals, however, were observed to have sperm plugs in the vulvae, a sign of recent mating. The smallest individual observed to have been mated was 89 mm CW. With the exception of two individuals, all females were collected from the months of May–September.

In situ *Density Estimates*

A total of 1,324 *Cancer borealis* were sighted over 583,480 m^2 of bottom surveyed during submersible transects within the three study areas in 1986 and 1987 (Table 2). Densities (no. individuals/1000 m^2) were significantly different among habitats (Kruskal-Wallis, $\chi^2 = 54.3$, $P < 0.001$), with highest values over bottom characterized by soft-bioturbated sediment (8.5/1000 m^2) and dune (6.5/1000 m^2) topography (Fig. 4). Forty-three percent of *C. borealis* sighted along transects occurred in the soft-bioturbated habitat, which comprised only 12% of the total area surveyed. Fewer individuals (9%) were sighted in the dune habitat, which constituted only 3% of the area surveyed. Lowest densities were associated with the coral mound habitat (0.13/1000 m^2). Densities in the remaining habitats were similar (0.8–1.9/1000 m^2).

Mean density of *C. borealis* differed significantly among 50-m depth intervals (Kruskal-Wallis test, $\chi^2 = 59.7$, $P < 0.001$), showing a trend of decreasing density with increasing depth (Table 6). The greatest number of individuals (83% of total) was sighted along transects at depths from 401–500 m.

Population Estimates

Estimates of crab populations for the three sub-areas were directly related to the proportions of habitat types observed, since calculations included habitat-specific crab densities that were generalized across all the fishing areas. The estimated crab population was greatest (117,400 crabs) within the 25.9 km^2 sub-area of fishing Area B (Table 7); however, differences in estimates between sub-areas in areas A (84,900 crabs) and B may have resulted from unequal sampling of the different habitat types. Data on which to base estimates for the 28.4 km^2 sub-area in Area A were lacking from habitats shallower than 400 m. Thus, a total population estimate equal to or greater than that of the sub-area in Area B may have resulted if the unconfirmed sections of the sub-area in Area A (<400 m) were the same soft-bioturbated habitat of high crab density found at similar depths in the sub-area of Area B.

Population estimates by habitat type within sub-areas indicate the relative importance of specific habitats to the total crab population of the continental slope. The habitat type of greatest crab density, soft-bioturbated ooze, contained ≥47% of the crabs in all three areas and >85% of the crabs in Area B. Yet, the soft-bioturbated habitat represented less than 26% of the three sub-areas combined. The coral mound habitat, which contained less than 1.0% of the crab population of each area, represented more than 14% of the three sub-areas combined. Two habitat types, flat ooze and dune, varied greatly in their relative crab population size

TABLE 4.

Frequency of male and female *Cancer borealis* within each depth stratum.

	Strata (m)						
Sex	**274–366**	**367–457**	**458–549**	**550–640**	**641–732**	**733–823**	**>823**
Male	*635	*1185	*110	*11	0	0	0
Female	73	25	0	0	0	0	0

Asterisks denote significant deviation ($P < 0.05$) from 1:1 by Chi-square analysis.

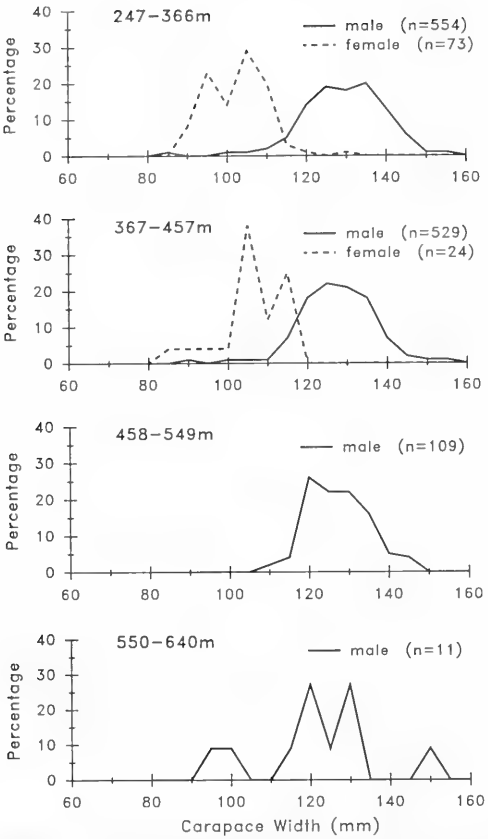

Figure 3. Width frequency distributions of *Cancer borealis* by depth strata.

among areas. The flat ooze habitat represented the largest (29.9%) proportion of the sub-area of Area A and contained a large (17.3%) proportion of the total crab population in that sub-area, while it did not exist in the sub-area of Area C. Only in the sub-area of Area C was the area of the dune habitat large enough to contain a sizable proportion (29.4%) of the areas' total crab population.

DISCUSSION

Catch rates from traps suggest that stocks in the study area may be greater than those reported for waters off New York (Briggs and Mushacke 1982) and Maine (Krouse 1980). Our greatest catch (9.5 crabs/trap) occurred in the shallowest stratum (274–366 m) and density estimates of 7.5 crabs/1000 m^2 were obtained for transects at depths of 351–400 m. Trap catches of Jonah crab off the south shore of Long Island averaged 0.8 crabs/trap (Briggs and Mushacke 1982), while Krouse (1980) reported 0.07 crabs/trap at 3–20 m near Boothbay Harbor, Maine. In a survey of Jonah crabs taken by lobster traps in Baltimore, Washington, and Norfolk canyons, and an area northeast of Oregon Inlet, North Carolina, catches of 6–8 crabs/trap occurred at depths of 180–260 m. Catch rates from Norfolk and Washington canyons produced the smallest average catches (<0.5 crabs/trap) (R. Harris and W. A. Van Engel, unpublished data report, September 1977, Virginia Institute of Marine Science).

TABLE 5.

Ovarian condition, as described by Haefner (1977) and Carpenter (1978), and size statistics for female *Cancer borealis*.

Ovarian Condition	n	Carapace Width Min.	Max.	x̄
Slight—Ovary visible macroscopically; colorless to white	1	109	109	109
Moderate—Ovary about one-half volume of hepatopancreas; White, cream or light orange	4	95	110	105
Advanced—Volume of ovary sub-equal to hepatopancreas; Light orange to orange	15	91	115	105
Mature—Ovary is visibly dominant internal organ; bright orange in color; Ova are obvious	46	89	132	105

The presence of sperm in the seminal receptacles and mature and advanced stage ovaries in most of the females for the months of May–September suggests that mating had occurred. The lack of ovigerous females in our collections precluded definition of a spawning season, which has been reported to occur from March (Haefner 1977) through September (Smith 1879). Carpenter (1978) suggested that the spawning season occurs later in the year in the northern portion of the geographic range. Since we did not sample at depths <150 m where females are reportedly most abundant (Carpenter 1978), we can only speculate that ovigerous females were present at these shallower depths throughout the summer.

Male *C. borealis* dominated trap catches from all strata. Un-

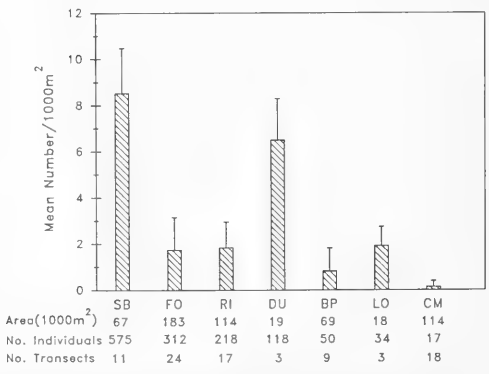

Figure 4. Density of Jonah crab among seven habitat types surveyed by submersible (Codes for habitat type are explained in Table 2). Error bars depict standard deviation.

TABLE 6.

Total number of individuals and density (No./1000 m^2) calculations of *Cancer borealis* among 50 m depth intervals.

Depth	No. Individuals	No. Transects	Density	
			x	s
351–400	87	2	7.49	0.67
401–450	520	19	4.11	2.09
451–500	583	20	4.44	3.69
501–550	125	37	0.48	0.65
551–600	9	7	0.16	0.22

equal sex ratios have been noted for *C. borealis* along the Maine coast (Krouse 1980) and in the Norfolk Canyon and adjacent slope (Carpenter 1978). The tendency for increasing number of females with decreasing depth in our study area supports similar observations by Carpenter (1978) and Stehlik et al. (1991) of increased female abundance at depths <200 m. Haefner (1977) had insufficient data to indicate depth preference by sex, but found that very large male crabs occurred in deeper water. Whether an inshore spawning migration occurs off the southeastern U.S., as has been suggested for populations near Boothbay Harbor, Maine (Krouse 1980) and those from the Norfolk Canyon area (Carpenter 1978), remains to be determined. In a multiyear trawl and dredge survey of the continental shelf from the Gulf of Maine to Cape Hatteras, Stehlik et al. (1991) found seasonal differences in sex ratios. Males were more numerous in spring, whereas females dominated in fall. They postulated that these seasonal changes in sex ratio could be due to single-sex migrations or differences in catchability related to the inactivity of reproducing females.

A possible explanation for the shallow bathymetric distribution of female *C. borealis* may be to enhance reproductive success. The soft-bioturbated ooze substrates found at shallower depths during our study would be more conducive to burying, an activity undertaken by females of the congener, *C. irroratus* Say (Krouse, 1980). Burying apparently facilitates attachment of eggs to the pleopods during spawning. Warmer temperatures and increased food available, attributable to seasonal upwelling on the upper slope (Blake et al. 1985), may favor oogenesis and vitellogenesis and, thereby, influence the distribution of female Jonah crab.

Although the carapace width of male *C. borealis* differed significantly among depth strata, no marked size-depth relationship was noted as has been reported in other studies. Haefner (1977) and Carpenter (1978) reported that smaller crabs (30–40 mm) were most abundant at depths <150 m, while the maximum abundance of larger crabs (>40 mm) occurred from 150 to 400 m. We did not sample with traps at depths <274 m, nor did we collect any Jonah crab smaller than 87 mm. The lack of small *C. borealis* in our collections may reflect either the fact that small crabs are absent from the depths we sampled, or, that our traps were biased for capture of large individuals. A similar minimum size of 85 mm for *Chaceon fenneri* collected by Wenner et al. (1987) using the same traps suggests that this may be the minimum size at which crabs are vulnerable to this particular gear.

Habitat and depth are important variables that influence catch rates of *C. borealis* within and among geographic areas. Although we did not sample at depths <274 m, the maximum abundance in our study occurred within the depth range of 150–400 m where greatest abundance was reported by Haefner (1977) and Carpenter (1978). Off North Carolina, *C. borealis* belonged to the group of large epibenthic invertebrates of the upper slope (200–1000 m) which exhibited narrow ribbons of distribution along depth contours that were related to sediment size (comparable to habitat types) and temperature variations (Rowe and Menzies 1969). Dense populations of Jonah crabs between 327 and 599 m off Cape Hatteras were similar in depth distribution to that of fine mud sediments (400–500 m) with numerous large biogenic excavations (Blake et al. 1985). Our definition of a soft-bioturbated habitat type appears synonymous with, and possibly continuous with, those of similar depths off North Carolina. The coral mound habitats of our study, where the lowest densities of *C. borealis* occurred, were similar to the discontinuous banks noted by Rowe and Menzies (1969) along the 450 m isobath off North Carolina. While depth-related patterns of habitat and Jonah crab distributions appear to exist along the North and South Carolina coast, habitat distributions within submarine canyons are quite different. Although Jonah crabs were relatively abundant at 395 m in the Hatteras Canyon, canyon species and faunal zonation were considerably different than those found along the continental margins (Rowe 1971).

Maximum trap catches coincided with depths where sedimentary features were characterized by globigerina ooze, current-generated ripples, or dunes. The apparent preference of *C. borealis* for rocky substrates off New England (Krouse 1980) contrasts with our observations of greatest density on soft-bioturbated sediments of globigerina ooze. The shallow depressions, mounds and burrows that typified this ooze habitat may have resulted from the excavating and foraging tactics of *Cancer borealis*. Auster and

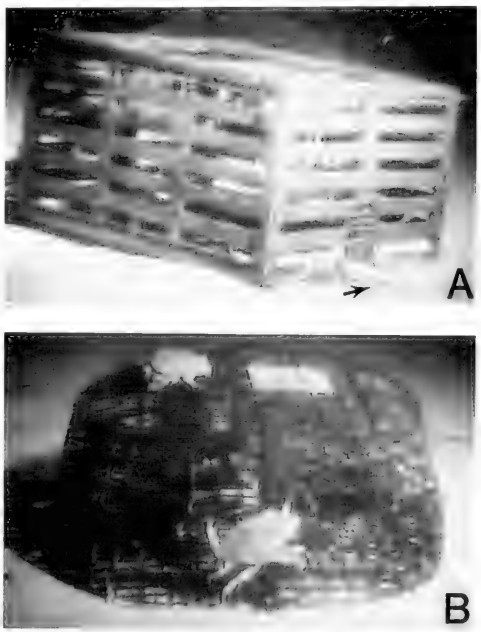

Figure 5. A) Florida trap with *C. borealis* at base of side, B) Fathoms Plus trap with *C. borealis* scaling side.

TABLE 7.

Estimates of *Cancer borealis* abundance by habitat type for subareas within fishing areas A, B and C (habitat abbreviations as in Table 2).

Area	Habitats							Total
	FO	CM	RI	DU	BP	LO	SB	
Sub Area A								
Habitat Area ($m^2 \times 10^6$)[a]	8.5	0.8	4.7	2.4	7.3	—	4.7	28.4
Abundance[b] ($\times 10^3$)	14.7	0.1	8.6	15.6	5.9	—	40.0	84.9
% of Area's population	17.3	0.1	10.1	18.4	7.0	—	47.2	100.1
Sub Area B								
Habitat Area ($m^2 \times 10^6$)[a]	4.6	6.4	1.4	0.7	0.8	0.2	11.8	25.9
Abundance[b] ($\times 10^3$)	8.0	0.8	2.5	4.5	0.6	0.4	100.5	117.4
% of Area's population	6.8	0.7	2.2	3.9	0.6	0.3	85.6	100.1
Sub Area C								
Habitat Area ($m^2 \times 10^6$)[a]	—	4.5	3.2	3.2	8.5	1.8	3.9	25.1
Abundance[b] ($\times 10^3$)	—	0.6	5.8	20.8	6.9	3.4	33.2	70.7
% of Area's population	—	0.8	8.2	29.4	9.7	4.8	47.0	99.9

[a] Habitat Area = arbitrary width of 4000 m × habitat distance along across-isobath transects between limits of data: Area A, 414–555 m depth; Area B, 293–540 m; Area C, 320–539 m.

[b] Abundance = Area of habitat × mean density (all areas) for that habitat.

Crockett (1984) observed that *C. borealis* digs vertical burrows or pits to expose potential prey items, and these pits attract secondary excavators that further disturb sediments or inhabit burrows abandoned by the primary excavator. Auster and Crockett (1984) noted that excavations at shallow depths were ephemeral, being eroded over the course of several tidal cycles. We are uncertain whether the highly excavated nature of the sediment reflects intense short-term activity or whether excavations are preserved over a long time period in the presence of a comparatively slow-moving bottom current of 0.4–0.6 km hr^{-1} at depths >200 m.

Observations of lower catch rates for *C. borealis* in the Florida trap may reflect differences in trap design (Miller 1980) or behavioral interactions with other species (Richards et al. 1983). Jonah crab may have had difficulty scaling the vertical sides of the Florida trap (Fig. 5A & B) which would explain its lower catch. Significant differences in catch between the two trap types in the 367–457 m stratum may have been influenced by high numbers of golden crab which were caught by the Florida trap at those depths (Wenner et al. 1987). Such density-dependent effects in catchability have been reported by Miller (1978) for *C. productus* (Randall) and *C. magister* Dana and by Richards et al. (1983), who observed that stocking of lobster traps with *Homarus americanus* H. Milne Edwards reduced entry and increased the escape rate of *C. borealis*.

Estimation of the relative contribution of different habitat-specific crab populations (Table 7) to the total crab population of a sub-area within each fishing area (A, B and C) indicated that the soft-bioturbated habitats supported most of the crab population within an area. The estimated population sizes ranged from 70,700 (sub-area C of 25.1 km^2) to 117,400 crabs (sub-area B of 25.9 km^2) in the three sub-areas surveyed. Estimates were an order of magnitude greater than populations of the coexisting *Chaceon fenneri* in the same sub-areas (4,300–5,800 crabs; Wenner and Barans 1990). In general, the soft-bioturbated habitat of greatest Jonah crab abundance was the predominant bottom type in waters less than 465 m depth in Areas A and B, while the coral mound habitat, where crabs were least abundant, was predominant at depths beyond 525 m. The relationship between *C. borealis* abundance and habitat type is consistent with the relationship between water depth and size of trap catches. In Area C, the soft-bioturbated habitat occurred between 330 and 400 m during observations to 320 m. The relatively narrow zone of habitats between 400–465 m and 525 m contained low densities of Jonah crabs except in the intermittently occurring dune habitat, which was inshore of rippled habitats. Estimates of population sizes were considered conservative because some Jonah crabs that were partly buried at the edge of the visual range may have occasionally not been counted.

Before further comparisons of trap catches among areas, depths and habitats can be made, the selectivity of each trap type and the catchability coefficients for specific habitats should be determined (Miller 1990). The bottom type or habitat fished by traps necessary for the above calculations, might be confirmed by observations from a submersible, remote video recordings, or a trap-mounted bottom sampling device.

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DEPURATION RATES OF NORTHERN QUAHOGS, *MERCENARIA MERCENARIA* (LINNAEUS, 1758) AND EASTERN OYSTERS *CRASSOSTREA VIRGINICA* (GMELIN, 1791) IN OZONE- AND ULTRAVIOLET LIGHT-DISINFECTED SEAWATER SYSTEMS

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ABSTRACT The relative elimination rates of a diverse group of indicator microorganisms from hard-shelled clams (*Mercenaria mercenaria*) and eastern oysters (*Crassostrea virginica*) were evaluated in ultraviolet light and ozone-disinfected seawater systems. The indicator organisms included vegetative bacteria (fecal coliforms, *Escherichia coli*, and enterococci), a spore-forming, anaerobic bacterium (*Clostridium perfringens*), and a group of bacterial viruses (male-specific bacteriophages). The addition of ozone to seawater resulted in the rapid inactivation of both the vegetative bacteria and viral indicators. Of the indicators used in this study, *C. perfringens* was the least affected by ozone. Inactivation and elimination rates, however, were not higher in shellfish placed in ozonated tanks than in shellfish in the ultraviolet light treated control tanks. Bacteriophages were eliminated at a much slower rate than were vegetative or spore-forming bacteria. Regardless of the disinfection system, bacteriophages persisted in depurating shellfish for more than 2 weeks.

KEY WORDS: ozone, depuration, clams, oysters, ultraviolet light

INTRODUCTION

Purification of contaminated molluscan shellfish by depuration was first practiced with oysters in the late 1800s (Herdman and Boyce 1989). Since that time, depuration has been used for purifying other types of shellfish, including clams, mussels, and cockles. For commercial operations, the success of depuration depends on the ability of shellfish to rapidly and efficiently eliminate biological contaminants. The effectiveness of depuration is determined by monitoring shellfish meats for decreases in the densities of fecal coliforms, the standard sanitary indicator group (U.S. Food and Drug Administration 1988). When fecal coliforms are reduced to a shellfish species-dependent endpoint (U.S. Food and Drug Administration 1988), the animals are considered acceptable for human consumption. This cleansing process generally is practiced for 48 h in commercial operations.

In the United States, waters used for depuration are commonly disinfected with ultraviolet (UV) irradiation (Richards 1988). However, the effectiveness of depuration with UV-disinfected seawater has been questioned. Fecal coliforms (bacterial indicators) have elimination characteristics different from those of several pathogens (notably viruses) of primary public health concern (Grohmann et al. 1981, Gunn et al. 1982, Sobsey et al. 1991). These pathogens include the Norwalk and hepatitis A viruses. These findings cast doubt on the use of UV light disinfection systems for the timely elimination of these enteric pathogens and on the commercial depuration process.

As an alternative to UV irradiation, ozone has been suggested as a more effective disinfecting agent for purifying molluscan shellfish. Ozone is currently being used in commercial plants in France (Fauvel et al. 1979) and Australia (Richards 1988). It has been tested periodically in the United States, although ozone purification is not approved by the U.S. Food and Drug Administration. Ozonated seawater has also been used to effectively reduce the concentrations of marine biotoxins in soft-shelled clams (*Mya arenaria*) (Blogoslawski 1988) and *Vibrio vulnificus* in hard-shelled clams (Schneider 1987). Ozone can rapidly inactivate microbial indicator organisms (Blogoslawski et al. 1975) and certain enteric viruses (Snyder 1974) in water. Consequently, ozone is now being used to disinfect waters in whirlpools and has been investigated, on an experimental scale, for disinfecting sewage effluents (DenBlanken 1985, Meckes et al. 1983). Unlike UV disinfection, which only provides high-quality influent tank water, ozonated seawater contains a residual oxidant that bathes the depurating animals. Thus, microorganisms are inactivated in the tank waters, on the shellfish surfaces and, potentially, within the animals themselves.

In this study we sought to determine the effectiveness of ozone for inactivating fecal contaminants in depurating bivalve mollusks. The elimination rates of several microbial indicator organisms were determined in two commercially important shellfish, hard-shelled clams (*Mercenaria mercenaria*) and eastern oysters (*Crassostrea virginica*). We performed parallel experiments with ozone and UV light to compare the effectiveness of the two methods in decontaminating molluscan shellfish.

MATERIALS AND METHODS

Inactivation of Indicators in Seawater

Initial experiments were conducted to determine indicator inactivation rates in seawater and in seawater supplemented with ozone. Trials were conducted in rectangular fiberglass tanks (210 L capacity) containing seawater obtained from Narragansett Bay, RI. Temperatures were maintained at 15°C. Ozone, produced with a photoionization chamber (Water Management, Inc., Englewood,

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CO), was added to one tank through a polyvinyl chloride diffusion tube. We determined ozone concentrations by the *N'N*-diethyl-*p*-phenylenediamine (DPD) method using a commercial kit (Model LP-28, LaMotte Chemical Products Co., Chestertown, MD); ozone concentrations were maintained at 0.05–0.20 ppm. After ozone equilibration, 10.0 L of untreated municipal wastewater obtained from a local treatment facility (East Greenwich, RI) was added to both tanks (with and without ozone) and mixed thoroughly. At regular intervals thereafter, seawaters were collected in sterile, 500 ml polypropylene screw-cap bottles (Nalgene Laboratories Inc., Rochester, NY) containing 1 ml of a 10% solution of sodium thiosulfate for ozone inactivation. Test samples were held on ice and examined within 2 h of collection. The mTEC procedure (Dufour et al. 1981) was used to enumerate fecal coliforms and *Escherichia coli* in the wastewater and seawater. We determined enterococci densities with a modified mE procedure (Dufour 1980, Levin et al. 1975) and *Clostridium perfringens* densities with the mCP procedure (Bisson & Cabelli 1979). Concentrations of male-specific bacteriophages were determined with a modified double-agar overlay method (DeBartolomeis 1988). The host cell used for enumerating male-specific bacteriophages was *E. coli* strain HS[pFamp]R obtained from V. J. Cabelli (University of Rhode Island).

Shellfish Purification

We used laboratory-contaminated shellfish (either hard-shelled clams, *M. mercenaria,* or eastern oysters, *C. virginica*) for these depuration studies. Before all trials, shellfish were contaminated for 72 h in circular fiberglass tanks (50 L working volumes) that received a continuous flow of both ambient seawater (1–3 L/min) from Narragansett Bay (salinity 29–30 ppt) and untreated municipal wastewater (1.0–2.8 ml/min). Wastewater was delivered from refrigerated carboys of sewage by a proportioning pump (Technicon Corp., Tarrytown, NY). Water temperatures and salinities in the contamination tank were monitored daily with an electrodeless induction salinometer (Model RS 5-3, Beckman Corp., Cedar Grove, NJ). Temperatures ranged from 15 to 23°C and salinities remained generally stable at 29–31 ppt.

After contamination, the shellfish were rinsed with dechlorinated tap water and then split into two groups of 120 animals each. Both groups were placed in recirculating natural seawater systems; one disinfected with ozone and the other disinfected with UV light. These systems consisted of rectangular tanks (210 L capacities) identical to those described above. Shellfish were placed in each system in a monolayer on galvanized hardware cloth (0.25 inch mesh) suspended 10 cm from the tank bottom and ozone was diffused into the seawater (Fig. 1). Trials were conducted at three different ozone concentration ranges (0.10–0.20 ppm, 0.25–0.35 ppm, and 0.40–0.55 ppm). Seawater was recirculated at a rate of 6 L/min in all trials. UV light disinfection (Fig. 2) was achieved with a 4-bulb (15 watt germicidal lamps, General Electric Co., Cleveland, OH) Kelly-Purdy unit (Kelly 1961). A submersible pump (Model 1, Little Giant, Oklahoma City, OK) recirculated seawater through the UV disinfection unit at a rate of 6 L/min. Water samples were taken daily from each depuration system and examined for each of the microbial indicators.

Assays of Shellfish for Microbial Indicators

Throughout the parallel depuration trials, 30 animals were removed from each system at regular intervals and held in polypropylene bags on ice until examined (generally within 4 h). Shellfish were scrubbed to remove external debris and were randomly divided into three subgroups of 10 animals each. Each subgroup was shucked into separate sterile blender jars (Waring Corp., Corning, NY), blended at high speed for 2 min, and then analyzed for microbial indicator concentrations.

We determined fecal coliform concentrations using the procedure for shellfish recommended by the American Public Health Association (APHA 1970). Lauryl tryptose broth (Difco, Detroit, MI) was the presumptive test medium in a 5-tube, multiple dilution most probable number (MPN) procedure. Presumptively positive tubes were confirmed for fecal coliforms and *E. coli* in EC-MUG media (Difco) (Rippey et al. 1987). Azide dextrose broth

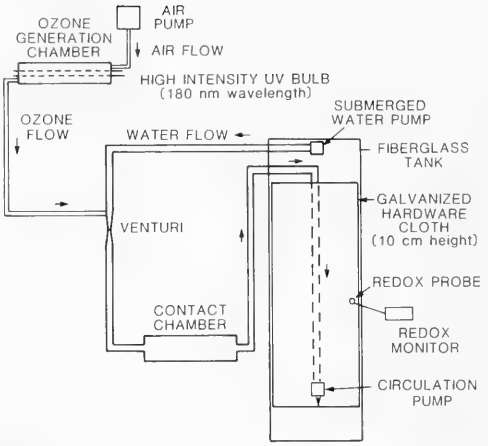

Figure 1. Schematic of the ozone-disinfected seawater depuration system.

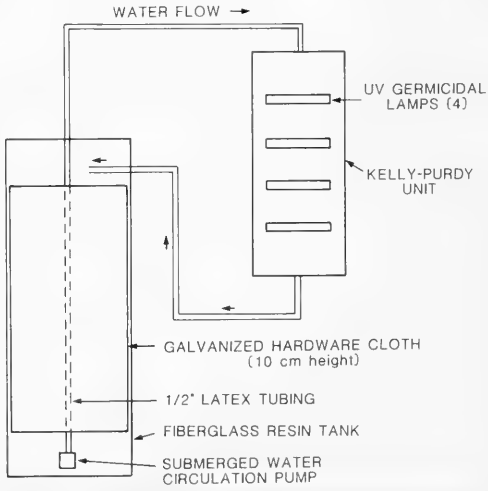

Figure 2. Schematic of the ultraviolet light-disinfected seawater depuration system.

TABLE 1.

Inactivation of indicators in seawater and seawater with ozone (0.05–0.20 ppm).

Time (min)	Percent Decrease of Microbial Indicator Densities in Seawater Alone				Percent Decrease of Microbial Indicator Densities in Ozonated Seawater			
	Fecal Coliform	Enterococci	*C. perfringens*	MS Phages	Fecal Coliforms	Enterococci	*C. perfringens*	MS Phages
20	39.1	4.5	50.0	9.3	39.1	59.2	7.1	96.8
45	45.0	13.6	52.7	45.3	99.3	97.7	46.5	99.2
60	52.2	22.7	62.2	30.9	99.950	99.2	48.0	>99.92
120	78.2	45.5	71.6	36.4	99.995	>99.92	49.3	>99.92
240	80.4	63.6	82.4	38.6	>99.999	>99.992	67.9	>99.92
360	82.6	68.2	71.6	45.3	>99.999	>99.992	65.6	>99.92
1440	95.9	96.8	78.4	65.4	>99.999	>99.992	99.0	>99.92

Abbreviations: MS phages, male-specific bacteriophages.

(Difco) was used as the presumptive medium for enterococci. In tubes that showed growth after 24 and 48 h at 35°C, approximately 0.1 ml of the suspension was streaked with a sterile applicator stick onto the surface of a 0.7 μm membrane filter (HC filter, Millipore Corp., Bedford, NH); the inoculated filter was placed on modified mE agar (Dufour 1980) plates. Plates were incubated for 24 h at 41°C. Cultures (and corresponding tubes) were scored positive for enterococci if the streaks produced blue growth. *C. perfringens* concentrations were determined with the iron milk method (Abeyta 1983) in a 5-tube, multiple dilution MPN procedure. Male-specific bacteriophage densities in shellfish were determined by a modified double-agar overlay procedure described by Cabelli (1988).

RESULTS

At low concentrations (0.05–0.20 ppm) in seawater, ozone was highly effective for inactivating fecal coliforms, *E. coli* (data not shown), and male enterococci-specific bacteriophages (Table 1). Reductions in the initial densities of each of these indicators exceeded 99% within 60 min; inactivation of these indicators was approximately at the same rate. In contrast, nearly half of the initial densities of these indicators remained viable in untreated seawater after 1 h. *C. perfringens* spores were significantly more resistant to the ozone concentrations used in this study than were the male-specific bacteriophages and the vegetative bacteria. In fact, the inactivation of *C. perfringens* by low concentrations of ozone was not different from that occurring in seawater alone.

Results from depuration experiments conducted with *M. mercenaria* held in recirculating seawater systems disinfected with ozone (at 0.10–0.20 ppm, 0.25–0.35 ppm, and 0.40–0.55 ppm) and UV irradiation are shown in Table 2 and Figure 3. Fecal coliforms, *E. coli* (data not shown), and enterococci generally were eliminated at similar rates when disinfected with ozone at the lower concentrations (0.10–0.20 ppm and 0.25–0.35 ppm) and UV light. Initial concentrations of each of these vegetative bacterial indicators were reduced by 90% within 24 h and 99% within 42 h. In contrast, the elimination rates found for *C. perfringens* and male-specific bacteriophages were extremely protracted. Of these, the male-specific bacteriophages were the most refractory to elimination, requiring as long as 11 days to achieve 90% reductions from initial densities. Ozone concentrations of ≥0.40 ppm adversely affected the elimination of all indicators, and significantly longer periods were required to reduce concentrations of indicator organisms in clams.

Results of depuration trials with oysters (Table 3 and Fig. 4) were similar to those found with hard-shelled clams. Fecal coliforms, *E. coli* (data not shown), and the enterococci were eliminated significantly more rapidly than either *C. perfringens* or

TABLE 2.

Elimination of indicator organisms in *Mercenaria mercenaria* by ultraviolet (UV) light and ozone disinfection systems.

Indicator Organism	Mean Elimination Time (h)[a] Concentrations of Ozone in Seawater 0.10–0.20 ppm T_{90}	0.10–0.20 ppm T_{99}	0.25–0.35 ppm T_{90}	0.25–0.35 ppm T_{99}	0.40–0.55 ppm T_{90}	0.40–0.55 ppm T_{99}	UV Irradiation Control Trials T_{90}	UV Irradiation Control Trials T_{99}
Fecal coliforms	15.9	31.7	24.6	36.9	336.2[b]	670.9[b]	22.3	42.8
Enterococci	16.8	33.5	18.4	39.0	241.5[b]	515.4[b]	21.5	40.6
C. perfringens	37.2	74.4	33.4	86.4	576.2[b]	1080.7[b]	36.3	71.7
Male-specific bacteriophages	99.3	198.6	267.1	510.2	856.6[b]	1717.9[b]	141.9	305.2

Abbreviations: T_{90}, time for 90% reduction from initial concentrations; T_{99}, time for 99% reduction from initial concentrations.

[a] Mean determined from the results of replicate depuration trials (ozone, $n = 2$ per concentration; UV irradiation, $n = 6$.

[b] Significantly ($P < 0.05$) longer elimination than that of the UV system.

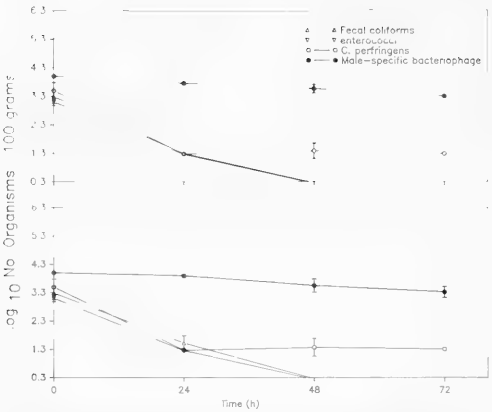

Figure 3. Hard-shelled clam (*Mercenaria mercenaria*) depuration by ultraviolet light-disinfected (top) and ozone-disinfected (bottom) seawater depuration systems. Values shown are the geometric mean concentrations determined from the assay results of triplicate subgroups (10 animals each); bars denote 95% confidence limits.

male-specific bacteriophages. Approximately 36 h was required to reduce the vegetative cell densities by 99%; the reduction of male-specific bacteriophages to comparable levels required 11–12 days. The inactivation and elimination rates found for each of the indicators in ozone-disinfected (≤0.35 ppm) and UV light-disinfected depuration systems were not significantly different. However, when ozone concentrations were elevated (≥0.40 ppm), the elimination of vegetative bacterial indicators required significantly longer periods than was required for elimination in a UV light-disinfected system or at lower ozone concentrations. The times required to eliminate *C. perfringens* and male-specific bacteriophages at the elevated ozone concentrations, although not significantly different, were consistently longer than those found in UV depuration trials.

DISCUSSION

Many investigators have questioned whether viruses can be eliminated in a timely manner in molluscan shellfish by UV light-disinfected seawater systems (Grohmann et al. 1981, Gunn et al. 1982, Sobsey et al. 1987, Sobsey et al. 1991). One problem is that UV light produces no residual disinfectant. When ozone is used, animals are bathed in residual oxidant, which works on epiphytic agents and agents expelled by physiologically active animals. These expelled agents are presumably inactivated in the ozonated seawater and cannot be reaccumulated by the shellfish. Ozone is transported into the animal and the disinfectant activity may be maintained over time. If this is the case, disinfection would occur internally. This *in vivo* inactivation would, in theory, greatly facilitate depuration.

Ozone effectively and rapidly eliminated certain microbial indicator organisms in seawater. Ozone treatment may improve depuration efficiency for both viral and bacterial contaminants. At ozone concentrations of 0.05–0.20 ppm, reductions of over two orders of magnitude were observed in fecal coliforms, *E. coli*, enterococci, and male-specific bacteriophages within 1 h of contact. Similar reductions were not found for *C. perfringens* spores at these ozone concentrations.

We examined three ranges of ozone concentrations using a model shellfish depuration facility. Two ranges of ozone concentrations ≤0.35 ppm (0.10–0.20 ppm and 0.25–0.35 ppm) provided similar results for both *M. mercenaria* and *C. virginica*. The elimination rates of the indicator organisms were similar to those obtained in depuration trials conducted with UV light-disinfected seawater. However, in depuration trials conducted with ozonated seawater at ≥0.40 ppm, we observed a significantly lower rate of elimination for each of the indicator organisms in hard-shelled clams and oysters. The animals were apparently sensitive to these higher concentrations of oxidant because their normal filter-feeding activity was retarded, as evidenced by reduced siphon extension activity. At all three concentrations of ozone used in these trials, any oxidant entering the animals was not effective in reducing the microbial contaminant concentrations. Higher disinfectant concentrations might have been more effective *in vivo*, but animal sensitivity precluded these investigations.

Our results support earlier findings that in shellfish, elimination rates are longer for *C. perfringens* and male-specific bacteriophages than for vegetative bacteria (fecal coliforms, *E. coli*, and enterococci). It is highly probable that neither fecal coliforms nor *E. coli* reliably index the sanitary quality of depurated shellfish. The results presented here strongly suggest that enteric viruses are

TABLE 3.

Comparison of ozone and ultraviolet (UV) light disinfection systems for elimination of microbial indicators from *Crassostrea virginica*.

Indicator Organism	Mean Elimination Time (h)[a]							
	Concentrations of Ozone in Seawater						UV Irradiation Control Trials	
	0.10–0.20 ppm		0.25–0.35 ppm		0.40–0.55 ppm			
	T_{90}	T_{99}	T_{90}	T_{99}	T_{90}	T_{99}	T_{90}	T_{99}
Fecal coliforms	14.0	33.6	9.9	31.8	240.3[b]	379.2[b]	12.1	35.7
Enterococci	15.4	35.9	33.0	49.7	542.4[b]	680.5[b]	17.7	36.9
C. perfringens	58.2	169.5	79.0	246.8	147.4	261.1	59.61	53.0
Male-specific bacteriophages	140.2	292.3	113.8	276.1	219.1	492.8	120.1	268.9

Abbreviations: T_{90}, time for 90% reduction from initial concentrations; T_{99}, time for 99% reduction from initial concentrations.

[a] Mean determined from the results of replicate depuration trials (ozone, $n = 2$ per concentration; UV irradiation, $n = 6$).

[b] Significantly longer ($P < 0.05$) elimination than that of the UV system.

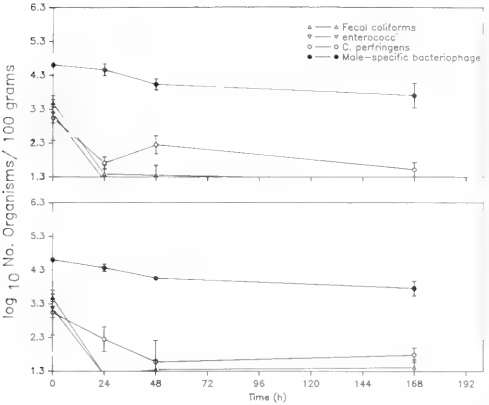

Figure 4. Eastern oyster (*Crassostrea virginica*) depuration by ultraviolet light-disinfected (top) and ozone-disinfected (bottom) seawater depuration systems. Values shown are the geometric mean concentrations determined from the assay results of triplicate subgroups (10 animals each); bars denote 95% confidence limits.

much more refractory to the purification process. Some of these viruses, such as hepatitis A and Norwalk, are currently the greatest health threat to consumers of raw shellfish (Rippey 1991).

Depuration of viruses does not occur in a commercially realistic time period (generally within 48 h) with either UV light- or ozone-disinfected seawater systems. Therefore, depuration may not be a reliable process for protecting the public health. These studies demonstrate that a minimum of 8.3 days was required for a 99% reduction of viruses in hard-shelled clams at ozone concentrations of 0.10–0.20 ppm, and a maximum of 21.3 days was required at ozone concentrations of 0.25–0.35 ppm. For oysters, as few as 4.7 days were required for a 99% reduction in viruses at ozone concentrations of 0.25–0.35 ppm, whereas as many as 12.2 days were needed at ozone concentrations of 0.10–0.20 ppm.

Several questions concerning the depuration of viruses remain. The achievable rates for eliminating viral pathogens from shellfish need to be determined, particularly those for hepatitis A and Norwalk viruses. Also, the maximum initial concentrations of viral pathogens that can be fully eliminated by commercial depuration must be established. Accurate, sensitive, and reliable methods for quantifying these viruses are not yet available; once such methods are developed, the best enteric virus indicators must be established. For now, depuration is probably not a fully effective barrier for safeguarding public health.

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THE ENVIRONMENTAL CONTEXT OF A *GYRODINIUM AUREOLUM* BLOOM AND SHELLFISH KILL IN MAQUOIT BAY, MAINE, SEPTEMBER 1988

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ABSTRACT In September of 1988 a shellfish kill occurred in Maquoit Bay, Brunswick, Maine where clams, mussels and worms on the shellfish grounds suffered an estimated mortality of 30–40%. The dinoflagellate *Gyrodinium aureolum* was the dominant phytoplanket in water samples taken a few days after this event. Alternative origin and development scenarios for the bloom were considered based on the location where log phase growth may have occurred. The spatial and temporal events associated with the bloom and shellfish kill were examined by analysing meterorological data for the months of August and September, the geomorphology of Maquoit Bay, tidal data, the current patterns in Casco Bay, and other hydrographic data for the period. Our analysis suggests that a *G. aureolum* bloom of offshore origin was transported shoreward by a period of southerly winds enhanced by tidal action. Algal cells were further concentrated to around 1.4×10^8 cells l^{-1} by onshore winds and reduced tidal flushing in Maquoit Bay, a location with unique characteristics that may make it susceptible to such events. High mortality of marine organisms in Maquoit Bay may have been caused by the exposure of animals on the shellfish grounds to very low oxygen concentrations during the early morning hours over a period of 5 to 7 days. Mucous and toxin production by *G. aureolum* may have played a secondary role in the kill. Rapid decomposition of dead shellfish probably contributed an additional oxygen demand to that already present on the shellfish grounds.

INTRODUCTION

The 20th century has been a time of considerable anthropogenic change in ecosystems throughout the world. The socioeconomic activities of man have altered the structure and function of ecosystems both by changing the magnitude and extent of their controlling factors and by introducing or removing ecosystem components such as plants and animals (Elton 1958). The increasing frequency of toxic dinoflagellate blooms in some coastal and shelf seas, associated with increased eutrophication and pollution of coastal waters, (Prakash 1987, Nixon 1989) is an example of the results of such alterations. The effects of these alterations may be further illustrated by the presence of toxic dinoflagellate species in waters where they were formerly unknown. For example, since 1966 blooms of the dinoflagellate *Gyrodinium* aureolum have become common occurrences in the coastal seas of northwestern Europe where there is no prior record of the organism or the distinctive shellfish, fish and worm kills caused by its blooms (Tangen 1977, Boalch 1987, Gowen 1987, Holligan 1987).

G. aureolum was first identified and described by Hulburt (1957) who found it in a coastal pond near Woods Hole, Massachusetts. Since 1957 it has been reported from the following locations in the United States: North Carolina estuaries (Campbell 1973), the Chesapeake Bay (Marshall 1980), and a Long Island estuary (Chang and Carpenter 1985). We are not aware of any previous report of a shellfish kill associated with a *G. aureolum* bloom in U.S. waters.

Since the first identification of *G. aureolum* in Norwegian waters in 1966, (Braarud and Heimdal 1970), numerous subsequent blooms have been reported in Europe (Boalch 1979, Blake et al. 1981, Richardson and Kullenberg 1987) in which *G. aureolum* has been implicated in mortalities of finfish (Jones et al., 1982) and other marine organisms (Helm et al. 1974, Tangen 1977, Ottway et al. 1979). No specific toxin was previously reported in association with these events (Turner et al. 1987), but Partensky et al. (1989) have recently identified a minor cytotoxin present in *Gymnodinium nagasakiensis*.

G. aureolum is phenotypically identical to *Gymnodinium nagasakiense* (Partensky et al. 1988), a common cause of "red tides" in Japan (Iizuka et al. 1989), where it has been associated with low oxygen events and shellfish kills (Ochi 1989). Partensky et al. (1988) noted that, despite the external similarity between these two species, there appear to be physiological and genetic differences between the two. The worldwide occurrences of such blooms has been summarized by Partensky and Sournia (1986), White (1988), and Shumway (1990).

Problem and Approach

On September 27, 1988 a shellfish kill in Maquoit Bay, Brunswick, Maine was reported to the Maine Department of Marine Resources (DMR). The softshell clam, *Mya arenaria* suffered 30–40% mortality on affected shellfish grounds. Marine worms of unspecified species and blue mussels, *Mytilus edulis*, were also affected. The extent of anoxia and advanced stages of decomposition observed in the dead shellfish suggested that the actual kill had occurred 7–10 days earlier (Brian Marcotte, pers. comm.). The lack of on-site observations before and during this event makes definitive determination of the actual cause of the mortalities impossible; however, the dinoflagellate, *Gyrodinium aureolum*, was the dominant algal species in the water of Maquoit Bay several days after the shellfish kill occurred (Selvin 1988, Haugen 1988). The presence of a dinoflagellate species, known to be associated with fish kills elsewhere, as the dominant phytoplankter just after a large shellfish kill is strong circumstantial evidence indicating its involvement in the incident.

In this paper we examined the spatial and temporal environment that produced a *G. aureolum* bloom in Maquiot Bay which in turn contributed to a shellfish and worm kill there. These results are used to reconstruct several possible scenarios that could ac-

count for this extraordinary event. The purpose of this research was to develop working hypotheses that can be used to guide future research on *G. aureolum* blooms in Maine and other U.S. coastal waters.

The analysis presented here was undertaken for several reasons. First, there are very few sets of observations that describe in detail the complete course of toxic dinoflagellate blooms and the observations which do exist are generally from the declining phase of the bloom making it difficult to unequivocally establish cause and effect (Holligan 1987). The technical and logistic difficulties associated with investigating ephemeral events (spatial scale of 10 km and temporal scale of 10 days) in coastal and shelf seas are in part responsible for this lack of detailed information on the physical environment of toxic bloom events (Holligan 1987). Second, the complex interactions of physical, chemical, and biological factors that control algar bloom dynamics are always hard to resolve and often require expensive field studies that have no guarantee of success. As a result of this lack of information, a general framework for assessing the physical context of these blooms cannot be developed at present (Holligan 1987). With so many difficulties preventing research on toxic and other algal blooms, scientists need viable working hypotheses around which to frame focused research proposals. Also, the economic impact of toxic and noxious algal blooms can be quite high, as it was to the shellfish industry in the case of the Maquoit Bay kill. Much higher costs may be incurred if the growing Maine salmon aquaculture industry is affected by *G. aureolum* blooms in the future. Therefore, high realized and potential economic impacts and the lack of a sufficient information base to establish, unequivocally, the cause of *G. aureolum* blooms make it necessary to use existing information and our knowledge of ecological principles to construct working hypotheses that could explain the Maquoit Bay kill.

Recognizing the variety of ways in which algal blooms can occur, we have developed two principal conceptual models to account for the presence of a *G. aureolum* bloom in Maquoit Bay. The Inshore Development model proposes that a bloom could have developed within the Bay as a result of local reproduction. A *G. aureolum* bloom which developed as a result of local reproduction was observed in the Carmens estuary of Great South Bay, Long Island in 1982 and 1983 (Chang and Carpenter 1985). The second, or Offshore Development, model, proposes the offshore development of a *G. aureolum* population associated with the stability and nutrient differences between stratified and mixed waters. In this case, the *G. aureolum* bloom must be transported into nearshore waters by favorable conditions of wind and tide. Holligan (1979) describes the processes that lead to *G. aureolum* blooms associated with density discontinuity fronts in the waters around the United Kingdom.

The Offshore Development model can, in turn, be divided into two alternative models based on whether log phase growth takes place on the surface or along subsurface density discontinuity fronts. Holligan (1979) describes a double peak pattern in the vertical distribution of chlorophyll which occurs when *G. aureolum* is brought to the surface and reproduces there. In the early summer, *G. aureolum* develops as part of the chlorophyll maximum flora on the stratified side of density discontinuity fronts in the western English Channel and is often found at the surface discontinuity between stratified and mixed waters (Holligan 1985). Alternatively, log phase growth could occur in the subsurface chlorophyll maximum layer as proposed by Richardson and Kullenberg (1987). Subsurface populations of *G. aureolum* occur at lower cell densities than those in surface waters and this submodel, therefore, depends on physical processes to concentrate the algal cells to bloom levels on the surface as well as to transport the bloom into nearshore waters. Lindahl (1987) presents evidence that offshore blooms of *G. aureolum* are concentrated in salinity fronts along the Norwegian coast.

METHODS

The methods used in this study were simple and direct. First, we compiled and reviewed existing bathymetric and hydrographic information on Casco Bay and Maquoit Bay. Second, we reconstructed the chronology of the shellfish kill from published accounts and conversations with eyewitness observers. Third, we assembled and interpreted existing information on temperature, wind, tide, and solar insolation to characterize environmental conditions before, during, and after the Maquoit Bay shellfish kill. This information was then used to develop two detailed scenarios based on the models described above.

RESULTS

Maquoit Bay

Maquoit Bay is an enclosed coastal embayment in the northeastern part of Casco Bay located at 70°00′ west longitude and 43°55′ north latitude (Fig. 1). The bay is shallow, the upper portion having a mean depth of 2–3 meters at mean high water (MHW) and approximately 81 hectares of mud flat are exposed at mean low water (MLW). The central portion of the bay has a mean depth of 3–4 meters at MLW. The bottom throughout the bay is predominantly soft mud, with eelgrass beds along the western and northern shores at or just below the MLW mark. Freshwater enters the bay from Bunganuc Brook on the western shore, which drains the western section of the town of Brunswick, including the now closed town dump, and from an unnamed brook which enters the head of the bay at Wharton Pt. at the northern extreme. A rather extensive marshy area exists on the northeastern shore which drains the Rossmore Road area.

The Bay is drained primarily through a channel between Sister Is. and Mere Pt. which is about 900 m wide. This opening is approximately 6% of the total perimeter of the Bay which is around 10 km^2 in area. When water leaves the Bay it tends to

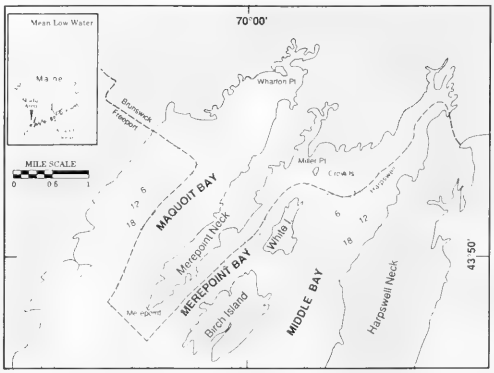

Figure 1. Maquoit and Middle Bay, Brunswick, Maine.

follow the depth contours shown in Figure 1, lying closer to the eastern side of the bay at low water. The water in Maquoit Bay is not flushed out as quickly as waters in neighboring Middle Bay which is widest at the mouth and becomes progressively narrower as it extends landward. Recent research by Gilfillian and others at Bowdoin College has produced a preliminary estimate of the flushing time in Maquoit Bay of 6 days, ranging from 5 to 15 days depending on stream flow and other conditions (Kresja 1990). Relatively slow flushing in Maquoit Bay may be a key to its high productivity, since phytoplankton are retained for longer times in the shallow, warm, fertile waters that are ideal for algal growth. The high productivity of Maquoit Bay is the reason for excellent growth of the resident shellfish population as well as the reason Maquoit Bay sediments are rich in organic matter with a shallow aerobic zone (Gilfillan et al. 1990). Historically, the Bay has been an extremely productive shellfish area for both the soft-shell clam, *Mya arenaria* and the northern guahog, *Mercenaria mercenaria*. Production in the bay for these species has been estimated at over $1 million annually (Alan Houston, pers. comm.).

Hydrography of Casco Bay

The current patterns of Casco Bay were characterized as part of a larger study to assist in the prediction of oil spill trajectories (Parker 1982). Unfortunately, the upper reaches of Casco Bay, specifically Maquoit and Middle Bays, were not included in this study. Flow patterns of surface currents for ebb and flood tides for those areas studied in Casco Bay are summarized in Figures 2 and 3.

Most of the water entering Maquoit Bay and Middle Bay originates offshore and passes principally through Broad Sound, with a small component passing through Luckse Sound, and along the western shore of Whaleboat Island. Current velocities in Broad Sound during flood tide are quite high, ranging from 18–91 cm/sec and averaging 25–30 cm/sec. In Luckse Sound current velocities range from 10–33 cm/sec with an approximate average of 15–20 cm/sec (Parker 1982).

Countercurrents exist during flood tide which flow in the opposite direction of that expected. Thus, flood tide in Casco Bay is of shorter duration than ebb tide and has slightly stronger average currents. Parker (1982) explains these unexpected reversals in current direction during the first two hours of the flood tide as being due to residual draining of the upper bays. Current flow patterns during ebb tide are more consistent with the expected direction. Southerly flowing water from both Maquoit and Middle Bays converge at the southern tip of Whaleboat Island and flow seaward, the principal flow passing through Broad Sound with a smaller flow exiting through Luckse Sound (Parker 1982).

Parker's late summer data also showed that a recirculating gyre occurred near West Cod Ledge and persisted throughout the tidal cycle. This gyre is characterized by strong vertical stability, a well defined thermocline between 5–10 meters, and low chlorophyll-a, which suggests nutrient deficiency and/or heavy grazing. Parker (1982) attributed the presence of this feature to the interference of West Cod Ledge with the north-south tidal flow and to the diversion of the southwesterly coastal current to the north and south of the ledge. The approximate position of this gyre is indicated by a plot of the temperature, density, stability, and chlorophyll-a fields (Fig. 4) in the vicinity of West Cod Ledge taken from Parker (1982).

Chronology, Observations, and Sample Analysis

On the morning of September 27th 1988 a report of a large clam, mussel, and worm kill in Maquoit Bay was received at the Maine Department of Marine Resources (DMR). Scientists were dispatched immediately and the area was surveyed and water samples taken that day. They estimated that shellfish in the Bay suf-

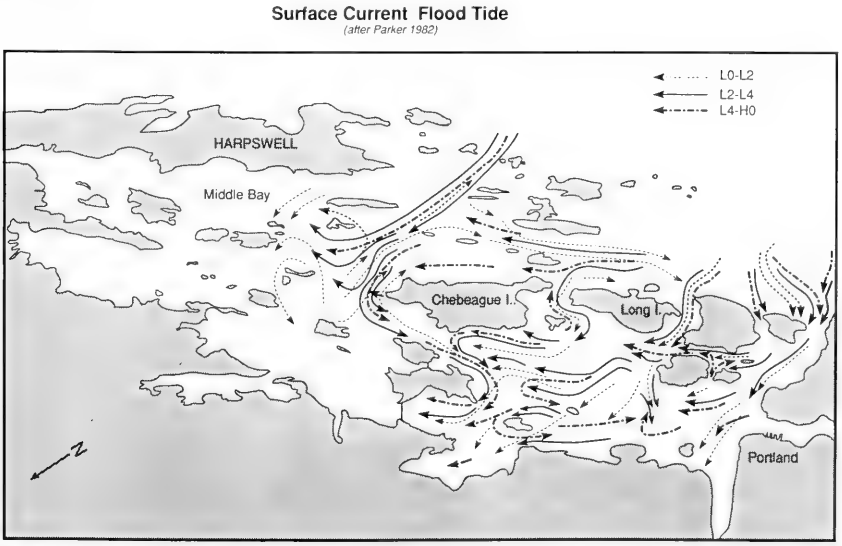

Figure 2. Casco Bay flood tide surface current patterns shown at two hour intervals from low water (LO) to high water (HO) (After Parker 1982).

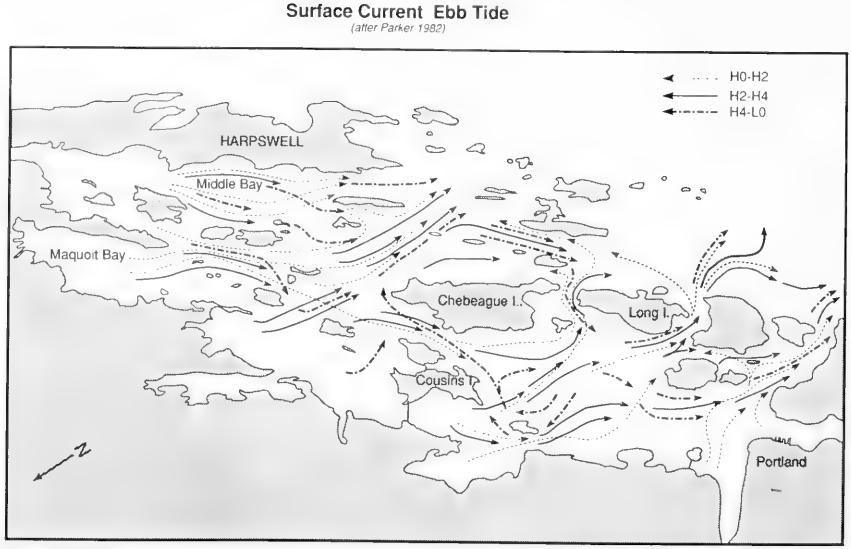

Figure 3. Casco Bay ebb tide surface current patterns shown at two hour intervals from high water (HO) to low water (LO) (After Parker 1982).

fered 30–40% mortality, and that most shellfish had been dead for a week to ten days. This estimate roughly places the time of the kill between the 18th and 21st of September. Later estimates by Alan Houston, Shellfish Warden for the town of Brunswick, indicate that the destruction of marine life was most severe on the eastern side of the Bay where almost all of the *Mya arenaria* were killed. On the western side of the bay around 30–40% of these clams were killed. Houston reported that many marine worms were also killed but that the quahog *Mercenaria mercenaria* survived fairly well.

The phytoplankton population present in Maquoit Bay on the 27th was independently analyzed by two researchers at the Bigelow Laboratory for Ocean Sciences. The results of these analyses are summarized in Table 1. Both analyses showed a predominance of a dinoflagellate identified as *Gyrodinium aureolum*, at cell concentrations of 1.8×10^6 cells l^{-1} (Haugen, 1988), and 1.0×10^6 cells l^{-1} (Selvin, 1988). The number of algal cells present in the mixed community of dinoflagellates and diatoms in Maquoit Bay on the 27th was not unusually high for Maine coastal waters in September (Haugen, 1988).

Eye-witnesses described patches of brown or coffee-colored water in bays and around the islands of Casco Bay. Around September 20th, a fisherman observed a red to rust-colored patch concentrated near the ferry landing in a small cove on Chebeague Island, renown for its eddies (Dana Wallace, pers. comm.). Chebeague Island is approximately 15–17 kilometers from the head of Maquoit Bay and does not provide conditions conducive to the development of a bloom. Similar patches of brown or coffee-colored water were observed in Merepoint Bay around Birch Island on the outgoing tide on the 24th (Jan Derby, pers. comm.) and along the southern and eastern shores of White Island in Middle Bay on the outgoing tide on the 25th (Charles White, pers. comm.). In both cases the patches were considered to be discrete

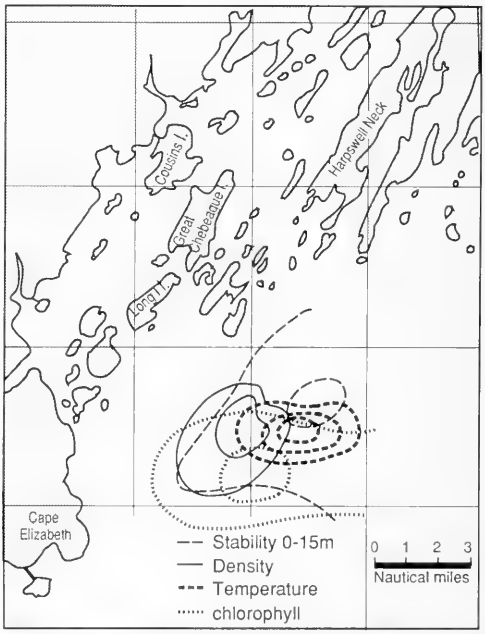

Figure 4. Approximate location of the West Cod Ledge gyre on ebb tide as indicated by stability, density, temperature, and chlorophyll-a fields (After Parker 1982.)

TABLE 1.

Phytoplankton analyses of Maquoit Bay samples.

	Rhonda Selvin	Elin Haugen
Analysis date	9/28–29/88	9/28/88
Species:		
Amphora sp.	Yes	—
Calycomonas sp.	Yes	—
Caetoceros *cf.* simplex	Yes	—
Chaetoceros spp.	—	Yes
Cryptomonad sp.	Yes	—
Dinophysis acuminata	Yes	Yes
Dinophysis sp.	Yes	—
Eutreptia lanowi	—	Yes
Gymnodinium sp.	Yes	—
Gyrodinium aureolum (Gymodinium nagasakiense)	Yes 1.0×10^6 l^{-1}	Yes 1.8×10^6 l^{-1}
Gyrodinium spirale	Yes	—
Gyrosigma sp.	Yes	—
Heterocapsa sp.	Yes	—
Katodinium rotundatum	—	Yes
Leptocylindrus sp.	Yes	Yes 2.5×10^5 l^{-1}
Navicula spp.	Yes	—
Nitzschia *cf.* closterium	Yes	Yes
Oxytoxum sp.	—	Yes
Prorocentrum micans	Yes	Yes 2.5×10^5 l^{-1}
Prorocentrum minimum	Yes	Yes 8.3×10^5 l^{-1}
Prorocentrum redfieldii	Yes	—
Protogonyaulax tamarensis	Yes	—
Rhizosolenia sp.	Yes	—
Scrippsiella sp.	Yes	Yes
Skeletonema costatum	—	Yes 2.8×10^5 l^{-1}
Tetraselmis sp.	—	Yes
Thalassiosira *cf.* pseudonana	—	Yes
Thalassiosira sp.	Yes	—
Total chlorophyll cells		
8μ filtration	—	8.6×10^6 l^{-1}
3μ filtration	—	9.0×10^6 l^{-1}
0.4μ filtration	—	1.2×10^7 l^{-1}
Total chlorophyll cells	—	3.0×10^7 l^{-1}
Total cyanobacteria	—	4.2×10^7 l^{-1}

in that they were surrounded by clear water at their peripheries. The reports of coffee-colored patches in the water described here are very similar to those reported for other *G. aureolum* blooms (Boalch 1979). Such patchiness would be consistent with the breaking up of a much larger algal mass as it passed around the numerous islands of Casco Bay during its shoreward movement; however, these algal patches could also have been produced by the leakage of cells on ebb tide from a population present in Maquoit Bay. For example, the patches observed around Merepoint on the 24th and in Middle Bay on the 25th could have resulted from the breakup and dispersion of a bloom in Maquoit Bay.

Environmental and Meteorological Conditions

Environmental conditions in Maquoit Bay on the 27th of September were not unusual for that time of year. Water temperatures were between 14° and 14.5°C, oxygen was 8 ppm, and salinity measured by a refractometer was around 33 parts per thousand. Sediments in Maquoit Bay were black and smelled of hydrogen sulfide. Tests conducted by the Pathology section of DMR found no diseases or parasites in tissue samples taken from the Bay that could have been responsible for the kill. The shellfish taken from Maquoit Bay did not contain saxotoxin (John Hurst, pers. comm.), which is the primary toxin present in *Alexandrium tamarensis,* the species responsible for paralytic shellfish poisoning along the Maine coast. Tests for heavy metals, pesticides, herbicides, volitile and non-volitile organics performed by the State of Maine Public Health Laboratory on water and sediment samples from the bay showed normal levels of all pollutants except at one station where there were elevated concentrations of a volitile organic compound (Stuart Sherbourne, pers. comm.).

Meteorological data from both the Portland Jetport and the Brunswick Naval Air Station were obtained from the National Climatic Data Center, Asheville, North Carolina. Table 2 summarizes the wind speed and wind direction observations and other meteorological data taken at the Brunswick Naval Air Station during the month of September, 1988. Figure 5 shows meteorological observations for the months of August and September taken at the Portland Jetport and surface seawater temperature recorded at Boothbay Harbor, Maine for the same period.

The air temperature during the first fifteen days of August averaged 5.78°C warmer than the long term average (Fig. 5a). During the remainder of August and during the month of September air temperature oscillated around its long term average value. The warmer air temperatures of the first half of August are reflected in peak seawater temperatures of 17.9°C recorded at Boothbay Harbor during this period (Fig. 5b). Surface seawater temperature remained warm (around 15°C) until the 13th of September when strong winds resulted in vertical mixing which lowered it to 12.7°C at Boothbay Harbor. From the 7th to the 16th of September, more than 90% of the possible direct solar insolation was received on eight of ten days. A four day moving average of solar insolation was calculated to reflect the light conditions physiologically distinguishable by phytoplankton (Fig. 5c). The average light supporting algal growth was high and constant during this ten day period. In addition, from the 24th of August to the 5th of September 12.7 cm of rain fell at the Portland Jetport (Fig. 5d).

Wind patterns from Brunswick Naval Air Station (Table 2) were similar to those observed at the Portland Jetport (Figure 5e). Wind speed and direction are slightly different, but the general pattern of high wind days in September is the same. Based on the estimated shellfish kill date, local wind conditions during the period of September 18 to September 22 are highly significant. Beginning at 18:25 on September 20, and continuing to 05:12 September 21, winds were recorded out of the south at sustained

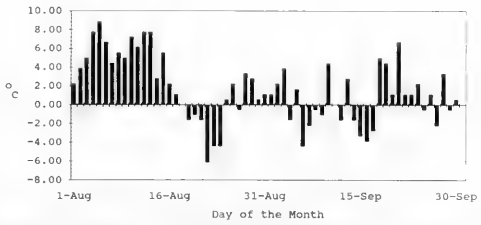

Figure 5a. Difference in air temperature from 30 yr. average for the period August 1 to September 30, 1988 at Portland, Maine.

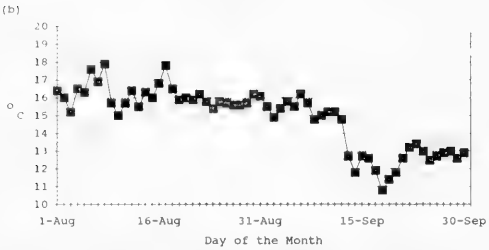

Figure 5b. Sea surface temperature at Boothbay Harbor, Maine for the period August 1 to September 30, 1988.

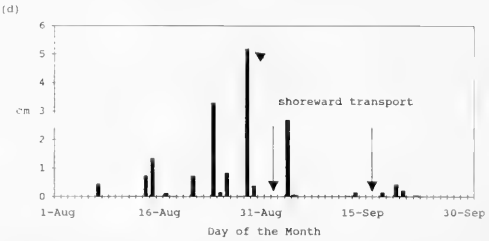

Figure 5d. Rainfall at Portland, Maine for the period August 1 to September 30, 1988.

speeds of 10–20 knots with frequent gusts to 27 knots. On September 22 winds were moderate out of the northwest at 10–18 knots with intermittent gust from 18–26 knots.

The pattern of tidal exchange during August and September in Maquoit Bay shows that, at the estimated time of the shellfish kill, the difference between the highest low tide and the lowest high tide was a minimum (Fig. 5f). Under these conditions tidal exchange is minimal and water coverage of the shellfish beds is at a maximum.

Possible Bloom Scenarios

The daily mean wind speed during August and September, 1988 at the Portland Jetport shows seven days on which the average wind speed exceeded 5 m/sec. Based on calculation on vertical mixing, as presented in Campbell and Wroblewski (1986), we determined that, on these days, the wind energy was strong enough to produce a surface mixed layer 7.5 m thick by overcoming the density stratification in the center of the West Cod Ledge gyre observed by Parker (1982). Thus, water from the thermocline and chlorophyll maximum layer at 5–10 m would have been brought to the surface during this period. The vertical mixing which occurred on August 24th, September 5th, September 11th, September 13th, and September 15th plays an integral role in explaining the *G. aureolum* bloom and shellfish kill in Maquoit Bay. In addition to the effects of vertical mixing, the strong winds out of the northwest on the 11th and 15th could have augmented the upwelling which normally occurs near the seaward islands of Casco Bay (Parker 1982) resulting in additional deep water being brought to the surface. Based on these events and their potential effects, three possible bloom development scenarios were developed.

Local Bloom Development

Several conditions which could have contributed to local bloom development may have been present in the late summer of 1988. A "seed" population of *G. aureolum* may have been introduced into the waters of Maquoit Bay either from the shoreward transport of a subsurface population found in stratified offshore waters, or by the resuspension of resting cysts present in the sediments of Casco or Maquoit Bays. However, the development of resting cysts by *C. aureolum* has never been demonstrated and we have no evidence that resting cysts of this organism exist in the sediments of Casco or Maquoit Bays, thus affording little support to the cyst resuspension alternative.

Examination of the wind data and calculations of Ekman transport show that a strong vertical mixing event could have occurred on August 24th followed by two periods of predominantly southerly winds from Aug. 26th to 29th and from September 1st to 4th. These latter winds could have resulted in shoreward transport sufficient to bring *G. aureolum* cells in offshore surface waters into Maquoit Bay. If a subsurface population of *G. aureolum* exists in the stratified waters of the West Cod Ledge gyre, cells might be regularly introduced into Casco Bay by routine upwelling events south of Jewell Island produced by the divergence of the flood tide surface flow as it enters Portland Harbor Channel, Hussey and Luckse Sounds (Parker 1982). Once transported into Maquoit Bay, these *G. aureolum* cells may have been subsequently retained in the bay if a sufficiently strong estuarine circulation pattern was produced in the bay by the 12.7 cm of rainfall from August 24th to September 5th. Persistent stratification within the Bay would have been required from September 5th through 17th to allow retention and growth of the population during the period of sunny

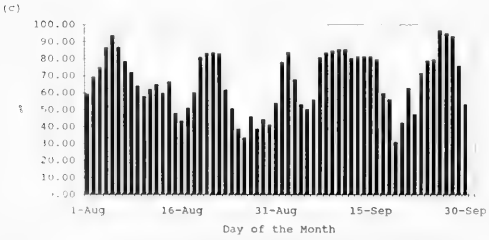

Figure 5c. Percent insolation at Portland, Maine for the period August 1 to September 30, 1988.

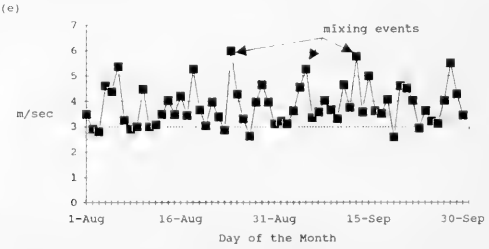

Figure 5e. Wind velocity at Portland, Maine for the period August 1 to September 30, 1988.

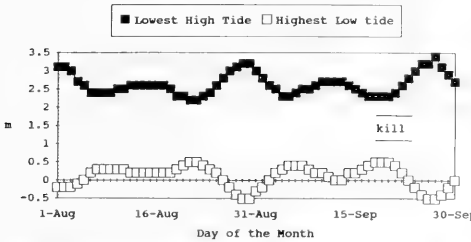

Figure 5f. Tidal amplitude at Portland, Maine for the period August 1 to September 30, 1988.

weather from September 6th to 17th. On September 17th a period of southerly winds began which could have resulted in the final concentration of *G. aureolum* cells at the head of Maquoit Bay. The resulting high cell concentration could, over a 5 day period of reduced tidal range, have caused a shellfish and worm kill either through oxygen depletion during nighttime respiration, mucous production, toxicity, or more likely, a combination of one or more of these.

TABLE 2.

Weather observations
Brunswick Naval Air Station (BNAS), Brunswick, Maine.

	September 1988						
	Wind*						
Date	Mean Speed	Mean Gust	Peak Speed	Direction (°true)	Temp. Max.	(°F.) Min.	Prec. (in.)
1	6.88	0.0	13	210	79	52	0.0
2	3.83	0.0	14	210	81	52	0.0
3	3.64	15.0	20	120	83	56	0.0
4	5.34	15.4	20	130	64	54	0.0
5	8.19	18.2	20	310	76	51	0.7
6	3.25	12.0	12	360	67	41	0.0
7	3.33	14.5	16	10	69	42	0.0
8	6.33	15.0	19	230	71	45	0.0
9	6.67	17.5	23	210	70	53	T
10	3.48	15.0	17	310	82	63	0.0
11	7.79	22.1	27	310	70	53	0.0
12	6.33	0.0	20	300	72	48	0.0
13	11.31	20.1	25	210	69	56	T
14	7.50	19.0	21	250	66	45	T
15	9.68	21.3	28	330	63	43	T
16	4.79	15.0	16	350	67	41	0.0
17	5.58	0.0	16	220	66	41	T
18	6.50	17.8	23	170	67	59	0.48
19	2.96	0.0	15	300	80	55	0.0
20	8.44	19.7	24	180	64	53	0.21
21	6.29	24.7	27	180	77	61	0.29
22	6.96	21.3	27	300	68	44	0.0
23	6.65	17.0	18	190	66	44	T
24	5.38	18.3	23	330	73	48	0.0
25	4.54	18.0	19	190	66	44	0.0
26	5.20	17.5	20	310	71	43	0.0
27	7.65	21.0	23	180	64	40	T
28	10.51	24.9	29	330	73	47	T
29	6.08	0.0	18	350	66	38	0.0
30	5.29	0.0	16	200	64	47	0.0

* Speeds in knots. T, trace amount.

Offshore Surface Growth

The offshore development of a *G. aureolum* bloom may have proceeded either as surface growth seeded by subsurface cells or by subsurface growth in the chlorophyll maximum layer. In the surface growth model, wind mixing events on September 5th, 11th, and 13th would have been strong enough to overturn the stratified waters of the West Cod Ledge gyre to a depth of 7.5 m bringing nutrients and *G. aureolum* cells into the surface waters where growth was subsequently stimulated by the prolonged period of high and constant light which occurred from September 6th to 17th.

Offshore Subsurface Growth

Subsurface growth of *G. aureolum* also would have been stimulated by high and constant light conditions present at the thermocline from September 6th to 17th. As Holligan (1985) points out, dinoflagellates in northwestern European waters take advantage of the sunlight penetrating through the upper, clearer stratum while thriving on the nutrients which diffuse across the thermocline from the nutrient-rich waters below.

On September 11th and again on the 15th, strong northwest winds, gusting to 28 knots, blew for periods of 9 and 15 hours, respectively. These winds, particularly those of the 15th, may have been sufficiently strong and prolonged to disrupt the West Cod Ledge gyre and enhance the tidal-based upwelling at the entrance to Casco Bay bringing a subsurface bloom to the surface. Upwelling of deep water as a result of the seaward displacement of wind driven surface water is one mechanism that has been proposed to explain how subsurface algal blooms could be carried to the surface (Tangen, 1977). Subsurface growth of *G. aureolum* does not appear to reach the cell concentrations found in surface blooms. Thus, the subsurface growth model depends on physical processes to concentrate algal cells from a large area thereby producing bloom concentrations. Such a concentration of subsurface cells could have occurred through upwelling of water from the thermocline of the West Cod Ledge gyre at the entrance of Casco Bay.

Shoreward Transport of Offshore Bloom and Final Concentration

Both offshore bloom development alternatives rely on wind-driven shoreward transport for the bloom's final arrival in Maquoit Bay. During August and September three periods of southerly winds occurred that could have produced a shoreward Ekman transport of sufficient magnitude to shift the position of the West Cod Ledge Gyre so that it abutted the mouth of Broad Sound. These transport periods occurred on August 25–29, August 31–September 2, and September 17–20 as shown in Fig. 5d. The potential movement of the gyre under the influence of southerly winds on the 17th and 18th of September (Fig. 6) was large enough to allow water from the gyre to be drawn into Casco Bay through Broad Sound on the flood tide. A similar position of the gyre was obtained for the two earlier periods calculating water movement as in Parker (1982). Based on the location of the West Cod Ledge gyre and the normal tidal current path through Broad Sound described by Parker, along with the wind directions over these periods, Maquoit and Middle Bays are the most likely points of concentration for materials transported in the surface waters.

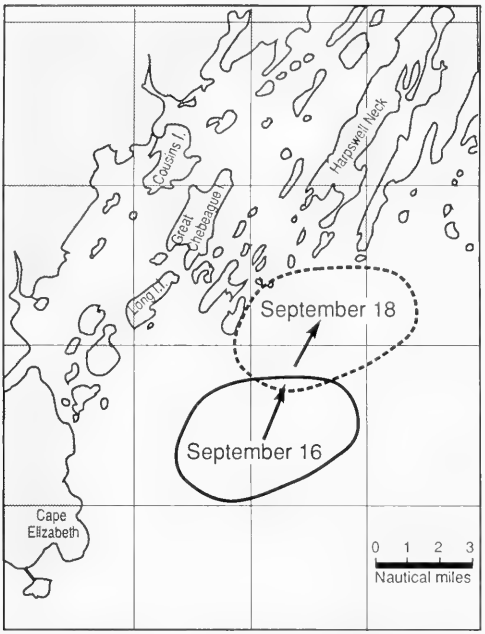

Figure 6. Estimated movement of the West Cod Ledge gyre under the influence of southerly winds on September 17–18, 1988.

In all growth scenarios, local wind patterns are responsible for concentrating *G. aureolum* cells on the shellfish grounds of Maquoit Bay. On the evening of September 20th strong southerly winds, with gusts between 24 and 27 knots, were recorded at Brunswick Naval Air Station and persisted through the early morning of the 21st. This period coincided with a +0.5 m low tide which would have afforded less than normal resistance to waters being moved by the opposing wind. These winds could also have pushed surface water up into the head of Maquoit Bay and over a large portion of the shellfish grounds, concentrating algal cells there. Winds from the northwest, gusting up to 27 knots on the 22nd, may have blown the concentrated bloom against the eastern side of the bay, thus accounting for the higher mortality found along that shore.

DISCUSSION

The association of phytoplankton with offshore gyres has been described by Pingree (1979). The West Cod Ledge gyre identified by Parker could provide ideal conditions for the development of *G. aureolum* blooms (Holligan, pers. comm.). The strong vertical stability of the gyre allows for the establishment of a discrete thermocline at a depth of approximately 5–10 meters between warm, nutrient-deficient surface water and cold, nutrient-rich water below. The transparency of the overlying water allows sunlight to penetrate deep into the upper layer. The combination of a discrete thermocline and adequate illumination provides the necessary requirements for the establishment of subsurface populations. Furthermore, the persistence of the gyre throughout most of the tidal cycle suggests the possibility of the existence of a resident, subsurface population of *G. aureolum* immediately offshore of Casco Bay. If *G. aureolum* proves to be a permanent resident in the subsurface phytoplankton community of West Cod Ledge, it could be regularly available for seeding into Casco Bay through the upwelling of deep waters southwest of Cliff Is., as well as through wind driven horizontal and vertical transport events as mentioned earlier.

In the absence of any evidence of cyst formation by *G. aureolum*, we must assume that an offshore subsurface population served as a seed source of cells for the local growth as well as offshore growth alternatives. The existence of this species as a member of the phytoplankton community in the Gulf of Maine should be confirmed by field research and its spatial and temporal distribution determined.

Local Bloom Development

Chang and Carpenter (1985) observed a dense bloom of *G. aureolum* during the summers of 1982 and 1983 in the Carmans estuary of Long Island, New York. These blooms had potentially toxic cell concentrations of 2.0×10^7 cells l^{-1}, however, no ill effects were observed. For comparison, *G. aureolum* cell concentrations on the Maquoit Bay shellfish grounds could have been around 1.4×10^8 cells l^{-1} based on an initial seeding of 10^4 cells l^{-1}, (a log phase growth increase of 2000× or approximately 1 division d^{-1}), and a wind driven volume concentration of 6.8× within Maquoit Bay. Chang and Carpenter (1985) used a model based on observed rates of cell division, vertical migration of algal cells, and water movements to show that cells must move downward at the mouth of the estuary by either convergence of water or vertical migration of cells for the bloom to be maintained in the estuary. A stratified water column and estuarine type circulation pattern were necessary for the development and maintenance of this local bloom. The balance between cell growth and estuarine flushing determined whether or not the bloom could be maintained in the Carmans estuary. Based on these observations, we believe that the weakest link in the chain of circumstances supporting local growth of a bloom in Maquoit Bay is the assumption that a stratified water column, sufficiently stable to allow retention of the *G. aureolum* cells during growth of the bloom, existed in the bay from September 5th to September 17th. Any stratification that was present in the bay must have been based on 12.7 cm of rain that fell between August 24th and September 5th. Stratification in Maquoit Bay has been minimal for all currently observed conditions, but to date, these observations have not been related to fresh water input (Kresja, pers. comm.). A flushing rate of around 6 days may be too rapid to allow cell growth to keep up with cell losses from flushing, despite optimum sunlight conditions over an 11 day log growth period. This question could be resolved by a growth versus flushing model similar to that of Chang and Carpenter (1985). Additional information on the stratification and flushing of the bay under various conditions of fresh water input would allow the development of a relatively simple model to predict whether or not a bloom could have developed in Maquoit Bay as a result of local growth driven by the environmental conditions that existed in September 1988. The development of such a local bloom is certainly possible (Chang and Carpenter 1985), however, we must consider it doubtful without further proof that conditions in Maquoit Bay during September 1988 were sufficient to allow for the retention and growth of a *G. aureolum* blooms.

Offshore Surface Growth

Offshore surface growth provides another alternative explanation for the development of the *G. aureolum* bloom. The offshore origin of coastal blooms has been discussed by Tyler and Seliger (1978) and Lindahl (1985). The surface growth alternative is favored because the growth of *G. aureolum* is not inhibited by strong light as is the growth of many dinoflagellates (Richardson and Kullenberg 1987). Thus, this species would not reach its highest growth potential until exposed to surface light conditions (Richardson and Kullenberg 1987). In this alternative a vertical mixing event on September 5th brought nutrients and *G. aureolum* cells to the surface from a subsurface population at concentration of 10^4 cells l^{-1}, similar to that commonly observed in European waters (Holligan 1979). A 2000× increase could have occurred through growth at the surface during 11 days of optimum light conditions (Iizuka et al. 1989) to result in an offshore concentration of 2.0×10^7 cells l^{-1} in the West Cod Ledge gyre. A further concentration of 6.8× by the southerly winds over Maquoit Bay could have resulted in an effective concentration of 1.4×10^8 cells l^{-1} on the shellfish grounds.

Offshore Subsurface Growth

The concentration of subsurface *G. aureolum* cells to bloom levels by physical transport processes has been proposed as a mechanism to explain some blooms in the waters of Northwestern Europe (Richardson and Kullenberg 1987). Starting with a subsurface population of 10^4 cells l^{-1}, growth and transport would have to increase cell concentrations to approximately 2×10^7 cells l^{-1} to produce bloom concentrations with visible coffee colored patches (Tangen 1977). We assume that subsurface growth can account for a 100× increase in cell numbers to account for a subsurface concentration of 1.0×10^6 cells l^{-1} similar to the maximum subsurface concentrations observed by Richardson and Kullenberg (1987). If all the water from the subsurface chlorophyl maximum layer was upwelled in the vertically mixed area in front of Casco Bay, this cell concentration would be increased approximately 4.5×. Cell concentrations could be increased an additional 6.8× to 3.0×10^7 cells l^{-1} as a result of the concentration of *G. aureolum* cells over the shellfish beds of Maquoit Bay by southerly winds.

There are several problems with the subsurface growth alternative in explaining the development and transport of the bloom. The primary area of tidal divergence and upwelling (Parker 1982) that would be affected by northwest winds is to the south of Jewell and Cliff Islands which means that *G. aureolum* cells would have been supplied primarily to the Middle and Maquoit Bays area by the small flow entering through Luckse Sound. However, if *G. aureolum* cells were concentrated in this manner, we would also expect cells to be carried into the southwestern portion of Casco Bay in considerable quantities, but this was not observed. Further, the final concentration of cells on the shellfish beds of Maquoit Bay obtained under the assumptions used here is about 5 times smaller than that obtainable in the local growth and offshore surface growth alternatives. This difference is primarily attributable to the comparatively slow log phase growth rates which were assumed to apply for subsurface growth. The most important uncertainty in explaining bloom development by the subsurface growth alternative is the rate of algal growth in the chlorophyll maximum layer. Research is needed to show whether or not growth rates of subsurface *G. aureolum* are faster or slower than our estimates of approximately 0.8 divisions d^{-1}.

The offshore surface growth alternative is a strong choice; however, it does not allow cells to reach Maquoit Bay as early as the subsurface growth option. The most uncertain assumption supporting the surface growth option is that sufficient nutrients would have been supplied from the September 5th mixing event to support 11 days of log phase growth. A more realistic picture may be provided if we assume that some combination of subsurface and surface growth occurred, perhaps with a double chlorophyll maximum as observed by Holligan (1979). The wind data shows that 4 vertical mixing events occurred during the 11 days of increased sunlight which could have kept surface nutrient concentrations high, but it would have redistributed algal cells according to the concentration difference between surface and bottom layers. Two of these wind events could have resulted in some enhancement of the upwelling at the entrance to Casco Bay. The transport of *G. aureolum* cells into Casco Bay may have been from both surface and subsurface growth regimes.

The Shellfish and Worm Kill

The mechanism by which shellfish and worms were killed in Maquoit Bay would have been the same for all growth alternatives. We can begin to understand how the kill occurred by examining more closely the environmental conditions which existed during the period from September 17th to September 23rd (Table 3). Marine organisms in Maquoit Bay could have been exposed to high concentrations of *G. aureolum* for five to seven of these days as discussed above. *G. aureolum* cells can negatively affect marine organisms by production of mucous, consumption of oxygen, and production of a weak toxin (Partensky et al. 1989).

Even though *G. aureolum* is a photosynthetic organism, oxygen consumption in the dark by dense algal concentrations has been shown to produce hypoxic conditions in shallow coastal waters (Odum and Wilson 1962). The normal cycle of diurnal oxygen concentration in shallow marine waters (Odum and Hoskin 1958) has a minimum in the early morning hours usually just after dawn. Table 2 shows that high tide occurred during the early morning hours from September 17th to 23rd and moderate southerly or southwesterly winds prevailed on six of seven days. Thus, a large proportion of the animals on the Maquoit Bay shellfish grounds were probably subjected to water loaded with highly concentrated, oxygen-consuming algae at the time of day when oxygen demand was highest. Under these conditions we may assume that sedentary

TABLE 3.

Summary of wind and tide data at the time of the shellfish kill.

	Tide		Wind Speed*	
Date	Time High Water	Minimum Range (m)	Resultant (m/s)	Direction (degrees)
Sept. 17th	0223	1.9	2.7	180
Sept. 18th	0310	1.8	2.8	180
Sept. 19th	0409	1.8	0.9	220
Sept. 20th	0514	1.8	4.2	170
Sept. 21st	0621	2.0	3.2	240
Sept. 22nd	0724	2.4	3.5	300
Sept. 23rd	0823	2.8	2.3	220

* Wind data from Portland Jetport; see Table 2 for local wind data.

marine organisms on the shellfish grounds were regularly exposed to severe oxygen stress over a period of 5 to 7 days. In addition, if *G. aureolum* cells settled out of the water column during slack water or migrated (Tangen et al. 1979) toward bottom waters on flood tide as implied by Chang and Carpenter (1985), the shellfish grounds would have been blanketed by a dense mat of algae with high oxygen demand. Since the aerobic sediment layer over much of Maquoit Bay is only a few millimeters thick (Maher 1991), we may assume this blanket of *G. aureolum* cells could have exposed marine organisms on the shellfish grounds to hypoxic conditions for up to six hours nightly over a period of 5 to 7 days. Mucous secretion and/or toxin production by such a mass of algae could have also contributed to the mortality of marine organisms during this time.

Hammen (1976) discusses the tolerance of many marine invertebrates to low oxygen levels. The time to 50% mortality under low oxygen conditions for various species tested ranged from less than one hour to 50 days. In general, crustaceans were most sensitive to low oxygen and mollusks least sensitive with marine worms in between. While mollusks are fairly tolerant of low oxygen conditions as a group, their tolerance for low oxygen is markedly less at higher temperatures. In addition, Theede (1973) demonstrated that the tolerance of marine invertebrates for oxygen deficiency decreased when the organisms were simultaneously exposed to hydrogen sulfide. We may assume that marine organisms on the Maquoit Bay shellfish beds were exposed to warm water (14.5–15°C), and to one or more of three additional stressors, hydrogen sulfide, mucous, and toxin. Given the potential synergistic effects of these adverse conditions we may further assume that marine invertebrates would have a minimum tolerance for low oxygen conditions on the grounds.

The toxic effects of *G. aureolum* on marine invertebrates have been demonstrated by Widdows et al. (1979), who challenged *Mytilus edulis* with high concentrations of *G. aureolum* in controlled tests of toxicity. In these experiments cell concentrations of 6.3×10^5 cells l^{-1} resulted in a marked decline in clearance rates along with cellular damage to the gut in experimental animals after only a few hours of exposure. This suggests that *G. aureolum* has a direct cytotoxic effect.

Turner et al. (1987) showed that *G. aureolum* produces a toxic substance which has cytological effects on fish. Nevertheless, considerable controversy exists concerning the exact mechanism involved in shellfish and other marine benthos mortalities associated with *G. aureolum* blooms. The prevailing view seems to be inclined towards deoxygenation of the water layer at the water-sediment interface resulting from the rapid decomposition of dead and dying cells, a well as the formation of mucoid slimes on the bottom, at the conclusion of a bloom (Helm et al. 1974, Tangen 1977, Tangen et al. 1979, Boalch 1979, Pybus 1980, Blake and Walker 1981). In the case of Maquoit Bay we must add low oxygen conditions brought on by a high oxygen demand produced by the wind-concentrated algal cells coincident with the normal diurnal oxygen minimum. Evidence of anoxic conditions was present in Maquoit Bay after the event in the form of black slime coating the bottom along the shore and the presence of the pungent odor of hydrogen sulfide, even though the water column was oxygen saturated. The fact that small clams and worms were seen scattered on the surface of the bottom suggests that conditions within the sediments became intolerable. Similar reactions have been reported in the lugworm, *Arenicola marina*, which appears to be particularly susceptible to the effects of *G. aureolum* blooms (Helm et al. 1974, Forster 1979). The severe anoxia that occurred in Maquoit Bay sometime before September 27 may have been exacerbated by the decomposition of clams and other benthic forms which succumbed to the initial effects of the bloom.

Finally, the detrimental effects of the *G. aureolum* bloom on marine organisms were not restricted to Maquoit Bay. Mortalities among marine worms in Merepoint Bay were reported on September 29 (Wallace, pers. comm.) and, although observations of the mussel beds in upper Middle Bay in late September indicated only limited impact on the area, fisherman harvesting these beds later in the fall reported evidence of significant mortality which was initially attributed to the unusually hot summer. However, the magnitude of clam and worm mortalities in Maquoit Bay was larger and the area affected more extensive than observed elsewhere. Based on the direction of wind driven transport, Maquoit and Middle Bays should have been affected equally by the bloom. This was not the case because the geomorphology of the two bays is different. Maquoit Bay is a largely enclosed, shallow bay that empties through a narrow channel and has a fairly slow turnover time, whereas, Middle Bay is deeper and funnel shaped allowing faster and more complete flushing.

CONCLUSION

There are several common factors that occur in all of the proposed explanations for the Maquoit Bay shellfish kill that, when taken together, can serve to identify conditions favorable to *G. aureolum* blooms in the future. Warm temperatures favor *G. aureolum* in competition with other dinoflagellate species as evidenced by the fact that *G. aureolum* is most abundant during the warmest time of the year (Holligan 1985). The warmer than normal temperatures of August 1988 may have favored *G. aureolum* in competition with *Alexandrium tamarensis* or other dinoflagellate species in the subsurface waters of the West Cod Ledge gyre. The 11 days period of high and constant light conditions may also have favored *G. aureolum* in competition because it does not experience photoinhibition at high light levels as observed for many other dinoflagellate species (Richardson and Kullenberg 1987). The existence of winds favorable to the concentration of algal cells inshore was a necessary factor. Southerly winds on the 20th and 21st concentrated algal cells at the head of Maquoit Bay, and northwest winds on the 22nd further concentrated the *G. aureolum* cells on the eastern side of the Bay where the greatest damage was observed. Reduced tidal range in Maquoit Bay occurred from September 17th to 21st which produced a minimum in the capacity for water exchange. Onshore winds during this period further reduced the exchange of waters between Maquoit and Casco Bays. The occurrence of high water in the early morning from September 20th to 22nd caused a maximum coverage of the shellfish grounds with concentrations of oxygen consuming *G. aureolum* (through dark respiration) at the time of day when oxygen supplies are usually at a minimum. Finally, all explanations require an offshore source of *G. aureolum*, and the dispersion of bloom concentrations by increased tidal flushing from September 23rd to 27th, possibly aided by the wind. A similar combination of physical events has been associated with large shellfish kills in the past as described by Nixon (1989) for the great Narragansett Bay fish kill.

These unique characteristics of Maquoit Bay are in part responsible for its high phytoplankton and shellfish productivity, but they also set the stage for high mortalities of marine organisms in the presence of high concentrations of a noxious organism like *G. aureolum*. Although the nutrient loading of Maquoit Bay, as a

result of point or non-point sources of pollution, may not have contributed appreciably to this algal bloom, this does not imply that it could not precipitate a similar event involving this or another algae sometime in the future.

Clearly, further study is required in order to understand the origin and evolution of such blooms. For the immediate future, it would be most valuable to determine if a resident, subsurface population of *G. aureolum* does indeed exists in the gyre off of Casco Bay. This is of particular interest in this case, for blooms of *G. aureolum*, in other parts of the world, particularly in Ireland and Norway, have historically repeated themselves in subsequent years after their initial observation.

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OYSTER LEASE TRANSFERS AND LENDING; ROLES IN REHABILITATION OF LOUISIANA'S OYSTER INDUSTRY

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ABSTRACT In states like Louisiana, where the majority of oyster production is derived from private grounds, it is logical to target this component of the harvesting sector for rehabilitation efforts. The information void regarding economic and financial aspects of this component, however, is seen to be one of the major obstacles confronting the oyster industry and management authorities in the planning stages of rehabilitation and expansion efforts. The purpose of this paper is to provide the information which can be used in rehabilitation and expansion efforts in Louisiana's oyster industry.

KEY WORDS: leasing, lending, Louisiana, oyster, rehabilitation

INTRODUCTION

The sharp decline in oyster production during the past several years has stimulated interest and subsequent discussion concerning alternative methods of stabilizing, if not reversing, the downward production trend. Two workshops sponsored by the National Sea Grant College Program, the National Marine Fisheries Service, the National Coastal Resources Research and Development Institute, and various state Sea Grant College Programs and state agencies were recently held and culminated in the document *A Plan Addressing the Restoration of the American Oyster Industry*. The document outlined several major areas and objectives, including economic and marketing, to be evaluated for oyster-industry restoration.

Unlike most U.S. fisheries, the oyster fishery is conducive to the issuance of private property rights. The species is sessile and as such is not the subject of conflict in ownership rights as long as territorial rights have been previously established. Furthermore, under certain environmental conditions (e.g. acceptable salinity and temperature ranges) oyster production can be enhanced by increasing the amount of suitable substrate. This entails the building of "oyster reefs" which are generally comprised of discarded oyster or clam shells. This being the case, the Sea Grant document explicitly recognized the need to examine the potential for institutional changes that would encourage the granting of property rights. The benefits of property rights in the oyster fishery have also been quantitatively established in an economic framework by Agnello and Donnelley (1975, 1976, 1984).

The enhancement of suitable substrate for increased cultivation of oysters is not a new concept. Florida, for instance, has maintained an aggressive shell-planting program since 1949. Other Gulf states will, depending upon funding availability, also plant shell. These activities, however, occur primarily on public reefs and benefit lease holders only indirectly when, for instance in Louisiana, public grounds provide a source of seed oyster for transplant to private grounds.

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 private grounds, it is logical to target this component of the harvesting sector for rehabilitation efforts.

The information void regarding economic aspects of the leasing situation in the primary leasing states, like Louisiana, 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. Individuals, for example, are hesitant to invest in rehabilitation activities without adequate economic knowledge. Financial institutions are also reluctant to provide funding for rehabilitation activities by the private sector without sufficient information. Finally, Federal and state legislatures and management agencies, responsible for the setting and direction of rehabilitation and expansion policy, on both private and public grounds, will almost certainly be hindered in the development of an "optimal" policy without prior information regarding the oyster fishery.

An analysis of the stability and economic and financial aspects of lease-based businesses is a precursor to reef rehabilitation efforts of privately owned grounds. Providing such an analysis serves as the basis for this paper. 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.

Background Information

Louisiana currently enjoys the highest annual oyster (*Crassostrea virginica*) production among all states in the nation. Its landings generally fall in the 8–12 million pound range though landings above 13 million pounds are not uncommon (Fig. 1).

As the current leader in production, Louisiana contributes significantly to the Gulf Region and national domestic oyster supply. As indicated in Figure 1, Louisiana generally contributes about

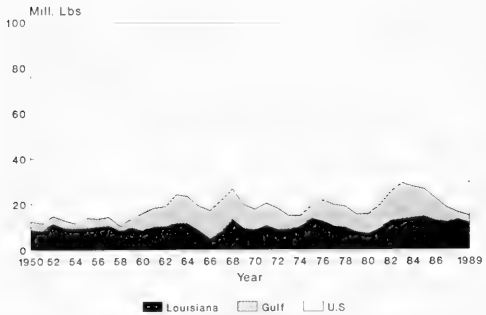

Figure 1. Louisiana, Gulf Region, and U.S. Oyster Production, 1950–89.

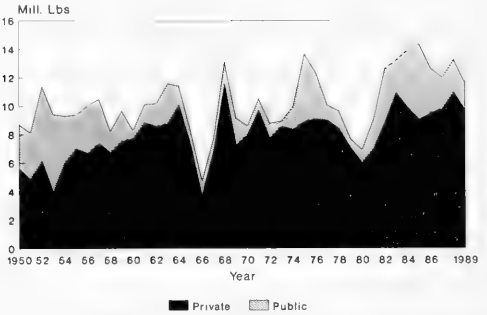

Figure 2. Louisiana Oyster Production from Private and Public Grounds.

50% of the oyster production in the Gulf Region and has contributed about two-thirds since 1986. At the national level, Louisiana typically supplies 15–20% of the total domestic oyster supply and has provided almost a third since 1986. Much of Louisiana's increasing share during the 1986–89 period is the result of a significant decline in production at both the Gulf and national levels (Fig. 1).

While Louisiana's vast wetland systems and conducive growing conditions help explain the state's large and relatively stable annual oyster harvests, the significance of the state's water bottoms leasing system cannot be overlooked. By providing a stable environment through its leasing policy, the state encourages industry investment and also provides impetus for the preservation, rehabilitation, and expansion of existing and potential oyster growing grounds.

And indeed, the privately leased grounds serve as the basis for the state's large and increasing share of the nation's domestic oyster production as well as its current number one ranking. Production from private grounds generally falls in the 7–10 million pound range and, with few exceptions, represents 70–90% of the state's total annual oyster harvest (see Fig. 2). Overall, the contribution to the state's total oyster production realized from its leased grounds appears to have fallen somewhat since the early 1980s. Further examination of the data suggests, however, that the decline is the result of increased production from public grounds, which has resulted in an increase in the state's total oyster production, rather than any decline in production from private grounds. In fact, production from private grounds since 1983 has been well above the 1950–89 average of 8.0 million pounds (Fig. 2).

There are several existing statutes which provide the basis and set the rules and regulations under which private leasing of the state's water bottoms is controlled (Dugas). Louisiana Revised Statute (La.R.S.) 56:3(B) entitles the state to ownership of "beds, bottoms of rivers, streams, bayous, lagoons, lakes, bays, sounds, and inlets bordering on or connecting with the Gulf of Mexico, within the territorial jurisdiction of the state, including all oysters and other shellfish and parts thereof, either naturally or cultivated and all oysters in the shells after they are caught or taken." This statute, in addition, states that ownership "shall be under exclusive control of the Wildlife and Fisheries Commission." La.R.S.56:425(A) discusses to whom the Secretary may lease any water bottoms and natural reefs in the water bottoms of the state while La.R.S.56:423 gives the lessee exclusive use of water bottoms and provides that a lease is heritable and transferrable. These statutes along with La.R.S.56:427, which describes the steps for initiating an oyster lease, and statute R.S.56:428, which describes the terms, the length, the provisions for lease renewal, and rental, legalize leasing and provide the basis for most leasing arrangements and activities.

The steps set forth in La.R.S.56:427 for obtaining a lease have been outlined by Richardson and are as follows. First, the potential leaseholder must identify a tract of water bottoms that is not within the public grounds and that is not already privately leased. Then, the potential lease holder files an application, including all pertinent information, with the Department of Wildlife and Fisheries and pays the appropriate application fee. The Department then registers the application, determines if the area is leasable, and, based on previous findings, will survey the area (the applicant can also employ a private surveyor). The lease can be granted once the survey is completed and plotted and must be filed in the parish to which the water area is adjacent.

As provided in La.R.S.56:428, all leases are for a duration of 15 years and the current leaseholders have the first right of renewal when the lease expires. La.R.S.56:432 limits the total acreage of water bottoms that any person, partnership, or corporation may lease to no more than one-thousand (with exceptions for canners); up significantly from earlier maximums of less than ten acres. The rental rate, set by legislation from the 1989 Louisiana legislative session, is fixed at two dollars per acre per year.

As noted, La.R.S.56:423 gives the lessee exclusive use of water bottoms and provides that leases are heritable and transferrable. This provision in the laws, plus the first right of renewal, gives lease holders an incentive to develop their leases and manage them in accordance with some long run profit enhancing criteria.

The conducive leasing laws have encouraged oyster leasing activities in the state and have permitted expansion within the industry (Fig. 3). In 1950, for example, about 32 thousand acres were under lease compared to 66 thousand acres just 10 years later. By 1970 total leased acreage had increased to more than 120 thousand and almost doubled to about 230 thousand acres by 1980. Leased water bottoms had expanded to more than 320 thousand acres in 1988. This increased leased acreage, it is noted, has brought forth only a marginal increase in production, indicating a significant decline in the production per acre leased. This could be due to (1) lower productivity among newer leases being put into

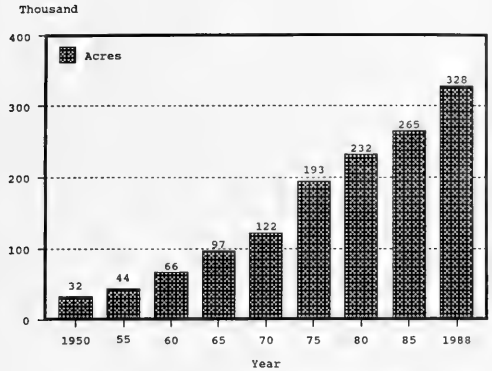

Figure 3. Acres of Louisiana water bottoms leased.

production, (2) declining productivity among the older, more established leases, or (3) a general decline in productivity throughout the industry in total.

Methodology

When transferring leases, current lease holders are obligated to notify the Louisiana Department of Wildlife and Fisheries (LDWF) of their intent. LDWF then draws up a "Certificate of Registration of Transfer of Lease," an example of which is given in Figure 4. Each Certificate lists the leases being transferred and

P-1

NO. 1241

Certificate Of Registration Of Transfer Of Lease

OFFICE OF

LOUISIANA WILD LIFE AND FISHERIES COMMISSION

NEW ORLEANS, LA.

THIS IS TO CERTIFY, That transfer of bedding ground contained in Lease No. 16085 - 17213 - 18016 - 18925 - 19298

evidenced by proper authentic act, has been made by Dominick Begovich and Frank Plaisance of the Parish of Orleans to Dorsey Burrell of the Parish of Tangipahoa on the 3rd day of November in the year 1969, in accordance with R.S. 56:424 through 430, both inclusive, that the said transfer has been registered on this 25th day of November in the year 1969, in lease Register No. 54 - 57 - 58 - 61 - 64, Folio 288 - 54 - 421 - 43 - 416; and that the said Dorsey Burrell is entitled to enjoy the profits, privileges and advantages of said oyster bedding ground until the expiration of said lease under the protection of the laws of this State, and the rules and regulations of the LOUISIANA WILD LIFE AND FISHERIES COMMISSION.

New Orleans, La., November 25, 1969 19

C. Rustevo
Chief Clerk.

Figure 4. Example of Certificate of Registration of Transfer of Lease.

the names of the transferor and transferee. In the example, five leases were transferred from Mr. Dominick Begovich and Mr. Frank Plaisance to Mr. Dorsey Burrell in November, 1969.

To evaluate stability in the Louisiana leased-based oyster operations, the authors researched all certificates during the 1950–89 period and collected information pertaining to all leases listed on each certificate. Data collected for each transfer include: (1) names of buyers and sellers, (2) number of leases in each transfer, (3) acreage of each lease transferred, and (4) location of each lease in the transfer (i.e. Parish and water body), and (5) terms associated with the transfer (e.g. condition of sale, monetary amount of transfer, conditions of payments, etc.). In the example previously cited, all leases were located in St. Bernard Parish and ranged in size from 19 to 500 acres. This information was then compiled and analyzed to examine the stability and value of Louisiana's oyster lease-based operations.

The paper also considers borrowing practices for reef rehabilitation and maintenance by the Louisiana oyster industry. Information for this aspect of the paper was collected from the Farmers Home Administration and private lending institutions.

RESULTS

The findings are presented in three sections. In the next section, stability of lease-based operations are examined. Then, the value of these operations is analyzed. Finally, borrowing practices for reef maintenance and rehabilitation efforts are documented.

Stability

As might be expected in accordance with the increased acreage of water bottoms leased during the past 40 years, the number of registered transfers has also risen. During 1960–69, for example, the Louisiana Department of Wildlife and Fisheries registered 380 transfers compared to 299 during the previous decade (Table 1). By the 1970s, the number of registered transfers had risen to 628 and increased sharply again to 1,184 during 1980–89.

The number of leases involved in each transfer ranged from one to more than twenty. On average, the number of water-bottom leases involved in each transfer remained relatively constant, at about four, during the 1950s, 1960s, and 1970s (Table 1). During the 1980s, however, the number fell to 2.92.

The total number of leases transferred during the 1950s, 1960s, and 1970s increased, by and large, in relation to the increase in the number of registered transfers. This reflects the stability in the number of leases in each registered transfer during 1950–79. For example, while the number of registered transfers increased by 30% between 1950–59 and 1960–69 (from 299 to 380), the total number of leases transferred increased by just over 30%; from 1,192 to 1,589 (Table 1). Similarly, as the number of registered transfers increased from 380 to 628 (65%) between the 1960s decade and the 1970s decade, the number of leases transferred increased by about 60% (1,589 to 2,511). While the registered number of transfers increased by about 90% (628 to 1,184) between the 1970s and 1980s, however, the number of transferred leases rose by less than 40%; from 2,511 to 3,462. This decline is the result of the sharp fall in average number of leases per transfer.

In addition to an increasing number of transfers during 1950–89, the average size of leases being transferred rose from 16.2 acres during 1950–59 to 46.5 acres during 1980–89 (Table 1). This likely represents an increase in lease size, at the aggregate level, as adjacent leases were merged throughout the earlier de-

TABLE 1.

Selected statistics related to Louisiana's registered oyster water-bottom transfers, 1950–89 ten year averages.

Time Period	Number of Transfers	Avg. No. Leases Per Transfer	No. of Leases Transferred	Avg. Acres Per Lease Transferred	Avg. Acres Per Transfer	Total Acres Transferred
1950–59	299	3.99	1,192	16.2	65	19,302
1960–69	380	4.18	1,589	25.6	107	40,706
1970–79	628	4.00	2,511	32.9	133	82,688
1980–89	1,184	2.92	3,462	46.5	136	160,893

Source: Compiled from unpublished data maintained by Louisiana Department of Wildlife and Fisheries, Oyster Division.

cades. The average number of acres per transfer rose rapidly during the 1950s and 1960s but has remained relatively stable, at about 130–140, in the 1970s and 1980s (Table 1). This is because the average number of leases involved in each transfer during the 1980s (2.92) fell from that observed during the 1970s (4.00) while the average number of acres per lease transferred during the 1980s (46.5) is larger than that observed during the previous decade (32.9). Finally, the total acres of transferred water bottoms has roughly doubled during each ten year period from the 1950s through the 1980s (Table 1). For example, about 19 thousand acres were transferred during the decade of the 1950s compared to 41 thousand acres during the 1960s, 83 thousand acres during the 1970s and 161 thousand during the 1980s. This increase roughly parallels the overall increase in the total acres of water bottoms leased by the state during the same period (Fig. 3).

Lease transfers occur for many reasons. Sometimes these transfers simply represent a name change rather than a change in ownership. Incorporation of a leasee's oyster operations, for example, requires the transfer of leases from the individual's name to the corporation's name. Similarly, creation of a partnership requires an act of transfer. Vice versa, dissolution of corporations or partnerships and name changes of corporations require notification of transfer. The transfer of leases from an estate to the legal inheritors also requires notification of transfer. The transfer of oyster leases among household members or closely related kin may also occur, possibly to circumvent acreage limitations set by La.R.S.56:432.

In an effort to evaluate in greater detail those transfers which represented a change in lease ownership, the lease transfer data were partitioned to isolate those transfers which represented a change in lease ownership. Partitioning of the data in this manner suggested that an estimated 259 of the 299 total registered transfers during the 1950s, or 87% of the total, represented a change in lease ownership (see Tables 1 and 2). During the 1960s, 1970s, and 1980s, the percentage declined to 75%–80% (300 transfers of the 380 total, 474 of the 628, and 873 of the 1,184).

Water bottom transfers which included ownership changes (i.e. outside the household transfers) historically involved fewer leases, on average, than what was represented by the total (see Tables 1 and 2), indicating that more leases were typically transferred when ownership rights did not change. This difference, however, appears to have diminished during the 1970s and 1980s. For example, transfers which represented a change in lease ownership averaged 3.28 leases per transfer during the 1950s compared to 3.99 leases among all transfers. By the 1980s, transfers representing ownership change represented 2.64 leases, compared to 2.92 leases for all transfers during the period, on average.

As noted, 1,192 leases were transferred during the 1950s compared to 3,462 during the 1980s (Table 1). About 70% of those transferred during the 1950s involved a change of ownership; 850 of the 1,192 (see Tables 1 and 2). Slightly more than 60%, or 985 of the 1,589 leases transferred during the 1960s entailed an ownership change. About two-thirds of the total leases transferred during the 1980s (2,305 of the 3,462 total) involved ownership change.

Of the 19,302 acres of water bottoms transferred during the 1950s, an estimated 14,218 acres, or 74% of the total, involved a change in ownership (see Tables 1 and 2). During the 1960s, an estimated 26.1 thousand acres, or about 65% of the total 40.7 thousand acres transferred, represented a change in ownership. From 65% to 70% of the acreage transferred during the 1970s and 1980s changed ownership.

The information provided above can be used to assess the annual rate of leased acreage turnover in the Louisiana oyster industry. A lower turnover rate would indicate increased stability. Furthermore, changes in the turnover rate through time can be compared to industry production and revenues to evaluate whether change in the later influence the rate of turnover.

TABLE 2.

Selected statistics related to Louisiana's registered oyster water-bottom transfers representing a change in ownership, 1950–89 ten year averages.

Time Period	Number of Transfers	Avg. No. Leases Per Transfer	No. of Leases Transferred	Avg. Acres Per Lease Transferred	Avg. Acres Per Transfer	Total Acres Transferred
1950–59	259	3.28	850	16.7	55	14,218
1960–69	300	3.28	985	26.5	87	26,114
1970–79	474	3.85	1,827	31.4	121	57,316
1980–89	873	2.64	2,305	46.9	124	108,203

Source: Compiled from unpublished data maintained by Louisiana Department of Wildlife and Fisheries, Oyster Division.

The information in Tables 1 and 2 suggests that an average of 1,930 acres were transferred on an annual basis during the 1950s of which 1,422 acres represented a change in ownership. Total number of acres leased on an annual basis during the 1950s, based on the information in Figure 3, was about 44 thousand (using 1955 acreage, i.e. midpoint, as average). Dividing the average annual number of acres transferred by the average annual number of acres leased indicated that total annual turnover during the 1950s equalled 4.4% while acreage turnover representing a change in ownership equalled 3.2%. Estimated annual turnover rate of all acreage and that representing a change in ownership during the 1960s, 1970s, and 1980s, is given as follows: 1960s (4.2% and 2.7%), 1970s (4.3% and 3.0%), and 1980s (6.1% and 4.1%). These numbers, provided in Table 3, indicate that the turnover rate of acreage representing a change in ownership has remained relatively constant during 1950–89 when evaluated on a ten year basis, except for a moderate increase during the 1980s. This indicates a high degree of stability in oyster lease based operations in Louisiana; approximately a 40% turnover of acreage (changing ownership) during the 1980s.

The relative stability in Louisiana's oyster lease based operations appears to be independent of a changing industry in aggregate. For example, the deflated dockside value of Louisiana's oyster production derived from the state's private grounds increased from $8.1 million annually during 1950–59 to $20.5 million annually during 1980–89 (Table 3) despite a much more stable production as measured by pounds harvested (Table 3). On a per acre basis, production from private grounds fell steadily during 1950–89, from 141 pounds per acre annually during 1950–59 to 34.6 pounds per acre annually during 1980–89 (Table 3). Because of an increasing deflated dockside price, however, the value (deflated) of production per acre fell significantly less than the pounds produced. In fact, virtually no change in the value of harvest per acre was observed since the 1970s despite more than 100 thousand acres being added to the total leased.

Analysis of Lease Value

As previously noted, lease holders are obligated to notify the LDWF when transferring leases. The information is then recorded and filed. This information often includes a "Bill of Sale." In collecting all transfer data, information pertaining to the conditions of sale was also collected.

Use of the "Bill of Sale" information required several modifications before analysis of the economic value of leased based operations could be conducted. These modifications included the following. First, all transfers not representing a change in ownership were deleted. Second, all "Bill of Sales" which stated a monetary value of sale plus "other considerations" were deleted. Third, all "Bill of Sales" which included assets in addition to leases, such as boats, were deleted. Fourth, all "Bill of Sales" which did not explicitly provide a monetary value were excluded. Finally, those transfers which did not include a "Bill of Sales" were excluded.

After making the aforementioned modifications, the "Bill of Sales" data were used to analyze the value of transferred oyster leases in Louisiana on a ten-year basis. Results are contained in Table 4. As indicated, the value of leases transferred in Louisiana declined from an estimated $127 per acre during the 1950s to $66 during the 1980s, or slightly less than 50%. Most of the decline occurred between the decade of the 1950s and the decade of the 1960s. The estimated value per acre among oyster leases transferred during the 1980s was only 15% below the estimated value during the 1960s. Furthermore, the estimated decline during the 1980s may reflect the 1981 increase in annual rental fees from $1.00 to $2.00 per acre.

While the estimated value among leases transferred in Louisiana declined from an average of $127 per acre during the 1950s to $66 per acre during the 1980s (Table 4), the average annual productivity per acre declined from $185 to $77 (see Table 3). Thus, the decline in the estimated value of leases transferred (48%) was somewhat less than the decline in productivity per acre (58%). This may be due to increased competition for available acreage as productive acreage is becoming increasingly scarce.

A conservative estimate of the value of Louisiana's leased acreage with respect to its use in oyster production can be derived by multiplying the estimated value per acre of oyster leases transferred (Table 4) by acreage leased for the different time periods. The results are considered conservative for at least two reasons. First, the estimated values in this paper have been derived solely from transferred leases. It is possible that more productive leases are transferred less frequently. Second, many of the transfers included assets other than leases. There were not used in the analysis because separating value of leases from other assets was impossible. It appeared, however, as though acreage values among these transfers were higher than the averages reported in Table 4. Sale of other assets in addition to leases suggests a dissolution of business in which case more productive leases would also be sold.

As indicated in Table 4, the estimated value of leased acreage to the production of oysters in Louisiana increased from $5.6 million during the 1950s to $17.2 million during the 1980s. This

TABLE 3.

Selected statistics related to Louisiana's oyster production, leased acreage, and turnover of leased acreage.

Time Period	No. of Acres Transferred as % of Acres Leased		Production from Private Grounds		No. of Acres Leased	Production Per Acre Leased	
	Total	Change in Ownership	1,000 lbs.	$1,000 (def)[a]	1,000s	lbs.	$ def.
	Avg. Annual						
1950–59	4.4	3.2	6,202	8,137	44	141.0	184.9
1960–69	4.2	2.7	8,076	10,575	97	83.3	109.0
1970–79	4.3	3.0	8,502	14,855	193	44.1	77.0
1980–89	6.1	4.1	9,177	20,486	265	34.6	77.3

[a] Value has been deflated based on the 1989 Consumer Price Index for all goods and services.

TABLE 4.

Estimated value per acre of oyster leases transferred in Louisiana and estimated total value of leases (1989 dollars).

Time Period	Value Per Acre[a] ($)	Value of Leased Acreage ($ mill.)
1950–59 avg.	127	5.63
1960–69 avg.	76	7.34
1970–79 avg.	76	14.69
1980–89 avg.	66	17.22

Source: Compiled from unpublished data maintained by Louisiana Department of Wildlife and Fisheries, Oyster Division.
[a] Values have been deflated based on the 1989 Consumer Price Index for all goods and services.

increase was the result of expansion in acreage leased. Private-grounds production of oysters expanded from $8.2 million annually to $20.5 million annually during the same period.

As indicated in Table 5, the estimated value per acre among registered transfers varied inversely with the total number of acres transferred.[4] During the 1950s, for example, transfers of less than fifty acres in total were valued at $186 per acre compared to $140 among transfers containing 50–100 acres, $108 among transfers of 101–500 total acres, and $13 for the one transfer including more than 500 acres in total. Estimated values per acre during the 1980s were all less than those estimated during the 1950s for the different transfer size classifications, except those in excess of 500 acres.

Leases Serving as Collateral

Louisiana laws governing the leasing of the state's water bottoms are conducive to the practice of using leases as collateral in the securement of loans. For instance, La.R.S.56:423 provides for the transfer of leases. Hence, a lending agency can take possession of leases in the event of default by a borrower. Also, the relatively long duration of leases and renewed rights, as described in La.R.S.56:428, gives an incentive to potential borrowers to secure loans for rehabilitation and expansion projects and also provides lending agencies additional security when providing loans.

The Farmers Home Administration was found to be the primary source of loans for oyster lease-based operations in Louisiana. In total, there were 26 outstanding loans with the FmHA as of January 1, 1991 (this figures does not account for loans that may have been paid back before date of final installment) with total principal of $1.56 million, or $60.2 thousand per loan. Three-hundred and thirteen leases totaling 12,007 acres, along with other assets, were used as collateral. This suggests that the maximum value placed on acreage used as collateral is about $130 per acre, on average. This is considered a maximum because other assets are also required in securing FmHA loans for oyster lease based operations.

In addition to the FmHA, private financial institutions were also surveyed to assess their participation in making loans to oyster lease-based operations. Personnel at FmHA provided the authors with a list of six financial institutions likely to be involved in such activities. The authors surveyed these six institutions and received five responses.

Overall, only one of the five financial institutions responding to the survey reported current outstanding loans to the oyster industry while three others reported that they had made loans previously but none were currently outstanding. Most banks reported that they did not consider an oyster lease as collateral on a loan but if so, only as secondary collateral. For confidentiality purposes, the dollar amount of loans outstanding for oyster harvesting related activities cannot be reported.

The relative paucity of private institution loans outstanding for oyster harvesting related purposes indicates that the FmHA may adequately serve the oyster industry's needs. Its lower interest rates and familiarity with the industry are likely "key" selling points to the industry when applying for loans, especially after disasters such as freezes. It is apparent, however, that significant leverage capacity is still available for future reef rehabilitation efforts.

DISCUSSION

While the results presented in the last section of this report are specific to Louisiana, they should also be of interest to other states in the Southeast as they plan their respective rehabilitation activities. Louisiana, it was shown, encouraged long-run maintenance and expansion of lease-based oyster operations by providing a climate conducive to leasing activities in the state. In particular, two state statutes, one that provides current leaseholders with the

[4]Monetary values in the "Bill of Sales" were generally not given for each individual lease, among those transfers containing more than one lease. Therefore, analysis could not be conducted which directly related estimated value to lease size.

TABLE 5.

Estimated value (1989 dollars) per acre transferred by size of transfer, 1950–89.

	Acres Transferred							
	<50 Acres		50–100 Acres		101–500 Acres		>500 Acres	
Time Period	$ Per Acre[a]	No. of Transfers	$ Per Acre	No. of Transfers	$ Per Acre	No. of Transfers	$ Per Acre	No. of Transfers
1950–59	185.8	151	139.8	20	108.2	22	13.4	1
1960–69	116.0	151	118.2	21	103.2	34	18.9	8
1970–79	138.3	217	90.1	53	75.1	65	56.4	20
1980–89	129.1	302	73.0	85	75.5	96	40.5	32

Source: Compiled from unpublished data maintained by Louisiana Department of Wildlife and Fisheries, Oyster Division.
[a] Values have been deflated based on the 1989 Consumer Price Index for all goods and services.

first right of renewal when the lease expires and one that provides that leases are heritable and transferrable, provide a climate in which leaseholders are able to make long-term investments and improvements in their leasing operations.

Some of the key findings reported in this paper include the following:

1. Turnover of leased acreage in the Louisiana oyster industry is relatively low and appears to be independent of economic conditions within the industry.
2. The estimated value of leases transferred in the state has declined sharply during the past four decades when evaluated on a per acre basis. This decline is in direct relation to declining oyster productivity (pounds harvested and dockside value) per acre.
3. The estimated value of oyster transfers appears to be inversely related to the number of acres transferred when evaluated on a per acre basis.
4. The Farmers Home Administration was found to be the primary lending institution for the Louisiana oyster industry. Private financial institutions had relatively few outstanding loans to the oyster industry.

As other Southeast states consider leasing activities as a means of rehabilitating their respective oyster industries, the findings discussed above can be used to evaluate possible outcomes associated with alternative actions.

ACKNOWLEDGMENTS

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INTERACTIONS BETWEEN SHELLFISH AND GROUNDFISH FISHERIES ON THE WEST COAST: IMPLICATIONS FOR SYSTEM MANAGEMENT

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ABSTRACT Fisheries are commonly defined and managed on a biological, single species basis. Linkages between stocks created by the operation of multipurpose fishing fleets create new fishery systems that cross species boundaries. Linkages between fisheries have both a biological and economic basis. This paper describes an important three-fishery system on the West Coast: groundfish trawl, shrimp trawl, and crab pots. The nature of fishery interactions is described. Factors linking the fisheries are detailed, including biological dynamics, economic dynamics, and risk reduction strategies. Implications of the three-fishery system for fishery management are discussed in the context of the timing of fishing seasons, interstate fishing effort, access limitation, and multifishery management.

KEY WORDS: multipurpose fishing strategies, groundfish trawl, shrimp trawl, crab pots, fishery linkages, fishery management.

INTRODUCTION

Fisheries are commonly defined and managed by the biological distinctions of fish populations. Biologically-based definitions of fisheries are an outgrowth of years of fisheries management in which quotas have been set according to best estimates of the reproductive requirements of a single stock. On the few occasions that groupings of stocks do occur in management, they are at most groupings of similar categories of species; e.g. finfish, shellfish.

Although undertaken for legitimate conservation reasons, biologically based definitions of fisheries miss an important element of fishery structure and function: system properties which result from the linkages between stocks created by the operation of multipurpose fishing fleets. Such linkages cross species and species category lines, and may include, for example, both shellfish and finfish species caught by multipurpose vessels using an array of gears.

The history of development in capture fisheries has been one of increasing degree of interactions between target fisheries. In the initial stages of fishery development, stock abundance may allow fishermen to target single stocks or assemblages of similar species. As the fishery develops, the increase in fishing effort and the concomitant decrease in population levels results in the need for fishermen to diversify their fishing strategies. In the West Coast groundfish fishery, for example, over time fishermen have diversified over the number of groundfish species as well as over the number of fishing activities in groundfish and other fisheries. (Carter 1981, Hanna 1991).

Once multispecies or multifishery fishing strategies are established, single-species biologically-based management approaches become obsolete, reflective of an earlier and simpler stage of interaction between human systems and fish populations. Multifishery fishing strategies define a new fishery environment which requires that management broaden its approach from biological considerations alone to a system of biological and economic components.

One implication of multispecies and multifishery fishing strategies is that components of fishing effort become more heterogeneous and therefore more difficult to link to a single population of fish. Wilen (1979) notes that because fishing technology is flexible, attempts to manage fisheries on the basis of restrictions placed on only a few components of effort are futile. Lieberman (1986) supplements Wilen's argument about flexible technology by arguing for an approach to fishery management that includes social as well as technical factors and is explicitly dynamic to accommodate for changing fishing patterns. Golden (1991) details the structure of "transfer capacity" in the West Coast trawl fleet which introduces complexity into the definition of a fleet.

Charles (1989) concludes that because one component of management is to predict the response of both fishermen and fish stocks to changing fishery conditions, management must account for the joint dynamics of both fish stocks and fishermen. Similarly, in earlier work I describe the increasing diversification of assemblage fishing in the West Coast groundfish trawl fishery and argue for using the economic and biological properties of groundfish assemblages as the structural basis for management (Hanna 1987). Mitchell (1982) notes that understanding the tradeoffs facing fishermen in their choice of fisheries is necessary for adequate control of multispecies fishing effort.

A common multifishery interaction on the West Coast exists in the Dungeness crab (*Cancer magister*), pink shrimp (*Pandalus jordani*), and groundfish (*Sebastes* spp., flatfish, and roundfish) fisheries. The dynamic relationship between these finfish and shellfish fisheries provides a classic example of fishery linkages which create a new definition of a fishery system.

This paper begins by describing the historical development and current status of groundfish, shrimp and crab interactions. Data from Oregon fisheries are used to illustrate the interaction. Next, the nature of these linkages are outlined through the identification and analysis of several factors which create the biological and economic dynamics of these fisheries. In the following sections, several management questions related to these multifishery interactions are discussed, including implications for system management in this fishery and others.

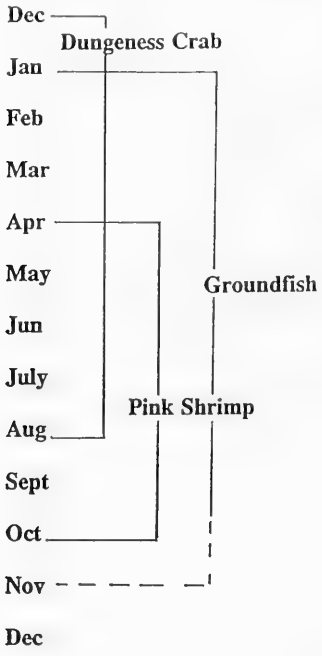
MATERIALS AND METHODS

Data on Oregon fisheries were acquired from several sources. Summaries of landings and gross revenue by fishery are published by the Oregon Department of Agriculture (1990). Unpublished data on individual vessel landings by gear type were provided by the Oregon Department of Fish and Wildlife. Data on individual vessels cover landings in Oregon from 1977–1990 by the three major gear types in the groundfish, shrimp and crab fisheries:

trawl and pots. Yearly summaries of Oregon crab and shrimp fisheries appear in a series of reports (Demory 1991, Bruneau 1980, Jones and Hannah 1991, Lukas 1978, Oregon Department of Fish and Wildlife 1984, Saelens et al. 1981, 1982, Saelens 1983, Saelens and Zirges 1985, Zirges and Robinson 1980). Histories of fishery development and regulation are presented in documents of the Pacific Fishery Management Council (1979, 1981, 1990).

Two major categories of fishery participation strategy are defined: specialists and generalists. The categorization of fishing strategy as specialist or generalist, advanced by Smith and McKelvey (1986), depends on the number of fishing activities in which a fisherman participates. Specialists pursue a single fishing activity and adapt to a changing environment by modifying this activity. Generalists adapt to change by adopting an array of fishing activities. Specialists are defined here as fishermen who in a given year pursue only one fishery within the three fishery system. Generalists are defined as fishermen who in a given year use a fishing strategy which includes more than one of the three fisheries.

Because this paper looks at data from the trawl and pot fisheries only, the strategy categories are defined within the context of these two gear types. The terms "specialist" and "generalist" are used in the context of this trawl-pot fishery system definition. Therefore crab fishermen who are defined as "specialists" are specialists within the context of the three target fisheries discussed herein. These same crab fishermen may in fact develop generalist fishing strategies which combine crab fishing with line fisheries such as albacore and salmon. For the purposes of simplicity of discussion, the paper is limited in focus to the three-fishery system of groundfish trawl, shrimp trawl and crab pot gear. However, the conclusions reached about management of this system also apply to other strategy combinations.



Data Source: Oregon Department of Fish and Wildlife 1991.

Figure 1. Oregon Fishing Seasons for Groundfish, Shrimp, and Crab.

TABLE 1.

Fishery participation strategies in three major Oregon fisheries, 1977 to 1990.

	Percent of Fleet		
	Range	Mean	Std. Dev.
	Specialists		
Crab pots	80–92%	81.8	1.92
Shrimp trawl	51–71%	60.4	6.83
Groundfish trawl	29–89%	49.2	20.63
	Generalists		
Groundfish/Shrimp	11–71% GF*	46.5	15.57
Shrimp/Crab	15–36% S**	25.3	9.86
Groundfish/Crab	28–30% GF*	11.8	3.03
Groundfish/Shrimp/Crab	5–15% GF*	9.9	3.31

* GF = Fleet landing primarily groundfish.

** S = Fleet landing primarily shrimp.

Data Source: unpublished fish ticket data, Oregon Department of Fish and Wildlife.

WEST COAST SHELLFISH-FINFISH FISHERY INTERACTIONS

One component of fishery interaction is the timing of fishing seasons. Existing Oregon fishing seasons for crab, shrimp, and groundfish are depicted in Figure 1 (Oregon Department of Fish and Wildlife 1991). The Dungeness crab ocean season (pot gear) opens in December and continues until August. The groundfish trawl fishing season begins in January and continues until quotas are filled in the fall, with specific closing dates varying by species. Season length is regulated to some extent by trip quotas which are set to prevent a glut of landings at the beginning of the fishing year. The shrimp fishing season opens in April and generally continues until October.

Figure 1 illustrates that for 10 months of the year, from January until October, fishing seasons of the three fisheries overlap. The existence of overlapping seasons means that fishermen with vessels capable of fishing any of the three fisheries can choose between fisheries. Fishermen will choose a fishery or combination of fisheries on the basis of expectations about earnings, expectations about stock abundance and prices, and costs of investing in or changing gear.

The proportion of the fleet using a specialist strategy varies from fishery to fishery. The proportion of specialists also varies within a fishery over time. Table 1 illustrates the range of proportion of each fleet which participated in a specialist strategy in the years between 1977–1990. Percentages represent the proportion of boats landing that species category in a given year which land only that category.

Within the three-fleet combination of groundfish trawl, shrimp trawl and crab, fishermen using crab pot gear tend to use more specialized strategies than either shrimp or groundfish trawlers: an

average of 81.8% of the crab fleet fished only for crab during the time period, with small variation about the mean. This high proportion of specialists is due in large part to a masking effect of the database. The crab fleet includes both trawl-pot boats which use trawl gear for shrimp and groundfish and pot-troll boats which use pot and line gear to pursue crab, salmon and albacore (Fletcher et al. 1988, Demory 1991). The pot-troll boats do not have the capability of using trawl gear to catch shrimp or groundfish. Of the boats which are estimated to be trawl capable, the percentage of crab specialists is much lower. For most commercial fishermen, the crab season is not long enough to sustain a specialist crab fishing strategy.

The proportion of specialist shrimp trawlers which landed only shrimp in a given year varied between 51 and 71% in the 13 year period, averaging 60.4%. The proportion of specialist groundfish trawlers landing only groundfish in a given year was the smallest (49.2%) of the three fisheries and had the largest variation about the mean. Some of the specialist vessels change their specialization from year to year; for example, a fisherman may fish only shrimp one year, only groundfish the next.

The most common combination of fishing strategies in the specified three fleet combination is the groundfish/shrimp combination (Table 1). The number of boats using this strategy is calculated as the number of boats whose landings are primarily groundfish, who also land shrimp. The pursuit of this fishing strategy varies widely, from 11 to 71% of the total number of groundfish boats over the time period. The shrimp and crab combination strategy comprises between 15–36% of the vessels landing shrimp. This combination is economically important to some fishermen who fill in the period of closed shrimp season with two or three months of crab fishing (Zirges and Robinson 1980). The current fishing season structure, based on times of biological availability, promotes groundfish-shrimp and shrimp-crab as the most compatible strategy combinations (Fig. 1).

Port differences influence the choice of fishing strategy. Oregon has three major fishing ports: Astoria, Newport, and Coos Bay. Astoria has the largest number of groundfish specialists. Severe winter weather prevents many Astoria fishermen from actively pursuing crab fishing. Newport is the most diverse of the three ports, with all fishing strategies represented. The most common generalist strategy in Coos Bay is the groundfish and shrimp combination (Smith and Hanna 1991).

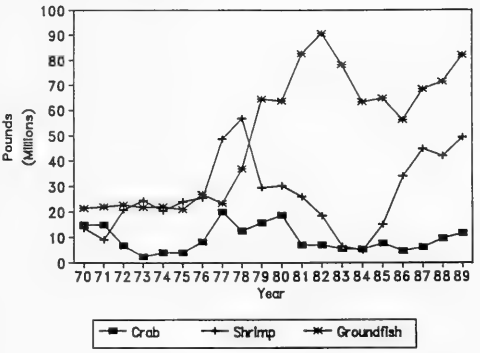
MULTIFISHERY LINKAGE FACTORS

The fishing strategies which link the three fisheries are the result of incentives created by both biological and economic dynamics.

Biological Dynamics

The changing patterns of landings of crab, shrimp and groundfish between 1970 and 1989 are a partial representation of the biological variation of stocks (Fig. 2). Landings of Dungeness crab reflect well-recognized but little understood population cycles. High points in landings occurred in 1970–71, 1977, 1980. Intra-year fluctuations are caused by variation in the timing of the molt period which influences both the catchability and the "fullness" of crab (Demory 1991).

Shrimp populations are also subject to cycles. Peaks in Oregon landings in 1977, 1978, and 1989 reflect recent cycle crests. Variation in the timing of spawning will also affect fishing, as seasons



Data Source: Oregon Department of Agriculture 1990

Figure 2. Landings of Groundfish, Shrimp and Crab into Oregon Ports, 1970–1989.

are set to protect gravid shrimp. Deterioration in flesh quality during spawning also influences the timing of fishing for shrimp (Zirges and Robinson 1980).

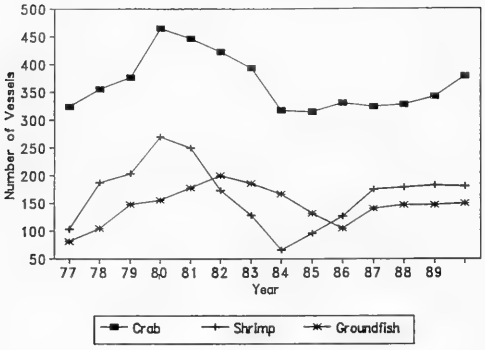
Groundfish landings increased continually between 1977 and 1982 as post-Magnuson Act fishing effort fished down the surplus of relatively unexploited stocks. Substantial in-year variation in landings exists as a result of seasonal onshore-offshore migration of stocks (Hanna 1987). Flesh quality of some species, e.g. Dover sole, is affected by season and water depth (J. Easley 1990). Community dynamics of groundfish assemblages, i.e. the structural compensation to selective harvesting of species, are still little understood (Tyler et al. 1982). Since 1982 various groundfish regulations have been put in place to maintain stocks at equilibrium yield levels, including single-species trip quotas and some initial attempts at assemblage trip quotas (Pacific Fishery Management Council 1990).

Population cycles, inter-year variability in stock location, and intra-year changes in location, spawning periods, and molt periods create a system of biological relationships characterized by high levels of variability. West Coast fishermen respond to this variability in their formation of expectations about biological influences on fishing opportunities in groundfish, shrimp and crab. Fishermen's expectations in turn underlie their planning and investment decisions which drive the distribution of fishing effort linking the three fisheries.

Economic Dynamics

Economic dynamics are also at work creating connections between the three fisheries. Some of these dynamics reflect the results of individual fishermen's decisions, others reflect economic forces at levels beyond the control of individual fishermen.

The influence of various economic factors in directing fishing effort between the three fisheries underlies patterns illustrated in Figure 3, which shows vessels landing in Oregon ports between 1977 and 1989. It is important to note that because vessels participate in more than one of these fisheries, a single vessel may be represented in the shrimp fishery in one year, and the crab fishery in another year. Fishermen using specialist strategies will be rep-



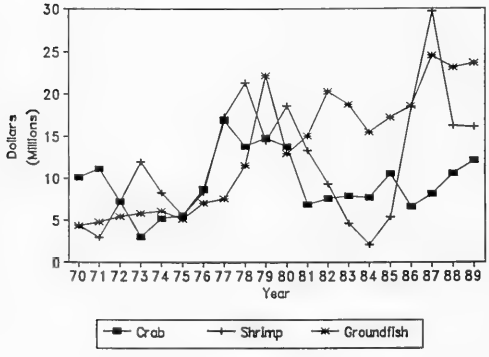
Data Source: Unpublished Data, Oregon Department of Fish and Wildlife

Figure 3. Vessels Landing Groundfish, Shrimp and Crab into Oregon Ports, 1977–1989.

resented in only one fishery in a given year, although they may be represented in different fisheries in different years. Fishermen using generalist strategies will be represented in more than one series in a given year.

A major driver of the economic dynamics of the fishery are individual fishing strategies developed to reduce the risk posed by a variable earnings environment. Changes in both landed quantity and exvessel price in the three fisheries lead to large variability in exvessel gross revenues to fishermen, illustrated in Figure 4. In any economic system, economic variability and the uncertainty it creates lead risk-averse people to adopt various uncertainty-reducing strategies. One of the common strategies used to reduce economic variability is to diversify over several sources of revenue. Multifishery fishing strategies are an example of such diversification.

Diversification usually, but not always, benefits fishermen in the form of reduced variability of landings and earnings. Table 2 illustrates one of these benefits: reduced year-to-year variability in production and earnings. Variability is measured as the coefficients of variation (c.v.) of landings and gross revenues (revenues before costs) for the various fishing strategies.



Data Source: Oregon Department of Agriculture 1990. Series deflated using Producer Price Index , Base Year 1980.

Figure 4. Real Exvessel Gross Revenues from Oregon Groundfish, Shrimp and Crab, 1970–1989.

Looking first at quantities landed, three specialist strategies of groundfish, shrimp, and crab show coefficients of variation in quantities landed of 49%, 53%, and 54% respectively. Groundfish fishermen who also fished shrimp (strategy G/S) were able to reduce the c.v. of their landings to 38%. By adding a third fishery to their fishing strategy, they realized a further reduction of the c.v. of their landings to 35%. Groundfish fishermen were able to stabilize inter-year production by increasing the number of fishing activities.

The reduction in variability of landings for shrimp fishermen through generalization of strategy showed a similar trend. Specialist shrimp fishermen had a c.v. of landings of 53%. Adding crab fishing to their strategy reduced the c.v. of landings to 48%. Fishermen who fished the groundfish/shrimp combinations had even lower c.v.'s; 38%. A generalist strategy which included all three fisheries lowered the c.v. to 35%.

For crab fishermen, adopting a generalist strategy reduced the variation of landings from 54% to 48% when shrimp fishing was added, to 35% when the fishing strategy included all three fisheries.

In terms of stabilized earnings, where earnings are represented here by average gross revenues, the benefits to fishermen of adopting generalized fishing strategies are mixed. In the case of shrimp fishermen, the addition of groundfish fishing to their strategy reduces variability in average gross revenues from 50% to 37%. Variability in earnings is reduced still further to 34% with the addition of the third fishery, crab, to the strategy.

For groundfish fishermen, the coefficient of variation of a combined groundfish/shrimp strategy (37%) is actually greater than for groundfish alone (35%), but is reduced slightly to 34% with the addition of the third fishing activity, crab, to the strategy. The addition of crab to the groundfish strategy also increases the c.v. of average gross revenues. This phenomenon is largely due to the lower variability of landings of groundfish and fairly constant exvessel prices for groundfish species over the time period examined. However, the continued existence of groundfish/shrimp generalists indicates that other factors besides stability in earnings are

TABLE 2.

Coefficients of variation (%) of quantities landed and average revenues earned using different fishing strategies in Oregon, 1977–1989.

A. Coefficient of variation (%) of quantities landed

Specialist Strategy*			Generalist Strategy*			
G	S	C	G/S	G/C	S/C	G/S/C
49	53	54	38	42	48	35

B. Coefficient of variation (%) of average revenues

Specialist Strategy*			Generalist Strategy*			
G	S	C	G/S	G/C	S/C	G/S/C
35	50	26	37	58	40	34

* G = groundfish; S = shrimp; C = crab; G/S = groundfish/shrimp; G/C = groundfish/crab; S/C = shrimp/crab; C/S/G = crab/shrimp/groundfish.
Data Source: Oregon Department of Agriculture (1990).

important to fishermen in this fishery. Despite the failure to reduce variability in earnings through this strategy of groundfish and shrimp, trawl fishermen reap benefits from the combination in the form of higher, albeit cyclical, earnings.

In contrast to the other two types of specialists, crab fishermen gain nothing in reduction of variability of revenues through the adoption of generalist fishing strategies. Variability in earnings from crab fishing alone was 26% over the time period, for crab combined with shrimp it was 37%, and for crab combined with shrimp and groundfish it was 34%. This phenomenon is due in part to the influence of the large number of very small specialized crab vessels in the "fleet" database which land small, unvarying quantities of groundfish throughout the season and from year to year.

A second major element of individual decision making which drives the economic dynamics of the groundfish-shrimp-crab fishery is the short-term effort responsiveness to changes in relative prices of groundfish, shrimp, and crab, changes in regulations, market conditions, and processor arrangements. Fishing effort is the outcome of a multicomponent choice set which includes expected net earnings of the primary fishery, expected net earnings of substitute fisheries. The choice set is modified by a constraint set which includes available technology, regulations, biological availability, processor limits, and general market conditions.

The short term responses between fisheries are extremely complex, as evidenced by the groundfish-shrimp interaction between 1979–1989. Figure 4 illustrates the changes. Between 1979 and 1983, real shrimp prices were generally increasing, while real groundfish prices were in general decline. However, volumes of available groundfish were high, and the increase in shrimp prices did not attract increases in the number of groundfish fishermen who landed shrimp. Processor constraints also played a role, as groundfish processors were reluctant to diversify their operations to include shrimp. In 1984, shrimp landings were low despite high prices, as the stock responded to the 1983 El Nino southern oscillation event (ENSO) (Jones and Hannah 1991). Between 1985–1987, average real shrimp prices increased 89%, from $.35 to .66. This price increase was followed by a corresponding increase in the proportion of groundfish vessels who fished the groundfish/shrimp combination activity: from 31% in 1985 to 58% in 1987. During this same period, groundfish prices were low as processors imported cheaper Canadian product. The proportion of groundfish specialists fell from 69% to 42%. Between 1987 and 1989, real shrimp prices fell 42% to an average of $.33, but the percent of groundfish vessels fishing the groundfish-shrimp activity remained at 58%. The price responsiveness of substitution between groundfish and shrimp was diminished over this period due to regulatory constraints in groundfish which introduced strict quantity controls on landings per trip.

Fishing in a single activity is often interrupted when price disputes between fishermen and processors cannot be resolved. For example, a mid-season downward price adjustment the 1989 Oregon shrimp fishery interrupted shrimp landings for a period of one month as processors and fishermen negotiated prices. Large landings early in the season left processors with large inventory holdings, leading processors to lower prices offered as a means to reduce inventory and cover storage costs (Jones and Hannah 1991).

Markets and general economic forces outside the control of individual fishermen are the second driver of economic dynamics. The exvessel market for groundfish, shrimp and crab is influenced by an array of economic factors. One is the buying patterns of fish processors. Oregon processors have at times objected to simultaneous landings of crab and shrimp, preferring to direct labor and equipment to one species (Zirges and Robinson 1980). In the period of early expansion of the groundfish fishery, many groundfish processors would buy only groundfish from groundfish trawl vessels, forcing a degree of specialization on vessels. In recent years, limitations on the supply of groundfish and shrimp have meant that processors have been more willing to buy both groundfish and shrimp from individual vessels (R. Young 1991).

Fluctuations in the exchange rate also influence the exvessel market for Oregon-caught groundfish, shrimp and crab. When foreign source groundfish are available at lower price than Oregon groundfish, West Coast processors have substituted imported groundfish for local groundfish in their production line. For example, in 1985 and 1986 a strong U.S. dollar in the Canada-U.S. exchange rate created a situation favorable to the import of relatively cheaper Canadian groundfish by U.S. processors. As Canadian groundfish were substituted for U.S. groundfish at the processing level, many vessels redirected their effort from groundfish to shrimp (Fig. 3).

Finally, in each of the three fisheries ex-vessel prices paid to fishermen are influenced by larger national or international markets characterized by their own substitution relationships. For example, Oregon wild shrimp compete in regional, national and international markets with cultured shrimp. Dungeness crab compete domestically with surimi-based crab analogues and in a European export market with other crab species and substitute shellfish species (Demory 1991). Groundfish compete at the retail level with other protein sources, namely poultry (P. Leipzig 1990).

IMPLICATIONS OF MULTIFISHERY LINKAGES FOR SYSTEM MANAGEMENT

Both generalist fishing strategies and inter-year changes in specialist strategies create linkages between the fisheries which in turn create unique management issues not faced in specialized fisheries. Major issues in the management of the West Coast groundfish, shrimp and crab fisheries include the timing of fishing seasons, the management of interstate fishing effort, the limitation of access to fishing opportunities, and the economic attributes of multifishery activities.

Timing of Fishing Seasons

The existence of an array of choices for fishermen who fish generalist strategies means that the composition of a fishing fleet is not static, but varies within and between seasons as fishermen combine fishing activities into strategies of their choice. Predictions of actual fishing effort on an individual stock are therefore difficult to make, but are assisted by an understanding of economic linkages and of the proportion of the fleet with transfer capacity. The timing of fishing seasons becomes a critical factor in the management of effort levels and in the time spacing of effort in a single fishery. Setting seasons on a per-fishery basis in isolation of other fisheries in the system guarantees that one fishery's season will generate external impacts on another fishery.

There are two general complementary outcomes of coordinated timing of fishing seasons across fisheries. The first is to maximize the probability of controlling the timing and level of fishing effort on various stocks. The second is to maximize the planning and coordination of the fishing year in ways that allow explicit consideration of enhancing the landed value of fish. Fishery interac-

tions require that for maximum management effect in these two areas, the timing of fishing seasons should be decided on a coordinated basis with both biological and economic linkages taken into account. Pre-season information on market conditions (demand, availability of substitutes, expected exvessel price) combined with information on biological conditions (stock size, recruitment, flesh quality, timing of molt) can be used to good effect in the planning of season timing.

For example, as Figure 1 illustrates, managers have the ability to lessen the impact of fishing effort on Dungeness crab in the early season by delaying the season opening one month to coincide with the groundfish season opening. By the same token, managers may pace crab landings throughout the season by avoiding the initial surge of fishing effort on December. The potential economic benefits of paced landings include the avoidance of landings gluts which depress market prices, and the harvest of "full" animals. The coincidence of the shrimp and groundfish seasons combined with the popularity of the shrimp-groundfish strategy combination provides the opportunity to alternate open and closed periods in these two fisheries if needed to lessen effort impacts on stocks or avoid market gluts. Joint groundfish-shrimp seasons could be planned on the basis of a market strategy to maximize joint value.

Interstate Fishing Effort

The crab, shrimp, and groundfish fisheries on the West Coast are managed under different jurisdictions in a mixed system of regulations. Although Dungeness crab is regulated separately by California, Oregon and Washington, no state's system of regulations operates in isolation. The timing and quantity of crab landings in a given state are influenced by the timing of seasons in a neighboring state. For example, in the years when Washington crabs molt late and are not available to the fishery at the December 1 opening, the Washington fleet shifts south for December fishing. The effect of effort location shifts is to increase the early-season fishing pressure on Oregon stocks as well as create landings gluts in Oregon ports and markets (Demory 1991, Heikkila 1991).

In the fall of 1990, a Tri-State Dungeness Crab Committee of crab fishermen was organized under the auspices of the Pacific States Marine Fisheries Commission to address the interstate effort issue and other concerns facing the crab fleet. In the organization of the committee, attempts were made to ensure representation from each port and vessel size class. Five issues were defined by the industry group for discussion and negotiation: early season opening date to be responsive to molt condition, late season closing date to respond to molt condition, a moratorium on new entry, enforcement of preseason gear placement regulations, and uniform gear marking (P. Heikkila 1991).

Shrimp have been managed under a license limitation system in Oregon since 1979 (Zirges and Robinson 1980, Saelens and Zirges 1985, Jones and Hannah 1991). No limitation on shrimp licenses exists in Washington or California. The Oregon license limitation program varies the number of permits available for purchase according to estimated of biological availability and includes a provision for commercial fishermen licensed to fish shrimp in other states to purchase up to six "single delivery" licenses per year for landing shrimp in Oregon ports. Since 1989 the number of permits has been limited to 187, with new permits to be sold by lottery only when the number of permits falls below that number. The single delivery provision creates a situation easily taken advantage of by cross-border fishing. Each fisherman in the Washington shrimp fleet, can fish in federal waters off Oregon and land in Washington, or buy 6 single delivery licenses to use for bad weather landings in Oregon (Oregon Department of Fish and Wildlife 1991).

Groundfish, under coordinated state and federal management jurisdiction, is managed consistently in California, Oregon and Washington. Nevertheless, the existence of mobile, multipurpose fleets means that the location of effort concentrations are difficult to predict. Under the current system, managers must account for a confusing array of sources of effort on the groundfish stock in question in the design of regulations to reach conservation goals for each stock. Within the three-fishery system, the practice of partial-system regulation has the effect of redirecting effort from one state to another as well as from one stock to another.

Access Limitation

The crab, shrimp, and groundfish fisheries have each reached a level of exploitation which will require strong effort controls. Unless managers and industry members are willing to continue down the path of increasingly strict effort controls in the form of short seasons or trip quotas, some form of access limitation is the obvious answer.

The current regulatory situation is a tangle of different controls, based on political boundaries. Shrimp is managed by one of the three states, Oregon, in a license limitation system of questionable effectiveness in controlling effort. No license limitation exists in California or Washington. Under the Oregon system, licenses are in fact limited in number, but the number of licenses outstanding and the provision for "single delivery" license purchase enables high levels of effort to be maintained. Supported by strong year classes, shrimp landings were at record high levels between 1988–1990. The concern has been expressed that the fishery is vulnerable to the continued strong recruitment of age 1 shrimp and that without it the stock will not support current effort levels (Jones and Hannah 1991).

A federal license limitation program has recently been developed for three major gears in the groundfish fishery in all three states: trawl, longline, and pots. This program, adopted by the Pacific Fishery Management Council in September 1991, will be implemented in 1993. Groundfish will continue to have an open access quota set aside for fishermen not qualifying under the license limitation program.

Although none of the three states has an access limitation program for crab, recent developments on coordinated interstate management by the Tri-State Dungeness Crab Committee look promising for effort control. A moratorium date which closes options for new entry has been adopted. A process will be initiated to consider the implementation of a limited access program. The Committee has agreed that the long-term goal of such a program is to reduce both the number of crab pots and the number of boats. An individual quota system for pots will be one of the alternatives for consideration (P. Heikkila 1991).

A striking characteristic of the development of the groundfish limited access plan and the establishment of the Tri-State Dungeness Crab Committee has been the coordination between industry and managers. Although the move toward cooperative industry-agency management planning is promising, the element of fishery

system planning is missing. Under current separate planning efforts, these three interrelated fisheries will have at best a piecemeal system of access limitation. Vessels excluded by a particular access limitation program will likely redirect fishing effort to substitute fisheries which remain open access. Regulatory inconsistencies between the three fisheries pose a further challenge to managers. The uncertainty created by the sporadic adoption of limited access programs has resulted in strategic positioning of fishermen to qualify in several fisheries in the expectation of continued limitation of their operations. Individuals fishing in new fisheries to qualify increase aggregate fishing effort, exacerbating the already difficult management task of controlling levels of exploitation.

Multifishery Management

As discussed above, there are various economic reasons why fishermen engage in multifishery activity. Stocks vary in availability as oceanographic conditions affect spawning, molt, and recruitment. Stock variability creates variability in earnings from the fishery which in turn creates the incentive to diversify over several target species. Market conditions affect relative prices of substitute species. Generalist fishing strategies result from the biological and economic variability which are a normal part of any ocean system. The ramifications of continuing these linked fisheries on the traditional individual fishery basis are many: inconsistently timed fishing seasons, external market effects, and unpredictable effort shifts in response to piecemeal restrictions on access.

Multifishery system management could address these problems. An explicit recognition of the three fisheries as a fishery system for management would allow the design of a regulatory system that internalizes external effects of single fishery management. The traditional practice has been that fishery management proceeds along single species lines. Although recognition of multifishery interactions exists, the inertia of habit and traditional thinking have slowed the evolution of management to reflect actual fishery operations.

The practical question of how to implement management planning at the system level remains. This is not a trivial question and requires systematic development of a procedure within the context of the system at hand. However, some basic components of system management can be outlined here: system definition, supporting data, the planning process, definition of management goals, and system evaluation.

Defining the system to be managed is an element which is obviously dependent on context. In the case of the Oregon crab, shrimp, and groundfish system discussed in this paper, multifishery management would require a broader system definition. While crab, shrimp, and groundfish are the large-volume components of the system, a critical role is played for generalist strategies by the albacore and ocean salmon fisheries as well. System definition requires a thorough knowledge of fishery operations which cannot be acquired through secondary data sources.

Data to support analysis and monitoring of both biological and economic components of the fishery system, once defined, are of course a basic need. Current systems of data collection and entry are based on the historical practice of single-species management. The definition of a data base along single fishery lines presents a practical barrier facing analysts of fishing fleet activity. The activity of multi-purpose vessels is thus included in several data bases which are often formatted in uncoordinated ways, prohibiting cross-fishery analysis. A vessel may "drop out" of a data base once it switches fishing activity. Another difficulty with the current system of data storage is the tracking and analysis of multifishery fishing effort. Effort units are often multiple-counted as separate data bases are analyzed.

The planning process for fishery system management is also critical, and should include pre-season information on both the biological and economic components of the system as well as a clear definition of the authorized planning parties. The definition of the legitimate "players" comes from the context of the fishery itself. Coordinated planning, to be successful, will have to involve representation of all the commercial fishery constituents; i.e. fishery managers, fishing industry, and consumers. Also necessary is a clear definition of objectives for the fishery system beyond the basic conservation goals.

Comanagement by managers and industry has taken various forms in the Oregon crab, shrimp and groundfish fisheries. Industry has played a strong and constructive role. Efforts to initiate discussions about limited access in the groundfish and crab fisheries originated in industry groups who felt that more effective management should be and could be implemented. Industry committees in both fisheries have been instrumental in outlining the problems to be addressed, defining management objectives, and discussing alternative means to reach goals.

One of the goals of multi-fishery management would be to avoid the problems illustrated by the case study of Oregon crab, shrimp and groundfish: uncoordinated timing of fishing seasons, interstate fishing effort shifts, and piecemeal regulatory programs. The coordination of multifishery seasons could minimize the surges in off-season effort in substitute fisheries. Interstate effort distributions could be stabilized through the coordination of state regulations and season timing. Bycatch issues, e.g. groundfish caught in shrimp trawls, could be explicitly addressed as components of a single system.

System evaluation will incorporate the other four elements: system definition, supporting data, the planning process, and the definition of management goals.

SUMMARY AND CONCLUSIONS

This case study of Oregon shellfish and finfish fisheries illustrate two principles which have application to other fisheries with multifishery activity. The first principle is that economic factors, although often not explicitly incorporated into management plans, are often instrumental in creating linkages between fisheries and so affect the efficacy of management plans. The second principle is that when linkages exist, management of fisheries must proceed on a level which recognizes these linkages and accounts for multifishery fishing strategies.

Fishery management on a system level which explicitly recognizes biological and economic linkages offers several potential benefits: coordinated timing of fishing seasons, more predictable control of the spatial and temporal components of fishing effort, and fewer unintended "external" impacts of regulations.

What we find instead in the management of these fisheries and indeed in the management of most capture fisheries is a jumble of biological controls mixed with political boundaries of jurisdictions, without an accounting of the economic forces creating interrelationships.

As populations of fishermen have increased and as fishing technology has become more sophisticated, fish populations have become more interconnected. The fishery is becoming smaller just as our political and economic world is becoming smaller. As the interconnectedness of fishery operations increases, it becomes increasingly important for fishery management to understand the heterogeneity of fishing strategies and their economic basis.

What is defined as a "fishery" has as much to do with human patterns of exploitation as with biological attributes of fish populations. The continuance of single-stock biological approaches to management is not sufficient to capture the full sphere of fishery components. It is also necessary to understand and account for the economic and social incentives facing people who catch and process the fish, and incorporate the human environment into a system of management. Defining new fishery systems is one approach.

ACKNOWLEDGMENTS

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ECONOMICS OF A LOUISIANA OYSTER SEED BEDDING FISHERY AND INFLUENCE OF LEASE YIELD ON EXPENSES TO OPERATE

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ABSTRACT Expenses associated with bedding oyster seed in the fall of the year and reharvesting for market by the following spring and summer were documented for eight oystermen (captains) and their vessels working in Barataria Bay, Louisiana. Labor was the highest variable expense (59%) followed by vessel and engine repairs (16%), fuel (12%), food and galley supplies (8%), and oil and grease (2%). Fixed expenses, such as oyster lease rental fees paid to the state, vessel depreciation, and insurance comprised 27 percent of total expenses (variable + fixed). Expenses were reported in 1988 dollars. Average daily operating expense was $337. Expenses associated with bedding seed oysters averaged $4.04 per Louisiana barrel (range = $2.52–$5.14). The expenses to bring a Louisiana sack of oysters to market the following spring averaged $4.77 (range = $2.98–$6.08). As lease yield increased, expenses associated with harvesting a sack of oysters for market decreased rapidly as bedding expenses were recouped.

KEY WORDS: economics, oyster, seed, Louisiana, fishery

INTRODUCTION

Privately leased oyster (*Crassostrea virginica* Gmelin) areas in Louisiana have contributed 65 to 95 percent of the state's annual landings from 1962 to 1981 and have averaged 74 percent from 1981 to 1988 (Keithly and Roberts 1988). This is significant when one considers that the average annual harvest from all sources in Louisiana waters is 4.7 million kilograms of meat. This private production comes from approximately 139,676 hectares of leased state water bottoms.

Although the exact percentage is unknown, much of private production is traditionally due to the harvest of public oyster seed and its subsequent bedding (replanting) on private leases for additional growth and fattening before reharvest for sale (Dugas 1982). This public-private relationship in Louisiana has been a stabilizing element in the industry this century (Perret and Chatry 1988). Private control of leases has helped to prevent wide swings in oyster prices and has allowed an oysterman to market his product when the public grounds are closed. This relationship has produced the largest privately leased acreage in the nation (Chew 1981) and rivals any oyster fishery found in the world (Bardach et al. 1972).

As important as private leases are to Louisiana and the northern Gulf of Mexico, knowledge of an oysterman's fishery-related expenses are limited. The only relatively complete information on a Louisiana seed oyster fishery dates back to 1928 (McConnel 1930). Korringa (1976) also documented a few expenses associated with a Louisiana seed fishery but took a more anecdotal approach by describing the activities and expenses of a Louisiana family who owned two oyster vessels (luggers). Pawlyk and Roberts (1986) documented the economic benefits to Louisiana of the public-private relationship, but with the data available to them could not document any economic information on an oysterman's fishing expenses.

In Gulf-wide studies, Centaur Associates (1984) could not find any recent data on a private oyster fishery in the northern Gulf of Mexico. They extracted information from other fisheries and estimated expenses for a Gulf of Mexico oyster dredging operation. More recently, Kearney/Centaur (1988) did an "exhaustive search" of the available literature and were able to document expenses and profits for 100 U.S. fisheries. However, the only data on oysters were for the tong fisheries of Mississippi, Alabama and Florida. Prochaska and Keithly (1986) also documented the tong fishery of Applachicola, Florida. Lea (1978) produced a prospectus on developing a four hectare oyster farm in Mississippi but relied on natural oyster spat sets, and not seed replanting, to restock the leases once cultch was established.

In contrast to the limited published economic information, the documentation of prevailing environmental conditions on the health of oysters is abundant in the literature, e.g., Breisch and Kennedy (1980). Published information, however, on how the environment impacts a Louisiana oysterman's efforts (Allen and Turner 1984) or how it influences private oyster production (Dugas and Roussel 1983, Melancon et al. 1987) is limited.

Louisiana's oyster fishery is entering a new era due to a sporadic supply of available oyster seed. Oystermen, private investors and state managers are looking intensely at hatcheries and other technologies to help supplement their oyster supplies. Information on expenses associated with the traditional methods of obtaining and growing Louisiana seed will allow comparisons to other methods and technologies being considered.

A Louisiana seed fishery can be described according to the (1) labor efforts to locate and harvest seed, bed, and reharvest the oysters for market, (2) environmental influences on lease production while the bedded oysters are exposed during the grow-out period, and (3) the associated operational expenses (Fig. 1). This paper documents the operational expenses associated with an oyster seed fishery in Barataria Bay, Louisiana, and how expenses to operate change with a change in lease production. A complete discussion of the conceptual model of a Louisiana oyster seed fishery (Fig. 1) can be found in Melancon (1990). The bioeconomic model describing all three components of a Louisiana seed oyster fishery can be obtained from the senior author by providing a PC-compatible 3.5 or 5.25 inch diskette.

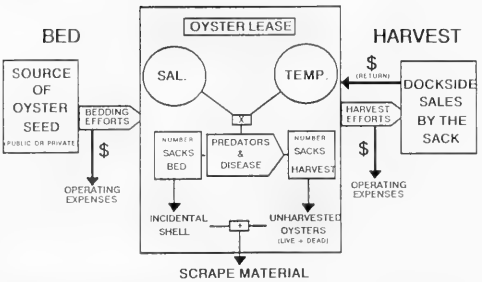

Figure 1. Conceptual Model of a Louisiana Oyster Seed Fishery (modified from Melancon 1990).

MATERIALS AND METHODS

Daily expenses to operate an oyster vessel came from information collected from a questionnaire developed by Melancon (1990) in which he interviewed eight Barataria Bay oystermen. Each captain (oysterman) and, if married, his wife were interviewed at their home as well as on their vessel. Expense data presented in this paper are in 1988 dollars. Average daily expenses along with high and low values are reported. Only average, high and low expenses are reported to insure each oysterman a degree of confidentiality and anonymity.

All reported expenses are water-related only. Ancillary land-based expenses such as for a truck, are not included. The captain as owner of the vessel was also not included in the labor expenses. Social Security, unemployment and worker's compensation taxes were included in the captain's costs for a deckhand. Each vessel was assumed to have two deckhands, the average number for a Bay lugger (Melancon 1990).

A bedding lease accounted for approximately 33 percent of an oysterman's annual fishing efforts (Melancon 1990). Therefore, whenever only annual expenses could be given by the oysterman, daily expenses were calculated by first taking one-third of that expense and then dividing by 60. A value of 60 was used because that was the average number of days needed to bed and later harvest-for-market a Bay lease during a three-year study of the Bay's oyster operations (Melancon 1990). Seventeen bedding leases were in the study.

Vessel repair and maintenance estimates accounted only for those expenses an oysterman considered routine during a year of fishing. Expenses for major repairs, such as an engine overhaul which could easily cost in excess of $10,000, were not included. Routine maintenance repairs included hull, engine and support equipment on deck. Considering the demand made on an engine and vessel during bedding operations, the values presented in this paper are probably conservative for maintenance expenses.

Regardless of age and length of time in service, the 1988 potential resale value of a vessel was used for estimating the average and high depreciation values. The low value, $0, represented a fully depreciated vessel. Vessel value included hull, engine, and all supporting equipment on deck. Both wooden and steel hulled vessels were represented. Vessel depreciation was calculated using a straight line method over 10 years as described by Etzold (1975).

Insurance was added to fixed costs. A value of $0 was used as the low estimate to represent those oystermen who did not carry insurance (3 of 8).

Van Sickle et al. (1976) estimated the average size lease in Barataria Bay to be approximately 34 acres and that was the acreage used for calculating the average and high rental fees to the state, which is $2 per acre per year. Three oystermen did not have bedding leases in the Bay but were allowed to use leases of other oystermen, at no charge. Therefore, the low rental was calculated at $0. The staked and bedded area was actually much smaller than the size of a lease and averaged 5.4 acres (Melancon 1990).

The daily expense data generated from the questionnaires were incorporated into a generalized oyster fishery operation in Barataria Bay. Fishing activities were developed from data by Melancon (1990) during a three year study of the Bay luggers. Waterborn expenses were based on a Bay model where 4,000 sacks (2,000 bbls) of oyster seed were bedded, the three year average. Lease yield was based on a volume displacement ratio of the number of sack harvested to the number of sacks bedded. The average lease yield ratio during the study was 1.1 sacks harvested for every sack bedded (yield ratio = 1.1:1). The increase in oyster volume was a function of shell growth. Bedding 4,000 sacks of seed in the fall of the year required 24 days of labor and vessel time. Harvesting 4,400 sacks in the spring and summer for sale required 36 days of labor and vessel time. The quantity of oysters harvested each day for sale from a Bay lease was considered to be constant at 122 sacks, the average daily harvest during the three year study.

RESULTS

Total labor costs, excluding the captain as owner, totaled $8,710, 59 percent of variable and 43 percent of total expenses (variable + fixed) (Table 1). Labor was the highest single expense.

Additional expenses of a hired captain and an extra deckhand would have increased labor expenses. A hired captain's salary averaged $90 within a range of $80–$100 per day. A hired captain would have added an additional average expense of $6,426. Some oystermen liked to hire an additional deckhand during bedding season. An additional deckhand would have added an average of $1,742 per lease during bedding operations.

Vessel and engine maintenance (based on ⅓ of annual expenses) totaled $2,307, 16 percent of variable and 11 percent of total expenses. Maintenance was the second highest variable expense after labor.

The price of diesel fuel oystermen were paying in 1988 averaged $0.17 per liter ($0.63/gal). Fuel expenses totaled $1,751, 12 percent of variable and nine percent of total expenses. Fuel was the third highest variable expense.

Grocery (food) and galley items were the next highest variable expense after fuel. Groceries and miscellaneous galley supplies totaled $1,236, eight percent of variable and six percent of total expenses. Oystermen were working virtually non-stop while bedding and food and galley supplies were used more rapidly. In contrast, during harvest operations in the Bay many of the crew lived close enough to port to go home at night. Five of the eight captains had lower daily grocery expenses while harvesting oysters from the Bay leases.

Oil and grease expenses were equal for all vessels. All captains stated that oil and grease were changed regularly every 100 hours of engine running time. Each engine oil and grease change, including filter, cost $35. Oil and grease expenses totaled $245, two percent of variable and one percent of total expenses.

TABLE 1.

Summary of expenses associated with harvesting oysters from Barataria Bay.

Item Variable Costs	Average Cost	Range Low	High
Bedding expenses for 24 days			
Labor to bed	3,484	3,427	3,713
Groceries and galley supplies	552	408	960
Fuel consumed while bedding	1,200	881	1,599
Oil and grease for engines	140	140	140
Butane and ice	75	0	108
Harvesting expenses for 36 days			
Labor to harvest	5,226	5,141	5,569
Groceries and galley supplies	684	360	1,440
Fuel consumed while harvesting	551	502	563
Oil and grease for engines	105	105	105
Butane and ice	144	0	206
Other expenses (⅓ of annual)			
Hull and equipment maintenance	2,307	1,523	3,317
Engine maint. (excl. oil & grease)	330	67	667
Subtotal	$14,798	$12,554	$18,387
Fixed costs (⅓ of annual)			
Willow poles to mark lease boundary*	54	0	75
Vessel and engine depreciation	2,896	0	4,166
Rental of bay leases from state*	68	0	68
Vessel and crew state licenses	47	30	53
Vessel and crew insurance	2,360	0	3,000
Subtotal	$ 5,425	$ 30	$ 7,362
Total	$20,223	$12,584	$25,749

* Annual expenses for willow poles and state lease rental fees.
Expenses are based on 1988 dollar values.

The average age of the vessels in 1988 was 18 years. The wooden hulled luggers were the oldest, averaging 25 years while the steel hulled luggers averaged eight years. Although initially more expensive to purchase, steel vessel required fewer hull repairs and less maintenance than wooden hulled vessel. Vessel depreciation (⅓ of annual), averaged $2,896, 14 percent of total expenses.

Insurance premiums amounted to $2,360, for 12 percent of the total costs (Table 1).

Variable expenses for bedding and harvesting along with fixed expenses were combined to produce an average daily expense of $337 to operate (range $210 to $429).

At $337 per day to operate, an oysterman spent an average of $4.04 (range = $2.52 to $5.14) to bed each barrel of oyster seed on his Bay lease (based on 24 days of labor to bed 2,000 barrels). The bedded oysters were wild seed harvested from the public reefs and were mixed in size. A Louisiana-bushel of seed oysters planted during the study contained approximately 480 oysters that averaged 69 ± 25mm in size. Only 52 percent were actually seed-sized oysters, 25–75mm in length. Since each barrel is equivalent in volume to three bushels, expenses to bed each Louisiana-bushel averaged $1.35 (range = $0.84 to $1.71).

If one looked at Bay harvest activities only, then each sack of oysters cost $2.75 (range = $1.72 to $3.51) to harvest (based on 36 days to harvest 4,400 sacks). Bedding expenses, however, can not be ignored when calculating the true cost to harvest each sack for market. Initial monetary returns to an oysterman were used to recoup bedding expenses. Combining the average cost to bed a sack of oysters with the average cost to harvest a sack produced a truer representation of an oysterman's average expense to bring a sack to market, $4.77 (range = $2.98 to $6.08) (Fig. 2). A Louisiana sack is equivalent to 1.5 Louisiana bushels. Therefore, to bring a bushel to market cost an average of $3.18 (range = $1.99–$4.05).

Expenses associated with a Bay seed fishery were influenced by lease yield (Fig. 2). When lease yield increased, expenses per harvested sack decreased rapidly as bedding expenses were recouped (Fig. 2). If an oysterman worked 24 days in the fall to bed 2,000 barrels of seed oysters, and worked only four days in the spring to harvest 400 sacks (0.1:1 yield ratio), then it would cost an average of $23.59 to harvest each sack. Conversely, if the same lease yielded 8,000 sacks (2:1 yield ratio) then it would take 66 days to harvest for market and the average expense to harvest each sack would be $3.79, an 84 percent reduction.

DISCUSSION

Using an exvessel price of $18 per sack as an example, an oysterman would not get a positive monetary return on a lease yield of 0.1:1, but show a loss of $2,236 for his bedding and harvesting efforts (based on 24 days to bed 4,000 sacks of seed and 4 days to harvest at an average cost of $337 per day). In contrast, a lease yield of 1.1:1 would generate $58,980 above water-related expenses. Subtracted from this $58,980 would be a salary for a captain (owner), a vessel's share for major repairs and all land-related expenses. Under this scenario, the average lease yield needed to recoup expenses associated with bedding operations would be a 0.14:1 ratio, equivalent to 560 sacks harvested and sold.

Oystermen will not see a return on their fall bedding expenses until it is time to reharvest in the spring and summer. During this interim period oystermen are dependent on the prevailing environmental conditions on the bedding lease and on the ability to avoid poaching or vandalism. For example, one lease during the study was vandalized by someone dumping chunks of sulfur on top of his oysters; no shed would buy them. The oysterman lost an estimated $44,190 in gross revenue. In another example, a lease was poached, and the oysterman lost an estimated $68,040 in gross revenue.

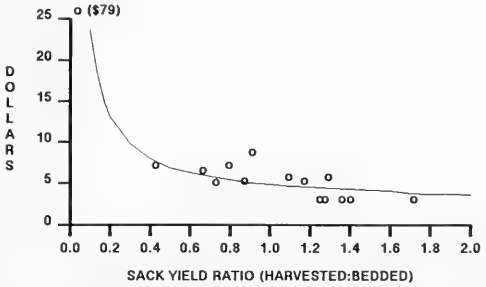

Figure 2. Expenses associated with harvesting a sack of oysters from Barataria Bay as a function of the lease yield ratio. (o = actual lease yields during study and subjected to an average daily operations expense of $337). (line represents bedding 4,000 sacks of oysters and varying the lease yield ratio).

TABLE 2.

Expenses and profits of a 1928 Barataria basin seed lease, 8 acres in size.

Equipment	Initial Cost	Depreciation	Maintenance
52 ft gas boat	$7,500	10%	$ 750.00
Two oyster skiffs	450	10%	45.00
Cabin and equip.	500	10%	50.00
Misc. tools, equip.	100	100%	100.00
Fencing	128.50	100%	128.50
Posts	50	50%	25.00
Operating expenses			
Salary for 3 men for 8 months at $120/month			2,880.00
Food and tobacco for 8 months at $75/month			600.00
Fuel—for 20 trips to natural reefs for seed			480.00
Canal fees for 20 trips			520.00
Insurance—fire, tornado, liability			200.00
Survey of bedding lease by state			11.00
Rental fee from state for 8 acres			8.00
Tonnage license from state			3.50
Dredging license from state			50.00
Total expenses =			$ 5,851.00
Production = 15,000 sacks at $1.00 per sack			
Gross profits =			$15,000.00
Net profits =			$ 9,149.00

Data from McConnell 1930.

The location of available seed for bedding and distance from the Bay determined bedding expenses. The data presented here reflects a composite of all locations fished for seed. The location of most of the harvested seed were the public grounds east of the Mississippi River in Breton and Mississippi Sounds, 65km from Barataria Bay. The farthest distance traveled to collect seed were the public areas of Grand Pass/Cabbage Reef also located east of the Mississippi River, 154km from Barataria Bay.

Oystermen when not operating in the Bay are fishing either other private leases or the public grounds for direct market oysters. As a result, the income from a bedding lease is often a substantial part of an oysterman's earnings for the year. When Bay income is spread over a year, average daily profits for his efforts are often reduced.

The oystermen of Barataria Bay are dependent on the public grounds for the majority of seed they transplant. During this study as many as 10 oystermen were bedding oysters in the lower Bay. However, by 1990, only two oystermen had bedded seed. The decrease was due to low supplies of seed available from the public grounds. When there are no oysters to bed, leases are left fallow.

Yield ratios during the three year study (excluding poached and vandalized leases) ranged from 0.41:1 to 1.68:1 (Melancon 1990). Mackin and Hopkins (1961) reported yield ratios in Louisiana between 0.89:1 and 1.52:1. However, Perret and Chatry (1988) estimated that yield ratios on some leases may reach 3.0:1 to 4.0:1. Dugas (1977) suggested that a reasonable average conversion ratio for a Louisiana bedding lease is 1.21:1.

An account of fishing expenses for a Louisiana bedding lease for the years 1927–28 was documented by McConnel (1930) (Table 2). A 1928 seed fishery centered around one lease that was worked for an entire year, not seasonal as in the seed fishery today. Nonetheless, the expenses (variable + fixed) between 1928 and 1988 produced interesting comparisons. Labor in 1928 accounted for 49 percent of expenses, six percent more than in 1988; fuel eight percent, only one percent less than in 1988; and grocery expenses 10 percent, four percent more than in 1988. Insurance premiums increased from three percent in 1928 to 11 percent of operating expenses in 1988. Lease rental fees doubled from $1 to $2 per acre and accounted for 0.1 percent in 1928 and 0.3 percent of expenses in 1988.

Since the 1988 survey, several captains have changed from paying a daily wage to paying deckhands a percentage of the daily harvest. This is an incentive for the deckhands to harvest more sacks per day. Each deckhand also becomes an independent fisherman and payment of all state and federal taxes now become his responsibility.

In reality, sack production per day was not constant. As the sack yield per day decreased toward the end of the season, oystermen had to decide when it was time to quit. Oystermen continued to work until harvest was down to 8–20 sacks per day. The dock-side price per sack influenced that decision. Korringa (1976) stated that a Bay lugger crew could, on a good day, harvest 100 sacks from a bedding reef. Oystermen during this study could easily harvest 200–250 sacks per day at the beginning of the summer, but were limited by the buyer's daily need. Oysters were concentrated on a relatively small area of water bottom and the dredge was easily filled at the beginning of the harvest season.

It is easy to see how important lease yield is to an oysterman's earnings. Unfortunately, leases in the Bay, especially in the southern region, have virtually no naturally occurring wild seed because of relatively high salinities and warm summer water temperatures which increase the abundance of predators and disease (Melancon et al. 1987). As a result, many of the Bay leases are restricted to having oysters bedded in the fall of one year and reharvested by the following summer before predators and disease increase. This is the trade off for planting in a high salinity area which produces fast growth and a good meat yield.

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CULTCHLESS EASTERN OYSTER (*CRASSOSTREA VIRGINICA* (GMELIN 1791)) CULTURE ON THE TEXAS GULF COAST: A FEASIBILITY ANALYSIS AND COMPARISON TO TRADITIONAL OYSTER FISHING

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ABSTRACT The feasibility of cultchless oyster (*Crassostrea virginica* Gmelin) culture was studied by a profitability analysis based on the production of 1.5 million market size oysters and was then compared to the economics of traditional oysters fishing. Costs of the production phase were estimated through trial grow-outs in Galveston Bay using laboratory reared larvae, the induction of cultchless spat via epinephrine treatment of larvae, and grow-out in floating tray culture. Losses at larval and spat stages were approximately 90%, but thereafter mortalities were slight (10%). Only 10% of the population was found to be lightly infected with the parasite *Perkinsus marinus*, even when they were grown in areas of high infection incidence (100%). Growth to market size was achieved in 10 months (range 6 to 15 months). The results suggested a small grower will need a minimum of four spawns to produce 1.5 million market size oysters. Hatchery costs varied primarily with the cost of algae production. Marketing costs varied with mortality, packing costs and alternate production options. The simulated net return after cash costs (NRACC) were achieved by selling the oysters in 200 count boxes as compared to the traditional boat sack. The costs of oyster fishing were analyzed in terms of production per boat per season and NRACC varied according to sale prices and number of boat sacks sold. We concluded that the NRACC of oyster culture could be higher than that of traditional oyster fishing.

KEY WORDS: oyster, culture, economics

INTRODUCTION

In the United States, the oyster industry has been shifting in recent years from natural harvesting toward mariculture (Catterall and Poiner 1987, Ortega 1987). *Crassostrea* and *Ostrea* are the two major genera of oysters used in mariculture. Two species of *Crassostrea* are of commercial importance, *Crassostrea gigas* (Thunberg 1793) and *Crassostrea virginica* (Gmelin 1791). *C. gigas*, imported from Asia, is primarily grown on the Pacific coast. *C. virginica*, native to the Atlantic coast, is cultured in Alaska on the West coast and from Canada to the Gulf of Mexico on the East coast. The form of mariculture most utilized is extensive, relying on hatchery produced seed or the management of specific seed beds to produce seed.

The Texas oyster industry however, still depends solely on gathering from the native population. It has had an estimated economic impact of $27.53 million in recent years (Quast et al. 1988) and Galveston Bay has contributed an estimated 80% of the total state production (Haby 1986). These contributions fluctuate annually and generally are declining due to anthropogenic and natural processes (droughts, floods, and diseases). It is therefore desirable to find an alternative to traditional oystering if Texas is to maintain or increase its market share.

A possible alternative to oyster fishing is the establishment of oyster mariculture. This approach has been successful in the United States and elsewhere. In the United States, this type of culture has been practiced for decades (e.g. Montane 1964, Shiappacasse and Niemeyer 1964, Speed 1969, Dillehay 1984, Ortega 1987) and it has proven to be technically and biologically sound (Hidu 1971). France, Japan, Britain, and the Philippine Islands have been successful in establishing oyster mariculture (Milne 1972). Typical culture methods include trays, bags, rafts, skates, Japanese lanterns, strings, tiles, pond and different types of bottoms for spat collection and grow-out (Milne 1972, Michael and Chew 1976, Ogle et al. 1978, Hidu et al. 1981, Walker and Gates 1981, Paynter and DiMichele 1990).

Previous studies have also demonstrated the economic feasibility of oyster mariculture. Im and Langmo (1977) have developed an economic analysis for the production of *C. gigas* on the West coast as means to supplement and increase the domestic oyster supply which fell by 32.4% from 1950–1975. They concluded that in the Pacific Northwest oyster mariculture can be practiced profitably. At the same time, Dupuy et al. (1977) produced a financial flow schedule suggesting the feasibility of oyster mariculture for the Chesapeake area. More recently, Myers and Boisvert (1990) arrived at the same conclusion in their economic study of algae and bivalve seed production in hatcheries.

Although the aforementioned studies indicate that oyster culture may be viable; the biological, technical, socioeconomic, and political conditions used were specific to particular areas. We hypothesized that cultchless oysters grown in floating tray culture might be particularly well suited to the environment and economy of coastal Texas. In this locale, the requirements for success include: rapid growth to market size (to avoid mortality due to the parasite, *Perkinsus marinus*), protection of young spat from predation; the ability to withstand seasonal coastal flooding and hurricanes, and the product's ability to compete with the natural fishery. The results of Paynter and DiMichele (1990) indicate that cultchless oysters produced by epinephrine treatment (Coon et al. 1986) and grown in floating trays have the required growth rate characteristics.

This study was conducted to determine the potential viability of

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this type of culture in Texas by running a pilot hatchery and grow-out area and developing a profitability analysis in comparison to traditional oyster harvesting. Our goal was to simulate the cost of producing 1.5 million oysters, *C. virginica*, to market size (7.5 cm); a target production rate we hypothesized might be feasible for a small business with access to non-navigable tidal waters where the use of floating trays would be permitted. It is also comparable to the production rate of a single oyster harvesting boat operating over one season. We conducted several production runs in 1988 through 1990. The hatchery used had the capacity to produce over two million spat, however our grow-out area was insufficient for the desired crop size. Therefore, we replicated the grow-out of approximately 50,000 oysters several times and extrapolated these costs.

MATERIALS AND METHODS

Oyster Production

Spawning and rearing of the larvae and cultchless spat took place in a hatchery on Galveston Island, Texas. A dock next to the hatchery and a site on East Galveston Bay near Smith Point, Texas were used for grow-out. Three stages of cultchless oyster culture were followed: i) mass spawning and larvae rearing, ii) cultchless spat "setting", and iii) grow-out.

Supplemental algal feed, *Isochrysis* aff. *galbana* strain T-ISO (Green), was provided during the larval and spat stages (Epifanio 1979, Laing and Millican 1986). The amount of feed varied depending on the amount of algae present in the incoming sea water (filtered to 5 μm) and ingestion rate of the larvae. Thus, the total daily food ration varied from 40,000 cells/ml at the beginning of the larval period to a maximum of 300,000 cells/ml at the "eyed" stage.

The hatchery set up was centered around 4 larval culture tanks (600 l ea.) and 2 (100 × 50 × 25 cm) spawning tables. Oysters from Galveston Bay were used as broodstock to produce mass spawns. They were kept in a flow-through system until they were ready for spawning. Oysters were induced to spawn according to Galstoff (1964) and/or modifications of Dupuy et al. (1977) and Hidu and Richmond (1977). Fertilized eggs were counted and placed in a 10 l bucket for 1 hr before they were stocked in the 600 l tanks at a density of 64 eggs/ml. Normal straight hinge larvae on day 2 were culled from the tanks and restocked at a density of 16 larvae/ml. A second culling was required on day 4 when larval density was reduced to a final density of 4 to 6/ml. Thereafter, the water was changed every 48 hours.

Once 50% of the larvae had reached the eyed stage (250–300 μm in length) and there were signs of metamorphosing spat (spat set) they were collected and induced to metamorphose by treatment with 0.1 mM epinephrine (Coon et al. 1986). Spat were initially kept in a closed downweller system and fed cultured algae. One or two weeks later, when the spat had reached 1 mm in length, they were transferred to a flow-through raceway (without additional feed) until they grew to 1 cm in length (approximately 2 months).

The oysters were then transferred to floating culture in Galveston Bay. Initially, the oysters were placed in spat bags (ADPI -cat. #'s: OBC 1, 2, and 3) containing a Styrofoam float, in floating plastic trays as described by Paynter and DiMichele (1990). The oysters were transferred to trays or bags of larger mesh size as they grew to assure adequate water movement and to avoid fouling. At each transfer, they were thinned out to prevent crowding and stunting. The mesh size used was determined by the average size of the oysters, i.e., a mesh size approximately one-half the length of the oyster (0.3 cm, 0.63 cm, 1.25 cm, 1.88 cm, or 2.5 cm). Measurements were taken periodically to determine growth rate and mortality during the grow-out period.

Economic Analysis

The economic feasibility of cultchless culture was determined through profitability analysis. These analyses were based on our pilot hatchery and grow-out facilities and the costs and revenues from the local oyster fishery. The production methods were then analyzed in comparable units. In both cases, opportunity costs, financing interest and insurance were excluded from the analyses since they vary greatly depending on the venture. Since our trial grow-outs were smaller than commercial size, these costs were extrapolated. Costs such as labor and vehicle usage were linearly extrapolated, while other estimated costs, such as material purchases, were adjusted to reflect the reductions in cost associated with increased size.

Cultchless Oyster Culture. Costs and the net return after cash costs (NRACC) were estimated for four production options: 1) producing spat for grow-out and purchasing algal monocultures for larval food production, 2) producing the spat for grow-out and maintaining the hatchery's algal monocultures, 3) purchasing the spat at $0.02 each, and 4) purchasing the spat at $0.05 each. Spat prices are estimates based on current prices of eyed larvae ($100–$400 per million larvae) and the percent survival experienced at the hatchery at one week ($0.02/spat) and at four weeks ($0.05/spat) after metamorphosis.

For the analysis of the various options, the costs of algae production and hatchery costs were based on an existent hatchery at Galveston, Texas. Grow-out costs were estimated by monitoring labor, time and materials used. Revenue was determined using regional market prices over the period 1988–1990.

Oyster Fisheries. Interviews of oystermen and seafood wholesales were used to develop a financial analysis of the oyster fishery. The capital, production and license costs per boat per season, number of boat sacks harvested daily per boat, duration of the season, and the market prices were taken from these interviews.

To produce a financial analysis for the oyster fishery comparable to the mariculture method, the yield of oysters per boat sack was determined. Revenues were then determined per 100 oysters and per boat (total annual revenue). Texas oystermen sell their product in "boat sacks" (approximately one and one half bushels each) which typically contain 165–350 "usable" oysters. They may also sell them as single oysters in counted boxes. Because cultchless oysters would be sold by the counted box, the NRACC of oyster mariculture and the fishery were compared in units of dollars/100 oysters produced/year.

RESULTS

Oyster Production

Oyster production involved three stages: mass spawning and larval rearing, metamorphosis, and grow-out. The duration of the three production stages varied within and among spawns. They

were also influenced by mortalities, growth rates, periodic storms and other environmental factors.

Mortalities occurred at all stages of the culture. Yet, most losses were experienced during the larval period. Survival of larvae varied among spawns and larval stages. Fertilization success and larval development from day zero (day of fertilization) to day two were especially variable among mass spawns (2.7%–30% survival, calculated from estimates of egg number). Large, but more consistent losses occurred between day 2 and 4. During this period, 50% to 70% of the surviving day 2 larvae died. Further losses, between day 4 and the eyed stage, ranged from 41% to 85%. Overall losses for the larval stage thus varied between 63% and 95%.

Losses during metamorphosis varied between 50% and 80%. By the time the spat had grown to an average size of 0.25 cm to 0.3 cm the average mortality increased to 90% (89 ± 5, SEM). Losses thereafter were small (10% total mortality to 7.5 cm) primarily due to handling and transferring of oysters to different mesh sizes. No mortality could be attributed to *Perkinsus marinus* (infection levels were light to negative, Martinez 1989).

Growth rate during the grow-out period varied depending on the time of year and oyster size. In general, oysters reached market size (7.5 cm) in 6 to 15 months. The mean grow-out time was 10 ± 1.5 (SEM) months.

Economic Analysis

In the analysis below, estimated costs for the cultchless oyster culture were based on the annual production or purchase of 2 million spat to produce 1.125 million market size oysters at the end of the grow-out period (i.e., we assumed a 25% loss of newly set spat at the hatchery and then a conservative 75% survival from the time of deployment in grow-out trays to market rather than the observed 90% survival). It was estimated from the pilot study that a minimum of four hatchery spawns are necessary to produce the desired spat and market size oysters in a system such as the one used for the study.

Hatchery and grow-out costs variations were modeled in the analysis by mortality and four different production options:

1. Production of spat and maintenance of algae monocultures for feed grow-out.
2. Production of spat and purchase of algae monocultures for feed grow-out.
3. Purchase of spat at $0.02 each.
4. Purchase of spat at $0.05 each.

Hatchery costs varied primarily with production costs of supplemental feed. Table 1 outlines the hatchery costs which varied depending on the two algal production methods. Details of algal production costs are outlined in Table 2.

The costs of grow-out varied primarily with mortality and packing costs. Table 3 indicates the costs involved and the change in cost due to variation in mortality as well as the different production schemes above. For example, with 75% mortality, packing cost and therefore total cost decreased $20,000 from the estimated cost at 0% mortality. On the other hand, the cost per 100 oysters increased.

Estimated revenues were modeled as a function of mortality

TABLE 1.

Hatchery costs associated with a grow-out facility[a] (based on an annual production of 2 million cultchless spat).

		Cost/yr		
	Cost	Depreciation (yr).	Purchase[b] Option	Maintenance[c] Option
I. Capital cost				
Land (1 acre)	$4,000	25	$160	—
Building[d]	$16,099	15	$1,073	—
Plumbing[e]	$4,115	15	$274	—
Aeration[f]	$1,536	3	$512	—
Tanks[g]	$2,200	8	$275	—
Miscellaneous[h]	$2,610	5	$522	—
II. Production cost				
Labor[i]			$6,464	—
Algae production[j]			$3,849	$6,816
Utilities[k]			$780	—
Maintenance			$2,500	—
Miscellaneous			$160	—
Total			$16,569	$19,536

[a] Assuming the hatchery will be operating only during the 4 months of larvae production.
[b] Option assumes hatchery purchases algal monocultures for grow-out as larval feed.
[c] Option assumes hatchery maintains its own algal monocultures for grow-out as larval feed.
[d] A 600 sq. ft. concrete block building.
[e] PVC lines running from water source through hatchery and from air pump through hatchery.
[f] Air blower, valves, tubing, etc.
[g] Four 600 l tanks ($195 ea.); two spawning tables ($160 ea.); two 1900 l reservoir tanks ($550 ea.).
[h] Items such as aquarium nets, refractometer, thermometers, glassware and buckets. Depreciation rate calculated as average of all such items.
[i] One temporary unskilled worker (808 hours at $8.00/hr.). Total hatchery labor includes the salaried worker accounted for in table 3.
[j] See table 2 for the break down of costs.
[k] Only includes the months of hatchery operation for larvae production.
— Same cost as that of the purchase option.

TABLE 2.

Algae production costs.

	Cost	Depreciation (yrs)	Cost/yr Purchase[a] Option	Cost/yr Maintain[b] Option
I. Grow-Out costs				
Sand filter	$320	10	$32	—
UV filter	$300	5	$60	—
Tanks	$3,300	12	$275	—
Blower		(same as hatchery)		
Lights	$500	5	$100	—
Microscope & supplies	$1,500	10	$150	—
Water pump		(same as hatchery)		
pH Meter		(same as hatchery)		
DO Meter	$500	7	$71	—
Plumbing	$500	10	$50	—
Diatomaceous water filter	$250	10	$25	—
Tygon tubing (500 ft.)	$300	5	$60	—
Clamps	$60	5	$12	—
Miscellaneous[c]	$571	5	$114	—
II. Production costs				
Employees		(same as hatchery and grow-out)		
Chemicals			$228	—
CO_2 bottles			$192	—
Artificial sea water (optional)			$1,125	—
Utilities (3½ months/yr.)			$575	$1,800
Algae culture			$1,200	$209
Set-up	$5,000	15	$333	—
Miscellaneous			$372	$412
Total			$3,810	$6,816

[a] Option assumes hatchery purchases algal monocultures for grow-out as larval feed.
[b] Option assumes hatchery maintains its own algal monocultures for grow-out as larval feed.
[c] Hemocytometer, carboys, glassware, etc.
— Same cost as that of the purchase option.

and sale prices for the half-shell market (200-count boxes) given by oystermen and wholesalers from the Galveston-Houston area. Table 4 indicates the NRACC obtained from each production option over a range of mortality rates at a typical selling price ($26.00/box). This analysis indicates that the cultured oysters can generate a positive NRACC if the business produces its own spat and the mortality rate is less than 50% or if spat are purchased at a $0.02 each. If spat were bought at $0.05 each, the business would have a positive NRACC only if the sale price per box was $32.00 and mortality was less than 28%. Our analysis also indicated that the cultured oysters could not be sold profitably by the traditional boat sack unless mortality is 0% (data not shown).

Our cash flow analyses indicated that the cumulative cash flow primarily varied according to sale prices and average mortality rates. Table 5 shows a cash flow analysis assuming 25% grow-out mortality and a sale price of $26.00/box for 10 years. If marketing prices are between $26.00 and $32.00 and mortality is less than 26%, the oyster grower will recover his capital investment after the first or second year. At a yearly average mortality of 50% the business will stay at a negative cumulative cash flow for six years. At higher mortalities it may take longer for the business to experience a positive cash flow or one many never occur. If boxes are sold at $21.00, the initial investment can only be recovered when mortality is nearly 0%.

A "typical" oyster fishing boat operation as practiced in Texas produces approximately the same number of oysters as the hypothetical business above (Table 6) and thus represents a good comparative model to determine the relative profitability of the aquaculture venture. It is necessary in this analysis to base costs and revenues in terms of "production/boat/season". Table 6 illustrates the cost included in the study and Table 7 indicates the various dockside sale prices and oyster yields per boat sack. Unlike in the oyster mariculture venture, NRACC in oyster fisheries varied according to sale price and number of boat sacks sold, not on the price of individual oysters. NRACC per 100 oysters thus fluctuated between $1.33–$2.75 (Table 8), but it did not affect the overall NRACC of $19,062/boat. This business begins to show a positive NRACC at an average boat sack price of $10.00 (assuming all 4,200 sacks produced during the season are sold) or if gross sales are above $60,738 (Table 8).

DISCUSSION

Hatchery Production

Our results indicate that it is biologically and technically feasible for our model business to profitably operate in Texas. Hatchery costs and the variance in larvae supply due to larval and metamorphic losses will most likely determine whether or not a business of our model's scale can survive from the technical standpoint.

TABLE 3.

Oyster grow-out costs (based on an annual stocking of 1.5 million spat).

			Cost/yr			
			Produce Spat[a]		Purchase Spat[b]	
	Cost	Depreciation (yrs.)	Purchase Option	Maintain Option	$0.02 ea.	$0.05 ea.
I. Capital cost						
Trays	$35,000	5	$7,000	—	—	—
Netting	$3,000	3	$1,000	—	—	—
Flotation			$2,000	—	—	—
Posts			$4,000	—	—	—
Boat	$2,700	10	$270	—	—	—
Vehicle	$14,000	5	$2,800	—	—	—
Building lease for packing			$24,000	—	—	—
II. Fixed production costs						
Employee			$25,000	—	—	—
Harvest and maintenance of posts and trays			$5,000	—	—	—
Spat			$16,569	$19,536	$30,000	$75,000
Boat operation and maintenance			$2,000	—	—	—
Vehicle operation and maintenance			$3,000	—	—	—
Subtotal			$92,639	$95,606	$106,070	$151,070

	% Mortality			
	0%	25%	50%	75%
III. Variable production costs				
Boxes	$19,500	$14,625	$9,750	$4,875
Labor	$9,375	$7,031	$4,688	$2,344
Subtotal	$28,875	$21,656	$14,438	$7,219
Total cost by production option:				
Produce spat at hatchery				
1) Algal purchase option	$121,475	$114,256	$107,038	$99,819
2) Algal maintenance option	$124,481	$117,262	$110,044	$102,825
Purchase spat at $0.02 ea.	$134,945	$127,726	$120,508	$113,289
Purchase spat at $0.05 ea.	$179,945	$172,726	$165,508	$158,289

[a] See Table 1 for costs breakdown. Purchase option assumes hatchery purchases algal monocultures for grow-out as larval feed. Maintenance option assumes hatchery maintains its own algal monocultures for grow-out as larval feed.
[b] $0.02 spat are younger than the $0.05 spat.
— Same cost as that of purchase option.

Fertilization success and overall survival of larvae varied widely among spawns. These variations were consistent with those from the literature (Davis and Calabrese 1964, Longwell and Stiles 1973, Stiles and Longwell 1973, Mallet and Haley 1983, Lipovsky 1984, Mallet and Haley 1984, Gallager et al. 1986, Gallager and Mann 1986, Nell and Holliday 1988). Although these early larval mortalities are high, they did not appreciably affect the economics of the hatchery operation because larvae had to be culled for optimum survival and growth (Lossanoff and Davis 1963, Dupuy et al. 1977, Hidu and Richmond 1977, Lannan 1980, Mallet and Haley 1983) within the constraints of the hatchery's size.

Unlike the early larval mortalities, the mortality associated with metamorphosis carries with it the loss of all the monies used to produce the eyed larvae. Our results indicate that an annual production of 1.5 million oysters will require the production of 20 million eyed larvae and necessitates the operation of the hatchery and associated algal production for approximately 2 months longer than would otherwise be necessary if the metamorphic mortality rate was half of its present value. Thus the cost to the business due to this mortality would range from $11,000 to $13,000. A possible solution to this problem would be to double larval production by purchasing 4 more 600 l larval tanks and 4 algal tanks for an additional $3,600 of initial capital investment. With a 10-year depreciation, the cost/year would increase by less than $400. The size of the model hatchery is suitable to accommodate the additional tanks. The time to produce the extra larvae and algae can most likely be integrated into the hatchery routine without other substantial costs being incurred (without additional labor costs in particular). However, any further increase in production capacity would necessitate a large increase in capital and operational expenses and the model business would be that of a larger scale operation than originally planned for a small oyster mariculture venture.

Economic Analysis

Cultchless oyster mariculture can be a profitable business. The use of the various alternatives for oyster mariculture depend primarily on the selected site and available funds. Some of these

TABLE 4.

Oyster mariculture. Profitability analysis of oysters marketed in 200-count boxes at $26.00/box.

	% Mortality[a]			
	0%	25%	50%	75%
I. NRACC/100 oysters if sold in 200-count boxes				
Produce spat at hatchery				
1) Algal purchase option	$4.90	$2.84	($1.28)	($13.63)
2) Algal maintenance option	$4.70	$2.58	($1.67)	($14.42)
Purchase spat at $0.02 ea.	$4.00	$1.65	($3.07)	($17.21)
Purchase spat at $0.05 ea.	$1.00	($2.35)	($9.07)	($29.21)
	% Mortality			
	0%	25%	50%	75%
II. Total NRACC				
Produce spat at hatchery				
1) Algal purchase option	$73,486	$31,955	($9,577)	($51,108)
2) Algal maintenance option	$70,519	$28,988	($12,544)	($54,075)
Purchase spat at $0.02 ea.	$60,055	$18,524	($23,008)	($64,539)
Purchase spat at $0.05 ea.	$15,055	($26,476)	($68,008)	($109,539)

[a] Zero percent mortality = 1,500,000 market size oysters, i.e., no mortality in excess of the early spat mortality at the hatchery.

alternatives may be less costly but riskier than the more expensive alternative. For instance, hatchery costs can be minimized by buying the algae monocultures required for algae grow-out (Table 1), but maintaining one's own algae monocultures minimizes the risk of buying contaminated or bad monocultures or failing to buy one at a critical time, thus decreasing the chances of losing a crop of larvae or lowering the crop's production. Also, if the farmer purchases spat, he can save initial production costs by buying newly set spat (<0.3 cm in length) but the chances of heavy mortalities may increase significantly. An older spat (>0.5 cm in length) may have greater chances of survival but it would most likely be sold at a higher price. This strategy would severely limit the amount of mortality that could be tolerated in grow-out and/or the range of profitable selling prices.

Choosing the right market and marketing technique is critical. Oyster mariculturists could emphasize high value added products such as fresh and half shell oysters and canned specialties. For the half-shell market, oysters can be boxed and marketed according to the price per boat sack, but it is highly unlikely the grower will make a profit if grow-out mortality approaches 25%. Moreover,

TABLE 5.

Cash flow and financial analysis for the oyster hatchery during the first 10 years of operation if production = 1.125 million market size oysters (25% mortality) and sale price per 200-count box is $26.00.

	Year									
	1	2	3	4	5	6	7	8	9	10
Total capital cost	$100,914	$536	$551	$3,837	$582	$60,180	$4,154	$715	$645	$4,470
Gross sales	$146,000	$150,380	$154,891	$159,538	$164,324	$169,254	$174,332	$179,562	$184,948	$190,497
Total direct expenses	$33,145	$28,506	$29,596	$32,726	$31,272	$34,008	$35,729	$33,730	$34,622	$38,130
Gross profit	$112,855	$121,874	$125,295	$126,812	$133,053	$135,246	$138,602	$145,832	$150,326	$152,366
Total operating costs	$49,692	$51,183	$52,718	$54,300	$55,929	$57,607	$59,335	$61,115	$62,948	$64,837
Net operating income before tax	$63,163	$70,691	$72,577	$72,512	$77,124	$77,640	$79,268	$84,717	$87,378	$87,530
Cash source net operating income after tax (21%)	$49,899	$55,846	$57,336	$57,285	$60,928	$61,335	$62,621	$66,926	$69,029	$69,148
Depreciation	$16,484	$16,979	$17,488	$18,013	$18,553	$19,109	$19,683	$20,273	$20,881	$21,508
Total cash [illegible]	$33,415	$38,867	$39,848	$39,272	$42,375	$42,226	$42,939	$46,653	$48,147	$47,641
Cum. [illegible]	($67,499)	($29,167)	$10,129	$45,565	$87,357	$69,403	$108,188	$154,126	$201,629	$244,799

TABLE 6.

Oyster fisheries costs (per boat)[a].

	Cost	Depreciation (Yrs)	Cost/Yr
I. Capital cost			
Boat	$40,000	5	$8,000
Dredges (1 season)			$480
II. Production costs			
Sack			$840
Crew[b]			$36,640
Boat operation and maintenance			$8,216
Miscellaneous (gloves, ropes, food, etc.)			$6,520
III. Licenses cost			$42
Total			$60,738

[a] Costs were based on the following data: Yield = 35 boat sacks/day; oyster season = 120 days; average total production = 4,200 boat sacks; price/sack (median) = $19; gross profit = $79,800. Insurance, taxes and financing interests are not included.

[b] 3 crew members whose salaries are 15% of gross profits each.

the price of boxing is about 10% of total cost of cultured oysters, therefore, it becomes unprofitable to sell them at boat sack prices. If oysters are sold by the boat sack, it is unlikely for the business to generate a positive NRACC unless: 1) the sack contains 254 oysters or less, 2) spat are produced at the hatchery or purchased at $0.02 ea. or less, 3) spat are bought at $0.05 ea. with 0% grow-out mortality, or 4) the sale price is above $22.00.

Single oysters are usually marketed in 200-count boxes when they are sold for the raw-bar trade. Premium prices are paid because they are "selected singles" rather than clumps of three or more found in boat sacks and additional costs such as boxes and labor are involved. This packing is preferred in oyster mariculture because revenue in this case is restricted by the number of individuals grown and their survival. For any culture alternative, profitability will then depend primarily on mortality and market price (Table 4). Assuming the grower produces his own spat and experiences negligible mortality, the NRACC can be as high as $118,525 if boxes are sold at $32.00 (the highest market price), or $73,525 if they are sold at $26.00 (an average recent price in Texas, Table 4). More realistically, at 25% mortality the NRACC could be $65,744 or $31,994 respectively.

Good records and trends of mortality and sale prices could determine the right marketing technique at the time of sale. Marketing cultchless oysters in boat sacks is preferred under certain circumstances and their sale in 200-count boxes under others. For example, if one experiences high mortality rates, oysters sold by the boat sack may generate a greater NRACC than those sold by the 200-count-box. The cost of boxing the oysters would then be excluded and a small positive NRACC thus obtained (e.g., if mortality is 75% and spat are produced at the hatchery, the grower can recuperate all his annual investment and obtain a small profit if he sells by the boat sack). This strategy holds true only when the boat sack comprises approximately 254 oysters or less. Note that the only time having 340 oysters per sack becomes profitable, even at 75% mortality, is at a sale price of $22.00 or above.

The 10 year financial and cash flow analysis (Table 6) implies the business will pay itself off according to the average mortality and sale prices per 200-count boxes. It may pay itself off within the first year ($32.00/box and 0% mortality) or it may always have a negative cash-flow ($21.00/box and 50% mortality).

Financing and insurance costs were excluded from our analysis, but these can be added to our schedule of costs to refigure a business plan. Another cost that may significantly impact the feasibility of our model business is depuration. The areas best suited for tray culture of oysters are most likely to be closed to shellfish

TABLE 7.

On-the-dock boat sack and 200-count box prices, average yield per boat sack, and estimate sale price/100 oysters based on the number and price per boat sack.

I. Prices

Date	Origin	Price/Boat Sack
1989		
Jan.	Port Lavaca	$16.00
Jan.	Galveston Bay	$22.00
Jan.	Louisiana	$17.50
Date	**Origin**	**Price/200-Count Box**
1989	Jeri's Seafood	$21.00
	Dutchman's Seafood & Lewis' Seafood	$26.00
	Dutchman's Seafood	$32.00

II. Average yield

	Doz. of Oysters Per Bushel	# of Oysters Per Bushel	# of Oysters Per Boat Sack*
Summer, 1988	14	168	210–252
	11	132	165–198
Winter, 1988	19	228	285–342

III. Sale price/100 oysters (based on 1988–89 data)

	Price/Boat Sack				
	$16.00	$18.00	$19.00	$20.00	$22.00
Oysters/boat sack					
165	$9.70	$10.91	$11.52	$12.12	$13.33
254	$6.30	$7.09	$7.48	$7.87	$8.66
342	$4.68	$5.26	$5.56	$5.85	$6.43

* One boat sack = 1.25–1.50 bushels.

TABLE 8.

Oyster fisheries. Profitability analysis per 100 oysters and per boat.

NRACC/100 Oysters	
Oysters/Boat Sack[a]	**Price/Boat Sack = $19.00[b]**
165	$2.76
254	$1.79
342	$1.33
Total NRACC	
Total cost	$60,738
Total revenue if sale price is $19.00/boat sack	$79,800
NRACC	$19,062

[a] Varies among seasons and origin of oysters (i.e., Galveston oysters give higher yields/sack).

[b] Median sale price/boat sack.

harvesting. This means that an additional depuration step will be needed in most cases. A simple and cost effective solution would be to simply relay the cultured oysters to an approved area before harvesting. However, relaying or using depuration plants to condition oysters for sale may be politically cumbersome as the regulations governing either process are unclear. Finally, security costs may also need to be considered in some areas as oyster poaching is a well known hazard.

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ECONOMICS OF RED CLAW (*CHERAX QUADRICARINATUS* (von Martens, 1868)) AQUACULTURE

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ABSTRACT Freshwater lobsters or crayfish in the genus *Cherax*, indigenous to Australia, are promising new aquaculture species. Fast growth and low feed costs in semi-intensive pond culture and high meat yield provide these species with the biological, production, and market characteristics necessary for a profitable aquaculture venture. Achieving profitable production of a new aquaculture species can require years of research and development. A variety of factors such as *Cherax* biology, aquaculture methods, environmental considerations, production economics, and market acceptance influence the direction of the research and development process. This article examines the economic considerations posed by "red claw" (*Cherax quadricarinatus*) development in the United States. A discounted cash flow analysis tied to biological, production, cost, and price variables describes the relationships between yield, market price, and production costs. The analysis has been used to help make decisions about research and development priorities, culture methods, and type of feasibility of commercial operation.

KEY WORDS: crayfish, *Cherax*, red claw, aquaculture, economics

INTRODUCTION

Australian freshwater lobsters or crayfish of the genus *Cherax* are promising new aquaculture species. Fast growth, semi-intensive pond culture, low feed costs, and high meat yield provide these crustaceans with the biological, physical, and market characteristics necessary for a profitable aquaculture venture. Looks like a lobster, grows better than a crayfish: it's easy to see why the aquaculture world has been intrigued. This article presents the economic considerations examined during a *Cherax* aquaculture pilot project conducted in South Carolina from January 1990 to December 1991.

Aquatic animals are being "brought into the barnyard" as the aquaculture industry seeks new species for culture. A small handful of shrimp, prawn, and finfish species are now cultured around the world. Can a *Cherax* species join the list? While initial commercial attempts at *Cherax* aquaculture are underway in Australia, developing profitable aquaculture methods and adapting the culture of an animal to any new environment can require years of research. Several critical questions needed to be addressed during research and pilot project phases:

- Will *Cherax* live, reproduce, and grow with economically attractive yields in pond or tank culture in the United States?
- Will the expected yields, market prices, and risk factors attract and justify research and then venture capital investment in *Cherax* commercialization?
- Will regulatory, environmental, and scientific protocol constraints affect the project's profitability? Possible risks to the new environment (escape, disease transfer) posed by *Cherax* and factors present in the new environment (pathogens, water quality, temperature) may adversely affect *Cherax* culture.

A financial investment analysis tied to biological, production, cost, and market price variables has been used to help answer these questions and to make decisions about research and development priorities, culture methods, location, and type and feasibility of commercial operation. The analysis describes the relationships between yield (growth and survival), market price, fixed and variable costs, and profitability indicators. Other decision making factors included research results and government agency, venture capital, and environmental considerations. The production, market, and environmental factors important to conducting the financial analysis are outlined in the following sections. Then, the inputs (production, cost, and price assumptions) and outputs (financial projections and sensitivity analyses) of the financial analysis computer spreadsheet program are summarized. Findings are presented in the final section.

BACKGROUND ON CHERAX DEVELOPMENT AND PRODUCTION

The largest crayfish in the world are indigenous to Australia. Of these, three species of the genus *Cherax* are cultured. Efforts started in the 1970s with the marron, *Cherax tenuimanus*, native to the streams of southwest Australia where it is a popular sport animal. Trials with the marron look-alike in Queensland, the red claw (sometimes called Queensland marron), *Cherax quadricarinatus*, began in the mid-1980s. Both animals grow to 40 g to 160 g in one year and can reach one kg in weight after several years (Morrissy 1979, Jones 1990a). The smaller yabbie, *Cherax destructor*, is also cultured and resembles the American crawfish (*Procambarus*). Although annual *Cherax* production in Australia remains limited (100 tons to 200 tons) by the small number of growout ponds constructed, production is expected to increase. There have been attempts to culture *Cherax* species in the United States, South Africa (Safriel and Burton 1984), New Zealand (Decker 1988), and South America (Hutchings 1991).

Red claw may emerge as the *Cherax* of choice for United States aquaculture. While marron has had limited success in Western Australia, it has failed to show adaptability to temperature changes and other environmental stresses in some other parts of Australia (O'Sullivan 1988) and in the Caribbean and United States (Rubino et al. 1990). Red claw, however, are farmed commercially in Queensland (O'Sullivan 1988, Gillespie 1990, Hutchings 1991) and have performed well in trials in South Carolina, Alabama, Texas, and Missouri (for a summary of initial United States results see Rouse et al. 1991). Red claw reproduces more frequently and tolerates higher summer temperatures than the marron. While these preliminary results with red claw appear encouraging, much work remains to be conducted on density, growth, feed, habitat, and hatchery/growout systems to achieve commercially profitable ventures.

The yabbie may have ecological and economic drawbacks for

culture in the United States. Yabbies burrow, making containment in ponds difficult (Mills 1983, Staniford, Kuznecovs, and Mills 1987); their deep burrows might also affect pond water retention. In addition, the yabbie's small size, hardiness, and niche requirements might make it a potential and ecological threat to domestic crawfish. Furthermore, the size range of cultured yabbie would likely put it in direct competition with domestic crawfish unless a specialized market was identified.

PRODUCTION FACTORS

Red claw is cultured in ponds using demonstrated technology adapted from crayfish and shrimp aquaculture. As red claw biology and production methods have been reported elsewhere (see Jones 1990a, 1990b, 1990c), only a summary is given here. The temperature tolerance for red claw culture ranges from 13°C to 33°C; they can survive temperatures down to 7°C for short periods of time. Trials have shown that they will die in outdoor ponds during winter in Alabama (Rouse et al. 1991) and South Carolina (Alon et al. 1991). Red claw tolerate a range of salinities and water hardness. The pond bottoms provide adequate feed at low stocking densities; higher densities call for supplemental feed in the form of planted grasses in the pond, hay, composted organic matter, and/or prepared feeds. Females spawn several times a year starting from less than one year of age. Larval development from egg to juvenile occurs on the underside of the female; when released, the juveniles are miniature adults. Spawning can occur in outdoor ponds and in indoor tanks. Red claw grow to 40 g to 160 g in one year and up to 250 g in two years. Survival rates of juveniles stocked in ponds are generally greater than 50 percent after one year under good pond management conditions. Red claw are harvested by trapping or pond draining.

Production results in aquaculture are expressed in terms of yield (kg per hectare per year); yield depends largely upon survival rate of juveniles stocked and growth rate or harvest weight. Yields of 2,000 kg/ha/yr to 5,000 kg/ha/yr are reported by Australian red claw aquaculture operations (O'Sullivan 1988, Jones 1990b, Hutchings 1991); reported research trials in Australia and the United States were about 1,000 kg/ha/yr (Jones 1990a, Rouse et al. 1991).

The biology of the red claw and research and commercial production results reported to date indicate that several factors will be critical to the economic viability of red claw aquaculture in the continental United States:

- Red claw exhibit size variation in populations of the same age. This poses harvest, size sorting, and labor cost considerations (trapping out big ones over several months may be labor intensive compared to pond drain harvest).
- Some red claw juveniles stocked in early spring may not reach market size of 80 g to 120 g by the onset of winter.
- Red claw will die during the winter in outdoor ponds at most locations in the continental United States.

These production issues, then, challenge red claw operations in the United States to overwinter animals and reduce harvest and other labor costs.

MARKET AND PRICE POTENTIAL

A wide range of market prices, market forms (fresh, frozen tails, different sizes), and consumer segments for red claw must be considered in planning a red claw aquaculture farm. "Lobster," "crayfish," and "prawns" are popular terms used to name a variety of fresh and saltwater crustaceans. Red claw market prices will be determined by 1) who it is sold to (pond, wholesale, retail), 2) market forms, and 3) prices of similar animals (lobster, lobster substitutes, and crayfish). Market categories for lobsters and their close market substitutes include American lobster (*Homarus americanus*), several species of spiny lobster (clawless saltwater lobsters) that are marketed live or as frozen tails, frozen small lobster tails ("langustino" or slipper lobster), giant prawns and shrimp in the 10–11 count to the pound size, and domestic red swamp and white river crayfish (*Procambarus clarkii* and *Procambarus acutus*).

While prices for red claw are expected to be similar to those for giant prawns, lobster tails, and whole lobster based on taste tests, test marketing, and current Australian market prices for *Cherax* species, no firm market data exists for red claw in the United States. The economic analysis described in this paper was used to identify the market price range that may be required for profitable red claw culture.

Red claw compares favorably to lobsters in terms of taste, tail to body ratio, and meat to total weight yield ratio. Tests conducted in Australia (Poole et al. 1990, Jones 1990a), by Auburn University, and by several restaurant chefs show that red claw's taste and texture are equal or superior to other lobsters and shrimp (Alon et al. 1991). The red claw tail is 35–42%, the claws 20%, of body weight and total meat yield is about 20%–50% of weight depending upon size, cooking method, and source of information (O'Sullivan 1988, Jones 1990a, Poole et al. 1990, Seafood Leader 1990). The retail price for red claw in Australia is A$20.00/kg to A$35.00/kg (US$16.00/kg to US$28.00/kg) (based on price quotes in Australia). They have been test marketed in New York at $24.25/kg to restaurants and received an enthusiastic response (Shnider 1990). Part of the marketing and pricing success of red claw will depend upon differentiating red claw from domestic red swamp crayfish. Red claw are different than domestic crawfish: red claw look like lobsters, grow to lobster sizes, have a much larger tail and about double the meat yield of crawfish, and have claws with edible meat unlike crawfish.

ENVIRONMENTAL CONSIDERATIONS

Possible risks to the new environment (escape, disease transfer) of a nonindigenous species and factors present in the new environment (pathogens, water quality, temperature) that might adversely affect red claw culture are two types of environmental concerns that must be addressed in red claw aquaculture. Actions taken to address these risks may increase capital and operating costs.

Precautions should be taken with the culture of nonindigenous species outside of their native habitats (Sindermann 1986, Thompson 1990). State regulations applicable to red claw culture in the United States range from no restrictions to prohibition. The precautions undertaken for red claw culture include environmental tolerance and species interaction research, disease screening, prophylactic treatment of red claw shipped from one pond location to another, escape prevention with fencing, and pond culture in areas where red claw would not survive the winter (Rubino et al. 1990, Rouse et al. 1991). Preliminary indications from research trials are that these measures reduce environmental risks (Rouse et al. 1991).

Red claw, like other crustaceans, are susceptible to opportunistic pathogens caused by stress (e.g., rapid temperature

changes). Pathogens that may be present in the southeastern United States have not yet been reported to harm red claw in aquaculture (Rouse et al. 1991). However, more work remains to be done and environmental, pathogen, and disease factors are a constant risk in any aquaculture operation.

FINANCIAL ANALYSIS

The economic (financial) components of production are analyzed with a discounted cash flow analysis dependent upon production, cost, and price variables for a model business (see Shang 1981, Gittinger 1984). The analysis can be used to trace and consider critical relationships between stocking density, harvest size, survival, and pond management factors that affect yield; capital and operating costs; and market prices. The analysis was conducted with a computer spreadsheet program that includes production, cost, and price assumptions; capital and operating costs (farm budgets); calculation of annual net cash balance for a ten year period, and financial indicators for a 100 pond-hectare red claw farm. The accounting perspective is that of a private company operating a red claw farm. In the case examined, pond construction is phased in over a three year period with 10 hectares of two hectare ponds built in year 1, 40 hectares in year 2, and 50 hectares in year 3. The size of the operation is based on reaching a production level large enough to attract venture capital investment, to take advantage of economies of scale of production and marketing, and to support the multidisciplinary staff necessary to operate a red claw farm. The discounted cash flow analysis projects cost and returns for a ten year period and assumes that the company is sold, say to a larger company, in year 10 at ten times net revenues. A seven to ten year time horizon is a typical investment period for venture capital investments (Gladstone 1988).

The components of the economic analysis include the following:

1. Production, Cost, and Price Variables

Table 1 lists the production, cost, and price factors that can be varied in the computer spreadsheet program. Yield or the quantity of red claw harvested annually is calculated by multiplying several biological and production factors:

$$Y = DSW$$

where Y is yield (kg/ha/yr), D is stocking density or rate (juveniles/ha), S is survival rate (percentage of juveniles stocked), and W is average harvest weight (kg). Capital and operating costs include both variable and fixed costs. Pond construction, feed, land lease, and crop insurance are examples of variable costs. Figures used in the "base case" run of the model for the production, cost, and price variables are listed in Table 1. These figures reflect current knowledge about red claw yield, production costs, and market price based on research and pilot project results in South Carolina and elsewhere in the United States during 1989–1991. Variations from the base case are considered in the sensitivity analyses.

2. Capital and Operating Costs

Variable and fixed capital and operating costs are summarized by budget category in Table 2. Fixed costs (e.g., building, trucks, labor) are all those costs which were not identified as variable costs in Table 1. Detailed assumptions for fixed costs and for depreciation (straight line method) are not listed but are based on production methods designed during research projects and actual costs of construction and operation in South Carolina.

TABLE 1.

Red claw farm assumptions and variable for cash flow analysis.

Production:	
Growout pond construction in ha: 10 ha in year 1, 40 ha in year 2, 50 ha in year 3	
Growout pond ha in production: 10 ha in year 1, 50 ha in year 2, 100 ha in year 3	
Small 200 m^2 ponds are built for half of needed juveniles held at 500/m^2	
Indoor tanks are installed for half of needed juveniles held at 3,000/m^2	
Stocking density juveniles/m^2	5.00
Survival rate	50%
Harvest weight in kg	0.08
Production or yield is a function of stocking density, survival rate, harvest weight, and ha in prod	
Production/ha/yr in kg	2000
Sale price:	
Sale price $/kg FOB live	8.82
Sale price $/lb FOB live	4.00
Capital costs:	
Pond new construction/ha	10000.00
Fencing cost/ha	500.00
Netting cost/ha	200.00
Aeration/ha	1000.00
Habitat/ha	100.00
Tanks/m^2	70.00
Small pond cover/liner	3000.00
Small pond cost	1000.00
Operating costs:	
Food conversion rate	0.70
Feed cost (US$/kg)	0.40
Labor fringe/overhead %	0.50
Lease/ha land for new ponds	200.00
Electricity $/kwh	0.10
Pond electr/ha/yr	435.49 (2hp/ha, 8h/day)
Crop insurance % sale	0.03
Equipment repair, maintenance 5% of capital cost per year	

3. Cash Flow Summary and Financial Indicators

The cash flow summary for the first four years of operation is presented in Table 2. The cost and revenue figures for years 5 through 10 are the same as for year 4 (net cash balance changes slightly in some years because of depreciation schedules). Net annual cash balance is derived by subtracting annual capital and operating costs and taxes from gross revenue. The analysis assumes that all startup capital and working capital costs are provided by an equity investment (cash). No assumptions are made about the financing of capital and working capital costs (or associated interest or opportunity costs).

Three financial indicators are calculated from the cash flow figures. The production cost per kg (or lb) of red claw produced (unit or average cost) is calculated by dividing the production cost

TABLE 2.

Summary of capital and operating costs, cash flow analysis, and financial indicators for red claw farm "base case" assumptions.

	Year 1	Year 2	Year 3	Year 4
Capital costs				
Growout ponds	100,000	400,000	500,000	
Fences, nets, aeration, electrical	18,000	72,000	90,000	
Equipment	19,500	20,000	6,500	
Trucks, tractors	10,000	10,000	34,000	
Hatchery build., greenhouses	30,000	10,000	10,000	
Hatchery tanks	29,167	29,167		
Small ponds & covers	50,000	50,000		
Contingency 10%	25,667	59,117	64,050	
Total capital cost	282,333	650,283	704,550	
Operating costs				
Feed	5,600	28,000	56,000	56,000
Electricity, fuel	6,355	24,774	50,549	53,549
Equipment replacement	2,125	11,975	23,500	28,500
Crop insurance	5,292	26,460	52,920	52,920
Travel, telephone	7,000	7,000	7,000	8,000
Miscellaneous	2,450	9,400	13,600	18,600
Land lease	3,000	15,000	30,000	30,000
Marketing cost (w/o labor)	7,500	15,000	43,000	57,000
Labor with fringe, overhead	117,750	267,375	322,500	430,500
Total operating cost	157,072	404,984	599,069	735,069
Gross revenue	176,400	882,000	1,764,000	1,764,000
Net operating revenue	19,328	477,016	1,164,931	1,028,931
Depreciation	23,109	68,680	117,202	117,202
Taxable income (loss carried forw)	(3,781)	404,555	1,047,729	911,729
Taxes (30%)	0	121,366	314,319	273,519
Net cash balance (Gross revenue − (capital cost + operating cost + taxes))	(263,005)	(294,634)	146,062	755,412
Financial indicators				
Production cost/kg in year 4	$5.59			
Production cost/lb in year 4 (Unit production cost = year 4 operating costs plus annualized capital cost at 10% interest divided by production)	$2.54			
Internal rate of return	74%			
Net present value in $	11,412,522			

Notes. IRR and net present value based on net cash flows for years 1–10. Sale of operation in year 10 at 10× net cash balance. All dollar figures in 1991 dollars. Straight line depreciation. Years 5–10 production and capital and operating costs same as year 4.

in year 4 (a full-scale production year) by the kg of red claw harvested. Production cost for this unit cost calculation includes annual operating costs and annualized capital costs (capital costs over a ten year period at 10 percent interest). Internal rate of return (IRR) and net present value (NPV) are based on the stream of net cash balance figures during the ten year investment period. The IRR gives the rate of return on money invested in the project over the ten year time period. The NPV is the present worth of the benefits (revenues) less the present worth of the costs of the project over the ten year period (see Gittinger 1982 for more definitions and calculation methods for IRR and NPV).

4. Sensitivity Analyses

Sensitivity analyses give preliminary indications of the effects on project financial indicators of various changes in production yield, cost, and market price. Table 3 presents the effect of changes in yield on unit cost of production (cost per kg of red claw produced) in year 4, a full-scale production year. Table 4 examines the effect of changes in yield and sales price on IRR. Table 5 lists the effect of percentage changes in pond construction costs on unit production costs and IRR.

TABLE 3.

Effect of yield on unit production cost.

Yield kg/ha/yr	Cost $ per kg	Cost $ per lb
1000	10.86	4.93
1500	7.35	3.33
2000	5.59	2.54
2500	4.54	2.06
3000	3.84	1.74
3500	3.34	1.51

TABLE 4.

Effect of variations in yield and price on internal rate of return.

Production kg/ha/yr	Price in $							
	2.00	2.50	3.00	3.50	4.00	5.00	6.00	Price/lb
	4.41	5.51	6.62	7.72	8.82	11.02	13.23	Price/kg
1000	ERR	ERR	−18%	2%	13%	29%	44%	
1500	−25%	7%	21%	32%	43%	66%	95%	
2000	10%	27%	42%	57%	74%	116%	176%	
2500	26%	45%	64%	87%	114%	191%	ERR	
3000	40%	63%	91%	126%	171%	307%	ERR	

Note: error terms indicate large negative or positive numbers.

IMPLICATIONS OF ECONOMIC INFORMATION AND RESULTS FOR RED CLAW AQUACULTURE

The results of the financial and sensitivity analyses indicate the following:

Projected Costs of Production

Average or unit cost of production ranges from $10.86/kg ($4.93/lb) at a production yield of 1,000 kg/ha/yr to $5.59/kg ($2.54/lb) at 2,000 kg/ha/yr to $3.84/kg ($1.74/lb) at 3,000 kg/ha/yr (see Table 3). At yields of about 1,250 kg/ha/yr, the unit cost of production comes close to the expected FOB (pond side) sale price of $8.82/kg or $4.00/lb used in the "base case." At higher yields, unit costs are less than the "base case" market price.

Table 3 shows that the unit costs of production per kg decline rapidly as yields climb to 2,000 kg/ha/yr and then decline at slower rates for higher yields. In other words, the rate of increase in profit per kg declines as yield increases. At some point, the increase in profit may be outweighed by the increased risks associated with the higher densities and operating costs (feed, aeration) required for higher yields.

Red Claw Aquaculture Profitability Is Very Sensitive to Yield and Price

The profitability of the 100 ha red claw farm will likely be sensitive to small changes in yield and price. In particular, Table 4 shows that profitability (measured by the IRR) will depend upon reaching consistent yields of at least 2,000 kg/ha/yr at a price of $6.62/kg ($3.00/lb) or a yield of 1,500 kg/ha/yr at a price of $8.82/kg ($4.00/lb). While production may still be profitable at lower sale price-yield combinations, these lower price-yield levels may not allow sufficient margin for risks. Profitability is also very sensitive to small changes in price at constant yields. The figures in Table 4 also show that red claw aquaculture is not likely to be profitable at domestic crawfish market prices (less than $4.00/kg). Red claw operations may be profitable if they can command sale prices in the $6.00/kg to $13.00/kg range (the lobster, lobster tail, and large shrimp/prawn price range).

TABLE 5.

Effect of increase in pond construction costs on unit production cost and internal rate of return.

Pond Construct Cost in $/ha	Unit Cost in $/kg	IRR
10000	5.59	74%
12500	5.82	63%
15000	6.04	55%
17500	6.26	49%

Yield and Price Levels Required to Attract Investment

A red claw venture will likely have to achieve results of at least the 2,000 kg/ha/yr and $8.82/kg ($4.00/lb) level or higher to attract venture capital investment. Investors, whether individuals, venture capital companies, or larger corporations, are looking for potential compound annual rates of return of 35 percent or better (five times their money invested back in five years) (see Gladstone 1988). Venture capital funds typically seek an average return of 20 percent. Because some of the funds' investments will fail, a higher return on the companies that succeed is required to reach a 20 percent return average. For example, in the base case example presented, suppose investors require a 50 percent equity ownership in the red claw farm in return for the approximately $2 million in startup capital and working capital costs (the other 50 percent of equity is reserved for the entrepreneurs). If an IRR of 74 percent represents the return to be split between investors and entrepreneurs, the investors will receive slightly more than their target 35 percent return. Therefore, a red claw venture will likely have to have the potential to achieve results of at least the 2,000 kg/ha/yr and $8.82/kg (project IRR of 74 percent) level to attract venture capital investment.

Investors will also expect the aquaculture operation to account for production and market risks. Expected yields and market prices have to be high enough to absorb yield and price cuts and still provide for exceptional investment potential. Reviewers of an aquaculture business plan are likely to examine "worst case" scenarios to consider the effects on profitability of crop failure, disease, market risks, and management problems. The sensitivity analysis results indicate that a red claw farm operating at the production levels reported in Australia can absorb significant yield and price cuts. As noted above, Australian red claw farms are reporting yields of 2,000 kg/ha/yr to 5,000 kg/ha/yr. If a production level of 3,000 kg/ha/yr and sale price of $8.82/kg ($4.00/lb) is feasible, a United States farm might remain profitable despite occasional problems that caused yields to drop by a third or even by half (IRR drops from 171 percent at a yield of 3,000 kg/ha/yr to 74 percent at 2,000 kg/ha/yr to 43 percent at 1,500 kg/ha/yr).

Modest Changes in Costs May Not Affect Profitability As Much As Changes in Yield and Price

A comparison of Table 3 with Table 5 shows that a 25 percent cut in yield or price affects IRR more than a 25 percent increase in pond construction costs: profitability is more sensitive to small changes in sales price and yield than to small changes in costs of production. This has several implications. While environmental considerations are a significant research and development cost, the costs of potential environmental requirements such as containment fencing are modest and are not likely to affect commercial profitability. Also, expenses for actions that increase yields, generate high sale prices, and maintain year round production and market supply may be worth the cost (up to a point).

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COST ANALYSIS OF FLOATING RAFT OYSTER PRODUCTION IN CHESAPEAKE BAY

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ABSTRACT Previous research has shown that oysters grow very rapidly in floating rafts in Chesapeake Bay. In order to assess the economic feasibility of floating raft culture in Chesapeake Bay, 400,000 clutchless spat (10 mm) were purchased from a local hatchery and grown to market size (>76 mm). 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 (MSX and dermo) 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 predators, primarily mud crabs, and moved into sequentially larger mesh cages and rafts as they grew. Twelve months later, when the animals reached 50–60 mm in height, 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 and alternative culture methods will be discussed.

KEY WORDS: oysters, cost analysis, aquaculture, Chesapeake Bay, growth

INTRODUCTION

The catastrophic decline in natural oyster populations in Chesapeake Bay has left the Maryland and Virginia oyster industries in near collapse. While the main cause of the historical decline (since the late 19th century) is most likely overharvesting, more recent dermo and MSX occurrences, caused respectively by the parasitic protozoans *Perkinsus marinus* and *Haplosporidium nelsoni*, have essentially wiped out remaining natural stocks and made traditional culture methods in the region unproductive. With natural stocks not available and traditional methods compromised, renewed interest in alternate oyster culture methods has occurred in the region. Most of these alternate methods involve suspended or off-bottom culture.

Off bottom culture has long been known to enhance bivalve growth rates. Truitt (1931) showed that lifting oysters off the bottom, even by only a few inches, increased their growth rate by 50 to 100%. More recently, Paynter and DiMichele (1990) showed that oysters raised in floating rafts exhibited very high growth rates (15 mm/month) and that oysters selectively inbred for faster growth grew more rapidly than their native counterparts. These observations led to the suggestion that large-scale intensive oyster aquaculture in floating rafts might be economically feasible in the Chesapeake Bay region.

In order to assess this possibility, 400,000 oyster spat of a selectively inbred line (see Brown and Paynter 1991) were purchased from a local hatchery in 1989 and raised in floating rafts to market size from 1989 through 1991. Records were kept which quantified the number of rafts and cages needed, supplies and associated equipment, as well as the labor performed in maintaining the animals. A cost model was constructed based on estimated labor costs and observed mortality and handling loss.

MATERIALS AND METHODS

The production site was located on the Wye River, MD, where an experimental oyster growing site had been maintained for the previous two years. This site was characterized by low salinity (8–10 ppt) and high chlorophyll *a* levels. It has supported good growth and had shown little, if any, prevalence of *P. marinus* during the study period. This was critical because *P. marinus* infection severely reduces growth in oysters (Andrews 1961, Paynter and Burreson 1991) and would significantly influence our estimation of production times. Furthermore, the site offered the water surface area and security required for the relatively large-scale operation.

The oysters were raised in floating rafts made of polyethylene mesh designed by F. Wilde (Chesapeake Bay Oyster Culture Co., Shady Side, MD). The rafts consisted of wooden frames with polyethylene mesh (12.5 or 21 mm) 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 × 61 cm wide × 20 cm deep. A 91 cm × 61 cm panel of extruded styrofoam wedged underneath the wooden frame was used to keep the tray afloat. The rafts were attached to long lines (6.35 mm diameter; approx. 183 m long) at 1.2 m intervals by a 1.2 m length of 3 mm diameter line with a brass snap at the raft end.

At the time of purchase (from Chesapeake Bay Oyster Culture

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TABLE 1.

Description of tasks and labor required for raising oysters in floating rafts. Tasks and time estimates are broken down by 4 groups of 100,000 oysters introduced over a 5 week period.

Group	Date	Description of Work	Men × Hours	Total Hours
1989-G1	25AUG89	100,000 Animals introduced	2 × 4	8
	30AUG89	Rafts cleaned	2 × 5	10
	14SEP89	Move up to ⅜″ cages	2 × 2.5	5
	22SEP89	Storm (Hugo) pre-check	1 × 1	1
	27SEP89	Animals bucket rinsed	2 × 1	2
	05OCT89	¼″ line work	1 × 1	1
	26OCT89	New ¼″ line set-up	2 × 0.5	1
	07NOV89	Animals hose-rinsed, some rafts replaced, snap work	2 × 4.5	9
	14MAR90	Rafts and animals hosed thoroughly	2 × 1.5	3
	03MAY90	Move up to ¾″ rafts, pressure-washing rafts and cages	2 × 7	14
	18MAY90	¼″ line and ⅛″ snap work	3 × 1	3
	22MAY90	¼″ line and ⅛″ snap work	2 × 1.25	2.5
	05JUL90	Animals and rafts bucket rinsed	1 × 7	7
	21AUG90	Move ⅜″ cages to ½″ rafts	1 × 4	4
	23AUG90	Pressure-washing cages and rafts	1 × 4	4
	02OCT90	Transferring animals into new rafts	2 × 7	14
	09OCT90	Transferring animals into new rafts	1 × 3	3
	12OCT90	Pressure-washing rafts	1 × 7	7
Total				98.5
1989-G2	05SEP89	120,000 Animals introduced	2 × 6	12
	14SEP89	Animals rinsed	2 × 1	2
	22SEP89	Storm (Hugo) pre-check	1 × 1	1
	25SEP89	Move up to ⅜″ cages	2 × 3.5	7
	05OCT89	¼″ line work	1 × 1	1
	26OCT89	New ¼″ line set-up	2 × 0.5	1
	07NOV89	Animal and tray check	2 × 0.5	1
	14MAR90	Rafts and animals hosed thoroughly	2 × 1.5	3
	18MAY90	¼″ line and ⅛″ snap work	3 × 1	3
	21MAY90	Move up to ½″ and ¾″ rafts	2 × 7	14
	22MAY90	¼″ line and ⅛″ snap work	2 × 1.25	2.5
	31MAY90	Pressure-washing cages and rafts	2 × 1.5	3
	24JUL90	Animals and rafts bucket rinsed	1 × 7	7
	22AUG90	Move ½″ rafts up to ¾″ rafts	1 × 7.5	7.5
	23AUG90	Pressure-washing cages and rafts	1 × 5	5
	19,20,21 SEP90	Move to Mobjack Bay (includes bringing animals in, rinsing them, putting them in clean rafts, trailering them to Mobjack, putting them overboard at Mobjack, and returning Pintail's trailer) (travel time included)	1 × 26 1 × 19	26 19
	13OCT90	Pressure-washing rafts	1 × 7	7
Total				122
1989-G3	14SEP89	100,000 Animals introduced, new ¼″ line set-up	2 × 2.5	5
	23SEP89	Storm (Hugo) post-check	1 × 1	1
	25SEP89	Animals rinsed, rafts replaced	2 × 1.5	3
	05OCT89	¼″ line work	1 × 1	1
	26OCT89	Move up to ⅜″ cages	2 × 4	8
	07NOV89	Animal and tray check	2 × 0.5	1
	14MAR90	Rafts and animals hosed thoroughly	2 × 1.5	3
	18MAY90	¼″ line and ⅛″ snap work	3 × 1	3
	22MAY90	¼″ line and ⅛″ snap work	2 × 1.25	2.5
	24MAY90	Move up to ½″ and ¾″ rafts	2 × 8	16
	31MAY90	Pressure-washing cages and rafts	2 × 1.5	3
	31JUL90	Animals and rafts bucket rinsed	1 × 7	7
	09OCT90	Transferring animals into new rafts	1 × 3	3
	10OCT90	Transferring animals into new rafts	2 × 7	14
	12OCT90	Move ½″ rafts to ¾″ rafts	1 × 7	7
	14OCT90	Pressure-washing rafts	1 × 7	7
	15OCT90	Pressure-washing rafts	1 × 2	2

continued on next page

TABLE 1.

continued

Group	Date	Description of Work	Men × Hours	Total Hours
Total				86.5
1989-G4	27SEP89	100,000 Animals introduced	2 × 4	8
	05OCT89	¼″ line work	1 × 1	1
	26OCT89	Animals hose-rinsed	2 × 1	2
	07NOV89	Animal and tray check	2 × 0.5	1
	14MAR90	Rafts and animals hosed thoroughly	2 × 1.5	3
	18MAY90	¼″ line and ⅛″ snap work	3 × 1	3
	22MAY90	¼″ line and ⅛″ snap work	2 × 1.25	2.5
	30MAY90	Move up to ⅜″ cages ½″ rafts	2 × 5	10
	31MAY90	Pressure-washing cages and rafts	2 × 1.5	3
	21AUG90	Move ⅜″ cages to ½″ rafts	1 × 3	3
	24AUG90	Pressure-washing cages and rafts	1 × 3	3
	15OCT90	Move ½″ rafts to ¾″ rafts	2 × 5	10
	15OCT90	¼″ line work	1 × 2	2
	16OCT90	Transferring animals into new rafts	2 × 7	14
	18OCT90	Pressure-washing rafts	1 × 10	10
Total				75.5
Extras	14SEP89	Working raft (section 1) built	2 × 2	4
	11,15,16,18DEC89	Ice checks	1 × 5.5	5.5
	21DEC89	Aeration system installed	1 × 6	6
	23,26,29DEC89	Ice checks	1 × 2.5	2.5
	04,08,10,22JAN90	Ice checks	1 × 3.5	3.5
	25JAN90	Expanding aeration system	1 × 2	2
	18MAY90	Working raft (section 2) built and connected	3 × 1	3
Total				26.5

Co., Shady Side, MD), the spat were approximately 10 mm long and were provided in groups of 100,000 at 1 week intervals. These oysters were first put into a rectangular polyethylene mesh (3 mm) cage (10,000/cage) which protected them from mud crab (*Rhithropanopeus harrisii*) predation. The cage was then inserted into a floating raft. After two to three weeks the oysters, which had more than doubled in size, were removed from the 3 mm mesh cages and placed in 9.5 mm mesh cages at lower densities (2,500/cage). The oysters remained in the 9.5 mm mesh cages for 4 to 6 weeks. After that time the animals were culled using 13 mm mesh. The oysters caught on 13 mm mesh were placed in rafts made from the same mesh (1,500/raft). At each step, the oysters which fell through the larger mesh were placed back onto the smaller mesh cages or rafts at similar densities. This treatment was used for each group of 100,000 oysters introduced in 1989. The oysters were maintained on the 13 mm mesh for the remainder of the growing season (until November) and the smaller animals which were not originally moved up were transferred into the 13 mm mesh rafts as they grew larger. The oysters were kept in the floating rafts throughout the winter months.

During the following March and April (1990) the animals were culled again using a 21 mm mesh. Oysters which were caught on this mesh were maintained in rafts made of 21 mm mesh (approx. 1,000/raft). Oysters which fell through the mesh were returned to the 13 mm mesh rafts. The culling procedure was performed every other month throughout the 1990 growing season. Fouling of the oysters and the mesh rafts required that both be rinsed and changed regularly. The raft changes generally coincided with culling efforts described above, but the rinsings occurred more often to discourage the sometimes rampant fouling problem. In mid-September a group of approximately 30,000 oysters which were 50 to 65 mm (2 to 2.5 inches) long were transferred to Mobjack Bay, VA, where they were deployed in a system similar to the one on the Wye River. The animals were transferred for two reasons: 1) to enhance the growth rates of the oysters and bring them to market size before the end of the growing season, 2) previous research had shown that oysters transferred to areas of high *P. marinus* prevalence (like Mobjack Bay) during September would acquire little disease but grow very well for several months. The animals were maintained at Mobjack Bay until they were sent to market.

Growth, condition index, mortality, disease status, and total number of the oysters were monitored closely. Activities associated with raising the oysters according to the description above were recorded along with the amount of time dedicated to a specific activity (Table 1). These activities included culling oysters, washing oysters, rafts and cages, changing rafts, making lines and checking the status of the rafts during inclement weather. Supplies and equipment needs were also documented so that a final list of labor, supply and equipment needs could be produced.

A cost model was constructed by incorporating labor cost (set at $10.00/hr), fringe benefit costs (25% of total wages), supply costs, equipment costs, and spat costs. The total of these costs was related to the estimated production of oysters for the time frame described.

RESULTS

Growth

The oyster spat grew relatively well for the low salinity (Fig. 1). By the end of the initial growing season (Nov. 1989), the average height of the spat had reached 25 mm. The next spring growth returned and the animals continued to grow well (9 mm/

month) throughout the growing season. When a subgroup of animals was transferred to Mobjack Bay on Sept. 15, 1990, growth rates increased nearly doubled (17 mm/month; Fig. 1). The average size of the animals at Mobjack Bay by December, 1990, was 98 mm (3.86 in) while the average size of the animals retained in the Wye River was 78 mm (3 in).

Mortality and Loss

Observed mortality was low throughout the growing effort; less than 1%/month on the average. However, mortality and/or handling loss resulted in the loss of 55% of the spat originally purchased from the hatchery. What happened to these spat is unknown. Observations indicate that the animals simply disappeared within 3 weeks of introduction in the 3 mm mesh cages. Neither empty shells nor decomposing tissue were evident in the cages. No mud crabs or flatworms (*Stylochus ellipticus*) were found inside any of the cages. We believe that the animals may have simply fallen through the mesh. Low rates of mortality and loss of the larger oysters continued throughout the second growing season. Although only 30,000 animals were moved to Mobjack Bay in September as an experiment, 150,000 oysters would have been moved had the success of the transfer been anticipated.

Labor

The labor involved with the culture effort was recorded as date, task description, number of individuals involved and hours spent (Table 1). As can be seen from the task descriptions, most of the labor involved the cleaning and changing of rafts with the concomitant culling or thinning of oysters within the rafts. Cleaning fouled rafts was another labor-intensive process. The labor involved in processing and cleaning the animals for market was not included in the cost analysis nor were management and marketing costs.

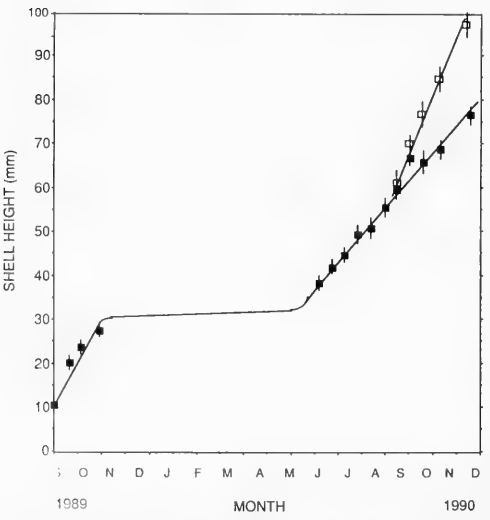

Figure 1. Increase in shell height (mm) of oysters grown at the Wye River (solid points) and Mobjack Bay (open points) between September 1989 and December 1990. Bars represent the standard error of the mean (SEM) of each point.

TABLE 2.

Costs associated with oyster culture in floating rafts.

	Actual	Annualized
Salaries		
500 man hours @ $10.00/hr	$5,000	$5,000
Sub-total	$5,000	$5,000
Fringe benefits (25%)	$1,250	$1,250
Total personnel	$6,250	$6,250
Supplies/Materials	$1,506	$1,506
Major equipment		
Rafts (600 @ $17 ea)	$10,200	$2,040
Cages (150 @ $3.85 ea)	$578	$115
Services		
Spat	$10,000	$10,000
Total direct costs	$28,534	$19,911

Annualized column represents costs in which equipment expenditures were spread over 5 years.

Supplies and Equipment

Rafts, rope, and cages comprised the bulk of the equipment necessary for the growout. Miscellaneous supplies including cable ties, brass snaps, gloves, boots, and tools were also needed. A large working raft (2.4 m × 7.3 m) was constructed from pressure-treated lumber and two 2.4 m styrofoam flotation logs (Read Plastics, Inc., Rockville, MD).

Costs and Estimated Returns

In estimating the cost of production we will assume that between 100,000 and 150,000 oysters would have been produced by the end of 1990. The budget used in estimating the cost of oyster production is presented in Table 2. For cost estimating purposes, labor was estimated at $10.00/hr. and fringe benefit costs were added to that figure. Labor hours were figured from Table 1 (409 hr) with 91 hr added to estimate the time needed for the transportation of all groups to Mobjack Bay. The most expensive equipment involved in the project was the purchase of the rafts ($10,200). Supplies and materials, including lines, snaps, cable ties, gloves, etc., cost $1,506. Seed oysters (spat) cost $10,000. Processing and shipping costs were not estimated. The total cost of the project was $28,534.

To estimate returns relative to the above costs, we assumed that the percentage of animals which grew to market size in group 1 (the only group moved to Mobjack Bay) was representative of the production if all oysters had been moved to VA. This assumption resulted in the prediction that 150,000 market size oysters would have been produced from the 400,000 originally planted 16 months before. Cost per oyster was then figured as:

$$\frac{\text{Total costs (\$28,534)}}{\text{Total oysters (150,000)}} = \$0.19/\text{oyster}$$

When major equipment costs were annualized over 5 yr (the estimated life of a raft without interest added), the cost was reduced to $0.133/oyster. If a higher mortality was factored in which resulted in only 100,000 oysters reaching to market size, then the estimated costs rise to $0.285 or $0.199/oyster, respectively.

DISCUSSION

The costs of oyster production as estimated in this study show that raft oyster culture in the Chesapeake Bay region is expensive.

Wholesale prices for premium half-shell quality oysters during the 1991/92 were $0.25–0.30 (S. Taylor, Capitol Seafood, Jessup, MD). While those prices may appear to provide enough profit over the $0.133/oyster cost estimated by this study to make raft culture in the Chesapeake Bay region feasible, it should be noted that this study estimated the costs of oyster production alone. A more thorough analysis of larger-scale capital, management, marketing costs and current market prices needs to be conducted in order to assess the economic feasibility of raft oyster culture. The production of oysters in floating rafts is a labor-intensive method and its economic feasibility depends on high turnover of oyster stocks and low mortality. This requires identification of good growing areas free from disease (specifically *Perkinsus marinus*), and intensive cultivation and care of the oysters.

Unfortunately, oysters grow best in areas where MSX and dermo are most prevalent (higher salinity). The strategy developed for this project was based on previous research (Paynter and Mallonee 1991, Paynter and Burreson 1991) and employed two distinct sites: a nursery site in a low salinity area with low disease prevalence and a high salinity area which had a high disease prevalence but supported extremely rapid growth (until the animals became infected). The research had shown that oysters introduced into Mobjack Bay in late August or early September would acquire little or no *P. marinus* infection and would grow very well until mid-December (Paynter and Burreson 1991). Furthermore, market research by World's End Aquaculture, Inc., concluded that the low salinity Wye River oysters were not as palatable as the oysters grown in Mobjack Bay, and that a premium half-shell oyster would require a higher salt content than that acquired at the Wye River site.

The growth rate observed in the oysters at low salinity was moderate. Experimental groups of oysters raised at the same site in previous years had grown about 30% faster. However, genetic and seasonal differences in growth rates are not unexpected. A higher growth rate would have resulted in more oysters getting to market size during the study period and lowered the cost per oyster. The mortality suffered in the early part of the study was unexplainable and recurred in 1990. We believe that many of the small spat fell through the mesh and were lost. In 1991, spat were maintained in upwellers for several weeks before deployment in cages and this lowered early losses considerably.

Most oysters grown in floating rafts in areas of high *P. marinus* prevalence failed to reach market size before dying (Paynter and Burreson 1991). This observation suggests that efforts to grow large seed oysters (30 to 50 mm) in areas of low disease prevalence might be more productive than attempting to "force" oysters to grow in areas of higher salinity where growth is much better but soon retarded by disease. The large seed oysters could then be "finished" in areas of higher salinity. This strategy has several advantages. First, low salinity nursery sites might not have to be in approved shellfish harvesting areas since they will not be marketed from that site. This removes a limitation on many of the available areas of low salinity in Chesapeake Bay. Second, it "diversifies" any individual farm so that oysters are in at least two areas at any given time. This might help in times of bad weather, pollution, and with disease problems. As shown in Table 1, the transportation costs are relatively small.

Unfortunately, there are many problems associated with raft culture as well. It is obviously a labor intensive operation which requires a great deal of time and management. It requires a relatively large capital investment and the floating rafts are vulnerable to weather (wind and ice) and theft. It is also likely that *P. marinus* infection, and maybe *H. nelsoni* as well, spreads more readily in highly concentrated groups of oysters. Hence, intensive oyster cultivation may be much more sensitive to disease and its effects. Recently, an oyster growing company in southern Maryland which utilized raft culture closed due to a great extent to the problems associated with *P. marinus* and intensive oyster cultivation (pers. commun., D. Bowers, St. George Oyster Co., Piney Pt., MD). However, if *P. marinus* continues to plague the Chesapeake Bay, many oysters grown on leased bottom will not grow to market size before succumbing to the disease (see Paynter and Burreson 1991). Finally, floating or suspended culture may be severely restricted, even prohibited, by legal restraints and permit regulations. These vary from state to state but represent a significant stumbling block to the development of large-scale alternative oyster culture in many states where alternative culture methods may be required for the survival of the oyster industry. In consideration of the impediments to oyster cultivation, the successful production of large numbers of oysters in an economically feasible way in the Chesapeake Bay region will be quite challenging.

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ECONOMIES OF SIZE AND INTEGRATION IN COMMERCIAL HARD CLAM CULTURE IN THE SOUTHEASTERN UNITED STATES[1]

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ABSTRACT Technological advances have enhanced the commercial feasibility of hard clam culture in the southeastern U.S. region. These improvements relate to all three phases of production: hatchery, nursery, and growout. Recent research indicates that at moderate output levels for stand-alone operations, the nursery and growout phases are profitable, while the hatchery is not. The current study suggests, however, that economies of size exist for larger levels of hatchery output and significant benefits to integration can be achieved when the hatchery is vertically linked with the nursery.

KEY WORDS: hard clams, aquaculture, economics, integration

INTRODUCTION

Considerable interest has developed recently regarding the commercial culture of the hard clam *Mercenaria mercenaria* (Linne) in the Southeastern U.S. This interest is the result of a number of significant technological advances in the culture process, as well as a growing demand and an expanding domestic market for seafood, including finfish and shellfish (Adams, Pomeroy, and Manzi 1991). These recent improvements in technique and method impact all three phases of the culture process—hatchery, nursery, and grow-out. Existing methods have been modified and new techniques have been developed that take advantage of the varied habitats found in the region extending from Virginia to the Florida East Coast.

A recent study examined the financial characteristics of various hard clam aquaculture hatchery, nursery, and grow-out systems (Adams, et al. 1991). The analysis assessed the pro-forma financial feasibility of a number of culture systems on a stand-alone and vertically integrated basis (i.e. combining a hatchery, nursery, and grow-out system into a single operation) for a given level of output. Two key findings emerged. One was that the stand-alone hatchery system was not profitable given the size of operation and other base assumptions used in the analysis. The other was that production cost reductions from integrating the hatchery and nursery were suggested. These findings are of interest for several reasons. First, the limited availability of hatchery seed for nursery conditioning has historically been a weak link in the further development of the hard clam culture industry in the Southeastern U.S. Therefore, potential investors will find a proposed clam culture operation less risky and more promising if the production system is vertically integrated into the hatchery, where seed availability will be controlled. Second, many states in the Southeastern region are considering placing strict limitations on the importation of hard clam seed across state lines for fear of transmitting disease to local oyster beds. As the industry expands in individual states, additional stand-alone commercial hatchery operations may be required to meet the growing local demand for seed, which may no longer be met by supplies from neighboring states. Third, a culture operation which wishes to genetically alter private stocks for purposes of growth rate enhancement, tagging for identification, or other reasons, will need to have complete control of the total reproduction system.

If so, two questions are relevant. At what size of operation does a stand-alone hatchery system become profitable? And, how do the cost characteristics of the seed clam production process change as the hatchery is combined with another level of production, such as a nursery operation?

This paper examines how costs of production for a hard clam hatchery and nursery change as (1) total output (i.e. size) changes and (2) integration between a hatchery and nursery is imposed. The findings allow insight into the inherent economies of size and potential benefits of vertical integration of hard clam culture in the hatchery and nursery phases of production.

MATERIALS AND METHODS

Applicable Economic Theory

Costs, Economies of Size, and Firm Behavior

Total costs of production for a firm include fixed and variable costs. Fixed costs are those which are "sunk" and do not change over a range of output levels (i.e. salaries, interest and depreciation on land, buildings, and equipment). Variable costs change directly with the level of output (i.e. labor, fuel, supplies). When combined and expressed on a per unit of production basis, these costs are referred to as average costs.

The average costs for a firm will change as output is changed, but will do so for different reasons, depending on whether the firm is being examined in a short-run or long-run setting. In the *short-run* as output is changed, the total number of fixed inputs may remain the same. Variable inputs, however, will change. An example would be increasing stocking density of a catfish farm, utilizing the same number of ponds, buildings, and pumps, but increasing labor and feed requirements. A short-run average cost

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curve (SRAC), therefore, describes changes in costs with different levels of output, with at least one input being fixed in quantity (Fig. 1). In a hypothetical *long-run* scenario, all fixed and variable inputs are allowed to adjust freely and optimally as output is changed, with all inputs perfectly divisible and changing proportionally. Long-run average costs (LRAC) represent the least cost combination of inputs per unit of production for each level of output (Fig. 1) (Maxwell, 1970).

The declining portion of the LRAC curve represents gains in the proficiency of labor and more efficient utilization of capital as output is increased. Over this range of output, the proportional change in output exceeds the proportional change in input cost (i.e. decreasing costs per unit of output) (Beattie and Taylor, 1985). "Economies of size" exist over the declining range of the LRAC and cost incentives motivate a firm to increase output over the long run. The rising portion of the LRAC curve represents "diseconomies of size". Adding additional labor or capital, for example, will actually increase average cost faster than output (i.e. costs per unit of output are increasing). A firm will have no long run cost incentive to expand into this range of output (Ferguson and Maurice, 1975). In real world cases, all inputs (particularly fixed inputs such as pumps, ponds, buildings, etc.) are not perfectly divisible. Input proportions representing least cost combinations may vary with the level of output. For example, an oyster depuration plant may be able to double volume while utilizing the same building, vats, set of pumps and UV filters, due to excess capacity of the existing plant over a range of output levels. In such cases, the LRAC curve may not be smooth, but rather "lumpy" as output is changed.

In the short run, or when a clam culture firm can not adjust all fixed inputs, profits are maximized where market price equals marginal cost. In long run competitive equilibrium, however, market price will equal the clam culture firm's minimum LRAC. In this case, pure economic profits (a return in excess of that obtainable elsewhere with the current investment) will be zero. The optimal firm size (Q1) will be where the minimum LRAC equals market price (P1). The clam culture industry as a whole will also be in long run equilibrium when all firms adjust to this optimal size. Profits are generated in the short run, however, if market price (P2) rises above LRAC, as shown in Figure 1. This may occur if, for example, the demand for seed clams increases through an increased demand for market clams. Short run profits will exist for clam culture firms whose size extends beyond output level "Q2". Profits for these firms are not maximized, however, until the marginal change in short-run costs for each firm just equals price. Although the individual firms may be in equilibrium, the industry is not. This is so because the presence of these profits may attract more clam culturists into the industry. These new firms will supply more seed clams and eventually bid the market price for seed clams back down to a new long run equilibrium where market price equals the minimum LRAC for each firm and the industry. This adjustment process may have some effect on costs of production (i.e., the shape and position of the LRAC) thereby resulting in a new optimal firm size.

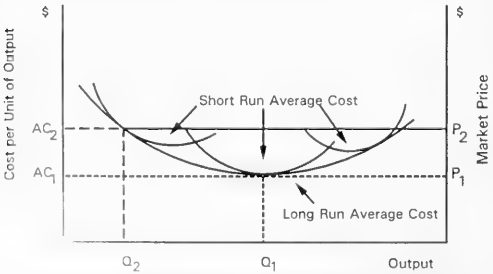

Figure 1. Hypothetical short and long-run average cost curves.

Vertical Integration

When integrating distinct and separate production activities into a single process, some costs of production may be shared. This is true for both variable and fixed costs. For example, land-based hard clam nursery and hatchery systems each require similar support equipment, such as pumps, water intake and treatment systems, heat exchangers, lab space, etc. Utilizing a single support system for a combined (integrated) hatchery/nursery system will likely produce fixed cost savings as compared to a separate support system for each the hatchery and nursery. Similarly, excess capacity and commonality in input use may provide for variable cost savings relative to labor, utilities, and other major expenses as the two processes are combined. If such benefits to vertical integration exist, the LRAC of the integrated system will be lower than the summation of the LRAC's for the separate hatchery and nursery systems. The potential benefits of vertically integrating the hatchery and nursery facilities can be assessed by examining the LRAC values for the stand-alone nursery (A) and integrated (B) systems. Positive differences in the value LRAC (A-B) over a range of facility sizes suggest cost benefits through vertical integration. These benefits to vertical integration may be substantial and result in a significant downward shift and altered rate of change in LRAC over varying output levels (Hay and Morris, 1979).

Financial Analysis

The financial characteristics of hard clam hatchery and nursery systems currently utilized in the Southeastern U.S. are examined. The study focuses on assessing pro-forma financial characteristics of each system on a stand-alone and integrated basis for a given set of hypothetical firm sizes or output levels. A number of assumptions are imposed pertaining to production technology, financial, and market related factors. These assumptions reflect recent research findings and current industry experience within the region. The analysis provides an estimate of least cost production for the hypothetical operation sizes examined.

Statistical and engineering-cost (SEC) methods are used to estimate costs of the hatchery and nursery operations at various hypothetical sizes. These methods are used because a sufficient number of appropriately sized firms to survey for the necessary cost data was not available. The statistical approach uses available information describing the actual costs of production for existing firms of similar size and configuration (Kittiampon and Faris, 1989). The engineering-cost approach utilizes relevant research findings, related technical and accounting data, and current input prices to approximate production costs for hypothetical operation sizes. Estimates are derived for fixed costs (i.e., salaries, overhead, interest, depreciation, etc.) and variable costs (i.e. labor, utilities, fuel, maintenance, operating interest, etc.). At each level of output, the lowest cost estimate derived by the SEC methods is accepted as a point on the LRAC curve (Hay and Morris, 1979).

Cost estimates were derived for six facility output levels for

each stand-alone and integrated system. These costs were estimated on a total and average basis. For the hatchery system, facility sizes of 24, 72, 120, 180, 240, and 480 million (harvest quantity) were utilized. For the nursery and integrated systems (assuming 50 percent mortality during the nursery stage), facility sizes capable of 12, 36, 60, 90, 120, and 240 million harvest quantity were assessed. The increments and range of facility sizes examined reflect reasonable facility size limits as indicated by current industry investment and available information. In addition, the authors have attempted to capture the underlying economic characteristics over the range of firm sizes with a minimum number of incremental shifts.

Hatchery and Nursery Systems Description

Hatchery

The hatchery spawns broodstock clams and raises the larval clams up through the post-set stage to 1 mm juvenile, or seed, clams. The process involves manipulating adult conditioned clams with alternating exposures to chilled and warmed sea water. After several cycles of exposure, the clams spawn. The eggs are sieved, collected, and placed in growing tanks where they develop into larvae. The larvae will "set" (i.e. metamorphose from larvae form to the adult form) after 7 to 14 days. The set are then placed in either upweller or downweller units through which algae enriched water is circulated on a continuous basis. The algae are cultured from unialgal flask cultures of preferred, but possibly non-indigenous, species in a controlled system. This procedure is referred to as the Milford Method. Depending on local water quality and temperature, the entire hatchery process can require 30 to 50 days to go from spawning to 1 mm seed clams ready for placement in the nursery system.

The Milford Method requires a substantial level of capital investment and technical expertise, yet produces a consistently high-quality algae supply which is less dependent on local conditions and algae species. The method is very suitable for high density culture and requires significant investment in a land-based facility, which would include an enclosed building, water treatment equipment, pumps, and algae culture equipment.

Nursery

The nursery is a critical link in the hard clam culture process. Placing hatchery seed clams directly into the field grow-out may induce unacceptably high mortality levels. The nursery provides a semi-controlled, intermediate step which nurtures hatchery-reared seed clams to a size less vulnerable to the stress of field grow-out. In addition, growing seed to the size required for the grow-out stage within the intensive hatchery environment would likely not be cost effective. Three primary alternative methods of nursery culture exist: land-based upflow, land-based raceways, and field nurseries. This study examined the upflow method, which has generally been accepted by the industry as a consistently reliable method. Upflow systems utilize ambient seawater, which is pumped to reservoir tanks and upflow cylinders. These units in turn bathe the seed clams with a vertical flow. The seed clams rest on a fine mesh screen as the water flows vertically through them. Several upflow units (open-ended cylindrical tubes of desired diameter) may share a common reservoir of water. The movement of water also serves to remove waste and prevent any accumulated siltation from suffocating the seed. The length of time required by a nursery to produce a 7 to 15 mm clam from 1 mm seed received through a hatchery will depend largely on water quality and temperature. The process requires from three to six months for the Southeastern region.

RESULTS AND DISCUSSION

Total Initial Investment and Annual Costs

Total initial capital investment, annual cash, and annual non-cash costs were estimated for each system. An overview of these total cost values are presented in Tables 1 through 3. A detailed listing of the various items in each cost category is not given for the sake of brevity, but is available from the authors upon request.

Hatchery

The initial capital investment cost estimate includes a support facility (i.e., building, seawater transmission and treatment system, heat exchanger, security system, etc.) and hatchery equipment (i.e., broodstock/post-set maintenance and algal/larval culture). Investment costs associated with the support facility represents the largest of the cost categories. The total of these investment costs increased from $243,000 to $396,000 over the range of facility sizes (Table 1). This represents an increase of only 60 percent as production increases twenty-fold.

Total annual costs increased approximately two-fold over the range of facility sizes, with the largest components being salaries/benefits, variable costs, and depreciation. An increased need for technical assistance resulted in an increase in salary related costs, while variable costs rose primarily as a result of costs associated with algae production and utilities (i.e., pumps and building) increasing directly with the output level. Non-cash depreciation costs increased commensurate with the level of capital investment.

Nursery

In contrast to the hatchery system, the support facility represents less than half of the total initial capital investment cost. The nursery equipment (i.e., passive flow system, covered pad, forced active flow system, rack system, culling screens, etc.) accounts for the largest share. Total investment costs increased from approximately $171,000 to $219,000 over the range of facility sizes (Table 2). This represents only a 30 percent increase in investment costs. Nursery equipment cost increases, which increase at a more rapid rate than those for the support facility, are primarily attributable to the additional cylinders, reservoir capacity, plumbing, and filters required by the passive flow system as output is increased. Additional pumping capacity is also required. Support facility costs decrease relative to the hatchery due the lack of any need for algal culture equipment at the nursery stage.

Total annual costs increase from $156,000 to $1,762,000 over the range of facility sizes, attributable primarily to increases in variable costs, salaries/benefits, and operating loan interest. Variable costs increase drastically due to the need to purchase seed clam for stocking the stand-alone nursery. Assuming a 50 percent level of mortality, 24 million seed clams are purchased at .3 cents apiece for stocking the facility capable of producing 12 million clams. The cost for the seed clams is $72,000. A facility capable of producing 240 million clams will need to purchase 480 million seed clams, which represents a cost of approximately $1.4 million. An operating loan will be required, which results in an interest

TABLE 1.

Costs[1] associated with a stand-alone Milford hard clam hatchery system of various sizes.

Cost Category	Facility Size (× One Million Seed Clams)					
	24	72	120	180	240	480
Initial capital investment:						
Support lab	$198.6	$198.6	$244.3	$244.3	$307.9	$307.9
Hatchery equipment	44.5	44.5	65.0	65.0	88.6	88.6
Annual cash costs:						
Interest on capital[2]	14.6	14.6	18.6	18.6	23.8	23.8
Variable costs	30.5	32.8	37.5	40.1	44.3	58.2
Salaries/Benefits	36.3	36.3	58.1	58.1	80.0	80.0
Overhead	3.0	3.0	5.5	5.5	8.0	8.0
Interest on operating loan[2]	8.4	8.7	12.1	12.5	15.9	17.5
Annual non-cash costs:						
Depreciation[3]	24.1	24.1	34.5	34.5	42.0	42.0
Opportunity cost of owner equity[2]	11.0	11.0	13.8	13.8	17.5	17.5
Total annual costs[4]	127.9	130.5	180.2	183.1	231.3	247.0

[1] Units of $1,000.
[2] Computed on interest rate of 12 percent.
[3] Straight-line method.
[4] May not add due to a rounding error.

charge for the large facility of $184,000. Costs associated with salaries/benefits increase commensurately with the increased need for technical and management services as output level increases.

Integrated System

Given that the integrated system represents the combination of a hatchery and nursery facility, initial capital investment costs exceed that for either stand-alone operation. Investment costs associated with the support lab increase to reflect the needs of both a hatchery and nursery, although economies exist such that the total support lab cost is less than the cumulative total for the stand-alone hatchery and nursery. Major shared support facility costs include pumps, building space, plumbing, and a pickup truck (Table 3). Costs associated with equipment specific to a hatchery and nursery are also required. The total initial investment cost for the integrated system ranges from approximately $387,000 to $549,000 over the range of facility sizes.

Salaries and benefits represent the largest annual cost category. Variable costs are reduced significantly for the integrated versus the stand-alone nursery system as seed clam are produced by the hatchery and not purchased on the open market. Non-cash depreciation costs increase as a result of the combined investment in hatchery and nursery equipment. Total annual costs increase from $192,000 to $376,000 over the range of facility sizes.

TABLE 2.

Costs[1] associated with a stand-alone upflow hard clam nursery system of various sizes.

Cost Category	Facility Size (× One Million Clams)					
	12	36	60	90	120	240
Initial capital investment:						
Support lab	$ 77.5	$ 77.5	$ 77.5	$ 77.5	$ 81.5	$ 81.5
Nursery equipment	93.4	93.4	115.0	115.0	137.2	137.2
Annual cash costs:						
Interest on capital[2]	10.3	10.3	11.6	11.6	13.1	13.1
Variable costs	83.6	227.9	375.6	556.1	740.3	1,460.8
Salaries/Benefits	15.9	15.9	32.0	32.0	42.3	59.4
Overhead	4.0	4.0	6.5	6.5	9.0	9.0
Interest on operating loan[2]	12.4	29.7	46.7	71.4	94.9	183.5
Annual non-cash costs:						
Depreciation[3]	21.2	21.2	23.4	23.4	26.1	26.1
Opportunity cost of owner equity[2]	8.0	8.0	8.9	8.9	10.0	10.0
Total annual costs[4]	155.5	317.1	504.7	709.9	935.8	1,761.9

[1] Units of $1,000.
[2] Computed on interest rate of 12 percent.
[3] Straight-line method.
[4] May not add due to a rounding error.

TABLE 3.

Costs[1] associated with an integrated hard clam hatchery/nursery system of various sizes.

Cost Category	Facility Size (× One Million Clams)					
	12	36	60	90	120	240
Initial capital investment:						
Support lab	$249.8	$249.8	$286.4	$286.4	$322.9	$322.9
Hatchery equipment	44.5	44.5	61.0	61.0	88.6	88.6
Nursery equipment	93.4	93.4	115.0	115.0	137.7	137.7
Annual cash costs:						
Interest on capital[2]	23.3	23.3	27.7	27.7	32.9	32.9
Variable costs	43.1	45.6	54.1	57.2	65.5	79.9
Salaries/Benefits	52.3	52.3	90.1	90.1	122.2	139.4
Overhead	5.0	5.0	8.0	8.0	11.0	11.0
Interest on operating loan[2]	12.0	12.3	18.3	18.3	23.9	27.6
Annual non-cash costs:						
Depreciation[3]	39.0	39.0	50.0	50.0	60.9	60.9
Opportunity cost of owner equity[2]	17.1	17.1	20.3	20.3	23.9	23.9
Total annual costs[4]	191.8	194.6	268.5	271.9	340.4	375.8

[1] Units of $1,000.
[2] Computed on interest rate of 12 percent.
[3] Straight-line method.
[4] May not add due to a rounding error.

Long-run Average Costs of Production as a Function of Facility Size

Long-run average costs (LRAC) are quantitatively defined as total annual costs expressed on a per unit of production basis. Tables 4 through 6 provide LRAC for each system, with the various components of LRAC expressed as a percentage of the total over a range of facility sizes. These values are graphically depicted in Figures 2 through 5.

Hatchery

Estimates for total LRAC decrease from $5.33 to $0.52 over the range of facility sizes (Table 4). Note that LRAC is expressed per one thousand one mm seed clams, which is how hatchery reared product is typically marketed. Facility size, however, is expressed in terms of total number of one mm seed clams produced. Opportunity cost of owner equity represents about 9 percent of the total LRAC, with the other cost components represents approximately equal shares of 30 percent. LRAC decreases from $5.33 to $1.81 as facility size is increased from 24 to 72 million, respectively. With a current market price of approximately $3.50 per thousand one mm seed clams, the stand-alone hatchery facility becomes profitable above annual costs at a facility size somewhere between 24 and 72 million. Assuming no market price effect, the profit margin for the stand-alone hatchery increases throughout the remaining range of facility sizes examined. The percentage distribution of LRAC component remains virtually constant, with salaries and overhead representing a slightly larger share for facility sizes of 120 million and larger.

The declining LRAC is shown in Figure 2. The curve depicted for the range of facility sizes examined represents the declining portion of the theoretical LRAC curve, indicating increasing economies of size as facility size is increased. Most of the economies of size are achieved as output level approaches the 180 million level, although the LRAC continues to decline thereafter. However, LRAC beyond the 180 million facility size denotes that approximate constant returns to size exist.

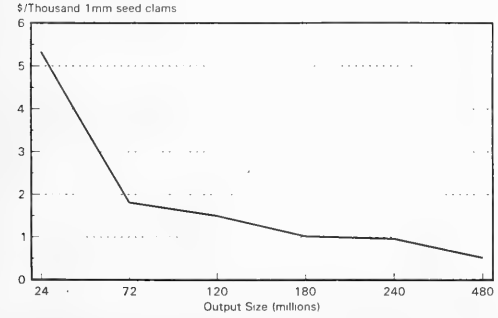

Figure 2. Long run average cost curve for hard clam hatchery (Milford) system.

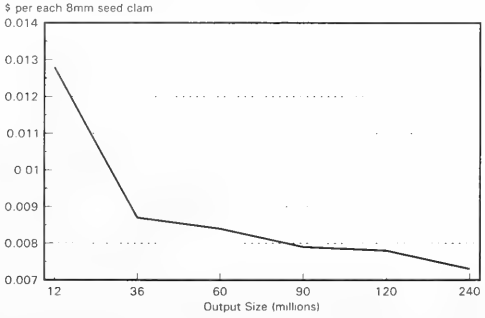

Figure 3. Long run average cost curve for hard clam nursery (upflow) system.

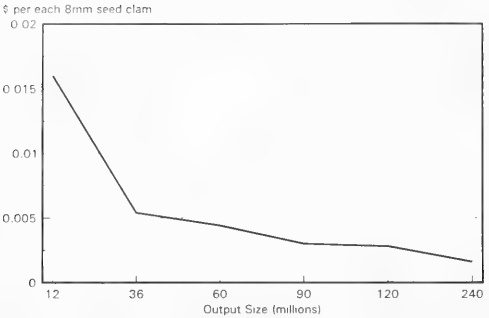

Figure 4. Long run average cost curve for hard clam hatchery/nursery integrated system.

Nursery

Long-run average costs for the stand-alone nursery operation are presented in Table 5. Given that the nursery must purchase seed clams on the open market, variable costs represent the largest share of the total cost per clam. The other cost components decline as a percentage of total cost due to the increased volume of seed clams required for successively larger facilities. LRAC declines from $0.0128 for the 12 million clam facility size to $0.0087 for the 36 million clam facility. For facilities larger than the 36 million clam capacity, LRAC is virtually constant. This indicates that most economies of size are achieved as the facility expands output from 12 to 36 million. Most economies of size are achieved at a smaller facility size than for the hatchery operation.

The LRAC curve for the nursery operation is depicted in Figure 3. As for the hatchery operation, the LRAC estimates are contained on the declining portion of the LRAC curve. Given a current market price of $0.02 each for eight mm seed clams, as would be produced by the nursery operation, the operation is profitable at all facility sizes. Assuming no market effects on local price from increased levels of production, the profit margin increases from $0.004 to $0.0184 per eight mm clam over the range of facility sizes.

Integrated System

The percentage distribution of cost components of the LRAC for the integrated system change somewhat with facility size (Table 6). The variable cost component remains relatively constant over the range of output levels. Interest on capital investment, however, decreases as a percentage of LRAC by over seven percent. In addition, salaries and overhead costs increases from 30 to 44 percent over the range of facility sizes. Opportunity costs of owner equity remains less than ten percent. Estimated total LRAC decreases by 90 percent from $0.0160 for the 12 million output level to $0.0016 for the 240 million output level. Although most economies of size are realized between the 12 and 36 million output levels, LRAC continues to decrease, though at a reduced rate, throughout the remaining range of facility sizes (Fig. 4).

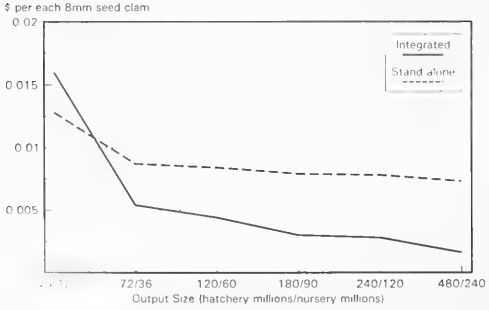

Figure 5. Long run average cost curves for hard clam stand-alone nursery and integrated systems.

TABLE 4.

Estimated long-run average costs of production for hard clam hatchery system of various sizes (Milford system).

Cost Category	Facility Size (× One Million)					
	24	72	120	180	240	480
V[1]	30.5%	31.7%	27.5%	28.7%	26.0%	30.6%
S[2]	30.7	30.2	35.3	34.7	38.0	35.6
C[3]	30.2	29.6	29.5	29.0	28.4	26.6
O[4]	8.6	8.5	7.7	7.6	7.6	7.2
T[5]	$5.33	$1.81	$1.50	$1.02	$0.96	$0.52

[1] Operating capital interest and variable costs.
[2] Salaries and overhead.
[3] Interest on capital investment.
[4] Opportunity cost of owner equity.
[5] Total cost per 1000 one mm seed clams.

Cost Benefits Through Vertical Integration

The previous discussions focus on the changes in LRAC as facility size (number of clams produced) is changed. Cost characteristics of the firm can also be affected through integration of separate phases of production. The following discussion addresses findings related to changes in LRAC resulting from vertical integration of the hatchery and nursery facilities over a range of facility sizes.

Except for the smallest facility size, LRAC for the stand-alone nursery exceeds the LRAC for the integrated system (Fig. 5 and Tables 5 and 6). The initial capital investment for the integrated system exceeds that for the stand-alone nursery for all facility

TABLE 5.

Estimated long-run average costs of production for hard clam nursery system of various sizes (upflow system).

Cost Category	Facility Size (× One Million)					
	12	36	60	90	120	240
V[1]	61.7%	81.6%	84.5%	88.5%	89.7%	94.5%
S[2]	12.5	5.7	7.1	5.1	5.1	2.7
C[3]	20.3	10.4	7.1	5.1	3.8	2.7
O[4]	5.5	2.3	1.3	1.3	1.4	0.1
T[5]	$0.0128	$0.0087	$0.0084	$0.0079	$0.0078	$0.0073

[1] Operating capital interest and variable costs.
[2] Salaries and overhead.
[3] Interest on capital investment.
[4] Opportunity cost of owner equity.
[5] Total cost per each 8 mm clam.

TABLE 6.

Estimated long-run average costs of production for hard clam integrated hatchery/nursery system of various sizes.

Cost Category	Facility Size (× One Million)					
	12	36	60	90	120	240
V[1]	28.8%	29.6%	27.3%	26.7%	25.0%	25.0%
S[2]	30.0	29.5	36.3	36.7	39.3	43.8
C[3]	32.5	31.5	29.5	30.0	28.6	25.0
O[4]	8.7	9.4	6.9	6.6	7.1	6.2
T[5]	$0.0160	$0.0054	$0.0044	$0.0030	$0.0028	$0.0016

[1] Operating capital interest and variable costs.
[2] Salaries and overhead.
[3] Interest on capital investment.
[4] Opportunity cost of owner equity.
[5] Total cost per each 8 mm clam.

sizes. This is reflected in the LRAC for the small integrated facility by relatively large estimates for capital interest, depreciation, and opportunity cost. The contribution of these costs causes the LRAC estimate for the integrated system to exceed that for the nursery at the small facility size. However, as facility size increases, costs associated with the required capital investment for the integrated system increase less rapidly (on an average basis) than do the variable costs for the nursery system (since the integrated system does not need to purchase seed clams). Therefore, LRAC for the stand-alone nursery system exceeds the integrated system at all facility sizes above the 12 million output level. At the 36 million output level, LRAC for the integrated system represents a 38 percent reduction in the LRAC associated with the stand-alone nursery. At the 240 million output level, LRAC for the integrated system represents only 22 percent of the LRAC for the stand-alone nursery. Therefore, benefits to vertically integrating the hatchery and nursery systems exist at facility sizes which exceed 12 million clams. These benefits continue to increase at a slightly increasing rate as facility size is further increased to 240 million clams.

CONCLUSIONS

The stand-alone hatchery operation becomes profitable at some long run output level of between 24 and 72 million clams, given current market prices per thousand seed clams. Additional research needs to be conducted for firms within this range of facility sizes to refine the lower limit of profitability. Alternatively, the stand-alone nursery is characterized by long run average costs that lie below current prices at all facility sizes. The output levels at which profit *maximization* occurs in the short run for the hatchery and nursery were not determined. Further research into the nature of marginal costs (i.e., the change in variable costs with changes in output) is required.

For the hatchery and nursery stand-alone systems, most economies of size are achieved at the 72 and 36 million clam output level, respectively. Approximate constant returns to size are exhibited beyond these output levels, although LRAC does continue to decrease throughout the range of facility sizes. Diseconomies were not observed over the range of facility sizes examined.

Significant cost reductions are achieved through vertical integration of the hatchery and nursery facilities at output levels of 72 and 36 million clams and beyond, respectively. These benefits to vertical integration may provide incentives for local investors to become less dependent on non-local supplies of seed clams. This will be particularly true for relatively larger facilities. For stand-alone nursery operations with an output capacity below 36 million clams, no cost incentive exists for vertical integration into hatchery operations.

An underlying assumption in this analysis is that market prices are not sensitive to industry output. However, potential investors must assess the impact to local market prices resulting from large production levels. The analysis assumes a constant market price, which may not be valid as the production volumes from large scale operations are released onto the market. Brown and Folsom (1983) and Hsiao, Johnson, and Easley (1986) have suggested that hard clam prices are sensitive to changes in hard clam supplies. In general, hard clam prices decrease (increase) as hard clam landings increase (decrease). This relationship is not incorporated into the preceding analysis, which focuses primarily on cost changes as output levels vary. Investors in hard clam aquaculture should be aware of the potential negative effects on market prices as firm size and industry output levels increase.

Although the theoretical equilibrium for clam culture firms and the industry will likely never be achieved, the process set in motion as firms and the industry adjust toward equilibrium is a "real world" phenomenon and can be observed. Given the cost curves estimated and the current market prices, profits are currently being generated by at least some firms in the seed clam culture industry. With the exception of stand-alone hatchery operations of between 24 and 72 million seed clam output, profits are being accrued by both the hatchery and nursery operations over the entire range of estimated facility sizes. The existence of these profits will likely attract new firms. In addition, existing firms may increase their size of operation to take advantage of further economies of size. Such industry development may eventually bid down prices for seed clams in the face of a fixed level of demand. Also, prices for certain variable and fixed inputs and services may be bid up, as the demand for these factors by clam culturists increases. What may result is a market price that is falling simultaneous to a rising cost structure. This adjustment process may stabilize the rate of investment in the industry and better define the "typical" size for a clam hatchery and nursery operation. If improvements to the current technology occur, the cost structure will be affected and the adjustment process will begin anew. The adjustment process may also be reinitiated if market developments occur that increase market prices and, therefore, profits.

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MARINE AQUACULTURE ENFORCEMENT: PASSING THE BUCK

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ABSTRACT All states 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. The principal argument supporting maintenance of size restrictions for wild and cultured shellfish in some states 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. This paper discusses this issue in more detail and presents simulated estimates of financial losses due to harvest size restrictions for a hypothetical oyster (*Crassostrea virginica* Gmelin) aquaculture business. Estimates of the economic cost of enforcing a minimum size regulation on oyster aquaculture producers in Florida are presented.

KEY WORDS: aquaculture, enforcement, regulation, *Crassostrea virginica*

INTRODUCTION

Shellfish resources like other marine fisheries are subject to the "tragedy of the commons." Hardin (1968) described the "tragedy" as one in which the absence of property rights, or equivalently, the inability to control access leads to the ultimate destruction of a resource. However, in point of fact, property rights do typically exist for shellfish resources. All states do claim a territorial right to their coastal waters and access to harvest shellfish resources is restricted in some fashion. Thus, some form of weakly specified property rights are exerted over shellfish resources. Unfortunately, these rights simply grant access to harvest but not to produce shellfish. With no ability to establish property rights to an individual's production activity, no one has the incentive to conserve shellfish resources. Under these conditions, the outcome has been over-harvesting and the typical public policy response has been regulation.

The purpose of marine shellfish regulations—and from here on out we will focus on molluscan shellfish—is to protect public shellfish resources. In most cases this management objective is accomplished by controlling harvest levels, harvesting effort, harvesting technology or, more likely, some combination of all of these. Some common regulatory instruments include, but may not be limited to, daily bag limits, restrictions on areas, seasons or time fished, limits on allowable gear, and minimum size limits.

The conditions that lead to over-harvesting of public shellfish resources encourage the development of private culture on leased bottoms. With a strong demand for seafood and depletion of wild resources, aquaculture of a variety of shellfish species has become an increasingly attractive investment opportunity. In states such as Virginia and Louisiana, with a long history of shellfish aquaculture, private entrepreneurs are exempt from most shellfish harvest regulations that are enforced on public resources. In other states, Florida among them, private aquaculture businesses are subject to some if not all regulations that are designed to protect wild shellfish stocks.

The principle argument made to support regulation of marine aquaculture is that marine enforcement agencies cannot distinguish between wild and cultured product. Therefore, effective management of wild shellfish resources requires that all sources of product be subject to the same rules. Unfortunately, shellfish regulations tend to restrict the ability of private shellfish producers to operate in the most efficient manner possible. Consequently, private aquaculture producers either operate at a higher cost or a lower level of productivity or both. In any case, lower levels of profitability result as the burden of payment for enforcement of regulations designed to protect public shellfish resources is shifted—the buck is passed—to aquaculture businesses.

The objective of this paper is to demonstrate the economic cost of marine shellfish regulations to aquaculture businesses. For demonstration purposes a minimum size limit on oysters (*Crassostrea virginica* Gmelin) in Florida will be used as a case study. Our purpose is to; 1) show how regulations whose purpose is to protect public shellfish resources can indirectly affect aquaculture profitability, hence growth in the shellfish aquaculture industry, and 2) demonstrate the potential gains from designing alternative mechanisms for marine shellfish regulation.

OYSTER AQUACULTURE PRODUCTION SYSTEM

For demonstration purposes a case study of an aquaculture business using a flexible belt production system will be analyzed. The design and production methods for the flexible belt system are documented in Creswell et al. 1989, and in Vaughan et al. 1988. The flexible belt looks much like a ladder with flexible joints at each rung with a nylon mesh bag containing the oysters placed in the space between. The belt is anchored to the bottom with screw anchors that are removed when harvesting or handling. The flexible belt system offers several distinct advantages.

1. The entire belt is constructed of rope, PVC, and nylon bags.

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This makes the belt light, durable, corrosion resistant, and easy to handle.

2. The oysters can be moved or handled simply by bringing the belt to the surface offering operational ease in cleaning, sorting or sampling oysters.
3. Harvesting can be accomplished by raising the belt and removing the oysters from each bag. Harvesting in this manner reduces labor requirements and reduces harvest costs.
4. The oysters are retained at all times in a nylon mesh bag and are protected from benthic and free-swimming predators.
5. The flexible belt system produces a cultchless oyster that is uniform in size and shape. Such an oyster may be regarded as a premium product for restaurants and the raw bar trade.

Table 1 presents a production schedule used for the flexible belt production system. Seed oysters 8 mm in size are placed in 2 mm nylon mesh nursery bags at the beginning of the production cycle at a stocking rate of 10,000 oysters per bag. Management of the flexible belt system requires periodic cleansing of the belt and monitoring of the growing oysters to assure proper stocking densities and optimal growing conditions. While in the first nursery bag the oysters are inspected and the bags are cleaned twice each week. When the oysters reach a 6.25 mm size they are restocked at a rate of 4,000 oysters per bag into a second nursery bag having a 3.25 mm mesh size. Inspection and cleaning of the second nursery bag is also done twice weekly. The production cycle continues as indicated in Table 1 with the oysters being sequentially restocked in three growout bags of 6.25, 12.5, and 18.75 mm size mesh until being harvested.

PROCEDURES

The state of Florida enforces a 75 mm (three inch) minimum size limit on oysters harvested from state waters. To evaluate the economic cost of enforcing a 75 mm minimum harvest size a baseline condition must be established. Therefore, the first step in the analysis is to estimate costs and returns for an oyster aquaculture business subject to a 75 mm minimum harvest rule. Once baseline profitability has been established, the growout period can be varied to estimate the difference in profitability for different harvest sizes. For comparative purposes, growout periods for 75 and 62.5 mm oyster sizes were used. Field trials under Florida growing conditions indicate that, on average, oysters reach a 62.5 mm size in approximately 12 months and take an additional 3 to 6 months to reach the legal 75 mm size. Therefore, there is a 3 to 6 month period over which Florida producers incur mortalities (hence losses in revenue) and higher operating costs than would be the case if oysters were harvested at the 62.5 mm size. To estimate these losses, an economic analysis using a spreadsheet computer program, of a representative Florida oyster aquaculture business using the flexible belt system was developed.

TABLE 1.

Production schedule for flexible belt.

Production Activity
1) Stock 8 mm seed @ 10,000/bag in 2 mm mesh nursery bags
Clean/Inspect bags twice weekly
2) Stock 6.25 mm seed @ 4,000/bag in 3.25 mm mesh nursery bags
Clean/Inspect bags twice weekly
3) Stock 18.75 mm seed @ 1,500/bag in 6.25 mm mesh growout bags
Clean/Inspect bags weekly
4) Stock 25 mm seed @ 500/bag in 12.5 mm mesh growout bags
Clean/Inspect bags weekly
5) Stock 37.5 mm seed @ 250/bag in 18.75 mm mesh growout bags
Clean/Inspect bags monthly
6) Harvest oysters

Economic Analysis

Figure 1 depicts the structure of the spreadsheet program and how the different elements are linked. The analysis provides an economic framework from which to analyze a representative oyster aquaculture business on a monthly basis from start up and continuing for a five year period. The economic analysis consists of a series of linked budgets beginning with a "process" budget. The process budget provides an itemized monthly record of all production activities (listed in Table 1) and the resources required to conduct those activities. Listed in the box labeled "Process Budget" in Figure 1 are the choice variables that define the technical production conditions used in constructing the process budget. According to field trials, survival rates for a 15 month production period have ranged between 85 and 90 percent. A simplifying assumption of a 99% monthly survival rate is made (a 99% monthly rate results in an 86% survival rate at harvest for a 15-month production period). In practice, monthly survival rates fluc-

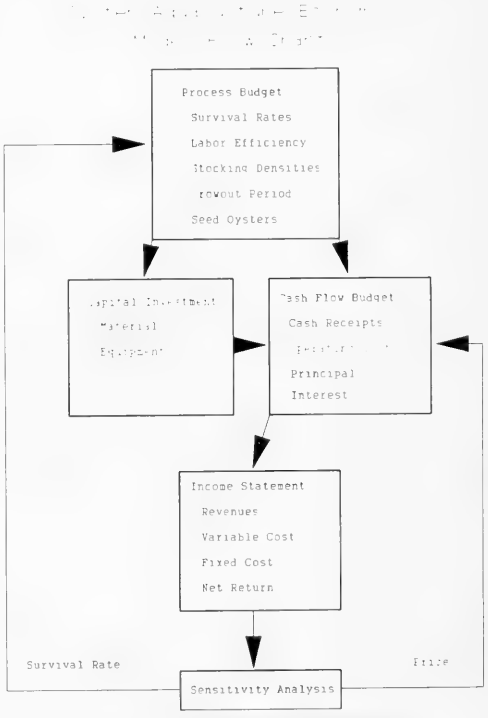

Figure 1. Computer spreadsheet program flow.

TABLE 2.

Five year total financial and resource requirements by growout period (99% MSR and $.20 per oyster).

	Growout Period				
	Twelve Month	Fifteen Month	Eighteen Month	Twelve/Fifteen Month	Fifteen/Eighteen Month
Harvested oysters (each)	4,933,638	3,679,310	2,745,120	4,217,648	3,168,762
Operator labor (hours)	6,445	6,162	6,048	6,177	6,020
Capital investment ($)	35,398	35,386	35,466	35,373	35,430
Cash receipts ($)	1,051,079	735,862	549,024	871,451	63,375
Operating interest ($)	1,865	2,649	3,624	2,036	2,956
Fixed interest ($)	9,292	9,288	9,309	9,285	9,300
Total cost ($)	170,564	146,130	132,383	156,297	138,407
Net return ($)	880,514	589,732	416,641	715,152	495,344
Total seed (each)	7,260,000	5,580,000	4,590,000	6,300,000	5,040,000
Seed per month (each)	121,000	93,000	76,500	105,000	84,000

tuate due to a variety of environmental factors such as weather conditions, predators, and diseases. The principal concern, however, is the net survival at harvest. Alternative survival rates are addressed later in a sensitivity analysis. Labor efficiency is assumed to be 3 bags handled per minute or one complete belt handled in 48 minutes. This labor efficiency applies to all required tasks; cleaning, inspecting, sorting, and harvesting. Stocking densities are the same as that indicated in Table 1. Harvest at the 75 mm size takes place at the end of the 15th month. Seed oysters are planted on a monthly basis to simulate a business that provides product on a sustained year-round basis. The number of seed oysters planted depends upon the size of the operation and the length of the growout period. For any given plant size (one acre or 18 belts in this case) the faster the oysters can be grown and harvested the greater the number of seed oysters that can be planted on a sustained basis. Therefore, once the growout period has been selected and all other factors are held constant, the spreadsheet program selects the maximum amount of seed that can be planted subject to the 18 belt limit.

The process budget provides an itemized list of labor, oysters planted, oysters growing by size class, and the number of bags by size class. The latter information is used to compute the cost of materials for the capital investment schedule. Additional equipment such as a truck, boat, motor etc. are also included in the capital investment schedule. Of the capital investment needs, all equipment except the materials for the flexible belts are invariant with respect to growout period.

The cash flow budget includes cash receipts from the sale of oysters, operational cash costs, and principal and interest payments on operating and capital investment loans. The cash flow budget is linked to the process budget in that the number of harvested oysters and required monthly hired labor come from the process budget. Similarly, the cash flow budget is linked to the capital investment schedule in that the level of principal and interest payments on capital loans are ultimately determined by the capital investment schedule.

The income statement is linked to the cash flow budget and provides an annual statement of all costs and earnings and net returns to operator labor, management and risk. The sensitivity analysis determines the effect on net returns under different oyster price and survival rate conditions.

The economic cost of enforcing a minimum size on aquaculture businesses is the difference between net returns under a minimum size restriction and that of no restriction. The current Florida minimum size limit for oysters is 75 mm. Anecdotal evidence from oyster aquaculture industry representatives indicates that a 62.5 mm oyster could be marketed. For the baseline scenario it is assumed that each size is marketed at the same price. The possibility that a smaller oyster will only be marketed at a discounted price will be examined through a sensitivity analysis. Field trials under Florida growing conditions indicate that oysters reach a 62.5 mm size in 12 to 15 months and a 75 mm size in 15 to 18 months. Therefore, net returns for an oyster aquaculture business are estimated for five different growout periods. First, 12 month, 15 month, and 18 month growing periods are analyzed. Given the fact that not all oysters will mature at the same rate two additional scenarios are examined. In the first, 50% of all oysters mature in 12 months and 50% of all oysters mature in 15 months while in the second, 50% of all oysters mature in 15 months and 50% of all oysters mature in 18 months. Many other combinations of maturation rates and growing periods could be examined. The differences in net returns for any two scenarios is a measure of the economic loss associated with a prolonged growing period due to a minimum harvest size restriction.

For each scenario the assumed initial conditions are; 1) a one acre production unit, 2) five year business horizon, 3) 99% monthly survival rate, and 4) $.20 per oyster price. A sensitivity analysis on net returns is performed for each growout period using oyster prices of $.10, $.15, $.20, and $.25 per oyster and monthly survival rates of .85, .87, .89, .90, .95, .97, and .99.[1]

The sensitivity analysis described above produces 24 estimates of net returns for each of the five different growout periods depending upon oyster prices and monthly survival rates. Computing the differences indicated previously, results in 72 estimates of the economic cost of enforcing a 75 mm minimum size limit. Using

[1]Total number of market sized oysters harvested as a percentage of seed planted is calculated as follows:

$$\% \text{ Oysters Harvested} = MRS^{n}$$

where: MSR = monthly survival rate

n = months to harvest

For example, a 97% MSR results in 69.4% ($.97^{12}$) of all seed oysters harvested at the end of a 12 month period.

TABLE 3.

Resource and financial losses (minimum size versus no minimum size regulations).

	Twelve–Fifteen Month	Fifteen–Eighteen Month	Twelve/Fifteen–Fifteen/Eighteen Month
Harvested oysters (each)	1,254,328	934,190	1,048,886
Operator labor (hours)	283	114	157
Investment capital ($)	12	−80	−57
Cash receipts ($)	315,217	186,838	237,699
Operating interest ($)	−784	−975	−920
Fixed interest ($)	4	−21	−15
Total cost ($)	24,434	13,747	17,890
Net return ($)	290,782	173,091	219,808
Total seed (each)	1,680,000	990,000	1,260,000
Seed per month (each)	28,000	16,500	21,000

these estimates as a data base, descriptive statistics such as ranges, means, and standard deviations can be used to describe the economic cost to a private oyster aquaculture business of complying with a minimum harvest regulation.

RESULTS AND DISCUSSION

Table 2 shows the five year totals for key financial variables for each of the five different growout periods. The number of harvested oysters and, therefore, value of cash receipts increases as growout period decreases, showing the effect on net returns of longer growing periods. The twelve month growout period requires the greatest amount of operator labor because of the greater volume of product being tended and harvested. Capital investment and interest payments on the initial investment loan under all scenarios are virtually constant. Interest payments on operating loans are lowest for the twelve month growout period due to the fact that operating loans are paid off sooner as compared to longer growout periods. Total cost increases as the production period decreases due to the fact that for shorter growout periods more product can be handled and, therefore, larger amounts of seed oysters are purchased. However, even though costs are higher the shorter the growout period, the increase in numbers of oysters sold more than offsets the cost of purchasing larger quantities of seed oysters. Net returns, therefore, are highest for the twelve month growout period.

Table 3 shows the computed differences for the resource and financial variables shown in Table 2 for different growout periods. For example, 1,254,328 more oysters are harvested over a five year period when harvested at the end of 12 months (at a 62.5 mm size) as compared to harvest in fifteen months at a 75 mm size. Under different assumed growout periods the difference in the number of oysters harvested under a 75 mm harvest regulation as compared to a 62.5 mm harvest size ranges between 934,190 and 1,254,328 fewer oysters. This difference results in a reduction in cash receipts of between $315,217 and $186,838 over a five year period.

TABLE 4.

Sensitivity analysis of year five net returns for price, survival rate and growing period.

	Growing Periods				
Price/Survival	Twelve Month	Fifteen Month	Eighteen Month	Twelve/Fifteen Month	Fifteen/Eighteen Month
$.15/85%	19,847	−50,729	−95,235	−15,434	−73,621
$.20/85%	62,457	−15,823	−73,696	31,131	−44,829
$.25/85%	101,715	19,084	−52,158	63,833	−16,038
$.15/87%	48,403	−9,498	−52,857	24,600	−30,898
$.20/87%	91,275	30,261	−27,013	60,994	1,749
$.25/87%	131,460	58,976	−1,169	96,077	34,321
$.10/89%	24,800	−17,376	−45,070	3,711	−31,222
$.15/89%	71,481	26,293	−14,982	48,810	5,753
$.20/89%	118,162	57,184	15,105	87,342	39,264
$.25/89%	164,844	87,482	42,701	125,873	65,091
$.10/90%	33,431	−3,275	−30,204	17,700	−16,923
$.15/90%	81,726	37,717	2,145	59,455	22,819
$.20/90%	130,021	69,837	33,776	99,742	5,2347
$.25/90%	178,316	101,956	57,017	140,028	80,114
$.10/95%	70,103	42,668	26,667	55,651	34,201
$.15/95%	126,842	82,974	57,650	103,826	69,568
$.20/95%	183,579	123,280	88,633	152,002	104,934
$.25/95%	240,317	163,587	119,615	200,177	140,300
$.10/97%	82,726	54,835	39,402	68,170	47,091
$.15/97%	143,090	98,529	74,079	119,927	86,334
$.20/97%	203,455	142,224	108,756	171,683	125,577
$.25/97%	263,819	185,918	143,433	223,440	164,820
$.10/99%	94,963	67,285	50,856	92,304	58,316
$.15/99%	159,315	115,276	89,160	155,700	101,019
$.20/99%	223,667	163,267	127,464	219,096	143,722
$.25/99%	288,019	211,258	165,768	282,492	186,425

TABLE 5.

Economic losses associated with a minimum size restriction (no price discount).

Price/ Survival Rate	Growing Periods		
	Twelve–Fifteen Month	Fifteen–Eighteen Month	Twelve/Fifteen–Fifteen/Eighteen Month
$.15/85%	70,576	44,506	58,187
$.20/85%	78,280	57,873	75,960
$.25/85%	82,631	71,242	79,871
$.15/85%	57,901	43,359	55,498
$.20/85%	61,014	57,274	59,245
$.25/85%	72,484	60,145	61,756
$.10/89%	42,176	27,694	34,933
$.15/89%	45,188	41,275	43,057
$.20/89%	60,978	42,079	48,078
$.25/89%	77,362	44,781	60,782
$.10/89%	36,706	26,929	34,623
$.15/89%	44,009	35,572	36,636
$.20/89%	60,184	36,061	47,395
$.25/89%	76,360	44,939	59,914
$.10/89%	27,435	16,001	21,450
$.15/89%	43,868	25,324	34,258
$.20/89%	60,299	34,647	47,068
$.25/89%	76,730	43,972	59,877
$.10/89%	27,891	15,433	21,079
$.15/89%	44,561	24,450	33,593
$.20/89%	61,231	33,468	46,106
$.25/89%	77,901	42,485	58,620
$.10/89%	27,678	16,429	33,988
$.15/89%	44,039	26,116	54,681
$.20/89%	60,400	35,803	75,374
$.25/89%	76,761	45,490	96,067

TABLE 6.

Economic losses associated with a minimum size restriction ($.05 price discount for 62.5 mm oyster).

Price/ Survival Rate	Growing Periods		
	Twelve–Fifteen Month	Fifteen–Eighteen Month	Twelve/Fifteen–Fifteen/Eighteen Month
$.15–.20/85%	35,670	22,967	29,395
$.20–.25/85%	43,373	36,335	47,169
$.15–.20/87%	18,142	17,515	22,851
$.20–.25/87%	32,299	31,430	26,673
$.10–.15/89%	−1,493	−2,394	2,042
$.15–.20/89%	14,297	11,188	9,546
$.20–.25/89%	30,680	14,483	22,251
$.10–.15/90%	−4,286	−5,420	−5,119
$.15–.20/90%	11,889	3,941	7,108
$.20–.25/90%	28,065	12,820	19,628
$.10–.15/95%	−12,871	−14,982	−13,917
$.15–.20/95%	3,562	−5,659	−1,108
$.20–.25/95%	19,992	3,665	11,702
$.10–.15/97%	−15,803	−19,244	−18,164
$.15–.20/97%	866	−10,227	−5,650
$.20–.25/97%	17,537	−1,209	6,863
$.10–.15/99%	−20,313	−21,875	−8,715
$.15–.20/99%	−3,952	−12,188	11,978
$.20–.25/99%	12,409	−2,501	32,671

Table 3 shows that hours of owner operator labor, the level of investment capital needed, interest payments on operating and long term loans, and total operating cost for a five year period varies little with respect to growout period. Economic losses as measured by the difference in net returns to owner operator labor, management and risk, however, is substantial and ranges from $173,091 to $290,782. The results shown in Table 3 are based on an assumed price of $.20 per oyster and a 99% monthly survival rate.

The results reported in Tables 2 and 3 are likely to be what may be called best case scenarios. Oyster survival rates and prices vary over the life of an aquaculture business. Furthermore, smaller sized oysters may not in fact be marketable at the same price as larger ones. To address both of these issues a sensitivity analysis was conducted. Table 4 shows the results of a sensitivity analysis using oyster prices ranging from $.10 to $.25 each and monthly survival rates ranging between 85 and 99%. Reported in Table 4 are the year five net returns to operator labor, management, and risk. Year five was selected because it represents a year for which the aquaculture business has stabilized and has achieved a sustained level of production. The results demonstrate that only under a 12 month growing period (harvest is at a 62.5 mm size) do all combinations of price and oyster survival rates produce positive net returns. However, with a few exceptions, for all growing periods, as long as monthly survival rates are equal to or exceed 87% net returns are positive for oyster prices even as low as $.10 each.

The estimated economic losses associated with enforcing a minimum 75 mm size rule are shown in Table 5. The economic losses range from a high of $96,067 to a low of $15,433 in year five. The average estimated loss if $49,052 and the standard deviation is 18,280. The results reported in Table 5 are based on the assumption that small and larger oysters are marketed at the same price. The effect of relaxing this assumption will be demonstrated momentarily. As long as oysters are marketed at the same price regardless of size, marketing a smaller oyster is always more profitable than marketing a larger one. The reasons are the same as stated earlier; lower mortality, and the product can be turned over more rapidly.

The possibility that an oyster will sell at the same price regardless of size cannot be substantiated due to the lack of the ability to market an oyster less than 75 mm in size. To address this concern an analysis of the effect on net returns of a differential oyster price was conducted. The sensitivity analysis results reported in Table 4 were used to compute the difference between year five net returns for an aquaculture business selling 62.5 mm oysters at a $.05 price discount as compared to the market price for a 75 mm oyster. These results are presented in Table 6. When monthly survival rates are low, (85% or 87%) harvesting earlier at a smaller size yields higher net returns even though the oysters are sold at a lower price. In these cases, the amount of lost revenue due to mortality exceeds the unit price differential, making it irrational to hold out for a better price. The results are less clear as survival rates increase. At low oyster prices ($.10) and higher survival rates (89% to 99%) the price premium paid for a larger oyster more than offsets mortalities associated with a longer growing period required for a .75 mm oyster. Thus, under certain combinations of price, survival rate, and growing period net returns are improved by marketing a larger oyster. In the majority of cases, however,

net returns are higher when a 62.5 mm oyster is marketed even though they are marketed at lower price.

CONCLUSIONS

The economic analysis showed that economic losses to an oyster aquaculture business due to a minimum harvest size restriction averages between $49,052 and $6,892 per year taking into account differences in assumed survival rates and growing periods and whether a price discount is applied to 62.5 mm oysters. Assuming a continuously operating business for a ten year period and a 10% discount rate the present value of such an economic loss is between $331,522 and $46,580 for a single aquaculture business. According to the most recent estimates there are 24 active oyster producers in Florida (Florida Agricultural Statistics Service, 1990). The present value of the economic loss of enforcing a minimum harvest size on private aquaculture businesses ranges between $7,956,528 and $111,792 on an industry-wide basis.

The analysis presented herein was for demonstration purposes and a hypothetical oyster aquaculture business was used as the unit of analysis. Nevertheless, the point that enforcement of shellfish harvest regulations designed to protect public resource stocks effectively may shift a part of the burden of payment for such policies to the private sector was demonstrated.

The state of Florida and many other coastal states have begun to explore and promote the potential of shellfish aquaculture as a way of reviving or diversifying depressed coastal economies. The need to protect and conserve public shellfish resources for those who wish to continue to harvest them is a real and legitimate justification for regulation. However, the need to examine the effects of public shellfish resource management policy on private and public initiatives to promote shellfish aquaculture industry development was demonstrated in this paper.

The principal reason for uniform regulatory treatment of public and private shellfish producers in the state of Florida remains the inability to tell the difference between wild and cultured product once the oysters leave the water. The findings of this study indicate that returns to innovative policies such as tagging or monitoring programs may be in the best long term interest for both private and public concerns.

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ABSTRACTS OF TECHNICAL PAPERS PRESENTED AT AQUACULTURE '92

Triennial meeting of:

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FISH CULTURE SECTION OF THE AMERICAN FISHERIES SOCIETY

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Orlando, Florida

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Editor's Note: Since this meeting was topic-oriented rather than society-oriented, papers presented here represent only those whose author(s) signified membership in the National Shellfisheries Association.

CONTENTS

OYSTER LARVAL SETTLEMENT AND FEEDING: A VIDEO. Shirley M. Baker, Virginia Institute of Marine Science, Gloucester Point, VA 23062.

The objective of this study was to film the settlement and feeding behaviors of *Crassostrea virginica* larvae. Settlement behavior of oyster pediveliger larvae was induced with 1% dimethyl sulfoxide (DMSO). Settling larvae were observed and filmed using a Zeiss IM 35 inverted microscope, a DAGE-MTI video camera and video recording equipment. Larvae and spat were fed 4.5 μm diameter Flouresbrite carboxylate microspheres (Polysciences, Inc.) which were suspended in an algal extract to enhance phagostimulation. The Zeiss IM 35 was converted to a fluoresence microscope by addition of a microscope illuminator with high-pressure mercury source. Oyster larvae show several settlement behaviors including swimming with the foot extended, gliding on the settlement substrate, pivoting on the foot in decreasing circles, and cementing. Oyster larvae feed until gliding behavior begins and the velum is withdrawn. After attachment, velum feeding may continue. As the oysters metamorphose, there is little or no time between the end of velum feeding and the beginning of gill feeding.

ROLES OF PHYSICAL CUES IN OYSTER LARVAL SETTLEMENT SITE CHOICE. Patrick Baker, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Settling oyster larvae use environmental cues to help determine final settlement site choice. Physical cues examined in this study include gravity and small-scale rugosity. Oyster (*Crassostrea virginica*) larvae were allowed 24 hours in darkness to settle onto either side of small settlement substrate chips, cut from adult oyster or scallop shells. Oyster shells were ground smooth on both sides, but one side of the scallops shells had parallel ridges about 700 μ across and 150 μ high. Oyster larvae settled more onto rough than smooth surfaces, regardless of substrate orientation (rough surface facing up, down, or sideways). Gravity had no detectable effect on settlement site choice, either when the larvae were given a choice of rough versus smooth surfaces, or when given only smooth surfaces to settle onto. This study was conducted in the absence of water currents, which have been shown by other researchers to interact with other settlement cues in some species. Tidal currents may account for apparent differences between the results of this study and some field settlement observations.

OPTIMIZATION OF TRIPLOIDY INDUCTION FOR THE OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN). Bruce J. Barber* and **Roger Mann,** Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062; **Standish K. Allen, Jr.,** Haskin Shellfish Lab, Rutgers University, Port Norris, NJ 08349.

Standard techniques developed for producing triploid oysters (*Crassostrea gigas*) in the northwestern United States cause >90% mortality of *Crassostrea virginica*, which is unacceptably high considering the scale of most east coast hatcheries. We examined the effect of cytochalasin B (CB) concentration, timing of treatment after fertilization, and duration of treatment on survival and triploidy induction of *C. virginica* embryos. Survival to D-stage was inversely related to CB concentration and length of treatment. Induction of triploidy was CB-dose dependent. Optimal treatment of 0.25 mg/l CB for 10–15 min beginning when half the embryos were observed to be undergoing meiosis I yielded mean survival to D-stage of 84% of control values with a mean of 96% triploid production.

DETERIORATION OF THE Y-ORGAN IN MALE SNOW CRAB *CHIONOECETES OPILIO* AND ITS POSSIBLE IMPLICATIONS WITH REGARD TO THE TERMINAL MOLT. E. Bataller* and **A. D. Boghen.** Université de Moncton, E1A 3E9, New Brunswick, Canada.

The Y-organ represents an integral part of a neuro-endocrine complex in Crustaceans and is believed to be primarily responsible for triggering the molt, hence the name "molting gland."

It measure approximately 2 × 3 × 4 mm, and consists of a mass of irregularly arranged cellular cords which are enclosed within a thin coat of fibrous tissue. As previously described for *C. opilio* females, in males, the organ is located along the latero-ventral plane within the anterior half of the cephalothorax.

Histological and ultrastructural studies of immature and mature crabs, demonstrates that there are important modifications which occur to the organ, as the animals age (global rarefaction, pycnotic nuclei, presence of membranous whorls in the cell, etc), and that these eventually translate themselves into widespread and massive degeneration. Such findings are also partly supported by quantitative investigations which show that the surface areas of nuclei sampled from Y-organs removed from immature crabs are, on the average, greater than those measured from mature ones.

Contrary to that which has been reported for other Crustaceans, our work suggests that the Y-organ does not become totally inactive after the animal's molt to maturity, thus raising the possibility, that if indeed there is a terminal molt in snow crab, it takes place later on in life.

PREY PREFERENCES OF THE BLUE CRAB (*CALLINECTES SAPIDUS*) FEEDING ON THREE BIVALVE SPECIES. Elizabeth L. Bettendorf and **Victor S. Kennedy,*** Horn Point Environmental Laboratory, University of Maryland, Box 775, Cambridge, MD 21613.

An energy maximization model correctly predicted that the blue crab, *Callinectes sapidus*, preferred the energetically profitable soft clam, *Mya arenaria*, over the low profitability brackish water clam, *Rangia cuneata*. The model did not predict clearly any preference between the soft clam and the hooked mussel, *Ischadium recurvum* (an epifaunal species, in contrast to the other two

species which are infaunal). Deep sand provided a more significant refuge for *Mya arenaria* than for *Rangia cuneata*, and a more significant refuge for *M. arenaria* than clustering provided for *Ischadium recurvum*. Prey location significantly affected consumption of prey in shallow sand, but not in deep sand. We conclude that blue crab feeding behavior cannot be understood based solely on the distance between the crab and the prey. Handling times, prey density, and search times due to prey refuges can significantly affect prey preferences of blue crabs and overall mortality of prey.

THREE-DIMENSIONAL HYDRODYNAMIC MODELING OF GALVESTON BAY. R. C. Berger, USAE Waterways Experiment Station, 3909 Halls Ferry Road, Vicksburg, MS 39180.

The effects of the enlargement of the Houston Ship Channel through Galveston Bay have been evaluated through a series of models and methods. The final model will be linked to an oyster population dynamics model to determine the effect of channel enlargement on the oyster populations of Galveston Bay. The hydrodynamic and salinity regimes, that are the primary input for each subsequent model, are provided by a 3-dimensional finite element program. A description of this model, its verification and the evaluation of the channel enlargement will be presented. The changes in the flow and salinity field associated with the channel enlargement and the lessons learned for this application will be discussed.

***PERKINSUS MARINUS* INFECTION INTENSITY AND PREVALENCE IN A 6 YR TIME SERIES AND AN OBSERVATION ON REEF-TO-REEF TRANSMISSION RATES. Stephanie A. Boyles, M. Kathleen Phillips, Eric N. Powell, James M. Brooks,** and **Roger R. Fay,** Dept. Oceanogr., Texas A&M Univ., College Station, TX 77843.

Oysters (*Crassostrea virginica*) were collected each winter from 1986 to 1991 from >60 sites in the Gulf of Mexico as part of NOAA's Status and Trends program. Mean infection intensity for *P. marinus* was relatively high (1.1) in yr 1, particularly in south Florida, remained low throughout the Gulf in the next 2 yr (0.7 and 0.8), rose to a 6-yr high in yr 4 (1.9) and then declined in yr 5 (1.5) and 6 (1.0); the exception being south Texas which remained at levels typical of yr 4. Prevalence was high in yr 1 (88.9), decreased during the next 2 yr (67.8 and 65.8), rose again in yr 4 (80.5), then decreased in yr 5 (74.9) and 6 (73.8). These trends indicate that mean infection intensity reacts quickly to climate change, but prevalence reacts slower and thus is more resistant to climate change. In yr 3, all oysters in San Antonio Bay died. Oysters did not reoccupy the bay until yr 5. In yr 5, *P. marinus* infection intensity and prevalence were lower than the average regional value, but, by yr 6, levels were back to normal. Recovery of *P. marinus* lagged behind the recovery of the oyster population by no more than 1 yr.

COMPARISON OF THREE GROWOUT ENCLOSURES AND TWO LOCATIONS FOR *ARGOPECTEN IRRADIANS* ON VIRGINIA'S EASTERN SHORE. Mark J. Brotman* and William DuPaul, Advisory Services, College of William and Mary, Virginia Institute of Marine Science, Gloucester Point, VA 23062.

Three gear types—'oyster' bags, polyethylene trays and four-tiered lantern nets—were deployed at two locations on Virginia's Eastern Shore (bayside and seaside) to examine suitability for *Argopecten irradians* growout. Animals spawned in April, 1991 were caught on an 18 mm screen in August and subsequently deployed at 65 ft^{-2}. Site comparisons were based on the environmental parameters of salinity, temperature, chlorophyll-a and current speed. Shell length (SL) and volume measurements and a live animal count were made on each unit every 2–3 weeks over the 3 month growout period. Harvested shell lengths and volumes were measured, and samples sacrificed for adductor and somatic weights. Multiple analysis of variance testing revealed differences in growth rates, final shell lengths and volumes between enclosure types and sites. The greatest and least average growth rates, proportion above a minimum market size of 45 mm and survivorship were in bayside trays (0.22 mm/day SL, 58.4%, 59.9%), and the top tier of the seaside lantern nets (0.07 mm/day SL, 0%, 35.0%), respectively. Mortality was apparently due primarily to predation by crabs (*Calinectes sapidus* and *Labinia* sp.) and handling/exposure during sampling efforts. It is concluded that it is possible to raise bay scallops in Virginia waters from spawning to market size within eight months, only if careful attention is paid to gear and site selection.

***PERKINSUS* RACES: MANAGEMENT IMPLICATIONS AND INITIAL OBSERVATIONS OF GEOGRAPHICALLY DISTINCT ISOLATES. David Bushek,** Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349.

Pathogenic organisms often develop races with different levels of virulence or environmental tolerance. *Perkinsus marinus*, a major pathogen of the American oyster *Crassostrea virginica*, has been studied for more than 40 years yet there is virtually no information concerning the existence of races. Such information is critical for development of control strategies and resistant oyster stocks, and screening non-native species for resistance. Observations on the distribution and virulence of *P. marinus* in the field suggest races may exist that vary in salinity tolerance, temperature tolerance and possibly virulence. This study represents an initial comparison of geographic isolates of *P. marinus*. Oysters infected with *P. marinus* were collected from several sites in the Gulf of Mexico and along the Atlantic Coast of the United States. Growth and development of isolates were compared under standard thioglycollate culture at various temperatures and salinity. Production of zoospores was also compared. Results are discussed in the context of disease management and directions for future research.

EVALUATION OF ANTICOCCIDIALS EFFICACY IN CONTROLLING *P. MARINUS* INFECTIONS IN OYSTERS, *CRASSOSTREA VIRGINICA: IN VIVO* AND *IN VITRO* EXPERIMENTS. Gustavo W. Calvo* and **Eugene M. Burreson,** Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Six compounds, amprolium, arprinocid, lasalocid, monensin, sufadimethoxine, and a potentiated sulfonamide, were tested *in vivo* as follows: 2 replicate oyster groups (N = 25) were exposed to 0.1 mg/L chemical baths for 4 days and subsequently challenged to 2 doses of 10^7 *P. marinus* cells (one per day during 2 consecutive days). Six weeks after challenge, all oyster groups were sacrificed and assayed for *P. marinus* prevalence and intensity. Average intensity, did not significantly differ between groups including the untreated control group. In addition to the chemicals previously mentioned, cycloheximide and malachite green were included in an *in vitro* experiment. This study consisted of exposing 200 uL aliquots of *P. marinus* infected oyster hemolymph to 1 mL of chemical solutions containing 100 ug/mL of active ingredient for 1 day, and subsequently incubating parasite cells in fluid thioglycollate. Using this assay, 6 compounds including cycloheximide and malachite green were associated with a significantly lower number of prezoosporangia than the untreated control group. These results suggest that even though some of the chemicals can control *P. marinus* when applied *in vitro,* they fail to prevent and/or control infections when applied to oysters as baths.

NEW INSIGHTS INTO MUSSEL BOTTOM CULTURE THROUGH CLOSE INTERACTION OF MODELING AND FIELD RESEARCH. Daniel E. Campbell, Graduate School of Oceanography, University of Rhode Island, Narrangansett, RI 02882; **Carter R. Newell,** Great Eastern Mussel Farms Inc., Tenants Harbor, ME 04860.

Close collaboration between modeling and field research over a period of three years has lead to new insights about the factors controlling the growth of mussels on bottom lease sites in Maine. An initial model of mussel production on a lease site was formulated using the results from a one year pilot field research effort. This model was forced by temperature and offsite food concentration and hypothesized that tidal flushing and vertical mixing mediated by current speed and bottom roughness were the dominant processes determining the supply of food to mussels on the bottom. Growth of mussel biomass depended on the biomass, bottom food concentration and temperature, whereas, respiration and shell growth were determined by mussel biomass and temperature. The initial number of mussels m^{-2} was included in the model and mortality resulted in reduced numbers and appropriate losses of shell volume and mussel biomass. As the field research program continued and additional simulations were made we realized that production on good lease sites was a consequence of large volume flux over the site and not tidal flushing. This observation resulted in the development of a detailed physical model for the Mud Cove lease site. Calibrating this simple model using physiological measurements on mussels from Mud Cove and the output of the hydrographic model demonstrated that resuspension of POC and the return of POC in fecal material to the bottom water POC pool were necessary to account for observed concentrations of POC in the bottom waters. In addition, the vertical flux of POC caused by turbulent mixing was not sufficient to account for the observed mussel growth. An additional flux of POC to the bottom due to settling during periods of low flow was necessary to account for the observed growth in length and weight. Video observations of mussel gap at slack water and physiological measurements of scope for growth at this time also indicated that mussels utilized concentrations of suspended POC settling to the bottom. Validation of this model using site data and food and temperature curves from two additional sites showed that the quantity and quality of food entering a site were the most important factors determining mussel growth and thus time to harvest, individual mussel size, and meat yield. Food quality was represented by partitioning total POC into phytoplankton and detritus components, and the food quality of detritus was further estimated by using the ratio of detrital nitrogen to detrital carbon modified to account for the high quality of detrital carbon after phytoplankton blooms. An optimum window for seeding was identified extending from May to July which resulted in the harvest of marketable mussels from 40 mm seed within 8 months in a good food year and thirteen months when fall food was poor. The use of models to test the relative importance of ecological processes calibrated with field data resulted in new insights into the processes controlling mussel growth on bottom culture lease sites. Rapid feedback from the model facilitated gathering the critical pieces of information necessary to accurately simulate mussel growth.

MATURATION AND SPAWNING IN CAPTIVITY OF *P. DUORARUM* USING EYESTALK ABLATION. Heidy B. Cardona* and **Thomas R. Capo,** University of Miami Experimental Hatchery, Division of Marine Biology and Fisheries, 4600 Rickenbacker Causeway, Miami, FL 33149.

The pink shrimp, *Penaeus duorarum* Burkenroad, 1939, were maturated and spawned in captivity using eyestalk ablation, special diet and ambient temperature seawater. Broodstock was collected from the Tortugas grounds, transported to the University of Miami's Experimental Hatchery on Virginia Key and held in 6000 liter flow-through raceways. The density ranged from 30 to 70 animals per raceway with sex ratio of 1:3 (male:female). The animals were conditioned on a diet of squid, bloodworms, and shrimp maturation pellets. Temperature ranged from 15 to 33°C, oxygen 6.0–7.5 mg/l, salinity 27–37‰, pH 7.0–8.0 and ammonia 0.2–0.6 μg/l. During the period of June–Dec 91, 61 females were unilaterally ablated by the incision-pinching method. Approximately three weeks post ablation, twenty six sexually mature fe-

males (42%) from 30 to 40 g spawned in individual 300 liter conical tanks. Eggs were collected, fixed, and counted. Results from 16 spawns indicated that ablated females produced from 9,333 to 174,160 eggs per spawning episode and mean of 52,423 eggs. These results indicate *P. duorarum* will maturate and spawn over a wide temperature range and has potential as a cultured cold water specie.

CHANGES IN AGGLUTININS AND PARASITES (*PERKINSUS MARINUS* AND *HAPLOSPORIDIUM NELSONI*) IN THE HEMOLYMPH OF OYSTERS *CRASSOSTREA VIRGINICA*, HELD IN MARYLAND AND NEW JERSEY. Marnita M. Chintala,* Horn Point Environmental Lab, University of Maryland, P.O. Box 775, Cambridge, MD 21613; **William S. Fisher,** Environmental Research Laboratory, U.S. EPA, Sabine Island, Gulf Breeze, FL 32561; **Susan E. Ford** and **Kathryn A. Ashton-Alcox,** Haskin Shellfish Research Laboratory, Rutgers University, Box B-8, Port Norris, NJ 08934.

The ability of molluscan hemolymph to agglutinate foreign material has been proposed as a potential defense mechanism against pathogenic organisms. We tested this hypothesis by 1) exposing 100 oysters, *Crassostrea virginica,* to the parasites *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX) for one year in both the Chesapeake Bay (CB) and Delaware Bay (DB); 2) measuring hemolymph changes, over time, in the levels of infection of both parasites, the protein concentration, and the agglutinating ability for the bacteria *Vibrio cholerae,* horse RBCs, and human RBCs; and 3) comparing agglutinating ability with infection intensities and survival of individual oysters after parasite challenge. MSX infections were low throughout the year in both locations. Dermo infection increased from Sept. to Nov., and decreased from Nov. to May. The Dermo intensity in animals that survived until May was lower than those that died. Most of the DB animals died by October 1990, while about a third of the CB animals survived until the end of the experiment (May 1991). Agglutinating ability changed seasonally and each location exhibited different patterns. There was no indication that agglutinating ability was related to an effective defense (as measured by infection or survival) against these pathogens. This is NJAES publication number K-32901-1-92.

REPRODUCTION AND SPAT SETTLEMENT OF SEA SCALLOPS (*PLACOPECTEN MAGELLANICUS*) IN THE MAGDALEN ISLANDS (QUEBEC, CANADA). G. Cliche,[1] M. Giguère,[2] et **P. Picard,[3]** [1]Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, C.P. 658, Cap aux Meules, Iles de la Madeleine, P. Québec, Canada, G0B-1B0; [2]Institut Maurice Lamontagne, Ministère des Pêches et Océans, C.P. 100, Mont-Joli, Québec, Canada, G5H-3Z4; [3]Roche Lt, 3075, Ch. Quatre-Bourgeois, Ste-Foy, Québec, Canada G1W-4X5.

The a scallop (*Placopecten magellanicus*) is the main scallop species fished commercially in the Magdalen Islands (Québec, Canada). Since 1990, data have been collected to study reproductive cycle and spatiotemporal variation of scallop spatfall.

Adult gonosomatic indices (GSI) were determined between April 1990 and August 1991. In August and September 1991, GSIs were also monitored in 5 regions to examine the degree of spawning synchronism among different beds. To verify that sea scallop has a single settling period in the Magdalen Islands area, six stations were monitored on a monthly basis from May 1990 to August 1991. Weekly sampling was also performed between September and November 1990 and 1991 to determine the peak settlement period. Finally, twenty stations dispersed over the southwest area of the Magdalen Islands were used to study spatial variation of spat settlement. These stations were sampled between September and December both in 1990 and 1991.

GSIs indicated that spawning took place simultaneously for male and female during the 7th to 13th September period in 1990 and slightly earlier (28th August to 3th September) in 1991. In 1990, maximum settling period for *Placopecten* was relatively short and occured between 12th and 26th October. However, in 1991, we noted two peaks of larval settling: 9th to 15th October in the southern region and 15th October to 3th November in the eastern region. There was important spatial variation in spat collection success over natural beds as well as over the total area under study in 1990. Spat number/collector varied from 4 to 1724 with a mean number of 272 ± 301. Significantly different mean spat lengths were also recorded among stations grouped *a posteriori* into four zones with mean length of .8 mm for westernmost area and 1.3 mm for easternmost area. Spat settlement was quite variable on collectors attached to the same line and only sea scallop spats appear to be collected in large quantities.

THE USE OF ALGAL SUBSTITUTES AND THE REQUIREMENT FOR LIVE ALGAE IN THE HATCHERY AND NURSERY REARING OF BIVALVE MOLLUSKS: AN INTERNATIONAL SURVEY. Peter Coutteau,* Karen Curé, and **Patrick Sorgeloos,** Laboratory of Aquaculture & Artemia Reference Center, University of Ghent, Rozier 44, B-9000 Gent, Belgium.

The mass-production of micro-algae has been recognized by several authors as the main bottle-neck for the culture of bivalve seed. This has prompted a search for alternatives to on site algal production, such as dried—heterotrophically grown algae, preserved algal pastes, microencapsulated diets, and manipulated yeasts. However, the extent to which these products have been tried, and rejected or retained, by hatchery operators is poorly documented. Also, the actual algal requirement and production cost of the bivalve seed industry is difficult to estimate. The present inquiry allowed the collection of data concerning the requirement for live algae and its associated costs encountered in 50 commercial and experimental hatcheries from all over the world. Furthermore, the hatchery operators were questioned about their experience with alternatives for live algae. Finally, this survey

offered the opportunity to collect some unique data on the quality and quantity of hatchery produced algae and bivalve seed, and the employment in this relatively small sector of aquaculture.

LABORATORY CULTURE OF THE PINK SHRIMP, *PENAEUS DUORARUM:* FECUNDITY, SURVIVAL AND GROWTH. Geraldine M. Cripe, U.S. Environmental Protection Agency, Sabine Island, Gulf Breeze, FL 32561.

A recirculating system using a 4′ fiberglass spawning tank produced an average of 3030 (±1560) viable pink shrimp eggs per day during fall, winter and spring. Culture of these eggs to post larvae in 10 days was successful using continuous delivery of mass cultured *Tetraselmis chuii*. A maximum of 81 percent of the eggs survived to post larvae and a group of the post larvae were grown under optimum conditions to assess potential growth rate. Fifteen days after metamorphosis to post larvae, the shrimp dry weight was 8.7 times that of Day 1 post larvae; 54 days after metamorphosis, they were 54 times that of Day 1 and 57 days after metamorphosis, the shrimp dry weight was 1514 times that of Day 1.

LARVAL REARING OF TWO DECAPOD FRESHWATER SHRIMP, *ATYA SCABRA* (LEACH) AND *A. LANIPES* (HOLTHUIS) IN THE LABORATORY. Sonia Cruz-Soltero* and **Dallas E. Alston,** University of Puerto Rico, Department of Marine Sciences, Mayaguez, Puerto Rico 00681-5000.

Research was conducted on *Atya scabra* and *A. lanipes* to provide basic information for the possible mass larval and juvenile culture for aquaculture purposes. Larval development was described from hatching until first juvenile stage for both species. These larvae were cultured in 30 ppt filtered seawater at 28 C temperature without aeration. They were fed with Zeigler's Ap-100 commercial food ad lib. The larvae of both *A. scabra* and *A. lanipes* hatched as free swimming zoeae of length 1.76 ± 0.21 (S.D.) mm, range 1.66–1.87 (n = 5) and 1.85 ± 0.22 (S.D.) mm, range 1.73–1.98 (n = 5), respectively. Immediately before metamorphosis into juveniles, they had attained a maximum size of 10.05 ± 0.65 mm (S.E., n = 4) for *A. scabra* and 11.2 ± 0.46 mm (S.E., n = 4) for *A. lanipes*. Twelve distinct larval developmental stages were observed for both species. Differences in length of larval development time ranged from 62 days for *A. lanipes* to 74 days for *A. scabra*. Evidence suggests that such long larval lives occur in the natural environment and are not the result of poor rearing conditions in the laboratory.

COMPARISON OF SURVIVORSHIP BETWEEN WILD AND HATCHERY-REARED JUVENILE QUEEN CONCH (*STROMBUS GIGAS* L.) FROM DECAPODA PREDATORS. Megan Davis, Department of Biological Sciences, Florida Institute of Technology, 150 W. University Blvd., Melbourne, FL 32901.

Queen conch, *Strombus gigas* L., a tropical marine gastropod, is an important fishery resource in the Caribbean region. It has suffered over-exploitation in many countries. Hatchery-reared juvenile conch are being cultured as potential stock enhancement species. Predation is one of the major obstacles to the successful restocking of this mollusk. The objective of this laboratory study is to determine if there are significant differences between hatchery-reared and wild juvenile conch in morphometrics and behavior, and whether these differences will affect their vulnerability to predation. The stone crab (*Menippe mercenaria*), box crab (*Calappa gallus*), and spiny lobster (*Panulirus argus*) are commonly found in conch habitats, and are known to consume juvenile queen conch. Size-specific predation curves, refuge sizes for conch, and predator consumption rates were determined for spiny lobster. Predation-prey interactions were observed and documented for these three crustacean predators. This research provides a basis for decisions on use of hatchery-reared stock for fisheries enhancement, and also improves knowledge of basic biology for this ecologically important Caribbean mollusk.

CONTROL OF OVERSET ON CULTURED OYSTERS USING BRINE SOLUTIONS. Gregory A. Debrosse* and **Standish K. Allen, Jr,** Rutgers University, Haskin Shellfish Research Laboratory, Port Norris, NJ 08349.

HSRL has a long standing program in oyster genetics and breeding. One of the worst scenarios for maintenance of broodstocks is overset by native oysters. Preliminary experiments in 1990 indicated that overset might be controlled simply by immersing animals in a concentrated brine solution; such treatments in 1990 resulted in 89–100% mortality of <1 mm spat. In 1990 field tests, overset on broodstocks was reduced to 3 spat/oyster using 200 ppt immersions compared to 22 spat/oyster in controls. In 1991 we refined the parameters for effective brine dips. First, we tested survival of oysters (potential substrate for overset) immersed for 2, 5, or 10 minutes in 200 ppt brine followed by either 3 or 6 hours aerial exposure. For juveniles, cumulative mortalities ranged from 3–6% compared to 5% in controls; for adults, 2–4% died after brine immersion and 2–3% died in controls. Second, we tested survival of hatchery set oyster spat immersed in 200 ppt brine. For spat with shell lengths <5.0 mm and immersed in 200 ppt brine for 2, 5, or 10 min, 57%, 70% and 83% died after 3 hr aerial exposure and 64%, 85%, and 86% died after 6 hr aerial exposure. Control mortality averaged about 23% in both 3 and 6 hr aerial exposures. For larger spat immersed in 200 ppt brine for 10 minutes, cumulative mortality was 47% and 88% for 3 and 6 hr aerial exposure, respectively, and 22% and 32% for controls. Results of 1990 field tests and 1991 experiments demonstrate that brine solutions will be effective and save considerable labor.

A PHYSIOLOGICALLY-BASED OYSTER LARVAL MODEL, CONSIDERING DIFFERING TEMPERATURE AND SALINITY REGIMES. Margaret M. Dekshenieks,* Center for Coastal Phys. Oceanogr., Old Dominion Univ., Norfolk, VA 23508.

A physiologically-based model, consisting of a system of or-

dinary differential equations, has been developed to demonstrate the response of oyster larval developmental stages (from trochophore to mature-eyed) to different temperature and salinity regimes. Parameterizations in the model account for temperature and salinity effects on larval growth and mortality. The model is calibrated using temperature, salinity and food concentration values typical of Galveston Bay, Texas. Simulations for Galveston Bay show that extremes in environmental conditions, such as periods of temperatures less than 25°C or salinities less than 7.5 ppt, retard larval growth rate. Thus, periods of increased fresh water discharge and/or reduced seasonal temperatures will extend the larval period and increase larval mortality. These types of episodic variations in environmental conditions may result in reduced recruitment to adult oyster populations. Additional simulations, with conditions appropriate for Apalachicola Bay and Chesapeake Bay, show the effect of latitudinal variations in environmental parameters on oyster larval growth and mortality.

DEPTH EFFECTS ON THE GROWTH OF *PLACOPECTEN MAGELLANICUS* IN SUSPENDED CULTURE: RESUSPENSION, FOOD QUALITY AND TEMPERATURE. Craig Emerson* and **Jonathan Grant,** Department of Oceanography, Dalhousie University, Halifax, N.S., Canada B3H 4J1.

As part of the Ocean Production Enhancement Network (a consortium of industrial, government and university researchers), we measured the growth of sea scallops in conjunction with numerous oceanographic variables to determine optimal conditions for culture in Atlantic Canada. Our specific goal was to determine whether resuspension of bottom sediments inhibits or enhances scallop growth. Juvenile and adult scallops were either ear-hung or placed in pearl nets at several heights over sand and mud. Data from optical backscatter sensors, transmissometers, fluorometers, current meters, thermisters and other instruments formed an extensive data base used to account for variation in monthly growth rates. We found that both growth and survival rates were often directly proportional to height above bottom. In addition, phytoplankton concentration was not always tightly coupled to growth; scallop growth remained relatively constant before, during, and after the fall phytoplankton bloom. A knowledge of local hydrography is necessary to optimize culture production, not solely to estimate advective food fluxes, but to determine the frequency and degree of resuspension.

SETTLEMENT, RECRUITMENT AND YIELDS AVAILABLE FOR THE SCALLOP *ARGOPECTEN CIRCULARIS* FISHERY IN BAHIAS MAGDALENA AND CONCEPCION, BAJA CALIFORNIA SUR, MEXICO. E. F. Felix-Pico,*[1] G. Bojorquez-Verastica,[2] R. Morales-Hernandez,[2] and **F. Garcia-Dominguez,[1]** [1]Cicimar-Ipn, Apdo. Postal 592, La Paz, B.C.S., 23000 Mexico, [2]Secretaria de Pesca, Dir. Acuacultura, Jalisco y Madero S/N, La Paz, B.C.S., 23000 Mexico.

Research on scallop populations in Bahias Magdalena and Concepcion have three major objectives: to determine the intensity and duration of spatfall on artificial collectors; to determine the abundance of recruits and residual stock in the bays prior to each fishing season; and reproductive biology and determination of the spawning season. Over the four years of this study, scallop spatfall was observed to occur over a short period (March–May) following the spring spawning in both bays, and only occur in another period (August–September) following the summer spawning in Bahia Magdalena. Spatfall one year can therefore be related to recruitment in the next. Years of high and low spatfall were reflected in subsequent differences in recruitment. For Bahia Concepcion in 1987, the settlement resulting from the minor spring spawning was 500 spat per bag and the stock was 70 million scallop. For 1988, the settlement increased to 40,000 spats and the stock was 120 million scallop. For 1991, the settlement decreased to 18,000 spats and the stock was near 50 million scallop. For Bahia Magdalena in 1989, the result from the major spring spawning was 10,000 spat per bag and the stock was 500 million of scallop. For 1991, the settlement was very low with only about 120 spat and the stock decreased to 2 million scallop.

SHELL COLOR POLYMORPHISM AND GROWTH IN THE CHILEAN OYSTER *TIOSTREA CHILENSIS*. Emilio Figueroa, Elisabeth von Brand,* and **Federico Winkler,** Departamento de Biología Marina, Universidad Católica del Norte Casilla: 117, Coquimbo-Chile.

In the chilean oyster, *Tiostrea chilensis,* matings were performed to determine the shell color polymorphism inheritance pattern of this species. Progeny was kept under equal conditions, and growth was measured for each morph separately. Three distinct morphs (unbanded, one valve banded, both valves banded) were observed. Results of the matings showed that the unbanded pattern is dominant over both banded ones, but no clear pattern could be determined for the one banded vs two banded morphs. The overall growth, for each morph did not show significant differences ($p > 0.05$), but comparing the growth rates seasonally, the unbanded morph grew faster in spring-summer (higher temperatures). The banded morphs, instead, grew faster in fall-winter (lower temperatures). The unbanded morph seems to be under control of a single locus, with two alleles. This phenotypic trait can be helpful to select adequate reproductors and seeds of chilean oyster for different environments.

BIOLOGICAL BIOFOULING CONTROL IN A FIELD BASED NURSERY FOR THE HARD CLAM, *MERCENARIA MERCENARIA*. G. E. Flimlin Jr.,* New Jersey Sea Grant Marine Advisory Service, Rutgers Cooperative Extension, 1623 Whitesville Road, Toms River, New Jersey 08755; **G. W. Mathis Jr.,** Mathis and Mathis Enterprises, Northfield, NJ 08225.

A field nursery is a low cost method of raising small (1.5 to 3 mm) hard clam, *Mercenaria mercenaria,* seed to field plantable size (8 to 15 mm). However, fouling by the tunicate *Molgula*

manhattensis can cause mortality of seed through entrainment, compete for nutrients in the water, and require significant time for removal and cleaning of the nursery box for the aquaculturist. The addition of four common mummichogs, *Fundulus heteroclitus*, in each layer of a stack of Nestier boxes lined with window screen significantly reduces squirt fouling. Although the *Fundulus* may slightly reduce the growth rate, the reduction in mortality and box maintainence is significant, and acceptable to the culturist.

RAFT CULTURE OF GREEN MUSSEL, *PERNA VIRIDIS*, IN SAPIAN BAY, PHILIPPINES. Wenresti G. Gallardo,* Giselle P. B. Samonte, and **Rolando S. Ortega,** Aquaculture Department, Southeast Asian Fisheries Development Center, P.O. Box 256, Iloilo City, Philippines 5000.

This paper describes raft culture of the green mussel, *Perna viridis*, based on data obtained from mussel farmers surveyed in Sapian Bay from September to November 1991. The raft method of farming green mussels in this area is different from the raft methods practiced in other countries. The raft consists of bamboo poles. No artificial floats are used. The culture process is divided into four stages: obtaining the seed, growing, thinning and harvesting. Seed are collected in the outer part of the bay using a 10 × 10 m raft consisting of about 50 bamboos, from October to January. After one to two months, the raft is hauled toward the inner part of the bay and refixed into 15–20 bamboo poles spaced at 1 m apart. The raft is fixed on the water column usually after 2–3 months when the mussels become heavy. Thinning is done once per cropping by attaching additional bamboo poles to those poles heavy with mussels. Harvesting is done 11–12 months after seed collection. Production averaged 22.3 kg/m^2.

A SOLUTION FOR THE MUSSEL SUMMER MORTALITIES IN THE MAGDALEN ISLANDS (QUÉBEC, CANADA) FROM A STOCKS × SITES EXPERIMENT. Jean Gaudreault* and **Bruno Myrand,** Dir. de la Recherche Scientifique et Technique Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec. C.P. 658, Cap-aux-Meules, Qué., Canada G0B 1B0.

Spat from 4 local populations were transferred to 5 growing sites in November '89 and then followed for the next 2 years. In June '90, mussels were placed in cages (6 per combination) at an initial density of 50 ind./cage. Thereafter cages were changed monthly between June and November to minimize fouling. On these occasions, dead and live mussels were counted and measured. Each November, cages from each combination were randomly selected and the mussels used for length-flesh dry weight and length-shell wt regressions. There were only minor differences between sites compared to the huge stock effects. With all sites combined, stocks from the Amherst lagoon (BHA) and the Bay of Pleasance (BP) exhibited higher survival rates (92.3% and 87.4%) in November '90 than stocks from the Great Entry (GE) and the House Harbour (HAM) lagoons (21.2% and 41.8%). At that time, we estimated that commercial production (flesh dry wt + shell wt from mussels ≥ 50 mm) was almost 10 times higher in BHA cages (170.7 g/cage) than in GE ones (17.4 g/cage). The higher mortality was recorded between the July 20 and the August 24 visits. In 1991, two yr-old mussels from the BP stock suffered high losses between July 05 and September 09 samplings. At the end of the study in November '91, survival rates of the different stocks (all sites combined) were: 82.1% for BHA, 11.1% for HAM, 6.7% for BP and 5.7% for GE. Estimates of commercial production are not yet available. The use of mussels from BHA in state of the currently used GE and HAM stocks could provide a cheap and easy solution to the summer mortality problem.

SPAT SETTLEMENT AND EARLY GROWTH OF *LYROPECTEN SUBNUDOSUS* (SOWERBY, 1835) IN LAGUNA OJO DE LIEBRE, B.C.S., MÉXICO, 1989–1990. F. Garcia-Dominguez,*[1] P. Castro-Moroyoqui,[2] and **E. F. Felix-Pico,[1]** [1]Cicimar-Ipn. Apdo. postal 592, La Paz, B.C.S., 23000, México, [2]Secretaria de Pesca, Dir. de Acuacultura, Jalisco y Madero S/N, La Paz, B.C.S., 23000, México.

Adult *Lyropecten subnudosus* spawn between September and November in Laguna Ojo de Liebre and larvae are present from September to December. Observations on spat settlement in collectors, however, indicate that the major settlement takes place during a period of about 2 months, September–October, and that the exact time of settlement varies from year to year occurring from September to November. Numbers of spat per collecting bag (37–38) were relatively constant from year to year but varied with depth. After settlement in November, spat reach a mean height of 17 mm by December and 76 mm by the next November in suspended culture. Natural bottom growth depends on depth and substratum.

EXPERIMENTAL STUDIES ON LARVAL HABITAT CHOICE IN THE MACTRID BIVALVE *MULINIA LATERALIS*. Judith P. Grassle, Institute of Marine and Coastal Sciences, Rutgers University, P.O. Box 231, New Brunswick, NJ 08903.

Still-water and flume flow experiments on habitat choice in larvae of the opportunistic coot clam, *Mulinia lateralis*, show that larvae always prefer organically rich mud over sediment lacking in organic matter in flow. The same significant preference is often but not always demonstrable in still water. An examination of larval competency and near-bottom behavior over the period of ~5 days in which larvae pass from pre-competent, to competent, then to spontaneously-metamorphosing stages, suggests that a choice is more readily made by late pediveligers when they are moved by the flow between different sediment patches. These results will be compared with what is known about settlement behavior in certain other bivalve larvae.

INCIDENTAL OCCURRENCE OF BAY SCALLOPS *ARGOPECTEN IRRADIANS AMPLICOSTATUS* IN AQUACULTURE RESEARCH PONDS IN TEXAS. **Joseph D. Gray,** Texas Parks and Wildlife Department, Star Route Box 385, Palacios, TX 77465.

Bay scallops, *Argopecten irradians amplicostatus*, were discovered in one 0.4-ha and one 0.8-ha earthen research ponds at the Texas Parks and Wildlife Department Perry R. Bass Marine Fisheries Research Station near Palacios, Texas. Ponds were used for growout and maturation of red drum (*Sciaenops ocellatus*), and common snook (*Centropomus undecimalis*), respectively. Ponds were filled with unfiltered Matagorda Bay water during April 1990, and were drained during September and October 1990, respectively. Mean surface water temperatures and salinities for the 0.4-ha pond were 27.1 C and 27‰, while mean surface water temperatures and salinities for the 0.8-ha pond were 26.6 C and 28‰. Mean total shell lengths of scallops harvested were 37 and 53 mm respectively. One thousand live scallops were moved to the bottom of another 0.4-ha pond for overwintering. An additional 200 were placed in four 6-mm mesh plastic cages and suspended in the water column. By April 1991, 50% of the caged scallops survived, with 0% survival by May. The pond was drained on December 6, 1991. There was no survival of the remaining 1000 scallops.

EFFECTS OF HARD CLAM HATCHERY MANAGEMENT PRACTICES ON PRODUCTIVITY AND ON BROODSTOCK QUALITY. **Nancy H. Hadley,** South Carolina Wildlife and Marine Resources Department, Marine Resources Research Institute, P.O. Box 12559, Charleston, SC 29422-2559.

Evidence from a large-scale breeding program with hard clams indicates that a number of hatchery management practices in common use may actually reduce productivity and result in broodstock with limited genetic variance. These potentially self-defeating practices include inadequate conditioning of spawners, strip-spawning, mass-spawning in common containers, spawning too few individuals, poor gamete storage procedures, culling of small individuals in the hatchery and nursery, and other intentional and/or inadvertent selection pressures. Potential problems arising from these practices include low effective parental numbers, detrimental inbreeding, selection for inappropriate traits, and overall reduction in genetic diversity which compounds the problems in the next generation. Hatchery managers should seek to maximize the number of parents contributing to spawns, equalize the contribution from individuals spawning, and retain individuals from as many parents as possible in the progeny population. Culling prior to field planting should be avoided since those individuals which do well in hatchery and nursery systems are often not the best performers in field growout.

VARIATIONS IN OYSTER SPATFALL AT DIFFERENT TIDAL HEIGHTS MEASURED HORIZONTALLY AND VERTICALLY IN SOUTH CAROLINA. **Nancy H. Hadley*** and **V. G. Burrell, Jr.**, South Carolina Wildlife and Marine Resources Department, Marine Resources Research Institute, P.O. Box 12559, Charleston, SC 29422-2559.

A major deterrent to oyster culture in South Carolina is the abundant and protracted natural spatfall which fouls cultured seed. In a preliminary experiment designed to identify zones of minimum spatfall, collector plates were deployed in an intertidal creek at different tidal heights. A vertical collector with base 1.5 feet below MLW held 5 horizontal plates at tidal heights of −1, 0, +1, +2 and +3 feet. Individual collectors holding single plates were placed at points on the adjacent shore corresponding to identical tidal heights. Plates were deployed in late June and replaced biweekly through October. All spat on the lower side of the plates were counted under a dissecting scope. Plates located onshore consistently had higher spatfall than corresponding offshore plates. 70% of all spat were observed on onshore plates. In weeks of peak settlement, onshore plates had up to 7 times as many spat as offshore plates. Offshore plates had higher rates of settlement of other organisms, primarily barnacles and bryozoans. Maximum spatfall of $3/cm^2$ was recorded at MLW onshore. Spatfall as high as $1/cm^2$ was observed even at +3 feet onshore. From 0 to +2 feet, spatfall at onshore collectors was high enough to preclude the possibility of growing cultchless single oysters in these zones. These results suggest that rates of spatfall relative to tidal height reported in the literature may be misleading, since most have been determined with vertical collectors. From this preliminary study, it would appear that culture of oysters in South Carolina will require innovative management practices to avoid spatfall.

USE OF GLASS PANELS IN A SPATFALL MONITORING PROGRAM FOR THE MANGROVE OYSTER. **Carl M. Hanson,*[1] Karen Roberts,[1]** and **Gary F. Newkirk,**[2] [1]Fisheries Division, Ministry of Agriculture, Kingston Jamaica and [2]Biology Department, Dalhousie University, Halifax, N.S. Canada B3H 4J1.

The culture of the mangrove oyster, *Crassostrea rhizophorae*, started to develop in Jamaica based on the collection of spat at one location. Spatfall monitoring is essential because oyster reproduction is seasonal and variable depending on the timing and intensity of rainfall. Furthermore, it is important to avoid the barnacle spatfall which usually occurs before the oyster set. Weekly monitoring of the spatfall is done by examining frosted glass panels placed at several locations in the bay. The farm manager is able to use the panel data to determine when the cultch should be dropped in the water. The data over the last 10 years shows a regular occurrence of spatfall twice each year, around July and again around December. However, the exact timing and intensity varies greatly from year to year but the monitoring program has been useful in providing enough advanced warning for the successful collection of spat for farmers.

MORTALITY AND UTILIZATION OF FUEL DEPOTS DURING METAMORPHOSIS OF *CRASSOSTREA VIRGINICA* GMELIN AND *CRASSOSTREA GIGAS* THUNBERG. **Maria C. Haws*** and **Leonard DiMichele,** Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843; **Steven C. Hand,** Department of Environmental, Population and Organismic Biology, University of Colorado, Boulder, CO 80303.

Epinephrine was used to induce synchronous metamorphosis in larvae. Mortality during metamorphosis ranged from 30 to 95% for *C. virginica* and 40 to 85% for *C. gigas* within 72 hours for both epinephrine treated larvae and untreated controls. Since the energetic demands of metamorphosis may exceed accumulated reserves, metabolic events during metamorphosis were examined. Calorespirometry indicated that oxygen consumption and total heat production decreased following epinephrine treatment, then rose to a new steady state just below that of the previous active swimming rate. Total aerobic energetic demand remained high. The calorimetric/respirometric ratio shifted from 480 ± 9.6 to 425 ± 9.6 kJ/mol O_2, indicating a shift from glycogen to glycogen/lipid metabolism. Lipid, glycogen and protein were measured prior to and 36 hours after the induction of metamorphosis. Pre-metamorphic glycogen levels were higher and more variable in *C. virginica* ($6.16 \times 10^{-2} \pm 2.78$ µg/larva) than *C. gigas* ($3.77 \times 10^{-2} \pm 1.44$ µg/larva). Similar results were obtained for pre-metamorphic lipid reserves which were higher and more variable in *C. virginica* ($6.16 \times 10^{-2} \pm 2.78$ µg/larva) than *C. gigas* ($3.77 \times 10^{-2} \pm 2.55$ µg/larva). Both lipid and glycogen utilization during metamorphosis were positively correlated with the pre-metamorphic levels, but final amounts differed. Regardless of the pre-metamorphic amount of glycogen, post-metamorphic levels were uniformly low and may represent the minimum utilizable level. However, post-metamorphic lipid content varied widely (2.0×10^{-2} to 1.2×10^{-1} µg/larva). Protein levels were initially higher for *C. gigas* ($1.83 \times 10^{-1} \pm 5.65$ µg/larva) than for *C. virginica* ($1.1 \times 10^{-1} \pm 5.47$ µg/larva). No correlation was found between protein utilization and initial protein levels. Thus, glycogen and lipid are heavily utilized during metamorphosis. Survival of metamorphosis may depend upon accumulation of sufficient fuel depots prior to metamorphosis, or the efficiency of utilization of reserves. These results indicate an interesting difference in strategy between and within species undergoing the same developmental process.

THE USE OF CEMENT STABILIZED GYPSUM AS CULTCH FOR THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA*, AND ITS EFFECTIVENESS AS COMPARED TO CLAMSHELL AND LIMESTONE. **Edward L. Haywood III,** Department of Biological Sciences, University of New Orleans, New Orleans, LA 70148; **Thomas M. Soniat,** Department of Biological Sciences, Nicholls State University, Thibodaux, LA 70310.

Cement-stabilized gypsum (gypment) was tested along with clamshell, *Rangia cuneata*, and Mexican limestone for its attractiveness to the larvae of the American oyster, *Crassostrea virginica*. Gypsum was previously found to attract larvae but its rapid dissolution in seawater precludes its use as cultch. Crushed gypsum was mixed in a 1:1 w:w ratio with Portland cement and poured into wooden molds. Once hardened, the gypment was broken into pieces and tested for solubility. Solubility tests showed no significant difference between initial and final weights of the gypment rock after exposure to seawater for two months. The three cultch materials were tested under both field and laboratory conditions. Field results showed that gypment and limestone attracted significantly ($P < 0.05$, Non-parametric ANOVA) more spat per dry liter of cultch than did clamshell. Laboratory results showed no significant difference among any of the cultches in their larval attracting capabilities. Gypsum, therefore, can be stabilized with cement and is good or better than clamshell at attracting spat.

MARKET ALTERNATIVES FOR SOUTH JERSEY SOFT-SHELLED BLUE CRABS. **N. R. Henderson** and **D. B. Strombom,** Rutgers Univ., New Brunswick, NJ.

New Jersey crabbers on Delaware Bay, operating independently and on a small scale, traditionally sell blue crabs in live and bait markets. Aquaculture ventures in which molting blue crabs are shed in tanks to produce soft-shelled crabs, a value-added product, are common in Chesapeake Bay, North Carolina, and the Gulf of Mexico. Two large-scale and several small-scale shedding operations have demonstrated that it is technically feasible to produce soft-shelled blue crabs in south Jersey. A shedding facility has been proposed for Salem County which would be constructed by county government on land donated by a public utility and leased to the private sector to be operated as a production, processing, and marketing cooperative. As part of an economic feasibility analysis for the proposed facility, market alternatives were evaluated using in-person surveys of seafood wholesalers and restaurants. Market alternatives identified are the sale of fresh, trimmed product to Philadelphia wholesalers and to local upscale restaurants during the summer tourism season, and of high quality frozen product in regional and selected international markets.

HETEROZYGOTE DEFICIENCY IN SEVEN POPULATIONS OF THE HARD CLAM *MERCENARIA MERCENARIA* (LINN.). **Vico Juste,** Department of Marine Sciences, University of Puerto Rico, P.O. Box 908, Lajas, PR 00667.

A study of the population genetics of the hard clam *Mercenaria mercenaria* (Linn.) from New York, Massachusetts (2), Delaware, Rhode Island, North Carolina, and Puerto Rico was done. Starch gel electrophoresis was used to analyze and identify the genotypes of certain alloenzymes (PGMA, PGMB, AK, MDH, PEPA, PEPB, TO, GPI, HKA, & HKB) in each population. The populations were found to be out of the Hardy-Weinberg equilibrium in at least 3 alloenzymes, and all showed heterozygote defi-

ciencies. The frequency of the most common allele ranged from 50.8% to 100% for all populations. All loci were polymorphic with the exception of MDH. Mean heterozygosity ranged, over all loci of all populations, from 20.8% to 26.5%. High positive values for Fit were found indicating a greater number of homozygous individuals relative to the expected when data were pooled for all populations. All hypotheses for heterozygote deficiency were considered for each population, but selection against heterozygotes gives the best explanation for the results.

GAMETOGENIC CYCLE OF THE SOUTHERN SURF CLAM *SPISULA SOLIDISSIMA SIMILIS* FROM ST. CATHERINES SOUND, GEORGIA. Amita Kanti,*[1,2] Peter B. Heffernan, and **Randal L. Walker,**[1] [1]Shellfish Research Lab, Marine Sciences Program, University of Georgia, P.O. Box 13687, Savannah, GA 31416; [2]Zoology Dept., University of Georgia, Athens, GA 30602.

The reproductive cycle of the southern surf clam was investigated for the first time in the southeastern USA using specimens collected from St. Catherines Sound, Georgia. Monthly (January 1990–July 1991) dredge samples were obtained from a site (7–11 M depth) north of St. Catherines Island. Specimens (N = 30/sample) were measured for shell length (SL) and processed for histology. Staging criteria and gonad indices (G.I.) (1 = Spent through 5 = Ripe) were employed to describe the gametogenic cycle. The unimodal gametogenic cycle began in September–October, with a rapid period of development through November (male G.I. = 4.00, female G.I. = 4.25) followed by a plateau through January (female) or February (male) prior to final maturation by March–April. Females (4.47 ± 0.13) achieved significantly higher G.I. levels (ANOVA, $p = 0.009$) than males (4.06 ± 0.06) in 1990. Peak maturity levels were significantly higher for both sexes during 1991 than 1990 (ANOVA, males $p = 0.0165$, females $p = 0.0004$). Spawning was from March–May (female) and April–May (male) 1990 and April–June (both sexes) 1991 (*ca.* 19°C). Sex ratios were 1:1 (Chi squared $p = 0.08$). There was no relationship observed between SL and stage of sexual maturity. In three monthly samples (from 17) size differences were detected, with the females significantly (ANOVA) larger on each occasion (November 1990, $p = 0.0005$; December 1990, $p = 0.0236$; January 1991, $p = 0.0355$).

MODELING OYSTER POPULATIONS. CRITICAL FEEDING PERIODS, GROWTH AND REPRODUCTION. Eileen E. Hofmann, John M. Klinck, Dept. Oceanogr., Old Dominion Univ., Norfolk, VA 23529; **Eric N. Powell,** Dept. Oceanogr., Texas A&M Univ., College Station, TX 77843.

A time-dependent population dynamics model for oyster (*Crassostrea virginica*) populations is used to examine the factors controlling reproductive effort. The timing of the spring and fall plankton blooms (the oyster's food supply) relative to the spring increase and fall decrease in temperature is crucial in determining reproductive effort over a spawning season. Delay of the spring bloom with respect to the spring temperature rise increases reproductive effort and affects the number and timing of spawning pulses. Simulations using environmental conditions appropriate for the Laguna Madre, Galveston Bay and Chesapeake Bay show that reproductive effort decreases with increasing latitude and that the timing of increases in food supply relative to rising temperature becomes more important. Reproductive pattern at higher latitudes should be characterized by discrete spawning pulses; continuous spawning should become more frequent at lower latitudes. Smaller oysters should spawn more, earlier, have a longer spawning season and more discrete spawning pulses. The characteristically wide range of reproductive efforts recorded in the literature may result from seemingly minor changes in the environment; a few degrees change in temperature or a small (2 to 4 week) shift in timing of the spring bloom.

MASS PRODUCTION METHODS FOR INDUSTRIALIZING SCALLOP CULTURE. Hiroshi Ito, Hokkaido National Fisheries Research Institute, Fisheries Agency, Japan Ministry of Agriculture, Forestry and Fisheries, 116 Katsurakoi, Kushiro, Hokkaido 085, Japan.

Recent information on scallop culture in Japan is reviewed. References of the Japan's scallop fishery usually refer to the Japanese scallop, *Patinopecten yessoensis,* fishery, which surpasses other scallop species in scale of landings. The major landings have always been around Hokkaido in northern Japan. Scallops caught in Hokkaido represented 77% of all Japanese scallop production from both wild and cultured stocks during the period 1910–88. During the end of the 1960s, industrial scallop cultures developed owing to an innovation in seed production using wild spats. In the 1980s, an excellently successful method for wild spat collection and an effective culture system have been developed with the new and excellent research operations. Due to the recent technical development, the scallop takes first place in molluscan shellfish production in Japan, with a twenty-three-fold increase in production and a forty-three-fold increase in value over the last 20 years of 1969–88. In 1988, the production of sowing culture and wild scallops amounted to 159,689 metric tons (t) and hanging culture reached 181,929t, for a total production of 341,618t. The value was 78,674 million yen (524 million U.S. dollars: calculated on the dollar = 150 yen).

***IN VITRO* CULTURE OF PRESUMPTIVE OYSTER NERVOUS TISSUE. S. J. Kleinschuster,* S. K. Allen, Jr., S. E. Ford,** and **S. Swink,** Rutgers University, Haskin Shellfish Research Laboratory, Port Norris, NJ 08349.

Long term survival of cultured molluscan (Pelecypoda) cells and tissues has long been possible. For example, dissociated cardiac tissue will adhere to most tissue culture substrates and remain viable for months. Deteriorating cardiac explants will also occasionally yield clumps of cells that adhere and beat temporarily.

Generally, epithelial cells will not adhere and grow, although mitotic activity has been reported in suspension culture. Anchorage-dependent cell or tissue types, other than those associated with hemolymph, have long been needed as models for acute and long term studies of both normal physiologic and pathologic responses of the organism. We report herein preliminary results of the *in vitro* culture of presumptive neuronal cells and tissues of the visceral ganglion of *Crassostrea virginica*.

MODELING OYSTER POPULATIONS. ADULT SIZE AND REPRODUCTIVE EFFORT. John M. Klinck, Eileen E. Hofmann, Dept. Oceanogr., Old Dominion Univ., Norfolk, VA 23529; **Eric N. Powell,** Dept. Oceanogr., Texas A&M Univ., College Station, TX 77843.

A time-dependent model of energy flow through post-settlement oyster populations is used to examine the factors influencing adult size and reproductive effort over the latitudinal gradient. Size and reproductive effort are determined by: (1) the allocation of net production into growth and reproduction and (2) the rate of food acquisition. Both are temperature-dependent parameters. Limitation on size comes from the balance between winter loss and summer gain in somatic tissue less the energy expended in reproduction. In the Gulf of Mexico, oyster size declines at lower latitudes because increased temperature decreases the allocation of net production to somatic growth. In some cases, smaller-sized populations may spawn more. Variations in temperature and food supply affect reproductive effort more strongly than adult size because (1) the rate of energy flow through the oyster is higher in warmer months when most net production is allocated to reproduction; (2) small changes in temperature substantially change the spawning season but vary the time of positive net production less; (3) the timing of the spring and fall blooms affects the total amount of energy available for reproduction more so than for somatic growth.

USE OF GENETIC MARKERS TO EVALUATE THE SUCCESS OF TRANSPLANTED BAY SCALLOPS. Maureen K. Krause, Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, NY 11794-5245.

New York populations of the bay scallop, *Argopecten irradians irradians*, suffered disastrous recruitment failures and mortalities due to brown tide events caused by *Aureococcus anophagefferens* in 1985, 1986, 1987, and 1990. In an attempt to rejuvenate the fishery, hatchery-produced seed were transplanted into Long Island waters on several occasions. This study describes the degree of genetic differentiation between introduced and native scallop stocks and uses this information to evaluate the relative contributions of transplanted and native animals to a successful recruitment event. Genetic variation at five enzymatic loci was detected by starch gel electrophoresis. Two techniques, multivariate discriminant function analysis and maximum likelihood estimation, were applied to these data to estimate proportional stock contributions to scallop recruitment in the fall of 1989. Although significant allele frequency differences were present among indigenous and transplanted scallops, these differences were not sufficient to separate the stocks using discriminant analysis. Maximum likelihood estimation of stock composition proved a more reliable approach and calculated that transplanted stocks contributed approximately 25% to recruitment.

THE EFFECT OF GPI POLYMORPHISM ON GLYCOLYTIC FLUX AND PRODUCTION-RELATED TRAITS IN THE BAY SCALLOP. Maureen K. Krause, Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, NY 11794-5245.

Only a few studies have clearly established the relationships among genetic variation for a specific enzyme, the mechanistic, biochemical expression of that variation, and its relevance to the whole organism. An integrated approach to examine the biological significance of genetic variation for a single enzyme involves detailing the effects of that variation on the overall rate of flux through the metabolic pathway in which the enzyme is embedded. The research presented uses this approach to attempt to establish a causal explanation for observations of a strong relationship between genotype for the enzyme glucose phosphate isomerase (*Gpi*) and production-related traits in the bay scallop, *Argopecten irradians*. The proposed mechanism for selection involves the differential inhibition of Gpi variants by an intermediate of the pentose shunt. The experiments discussed examine the expression of genetic variation by using radioisotopes to measure the rates of flux through *Gpi* when this enzyme is artificially inhibited with a product from the pentose shunt. Significant effects of genotype on flux were present, as were significant relationships between flux and overall size of the scallops, suggesting a possible mechanistic basis for the associations among *Gpi* genotypes and quantitative traits.

SEASONAL VARIATION IN THE UTILIZATION OF DIETARY PROTEIN BY THE MUSSEL, *MYTILUS TROSSULUS*. Daniel A. Kreeger and **Christopher J. Langdon,** Hatfield Marine Science Center, Oregon State University, Newport, OR 97365.

Seven samples of mussels (*Mytilus trossulus*) were collected from Yaquina Bay, Oregon, during the period December 1990 to November 1991. Mussels were fed a uniform diet of microalgae and ^{14}C-labeled protein microcapsules, and the radioactivities of ingested, digested, respired, defecated and incorporated fractions were determined for individual mussels. The assimilation efficiency for ^{14}C-protein capsules was calculated as the proportion of ingested ^{14}C that was respired or incorporated. Dietary protein was assimilated by *M. trossulus* with significantly ($p < 0.0001$) greater efficiency during December (26%), February (16%), April (31%) and November (19%) than during the summer months of May (10%), June (7%) and August (7%). The lowest ratio of ^{14}C-respiration to ^{14}C-incorporation and the highest ratio of oxygen

consumption to nitrogen excretion occurred during May, suggesting that greatest sparing of assimilated protein from catabolism occurred during early summer. Seasonal variation in the partitioning of protein between energy catabolism and tissue synthesis may be linked to mussel reproduction and growth cycles.

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.

The growth rates of quahogs (*Mercenaria mercenaria*) from two sites in Prince Edward Island are significantly different. A reciprocal transfer experiment was used to determine if these different growth rates are due to genetical or environmental differences.

A total of 120 quahogs, ranging in size from 25 mm to 45 mm in length, were collected from each site, and tagged. Half (60) of the tagged quahogs 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 during the ice-free period. Growth and mortality levels were monitored over an eighteen month period.

The growth increments at the end of the first four months in West River were 3.6 mm and 5.3 mm for quahogs originating from Pownal Bay and West River respectively, compared to 0.7 mm and 1.0 mm in Pownal Bay. Over the same period of time, mortality was higher in West River group at the West River site with 31 dead animals compared to an average of 6.3 from the three remaining groups.

***IN VITRO* INTERACTION OF *PERKINSUS MARINUS* WITH HEMOCYTES FROM EASTERN AND PACIFIC OYSTERS, *CRASSOSTREA VIRGINICA* AND *CRASSOSTREA GIGAS*. Jerome F. La Peyre, Fu-lin E. Chu,** and **Wolfgang K. Vogelbein,** Virginia Institute of Marine Science, School of Marine Science, The College of William and Mary, Gloucester Point, VA 23062.

This study was a first attempt to characterize and compare hemocyte responses of eastern and Pacific oysters to *Perkinus marinus in vitro*. Meronts (trophozoites) of *P. marinus* were isolated from infected eastern oysters. Hemolymph was collected from uninfected oysters kept in recirculating water systems at 15°C. Granulocytes, large hyalinocytes and small hyalinocytes were quantified. The percentage of large hyalinocytes was significantly greater in Pacific oysters. In phagocytosis assay, meronts were associated with granulocytes and small hyalinocytes in both species, whereas zymosan, a reference non-self particle, was predominantly associated with granulocytes. Chemiluminescence was not observed when hemocytes were exposed to meronts at a ratio of 1:80. Hemocyte chemiluminescence responses to zymosan in Pacific oysters were significantly greater than in eastern oysters. [illegible] on microscopy of meronts following incubation with hemocytes of eastern and Pacific oysters for 15 min and 12 hr indicated that the parasites were rapidly phagocytosed, and that a small percentage may be killed by 12 hr. Incubation of meronts with hemocytes affected their subsequent development. The number of meronts enlarging in fluid thioglycolate media, after incubation with hemocytes in plasma for one day at 15°C, was significantly lower than after incubation in plasma alone. Results suggest that intracellular killing of *P. marinus* occured.

VITELLIN PEPTIDE IN THE OOCYTES OF THE HARD CLAM, *MERCENARIA MERCENARIA*. Dr. Richard F. Lee,* Skidaway Institute of Oceanography, P.O. Box 13687, Savannah, GA 31416; **Dr. Peter B. Heffernan,** University of Georgia Marine Extension Service, Shellfish Laboratory, P.O. Box 13687, Savannaha, GA 31416.

Vitellogeneis involves the synthesis and assembly of vitellin, a major component of egg yolk. Vitellins serve the energy and nutritional needs of the developing embryo. A major component of clams oocytes was a very high density lipoprotein composed of a 56,000 dalton peptide and phospholipid. Lipoproteins were separated from other egg proteins by adjusting the cytosol density to 1.26 g/ml and ultracentrifugation at 117,000 g. The different peptides of lipoproteins were separated on sodium dodecyl sulfate-polyacrylamide gels and the major component, a 54,000 dalton peptide eluted from the gel. The purified 54,000 dalton peptide was used to prepare monoclonal antibodies. The clam egg peptide was injected into mice and after a high titer was achieved mouse spleen cells were fused with myeloma cells followed by cloning of the hydridomas. The clones were screened for their ability to generate antibodies which bound to the clam egg peptide. The clones which produced these antibodies were grown up in fetal calf serum. Using these monoclonal antibodies to clam egg peptide, we developed an Enzyme-Linked Immunosorbent Assay (ELISA) procedure to assay for clam egg peptide in various clam tissues. With this assay we are able to detect clam egg peptide at a concentration as low as 20 ng/ml. The ELISA procedure will allow us to follow changes in the concentration of egg vitellins that occur in both gonadal and non-gonadal clam tissues during vitellogenesis. We speculate that the digestive gland of *M. mercenaria* is responsible for the vitellin peptides found in the oocytes.

THE TOXICITY OF AMMONIA ON THE LARVAE OF THE BAY SCALLOP, *ARGOPECTEN IRRADIANS*. Guoming Lin, Institute of Oceanology, Ac. Sinica 7 Nanhai Road, Quingdao, P.R. China.

The lethal and sublethal effects of ammonia on the larvae of the bay scallop *A. irradians* were studied in the controlled condition (23°C, 31.6‰, pH 8.10). The 96h LC50s for the newly veligers (48h after fertilized), the D-shaped larvae of 110 μ in average shell length, and the umbo larvae were respectively 6.33, 7.84, 5.25 mg/L total ammonia-N, derived from the log-probit method in the

lethal experiment. The results of the sublethal experiment lasting 12 days, showed that the EC50 of total ammonia-N causing 50% reduction in growth was 4.04 mg/L, and 2.10 mg/L in the eye-spot appearance, 2.67 mg/L in metamorphosis. It is suggested that the newly veligers had lower tolerance than the elders, however the umbo larvae had the lowest. The particular sensitivity of the bay scallop to ammonia was observed in the stages of the eye-spot development and metamorphosis. The high ambient ammonia could damage the larval organs or tissues. We suggest the safe concentration of ammonia for the breeding of the bay scallop would be taken as 0.15 mg/L total ammonia-N (0.007 mg/L NH_3-N) in the above condition, and the applicable factor as 0.02 in extensive one.

INTEGRATED CULTURE OF GREEN MUSSEL (*PERNA VIRIDIS*) AND MARINE SHRIMP (*PENAEUS MONODON*). **C. K. Lin*** and **P. Ruamthaveesub, P. Wanuchsoontorn,** Asian Institute of Technology* and Charoen Pokaphand Feedmill Co., Ltd. Bangkok, Thailand.

The wastewater effluents discharged from intensive marine shrimp culture ponds contained an average of 1250 and 132 kg of dry organic matter and phytoplankton biomass, respectively, for 1 tonne of fresh shrimp production. The effluents with abundant nutrients and planktonic organisms are normally discharged to coastal waters, causing deterioration of water quality. To recover resources from and improve water quality of the wastewater, a mussel culture component was integrated with intensive shrimp culture ponds in a pilot-scale recycle system for a 4-month culture cycle. Mussels colonized on bamboo poles were suspended in the canals that received wastewater daily from shrimp ponds. The gross mussel production was approximately 2,000 kg with an average weight increased from 7 to 43 g/mussel, or 0.32 g/mussel/day. It is estimated that the mussels removed approximately 2,116 kg of solid organic matter, including 764 kg of phytoplankton biomass, from the effluents. With shrimp production of 17,581 kg in 2 ha of total pond area the financial gain from the mussel harvest was meager. But the mussel culture component played an invaluable role in sustaining shrimp culture in the recycle system.

***VIBRIO VULNIFICUS* INACTIVATION BY SELECTED SUBSTANCES IN SEAWATER.** **M. Logan,* J. Bemiss, J. Sample,** and **S. Price,** National Marine Fisheries Service, Charleston, SC.

Substances reported to have antibacterial activity were tested for their ability to inactivate *Vibrio vulnificus* in seawater in an attempt to identify potential agents for shellfish depuration. Filtered seawater spiked with 10^{-8}CFU/*ml* of environmental culture (4965) was used. *V. vulnificus* counts were determined by the spread plate technique. A total of 19 substances were screened and those causing total inactivation are listed.

Chemical	Amount/1	Sampling (hrs)	Percent Inactivation
Gallic Acid	0.062–5.0 g	24	100
Garlic Powder	5.0 g	24	100
Sodium Erythorbate	5.0 g	24	100
Tannic Acid	5.0 g	4	100
Vanillin	2.0–5.0 g	24	100
Apple Juice	100 ml	48	100
Carrot Juice	100 ml	48	100
Grape Juice	100 ml	48	100

These results suggest specific substances may be effective in enhancing inactivation of *Vibrio vulnificus* in shellfish within typical 48 hour commercial treatments.

FEEDING RESPONSES OF *PLACOPECTEN MAGELLANICUS* UNDER FIELD AND LABORATORY CONDITIONS. **B. A. MacDonald,* J. E. Ward, G. S. Bacon,** and **J. P. A. Gardner,** Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, N.F., Canada, A1C 5S7.

One objective of the Ocean Production Enhancement Network (OPEN) is to produce an integrated aquaculture production model for the giant scallop *Placopecten magellanicus* in Atlantic Canada. This will include extensive monitoring of physical oceanographic conditions and their influence the concentration and distribution of suspended particulate matter. Our specific objective is to provide realistic information on capture, ingestion, and conversion of suspended material into marketable biomass for this species over a wide range of environmental conditions. Laboratory studies, where food concentration and quality can be manipulated, are being combined with field studies, where ingestion rates and absorption efficiency can be measured using natural seston, to produce predictive equations for scallop feeding responses. To regulate ingestion at increasing particle concentrations this species reduces clearance rate and produces more pseudofaeces to maintain a constant absorption efficiency. This information will be incorporated into the production model to determine optimal stocking densities and the carrying capacity for different habitats.

THE SOUTHERN BAY SCALLOP, *ARGOPECTEN IRRADIANS CONCENTRICUS*, IN FLORIDA: STATUS OF KNOWLEDGE AND FUTURE RESEARCH. **D. C. Marelli,* W. S. Arnold, C. Lund,** and **C. P. Bray,** Florida Marine Research Institute, St. Petersburg.

The southern bay scallop, *Argopecten irradians concentricus*, occurs along the Gulf coast of Florida from Florida Bay north to Pensacola and has historically been an important recreational fishery species. Bay scallop distributions are closely associated with seagrass areas, but their abundance in these areas is spatially and

temporally variable. Fishery landings data are incomplete, but anecdotal evidence suggests that bay scallop populations in Florida have declined in recent times. This decline, coupled with the intense fishing pressure to which this species is subjected, makes the southern bay scallop a species that merits special attention. The status of the limited knowledge of this species is presented, and research plans for surveys and experiments designed to examine various aspects of bay scallop biology in Florida are outlined.

EFFECT OF DIFFERENT ENVIRONMENTAL CONDITIONS ON BIOCHEMICAL COMPOSITION OF POST-LARVAE AND GONAD OF THE SCALLOP *ARGOPECTEN PURPURATUS* DURING ITS RECOVERY AFTER SPAWNING. Gloria Martinez, M. Toryes, E. Uribe, M. A. Diaz, and **H. Perez,** Facultad de Ciencias del Mar, Universidad Católica del Norte. Casilla 117, Coquimbo, Chile.

Post-larvae of *Argopecten purpuratus* of 400 microns obtained from the same batch, were divided in two groups: one kept in hatchery, fed with a mixture of *Chaetoceros calcitrans* and *Isochrysis qalbana*, the other placed on pearl nets and hung into the sea. After 40 days, both groups were sized and analyzed for biochemical composition. Besides, adult scallops were induced to spawn, and spawned individuals were divided in two groups and tested the same way than post-larvae. Each 12 days, samples were analyzed for gonad index and biochemical components. Post-larvae reared in hatchery grew less, contained more carbohydrate, lipid and DNA, but less protein and RNA, and presented lower RNA/DNA index than the other group. Analysis of fatty acids showed that individuals in hatchery had higher content of C16:1n7 and C18:1n9, but much less of C22:6n3. Scallops kept in hatchery attained 48 days without showing complete gonad restoration, while the others spawned spontaneously before this date. Biochemical components showed similar behavior than post-larvae for both experimental conditions. These results showed that natural environment may be better than laboratory for growing and conditioning pectinid species for culture purposes.

POSSIBLE GENETIC INFLUENCES ON THE GROWTH RATE AND SURVIVAL OF TWO POPULATIONS OF *CRASSOSTREA VIRGINICA*. Mirella C. Martinez* and **Leonard DiMichele,** Department of Wildlife and Fisheries Sciences, Texas A & M University, College Station, TX 77843.

Laguna Madre (LM), Texas, has unique populations of oysters, *Crassostrea virginica*, that are genetically distinct from all other Atlantic populations. The LM is also a unique environment, characterized by extreme hypersalinities and high summer temperatures. We examined growth of LM oysters relative to Galveston Bay (GB) oysters to determine if this genetic differentiation is correlated with oyster physiology. Oysters were acclimated to a common environment and mass spawned to produce two pure populations (GB × GB = GG, LM × LM = LL) and the reciprocal crosses (GB × LM = GL, LM × GB = LG). Larvae were grown in 20 ppt and 40 ppt. The paternal GB crosses grown in 40 ppt died at the larval stage. Spat from surviving groups were grown in LM and GB. At both locations, LL_{40ppt} and LL_{20ppt} spat grew significantly faster than GG_{20ppt} spat. Growth of all reciprocal crosses were intermediate and showed significant parental effects. LL_{20ppt} growth was similar in both locations. GG_{20ppt} spat in LM grew faster than siblings in GB, but significantly slower than any LL cross. Some authors have speculated that LM populations have genetically adapted to their environment, while others have suggested that drift and isolation are responsible for the observed differentiation. These preliminary results are consistent with the isolation hypothesis but indicate that some physiological differentiation may have occurred as well.

RETENTION OF *VIBRIO CHOLERAE* O1 IN *CRASSOSTREA VIRGINICA* UNDER CONDITIONS OF CONTROLLED PURIFICATION. Rendi L. Murphree,* University of Florida, Gainesville, and Jacksonville State University, Alabama, and **Mark L. Tamplin,** University of Florida, Gainesville.

Vibrio cholerae O1, the causative agent of cholera, is known to persist in estuarine environments as autochthonous flora. The recent introduction of a virulent Latin American strain of *V. cholerae* O1 into Gulf of Mexico estuaries intensifies the need to determine the effect of controlled purification techniques which can reduce this pathogen in edible molluscan shellfish. Laboratory experiments were performed to define the parameters for uptake and retention of *V. cholerae* O1 in tissues of *Crassostrea virginica*. A mixture of three *V. cholerae* O1 Latin American strains was added to a tank of recirculating artificial seawater (25°C) containing freshly harvested oysters. Maximum uptake of *V. cholerae* O1 occurred within 4 h. Experimental conditions for controlled purification consisted of seawater which was recirculated through diatomaceous earth filters and ultraviolet light. Oysters meats were sampled over 6 days. Results showed that *V. cholerae* O1 levels were not reduced significantly after 2 d of treatment. At 3 d, *V. cholerae* O1 numbers declined approximately one $\log_{10}$ and remained stationary through 6 d. These experiments indicate that controlled purification techniques may not be effective for eliminating hazardous levels of *V. cholerae* O1 in oyster shellstock.

PRELIMINARY INDICATIONS THAT A SECOND SUMMER SPAWNING COULD BE RELATED TO THE SUMMER MORTALITY OF 2-YR-OLD CULTURED MUSSELS IN THE MAGDALEN ISLANDS (QUÉBEC, CANADA). Bruno Myrand* and **Jean Gaudreault,** Dir. de la Recherche Scientifique et Technique Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec. C.P. 658, Cap-aux-Meules, Qué., Canada G0B 1B0.

To understand the recurrent summer mortality of 2-yr-old cultured mussels in the Magdalen Islands lagoons, mussels that had

resisted the summer mortality of 1-yr-old mussels in 1990 were stripped off their socks on June 12, 1991. Then 96 groups of 50 mussels (50–60 mm) were placed in cages. Half the cages were placed in the Great Entry lagoon (IN) at 3 m depth while the others were transferred to open sea (OUT) at 15 m depth. On each sampling 3 cages from each site were pulled out. Live and dead mussels were counted and measured. Specimens were used for length-dry weight (mantle, total, shell) regressions, reserves (glycogen, proteins and lipids) measurements, or pathology and parasite observations. Water temperature was recorded at both sites. A first spawning occurred at both sites between June 12 (Condition Index = 25.8%) and June 27 (C.I.-IN = 17.6% and C.I.-OUT = 18.9%). After a short period of replenishment at both sites, a second spawning occurred in the lagoon when the C.I. dropped from 19.7% on July 16 to 14.2% on July 23. Mortality began the following week and about 50% of the mussels died by the end of August. There was no second spawning in the open sea and the C.I. remained around 20% through the end of the experiment. There was no summer mortality at this site. We hypothesize that although mussels were ready to spawn at both sites, environmental conditions only triggered spawning in the shallow lagoon where mussels were kept near the surface compared to those suspended much deeper in the open sea. These results appear to support the role of the spawning stress and the following exhaustion on the occurrence of summer mortality of the mussels.

OUTPUT OF A MODEL TO SEED MUSSEL BOTTOM LEASES TO THEIR CARRYING CAPACITY: CALIBRATION, VALIDATION AND SENSITIVITY ANALYSIS. **C. R. Newell*** and **D. E. Campbell,** Great Eastern Mussel Farms, Inc., Tenants Harbor, ME 04860, and U.S. E.P.A. Lab, Narragansett, RI 02879.

A model was developed using basic routines and an electronic spreadsheet to predict mussel growth under bottom culture in terms of shell length, seed to harvest yield, average meat size and shucked meat yield. The model was developed at a 30 hectare lease site in Maine, USA where extensive studies were performed over two years on current speed, seston supply and demand, and spatial and seasonal variations in food quality and mussel physiology. Model predictions were validated at two other contrasting sites, and against predicted effects of density on mussel growth. The importance of the spring phytoplankton bloom in supplying phytoplankton carbon and later on, high quality detrital carbon, is reflected in maximum mussel biomass at the site. Model results are presented for different seed sizes, spring and fall seeding, different food curves at the same site, and different sites.

The sensitivity of mussel growth to bottom density suggests a maximum bottom density of 150–350 mussels per square meter at low current sites (5 cm s-1 or lower bulk current speed) and about 600–900 mussels per square meter at higher current sites (over 10 cm s-1). Mussel seed distribution is critical to seed to harvest yields in bottom culture, and can make the difference between a commercial success and failure.

The model has general application to bottom culture of other shellfish species, especially those involved in large-scale seed transplantation, and has the potential of resulting in a doubling of seed to harvest yields and shucked meat yields.

This work was supported by NSF SBIR grant ISI8809760.

SHORT-TERM VARIABILITY IN SESTON FLUX AND PHYSIOLOGICAL RESPONSES OF BOTTOM-CULTURED MUSSELS (*MYTILUS EDULIS*) IN MAINE. **C. R. Newell*** and **S. M. Gallagher,** Great Eastern Mussel Farms, Inc., Tenants Harbor, ME 04860, and Woods Hole Oceanographic Institution, Woods Hole, MA 02543.

Mussel feeding physiology and scope for growth were monitored during spring (April, 1991) and fall (October, 1990) in efflux (flow-through) containers, benthic ecosystem tunnels, and with a time-lapse benthic video monitor over a 1–2 tidal cycles at a mussel bottom lease site in Stonington, Maine. Tidal cycle variations were observed in mussel shell gape, seston consumption, oxygen consumption and scope for growth. During periods of shell closure (correlated with low particle concentrations), oxygen consumption reduced to 25% of levels during active feeding. Tidal rhythms in feeding physiology indicate an important role of vertical flux at the site causing a period of maximal ingestion during high and ebb tides. Scope for growth calculations should consider reductions in time of feeding during periods of low seston concentrations in order to balance low respiration rates during those times. Spring vs fall comparisons indicate that mussel shell gape during periods of low food (fall) range from 30% to 100% while in the spring, gape varies from 70% to 100%. The time-lapse benthic video provides a cost-effective method for determining high frequency and seasonal changes in filtration activity of bivalves under undisturbed, field conditions, and can improve the prediction capabilities of production models.

This work was supported by NSF SBIR grant ISI8809760.

BIVALVE CULTURE AS A COMPONENT OF COASTAL AQUACULTURE IN DEVELOPING COUNTRIES: A REVIEW OF CONFLICTS AND CONSTRAINTS. **Gary F. Newkirk,** Mollusc Culture Network, Biology Department, Dalhousie University, Halifax, N.S. B3H 4J1 Canada.

Though bivalve culture is an attractive development option in tropical waters because of no feed inputs and rapid growth, over the past 30 years the only major developments have been mussel and oyster culture in Thailand and Philippines. However, there is a long list of failures to establish similar industries. The thesis of this paper is that many failures result from insufficient attention to the social and cultural aspects of the target communities and a failure to consider options other than the small-business model which requires major farmer involvement. Most people in coastal fishing communities depend on a diversity of livelihood sources.

In situations where natural bivalve production is high *and* there is a strong market for a moderately priced product (e.g., Thailand), the small business model may work. However, if production is variable and the return is low (e.g., Philippines), the small-business option is not attractive. However, in the latter case small scale, perhaps seasonal, investments using low cost techniques can be attractive as in the Philippines. The (truly) small scale option requires a very different approach from the small business option as technology, financing, extension, marketing, development period and project evaluation will be different.

TEMPORAL AND SPATIAL RECRUITMENT VARIATIONS OF *CRASSOSTREA VIRGINICA* WITHIN A GEORGIA ESTUARY. Francis X. O'Beirn,*[1,2] Peter B. Heffernan,[1] Randal L. Walker,[1] and **William K. Fitt,[2]** [1]Shellfish Research Lab., Marine Sciences Program, University of Georgia, P.O. Box 13687, Savannah, GA 31416, [2]Dept. of Zoology, University of Georgia, Athens, GA 30602.

Oyster recruitment was determined in Georgia (March–November, 1991), at House Creek (HC; low exposure; 25–32 ppt; 15.5–31°C), Skidaway River (SK; medium exposure; 17.7–30.5 ppt; 12.8–30.5°C) and Priest Landing (PL; high exposure; 15–28 ppt; 22.5–30°C). Sampling was conducted on a 14-day, 28-day and seasonal basis, subtidally (ST), at mean low water (LW) and two hours after mean low water (IT). The collectors were grooved ¼″ PVC tubes. Recruitment displayed unimodal peaks, at HC (mid-June to mid-October), SK (September) and PL (August). Maximum 14-day mean recruitment (spat/m^2) at each site was; HC (ST) 1880 ± 360 SE; SK (ST) 1210 ± 290 SE; PL (IT) 188 ± 50 SE. Maximum 28-day mean recruitment (spat/m^2) at each site was; HC (ST) 3660 ± 690 SE; SK (ST) 890 ± 270 SE; PL (IT) 220 ± 70 SE. Biweekly and monthly data showed consistent patterns at HC and SK, with ST = LW > IT. No differences were detected at PL. Maximum seasonal mean recruitment (spat/m^2) was; HC (IT) 7350 ± 570 SE; SK (IT) 1220 ± 170 SE; PL (ST) 600 ± 400 SE. At HC, IT > LW > ST; SK, IT > LW = ST and; PL, IT = LW = ST. Results revealed major site specific and temporal influences on recruitment with maximum levels recorded at the sheltered site (HC) and a reversal in the dominant level from subtidal to intertidal with time.

A REVIEW OF OYSTER FARMING TECHNIQUES IN AUSTRALIA. Dos (David) O'Sullivan, Key Centre for Teaching and Research in Aquaculture, University of Tasmania at Launceston, P.O. Box 1214, Launceston, Tasmania, 7250 Australia.

Edible and pearl oysters are an important part of the Australian aquaculture industry. In 1989–90 they represented almost 70% of the total value (farm gate) of Au$190 million. Several edible oyster species are cultivated in Australia although the majority of production is for the Sydney rock oyster (*Saccostrea commercialis*) which is cultivated in New South Wales and southern Queensland. Production of the Pacific oyster (*Crassostrea gigas*) is increasing rapidly from farms in Tasmania, South Australia and Victoria. The majority of pearl oyster production is from the 'silver lip' or 'golden lip' (*Pinctada maxima*) in Western Australia which produces the world famous 'South Seas Pearls,' although cultivation is also underway in the Northern Territory and Queensland. The variety of farming environments and species cultured has led to a number of innovative culture techniques including the use of racks, baskets, trays and longlines. The combined with an emphasis on continuous cleaning and grading of the oysters is leading to increased productivity as well as excellent quality products, particularly half-shell oysters and cultured pearls.

MERCURY BIOACCUMULATION IN OYSTERS, *CRASSOSTREA VIRGINICA*, BLUE CRABS, *CALLINECTES SAPIDUS*, AND *PENAEUS* SHRIMP IN A CONTAMINATED ESTUARY. Sally Jo Palmer,* Bobby J. Presley, and **Robert J. Taylor,** Department of Oceanography, Texas A&M University, College Station, TX 77843.

From 1966 to 1970 mercury was released from a chlor-alkali plant into Lavaca Bay, TX. Twenty one years later, high mercury levels persist in the sediment and organisms of the Bay. Caged animals (oysters, blue crabs, and shrimp) were exposed to ambient water and sediment of Lavaca Bay and a nearby uncontaminated estuary, Keller Bay. Samples were collected every 7 days over a month. Although natural populations of blue crabs in Lavaca Bay are greatly enriched in mercury when compared to shrimp, the study showed that shrimp accumulate mercury much faster than do blue crabs. Average shrimp mercury levels in Lavaca Bay increased from a baseline value of 336 to 1000 ppb (dry weight) after 29 days exposure. Caged oysters also accumulated mercury; average mercury values increased from 189 to 1336 ppb over 30 days. Average mercury concentrations in water, bottom sediment, and select infauna were consistently higher at Lavaca Bay compared to Keller Bay, suggesting that shrimp, crabs, and oysters have the potential to obtain mercury from both water and food.

A REVIEW OF MATHEMATICAL MODELS USED IN ASSESSING ENVIRONMENTAL IMPACTS OF SALMONID NET-PEN CULTURE. Vijay Panchang and **John Richardson,** Department of Civil Engineering, University of Maine, 456 Aubert Hall, Orono, ME 04469.

Considerable effort is being invested in determining the environmental impacts of net-pen aquaculture. Mathematical computer models can assist by estimating spatial variations within lease sites or the cumulative effects of several operations within an embayment. Tidal and wind-driven currents, waves, and the resulting fish-food/fecal matter dispersion processes dictate the environmental impact of aquaculture operations. Pioneering modelling efforts in this direction were made by Gowen & Bradbury (1987); although they included many processes, some studies in Maine (Swan's Island & Cobscook Bay sites) have found these modelling

techniques inadequate (e.g. Findlay, 1991). In our paper, we will review existing models by Gowen et al. (1989) and Parametric Inc. (Fox 1988). We will examine advantages and shortcomings, and describe our efforts to develop more sophisticated (yet practical) models. In particular, we will describe a 2-d hydrodynamic model for simulating wind- and tidal-flows, a simple model for establishing vertical variations in velocity, and a random-walk 3-d model for transporting the fish wastes. We will also review a wave model for studying resuspension by wave action, a problem in coastal Maine. Finally we will describe our efforts to use these models at two aquaculture sites in Maine (part of an ongoing NOAA/SK project).

MODELING OYSTER POPULATIONS. POPULATION CRASHES AND MANAGEMENT. Eric N. Powell, Dept. Oceanogr., Texas A&M Univ., College Station, TX 77843; **Eileen E. Hofmann** and **John M. Klinck,** Dept. Oceanogr., Old Dominion Univ., Norfolk, VA 23529.

A time-dependent model of energy flow through post-settlement oyster populations is used to examine how mortality affects oyster populations over the latitudinal gradient. Oyster populations are more susceptible to intense population declines when mortality is restricted to the summer months. Much higher rates of mortality can be sustained during the winter. Oyster populations are more susceptible to intense declines at higher latitudes. The association of declines with disease agents causing summer mortality and the increased frequency of long-term declines at higher latitudes is a product of the basic physiology of the oyster and the population dynamics that accrues therefrom. The simulations suggest that decisions on size limits, seasons and densities that trigger early season closure must differ across the latitudinal gradient and in populations experiencing different degrees of summer and winter mortality relative to the recruitment rate. In particular, the latitudinal gradient in susceptibility requires that management be more conservative at higher latitudes.

REPOPULATION DYNAMICS OF ADULT HARD CLAMS IN ESTUARINE HABITATS. Robert S. Prezant,* Harold B. Rollins, and **Ronald B. Toll,** Department of Biology, Indiana University of Pennsylvania, Indiana, PA 15705; Department of Geology, University of Pittsburgh, Pittsburgh, PA 15260; Department of Biology, University of the South, Sewanee, TN 37375.

Through a temporal series of defaunation mark and recapture experiments, we have examined the population dynamics of hard clams, *Mercenaria mercenaria,* along a high energy salt marsh of coastal Georgia. Previously, only larval or juvenile dispersal was considered as a means of recruitment into an established population. Habitat repopulation by adult clams, however, is now documented as an important means of dispersal and repopulation via passive transport. This can help explain observations of missing cohorts, especially younger groups, in clam populations along the mid-Atlantic coast. Harvested or environmentally stressed clam beds could readily be repopulated by reproductively viable adult hard clams in response to specific hydrodynamic events. This, however, results in reduced predictability of *Mercenaria* distribution, abundance and cohort structure. Larval drift remains the most significant means of repopulation over long distances, however, we must now consider adult clam drift as an integral part of hard clam population dynamics.

HISTOLOGICAL COMPARISON OF MASCULINIZED FEMALES AND ANDROGYNOUS MALES IN THE WEST INDIAN FIGHTING CONCH, *STROMBUS PUGILIS*. Shawna E. Reed, Department of Marine Sciences, University of Puerto Rico, Box 908, Lajas, Puerto Rico.

Gonad and other reproductive tissues were taken from masculinized females and sexually undeveloped individuals of the West Indian fighting conch, *Strombus pugilis,* and a masculinized females *S. gigas* for comparison to normal males and females of the same species. Gonads were fixed in Davidson's solution, and other tissues in Bouin's, prior to histological processing. All histological staining was with regressive hematoxylin and eosin. Masculinized females were indistinguishable from normal females except for the presence of a small, deformed penis which resembled, microscopically, that of a normal male. Sexually undeveloped specimens did not possess a penis or an egg groove. Microscopic examination of the sexually undeveloped gonad tissue revealed some spermatogenic tissue which was inactive, showing that these specimens were sexually undeveloped males.

ESTIMATION OF MUSSEL SEEDING DENSITIES BY MATHEMATICAL MODELLING. John E. Richardson and **Vijay Panchang,** Department of Civil Engineering, University of Maine, 453 Aubert Hall, Orono, ME 04469; **Carter Newell,** Great Eastern Mussels, P.O. Box 141, Long Cove Road, Tenants Harbor, ME 04860.

In order to maximize mussel production without adversely affecting their growth rates, it is necessary to estimate their optimum density in the areas used for their culture. This in turn depends on the availability and exchange of water over the mussel beds. A 2-dimensional, shallow-water, hydrodynamic computer model for simulating tidal flows is applied to Mud Cove (Stonington, Maine) and the Mount Desert Narrows (Mount Desert Island, Maine). The models are calibrated using field data and they provide (as output) a time record of surface elevations and depth-averaged water velocities. This information is then used in conjunction with known food densities and feeding rates to determine the proper seeding densities.

The numerical model provides an added degree of refinement for the determination of seeding densities compared to the simpler tidal exchange approach (Dyer, 1973). We find that the models are capable of describing the dominant flow characteristics, and that the analysis using the modelled velocities allows for detailed spatial description of seeding densities within the embayment. This

resolution is not possible with the tidal exchange method. The numerical models also provide convenient means for visualizing the complicated coastal flows. In addition, decreased data requirements make the modelling methods a cost-effective approach.

EFFECTS OF DIETARY ALGAL AND LIPID SUPPLEMENTS ON GONADAL AND LARVAL DEVELOPMENT OF *CRASSOSTREA GIGAS KUMAMOTO* (THUNBERG). **Anja M. Robinson,*** Department of Fisheries and Wildlife, Hatfield Marine Science Center, Oregon State University, Newport, OR 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 effect the 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.

ENHANCEMENT OF NATURAL SPAT SETTLEMENT IN THE SOFT-SHELL CLAM, *MYA ARENARIA*. **Shawn M. C. Robinson,** Department of Fisheries and Oceans, Scotia-Fundy Region, Biological Station, St. Andrews, NB, E0G 2X0, Canada.

The productivity of some clam-flats in southwestern New Brunswick is decreasing due to increased fishing effort. To combat this trend, an attempt was made (in conjunction with the local fishing industry) to alleviate some of the effects of fishing by increasing the natural spatfall on the flats in the hope of increasing the recruitment to the population. Three experimental plots (5 m × 5 m) with collectors and one control were set up. The plots were established in early July in 1990 and were sampled at regular intervals until the spring of 1992 using a new technique to separate spat from the sediment. Results indicated that the experimental treatments significantly enhanced the natural spatfall (up to 1,168/m^2) of *Mya arenaria* over the control plot (up to 522/m^2). Subsequent samples indicated the highest density plots had slightly slower growth rates than the control plots. Mortality rates for the 1990 cohort were similar for all plots until the summer months . . the mortality in the highest density plots increased dramat- This study demonstrated that enhancement of natural spatfall of soft-shell clam appears to be possible but, more work needs to be done on the subsequent survival of the juveniles.

ECONOMICS OF OYSTER (*CRASSOSTREA IREDALEI*) FARMING IN WESTERN VISAYAS, PHILIPPINES. **Giselle P. B. Samonte,* Wenresti G. Gallardo,** and **Reuel E. Tumaliuan,** Aquaculture Department, Southeast Asian Fisheries Development Center, P.O. Box 256, Iloilo City, Philippines 5000.

The slipper oyster, *Crassostrea iredalei,* is the most important commercial species of oyster being cultured in the Philippines. This paper describes the bottom, stake, rack hanging and raft hanging methods of farming oysters and evaluates each method in terms of economic efficiency. Data were collected from 72 oyster farmers in four provinces, namely Aklan, Capiz, Iloilo, and Negros Occidental from July to November 1991. The average oyster farm was 970 m^2. The stake method used bamboo and nipa petioles for cultch materials while empty oyster shells were substrate materials for the other three methods. Production was highest using the raft hanging method (41 kg/m^2), followed by the rack hanging method (22 kg/m^2), stake (14 kg/m^2), and lowest using the bottom method (11 kg/m^2). Operating costs for the bottom, stake, rack hanging and raft hanging methods were P1/m^2, P3/m^2, P15/m^2 and P40/m^2, respectively (US $1.00 = 27 Philippine Peso). Among the four culture methods, the rack hanging method was the most cost-efficient with net operating income of P34/m^2.

THE EFFECTS OF WATER TEMPERATURE ON THE SEXUAL DEVELOPMENT OF ADULT OLYMPIA OYSTERS, *OSTREA LURIDA*. **Jose M. Santos,* Sandra L. Downing,** and **Kenneth K. Chew,** University of Washington, School of Fisheries WH-10, Seattle, WA 98194.

Stocks of the native Washington State oyster, *Ostrea lurida* (Carpenter) are now so low that hatchery efforts will need to be utilized to better understand the gametogenesis, spawning, and alternate survivorship of spat. Thus a study was initiated to examine the broodstock conditioning in the hatchery under three temperature regimes (12, 18, and 21 C). Three hundred Olympia oysters were placed for conditioning under each regime for 3 months. Oysters were individually marked and samples taken at 15 days intervals during the first month, then weekly until the end of the experiment. At each sampling period, histological sections were taken from 10 oysters, for each treatment, and another batch of 10 were induced to spawn. The gender and number of oysters spawning were recorded, and fecundity estimated. *In vitro* fertilization was also attempted. In addition, live and dry meat weights were taken and condition indices determined. The gametogenic cycle for oysters at each temperature was established from the histological sections. Furthermore, parameters such as the maximum oocyte diameter (MOD), the percentage of gonadal area (PGA) occupied by follicles, and the percentage of follicle area occupied by gametes (PFA) were determined. This information

will help to improve the commercial hatchery production of this species.

TECHNIQUES TO DETERMINE GUT RETENTION TIME IN SUSPENSION FEEDING BIVALVES. **A. M. Scarratt,* B. A. MacDonald,** and **R. J. Thompson,** Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NF, Canada, A1C 5S7.

A new technique to determine the length of time organic and inorganic particles are retained within the bivalve gut has been developed using the soft-shell clam, *Mya arenaria*. A mixture of the green alga *Tetraselmis suecica*, known to contain a high proportion of chlorophyll *b*, and small inorganic silicon carbide particles (8–16 μm) were added to the diet of actively feeding clams for a 30 minute period. Samples of faeces were collected at regular intervals from clams exposed to an experimental diet and an initial "pulse" of the two markers. Presence of the organic marker in a series of sequential faecal samples was determined by the extraction of algal pigments and detection of the relative proportion of chlorophyll *b* using reverse phase HPLC. A peak of particles between 8 and 16 μm in diameter, detected with an electronic particle counter, in faeces indicated passage of the inorganic marker through the gut. These techniques may be useful in studies of physiological adaptations and feeding strategies in bivalves exposed to different environmental conditions.

CONFLICT ASSOCIATED WITH OYSTER AND MUSSEL FARMING IN WESTERN VISAYAS, PHILIPPINES. **Susana V. Siar,* Giselle P. B. Samonte, Wenresti G. Gallardo,** and **Rolando S. Ortega,** Aquaculture Department, Southeast Asian Fisheries Development Center, P.O. Box 256, Iloilo City, Philippines 5000.

This paper discusses an aspect of a bigger study on the economic and social analysis of oyster and mussel farming in Western Visayas. Slipper oyster (*Crassostrea iredalei*) and green mussel (*Perna viridis*) are the predominantly cultured bivalves in Western Visayas. There are approximately 2000 oyster and mussel farming households, majority of which are partially dependent on oyster and mussel farming for their livelihood. Oyster and mussel farms are usually found in bays and estuaries. Three types of conflict have been identified: (1) between oyster/mussel farmers and owners/operators of pumpboats; (2) between oyster/mussel farmers and fishpond operators whose fishponds usually surround oyster/mussel farms, and (3) among oyster farmers themselves who practice the bottom method. Starting January 1, 1992, municipalities have the exclusive authority of granting fishery privileges to erect fish corrals, oyster, mussel or other aquatic beds in the municipal waters (defined as 15 kilometers from the coastline) and impose rents, fees, or charges. In line with this, we recommend the following: (1) a review by the municipality of existing oyster/mussel farms; (2) a dialogue among the users of a bay or estuary to formulate rules and regulations, and (3) the setting up of farm size limits.

AN IMPROVED METHOD FOR MAPPING OYSTER BOTTOM USING A GLOBAL POSITIONING SYSTEM AND AN ACOUSTIC PROFILER. **James D. Simons** and **Eric N. Powell, Junggeun Song.** Dept. Oceanogr., Texas A&M Univ., College Station, TX 77843; **Thomas M. Soniat,** Dept. Biology, Nicholls State Univ., Thibodaux, LA 70310.

A method for rapidly and relatively inexpensively mapping oyster bottom is described. The method uses an acoustic profiler to differentiate substrate type, a fathometer to assess bottom relief and a global positioning system to accurately establish position. The method has the following desirable traits: use from a small research vessel, usable in most weather conditions, requires only a two-person crew, rapidly discriminates bottom type while underway, usable in shallow (<1 m) or deep (>10 m) water, accurate and precise navigation. The method has been used successfully to map the oyster reefs and oyster bottom of Galveston Bay, Texas, an area of approximately 1000 km^2.

COMMERCIAL TRIALS TO ASSESS THE GROWTH AND SURVIVAL OF REMOTE SET PACIFIC OYSTER (*CRASSOSTREA GIGAS*) LARVAE IN SHALLOW NURSERY PONDS. **Jeremy Thomas** and **Gavin Burnell,*** Aquaculture Development Centre, Zoology Department, University College Cork, Lee Maltings, Prospect Row, Cork, Ireland.

An increase in Irish Pacific oyster production from 100 tonnes in 1985 to 400 tonnes in 1990 has led to a 30% reduction in price for some growers. Since the purchase of hatchery seed is 40% of the production cost of a 70–100 g. oyster, remote settlement may help to reduce this figure. In three commercial scale (2–3 million per batch) experiments at Atlantic Shellfish Co. Cork, a settlement rate of 18% was achieved using mussel shell cultch and standard techniques. There was no significant difference in settlement between conditioned and unconditioned mussel cultch. After 3–5 days in the settlement tank the cultched spat were spread loose in nursery ponds (20 × 20 × 2 m) for 2–3 months and then transfered to oyster bags on trestles in the sea. Spat settled in June 1990 had reached 21 mm by January 1991 whereas those settled in August only achieved 8 mm. The estimated cost of producing spat by this technique was IR£3.40 per 1000 (2–10 mm) compared with IR£6 per 1000 for hatchery seed (4–5 m). The combination of cheaper remotely settled seed with the pond nursery give the grower the option of extensive ongrowing on the sea-bed rather than the current labour intensive bag-on-trestle techniques.

EFFECTS OF SUBSTRATE MODIFICATION ON NATURAL RECRUITMENT, GROWTH AND SURVIVAL OF HARDSHELL CLAMS IN WASHINGTON STATE. **D. R. Toba,*** 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; **K. K. Chew,** University of Washington, School of Fisheries WH-10, Seattle, WA 98195.

Over the past 15 years, the Washington Department of Fisheries (WDF) has constructed gravel plots on state beaches to enhance hardshell clam production. One site located on Hood Canal that was originally gravelled in 1983 is still well below the production goal of 10,000 pounds per acre. Gravel compaction and predation are believed to be two main causes of low clam productivity at this site. Two methods were used to alleviate gravel compaction, the existing gravel was tilled to a depth of 10 cm and a 5 cm layer of crushed oyster shell was tilled to 10 cm. In addition to reducing gravel compaction, crushed oyster shell is also thought to benefit natural recruitment and increase survival of newly settled clams. Predator netting was also used on half of each plot to limit the effects of predation.

Natural recruitment of Manila clams (*Venerupis japonica*) and native littleneck clams (*Protothaca staminea*) was low. Planted Manila clams were used on selected plots to test the effects of the substrate modification on their growth and survival. Clam growth was greater on the crushed oyster shell plots than the tilled only or control plots. Higher clam survival was recorded on netted plots. There was little difference between clam survival rates for the different treatments.

PROCEDURES FOR THE MAINTENANCE AND HATCHERY-CONDITIONING OF BIVALVE BROODSTOCKS. Susan D. Utting,* Ministry of Agriculture, Fisheries & Food, Directorate of Fisheries Research, Fisheries Laboratory, Benarth Road, Conwy, Gwynedd LL32 8UB, UK.

The hatchery production of seed bivalves began in the UK in the mid-1960s and most of the pioneering work was carried out at the Conwy Laboratory (Ministry of Agriculture, Fisheries & Food). Procedures for the maintenance and hatchery-conditioning of bivalve broodstocks have been developed at the Laboratory since that time and this paper is a review of those procedures. Introductions of non-indigenous bivalves into the UK for the shellfish industry are made through the Laboratory's quarantine facility. A range of indigenous and non-indigenous bivalve species are maintained on behalf of the industry at a site which is free from bivalve pests and diseases. Procedures for the hatchery-conditioning of broodstocks have centred on the quantity and quality of algae diets that are required to produce eggs and larvae with adequate food reserves to ensure good growth and survival. Diets include natural phytoplankton, a range of cultured algae species and spray-dried *Tetraselmis suecica* with particular reference being made to their polyunsaturated fatty acid content. Data from laboratory studies on the hatchery-conditioning of bivalve broodstocks, eg. *Ostrea edulis, Tapes philippinarum,* are of value for assessing gametogenesis of these species in the natural environment. Cryopreservation of eggs, embryos and larvae, as an alternative method for the long-term preservation of bivalve broodstocks, is under investigation.

SHELL FRAGILITY IN JUVENILE GEODUCKS (*PANOPEA ABRUPTA*) AND ITS IMPLICATIONS FOR THE GEODUCK ENHANCEMENT PROGRAM. D. E. Velasquez, University of Washington, School of Fisheries WH-10, Seattle, WA 98195.

Geoduck clams are being cultured so they may be planted subtidally to enhance recruitment to harvested populations in Puget Sound. Hatchery production is consistent; however problems have arisen in the nursery phase resulting in sporadic seed quality and survival. One problem is the tendency of the shells to break upon removal from the nursery, varying from 7–60% breakage depending on the nursery group and the harvesting method. The innate fragility of the shells of some nursery groups is primarily due to differences in shell thickness (between clams of equal shell lengths). Thin-shelled groups are less than half as thick as their thick-shelled counterparts. An experiment tested the performance of broken-shelled seed clams in a simulated planting. Populations of 100 seed clams were introduced into each of 6 chambers. Three populations possessed intact shells (IS) and three populations possessed broken shells (BS). Burial was monitored and exposure to crab predators (*Cancer productus*) followed. A staged excavation determined the number of surviving clams and their depth stations in the substrate. Results indicate the number of IS clams which avoided predation (63%) was significantly greater than BS clams (10%). Compared to the BS survivors, a greater proportion of IS survivors were found deeper in the substrate (76–152 mm and 152–228 mm depth stations). A strategy for reducing shell breakage is essential to increasing survival of planted juvenile geoduck.

LABORATORY STUDY OF REPRODUCTION IN *ARGOPECTEN CIRCULARIS*. Janzel R. Villalaz,* Centro de Ciencias del Mar y Limnología, Universidad de Panamá, Panamá.

A laboratory study was carried out in Delaware to observe changes in reproduction of *Argopecten circularis* by using relative dry weight in gonads, digestive gland, mantle-gill and adductor muscle. During 66 days, combinations of monocultures (50:50) of C-ISO and CH-1 were added daily to a tank with filtered and aerated seawater. Statistical analysis showed significant changes of water content in digestive gland, mantle-gill and gonad. No significant changes were observed of dry weights in digestive gland, adductor muscle, mantle-gill and gonad. This study is a contribution to the reproductive biology of *A. circularis* and mariculture of the tropical scallop.

GROWTH AND SURVIVAL OF EASTERN OYSTERS CULTURED IN AN EARTHEN POND. Richard K. Wallace,* Auburn University Marine Extension and Research Center, 4170 Commanders Drive, Mobile, AL 36615; **David B. Rouse,** Department of Fisheries and Allied Aquacultures, Auburn University, AL 36849.

In response to declining oyster production in the Gulf of Mexico region, the potential for growing oysters in ponds was evalu-

ated. Approximately 50,000 ($\bar{x}$ height = 5.6 mm) hatchery produced oysters set on microcultch (200–300 μm) were stocked into a 0.04 ha, fertilized pond equipped with an airlift system along a central divider. Oysters were placed in plastic mesh bags on racks at three levels and two densities. During the first 56 days standard density oysters grew more rapidly in height ($\bar{x}$ = 34 mm) than high density oysters ($\bar{x}$ = 28 mm). Thereafter, growth of both groups slowed and at the end of one year, standard density oysters were only slightly larger ($\bar{x}$ = 50 mm) than the high density oysters ($\bar{x}$ = 47 mm). Survival in the final grow-out bags was 74 percent and 65 percent respectively for standard and high density treatments. Standard density oysters on the top level of the rack were significantly ($p \leq 0.05$) larger ($\bar{x}$ = 53 mm) than the middle level ($\bar{x}$ = 47 mm) and the bottom level ($\bar{x}$ = 49 mm). Decline in growth rate after the first 56 days was associated with high water temperature (>30°C) and reduced phytoplankton (chlorophyll $a <$ 10 μg/l). Oysters nurseried in ponds, then moved to racks in Mobile Bay showed a similar growth pattern but more rapid growth in length and volume during the same period.

RECENT OCCURRENCE OF PARALYTIC SHELLFISH TOXINS IN OFFSHORE SHELLFISH IN THE NORTHEASTERN UNITED STATES. Alan W. White,[1] Julianne Nassif,[2] Sandra E. Shumway,[3,*] and David K. Whittaker,[2] [1]Northeast Fisheries Science Center, National Marine Fisheries Service, Woods Hole, MA, [2]State Laboratory Institute, Massachusetts Department of Public Health, Boston, MA, [3]Department of Marine Resources and Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME.

Since 1989, paralytic shellfish toxins have been detected in shellfish from the American sector of Georges Bank at levels exceeding the public health safety threshold, necessitating closure of shellfisheries in these offshore waters for the first time. Toxins reached maximum levels in shellfish during the summer of 1990, but have persisted at lower levels, still exceeding the safety threshold, in some species. Maximum toxin levels in whole animals, determined by mouse bioassay, were 6400 μg STX equiv · 100g tissue^{-1} for Atlantic surfclams (*Spisula solidissima*), 3900 μg STX equiv · 100g tissue^{-1} for sea scallops (*Placopecten magellanicus*), 3600 μg STX equiv · 100g tissue^{-1} for northern horsemussels (*Modiolus modiolus*), and 1200 μg STX equiv · 100g tissue^{-1} for ocean quahogs (*Arctica islandica*). High toxin levels have also been detected in carnivorous molluscs such as northern moonsnails (*Euspira heros*) and waved whelks (*Buccinum undatum*). In May and June 1990, eight fishermen were poisoned after eating blue mussels (*Mytilus edulis*) taken as bycatch from Georges Bank; mussels contained more than 24000 μg STX equiv · 100g tissue^{-1} before cooking. The source of the offshore toxins is presumed to be the dinoflagellate *Alexandrium tamarense*, although blooms of *A. tamarense* have not been confirmed in the Georges Bank area. The occurrence of high levels of toxins in offshore shellfish is apparently a new event.

VARIATION IN LEVELS OF PARALYTIC SHELLFISH TOXINS AMONG INDIVIDUAL SHELLFISH. Alan W. White,* Sandra E. Shumway, Julianne Nassif*** and David K. Whittaker,***** *Northeast Fisheries Science Center, NOAA National Marine Fisheries Service, Woods Hole, MA 02543; **Maine Department of Marine Resources and Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME 04575; ***State Laboratory Institute, Massachusetts Department of Public Health, Boston, MA 02130.

Monitoring studies of paralytic shellfish toxin levels in Georges Bank shellfish during 1990 and 1991 included 48 sets of assays of individual animals collected at six stations in the vicinity of Cultivator Shoal and Georges Shoal. At each station, 5 to 40 shellfish were taken at random from dredge hauls covering 200 to 700 m of ocean bottom. Mouse bioassay tests were conducted on individual, whole surfclams, ocean quahogs, moonsnails and sea scallops. Mean toxin levels of the data sets ranged from 71 to 3,356 μg/100 g. The coefficient of variation of the sets ranged from 19 to 99%. The overall mean coefficient of variation was 48.5%, which is consistent with the few reports of toxin levels in individuals of other shellfish species. There was a tendency for the degree of variation among individual surfclams to decrease as toxin levels increased. There was no significant correlation between toxin level and shell length of surfclams over the size range tested. Results indicate there are substantial differences in toxin levels among individual shellfish in the same area, emphasizing the need for including a large number of animals in composite samples.

TEMPORAL VARIABILITY IN FOOD AVAILABILITY TO NATURAL OYSTER POPULATIONS. Elizabeth A. Wilson* and **Eric N. Powell,** Dept. Oceanogr., Texas A&M Univ., College Station, TX 77843; **Sammy M. Ray,** Dept. Mar. Biol., Texas A&M Univ. at Galveston, Galveston, TX 77550.

Productivity in oyster populations is partially determined by food availability, which is dependent on the amount of food resources in the water and characteristics of the water flow making that food available to the population. Micro-current meters were used to measure flow at locations important to oysters, such as 1 to 3 cm above the bottom and at the surface. Flow measurements were made every 3 min for 24 hr, monthly, for 1 yr at Confederate Reef (Galveston Bay, Texas). Water was sampled every 3 hr to determine the amount of food resources available (particulate organic carbon and nitrogen, chlorophyll a and total seston). Fluxes of food (food cm^{-2}) were calculated and indicate high variability in available food on short temporal scales, depending on time of day and season. Important differences in food resources exist throughout the water column in x − y as well as z space. These results indicate that the flux of food, rather than filtration rate, limits oyster growth and reproduction in natural populations under certain water flow conditions and food resource availabilities. A computer model of oyster energetics was used to assess the magnitude of this restriction within x − y − z space on Confederate Reef.

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THE EARLY LIFE HISTORY OF ZEBRA MUSSELS: OVERWINTERING JUVENILES AND POST-SETTLEMENT MOVEMENTS. **Joseph Daniel Ackerman**[1] and **Renata Claudi,**[2] [1]Department of Mechanical Engineering, University of Toronto, Toronto, Ontario, Canada M5S 1A4, [2]Ontario Hydro.

Currently, there is no generally accepted nomenclature for the larval and post-larval stages of *Dreissena polymorpha*. This has led to confusion in the enumeration and reporting of zebra mussel occurrence and abundance. Morphological and behavioral observations of *Dreissena* larval and post-larval stages are, however, similar to marine bivalves. Based on these conserved traits, we have chosen to extend standard life history descriptions (e.g., Carriker 1961) to *Dreissena*.

The pediveliger and plantigrade are considered the most relevant planktonic post-larval stages from the perspective of mussel settlement. It is in the pediveliger stage that mussels are found swimming in plankton and crawling on hard substrates. Movement can occur between these two modes of existence until byssal attachments (primary settlement) are achieved in the plantigrade stage. Plantigrade post-larvae are not entirely sedentary and active movements occur on hard surfaces and in the water column following initial attachment (secondary settlement).

Post-larval zebra mussels can overwinter on hard surfaces and were collected during February and April of 1991 in Lake Erie. Moreover, pediveligers and plantigrades were detected in the plankton and on freshly developed substrates in the spring of 1991. There is reason to believe that these observations represented the movement of post-larvae from overwintering populations rather than the spawn of the 1991. This complexity in life history has implications for the understanding and control of zebra mussels.

RECRUITMENT AND ADHESION OF ZEBRA MUSSELS ON A VARIETY OF MATERIALS. **J. D. Ackerman, C. R. Ethier, D. G. Allen,** and **J. K. Spelt,** Department of Mechanical Engineering, University of Toronto, Toronto, Ontario, Canada M5S 1A4.

The recruitment of zebra mussels was monitored in Lake Erie (Nanticoke, Ontario) during the summer of 1991. Weekly photographic and visual surveys were conducted of 8 × 16 cm coupons made from copper, copper-nickel alloys, stainless steel, mild steel, aluminum, teflon, plexiglass, polyvinylchloride, marine plywood or concrete. Measurements of adhesion strength of the zebra mussels adhering to these coupons were undertaken using a wall jet device.

The first mussel recruitment occurred at the end of June and by the middle of July there was up to one mussel per square cm on some of the materials. At that time, three classes of materials (based on levels of recruitment) were identified: (I) materials with little or no mussel recruitment (copper and copper nickel alloys), (II) materials with some recruitment (<0.5 mussels per square cm; aluminum and concrete), and (III) materials with moderate levels of recruitment (>0.5 mussels per square cm; polyvinylchloride, stainless steel, plexiglass, and plywood). An increase in mussel densities was noted on most materials with time, including those from Category (I). For come materials, large increases were observed (e.g., concrete, mild steel, plexiglass). Generally, there were more mussels on roughened surfaces than smooth ones, although there were exceptions to this generalization.

Initially, the mussels were dispersed over the entire coupon. However, with time the mussels clumped and tended toward the edge of the coupons. In late July, the coupons were overgrown by an algal mat. Although the alga was several mm thick, it did not appear to affect the mussels.

The wall jet device produces a jet of water with well-defined fluid dynamic characteristics. The jet is used to detach mussels such that the adhesion force is related to the shear stress generated by the jet. Preliminary results indicate that it requires between 60 and 70 Pa of nominal wall shear stress to remove mussels from stainless steel and polyvinylchloride coupons. These results confirm our previous adhesion strength observations made with a rotating disk. Additional measurements are currently under way.

FACTOR INFLUENCING *DREISSENA* RECRUITMENT AND BIOMASS ACCUMULATION ON AN ARTIFICIAL SUBSTRATUM. **John F. Bailey** and **R. Douglas Hunter,** Department of Biological Sciences, Oakland University, Rochestor, MI 48309-4401.

Studies on *Dreissena* recruitment were done in 1990 at two locations in Lake St. Clair and one in northwestern Lake Erie. Artificial substrata consisting of 11 × 11 cm ceramic tiles were installed on racks at each site and subsequently examined according to one of two treatment protocols. One treatment "settlement rate tiles" (SETT), involved replacement of existing tiles every two weeks. The intent was to measure recruitment of larvae onto tile surfaces without the presence of settled adults. A second treatment, "cumulative tiles" (CUM), involved biweekly removal, measurement, and return of the same tiles to their site, providing an accumulating population of juveniles and adults over the entire summer. Each treatment had three replicates.

No settlement occurred on any of the tiles at the CR site (Clinton River, Lake St. Clair, Michigan) although a few individuals were observed on nearby surfaces. Settlement at the PM site (Pt. Mouillee, Lake Erie, Michigan) was very light with peak densities on CUM tiles reaching a maximum of only 337/m^2 and settlement averaging about 5/m^2 day. In contrast, settlement at the TR site (Thames River, Lake St. Clair, Ontario) was heavy, with a maximum density of 354,035/m^2 and settlement rates averaging 5,058/m^2 day. The SETT results at TR indicated one major recruitment on about July 10–24, and a lesser one on about September 4–17. Light recruitment occurred at other times beginning June

26. The last recorded recruitment was on November 13, after which the experiment was terminated.

The CUM tiles at TR showed a similar recruitment pattern to the SETT tiles but had densities that were about four times greater. This difference is likely due to the SETT tiles not having been aged (i.e., they were initially devoid of periphyton) hence they were less preferred substrata. A further difference was the growing presence of larger-sized mussels on the CUM tiles, which not only increases the surface area but provides optimal settling surfaces.

Evidence that tile removal, handling, and replacement is detrimental to growth was obtained from a subset of CUM tiles (CUM6) at TR which were left *in situ* for ten weeks before sampling (i.e., undisturbed from June 12 to August 21). These CUM6 tiles had *Dreissena* densities that were between one and two orders of magnitude higher than those on the biweekly sampled CUM tiles. For example, on September 4, the CUM tiles averaged 1,822/m^2 whereas the CUM6 tiles averaged 242,265/m^2. Lack of disturbance had positive consequences for recruitment as well.

Data from 1991 tiles indicate a major recruitment occurred around June 10–24, resulting in densities averaging 239,000/m^2, and consisting mostly of spat less than 1 mm. Based on density distribution of these newly-settled spat, a variety of natural surfaces (stone, bivalve shells, zebra mussel shells, etc.) are preferred surfaces compared to tile. It appears not to matter whether *Dreissena* shells on which the spat settle are live or empty.

PRELIMINARY INVESTIGATION OF THE SALINITY TOLERANCE OF ZEBRA MUSSELS, *DREISSENA POLYMORPHA*: IMPLICATIONS FOR CHESAPEAKE BAY. Bruce J. Barber, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

The potential for zebra mussels, *Dreissena polymorpha*, invading subestuaries of Chesapeake Bay was examined by exposing individuals to gradually increasing salinity and recording growth rate and cumulative mortality. Both experimental (in which salinity was gradually increased from 0 to 2.7 ppt) and control (in which salinity was maintained at 0 ppt) aquaria were constantly aerated at 15°C. One third of the water volume of each aquarium was changed every other day. Mussels were fed a daily ration of algal paste resuspended in deionized water. Cumulative mortality of both medium (7–16 mm) and large (19–22 mm) mussels in the experimental aquarium was 100% within 52 days while cumulative mortality of both medium and large mussels in the control aquarium was about 30%. Growth of medium mussels in both experimental and control aquaria was not observed in the first 38 days of the experiment. Cumulative mortality of *D. polymorpha* increased dramatically in the experimental aquarium after day 30 when salinity exceeded 1.5 ppt. Although many questions remain unanswered, *D. polymorpha* could potentially become resident in upper reaches of Chesapeake Bay.

SURVEILLANCE OF ZEBRA MUSSEL (*DREISSENA POLYMORPHA*), LARVAL DENSITIES, SETTLING, AND GROWTH AT A POWER PLANT ON WESTERN LAKE ERIE. Joseph R. Bidwell,[1] L. A. Lyons,[2] D. S. Cherry,[1] and **J. C. Petrille,[2]** [1]Department of Biology, Virginia Tech, Blacksburg, VA 24061, [2]Betz Laboratories, Inc.

Monitoring of zebra mussel densities, settling, and growth was initiated in June 1991 in the forebay of a power plant located on western Lake Erie. Densities of veligers were determined each week in water samples collected from depths of 2 and 4 m, while settled larvae were counted weekly on glass microscope slides which were suspended near the plant intake. Cement panels (10 cm × 20 cm) were also suspended in the forebay to further assess mussel accumulation and growth. Maximal veliger densities were observed in July, reaching as high as 530/L (530,000/m^3) on one sampling date. Veliger densities appeared to oscillate with temperature through July, with significant drops noted on two occasions when water temperatures approached 30°C. Larval settling reached 277 mussels/slide/week (74,000/m^2). When slides which were suspended on 6 July were examined after 30 days, the majority of those mussels greater than 1 mm in length fell into a 2 to 5 mm size range, with densities of 188/slide (50,000/m^2). Larger (>10 mm), transient mussels were also occasionally found on these slides. Cement panels from the forebay were completely covered with a layer of mussels by day 40. By day 60, the majority of these mussels ranged between 5 and 11 mm in length. A 24 cm^2 subsample from these panels contained 8.79 mussels/cm^2 (87,900/m^2) with a wet weight biomass of 1 gm/cm^2 (10 kg/m^2). Based on the data collected to date, July appears to have been the peak period in terms of veliger densities and rate of mussel accumulation on monitoring substrates at this field site.

EFFECT OF INTERMITTENT CHLORINE AND BROMINE TREATMENTS ON SETTLING, SURVIVAL, AND GROWTH OF THE ZEBRA MUSSEL, *DREISSENA POLYMORPHA*. Joseph R. Bidwell,[1] L. A. Lyons,[2] D. S. Cherry,[1] J. C. Petrille,[2] and **M. W. Werner,[2]** [1]Department of Biology, Virginia Tech, Blacksburg, VA 24061, [2]Betz Laboratories, Inc.

The effect of daily 2-hr treatments with chlorine (0.5 mg/L and 1.0 mg/L TRO) or bromine (0.5 mg/L TRO) upon growth and survival of two size classes (6–8 mm and 12–14 mm in length) of zebra mussels was assessed in a 30-day study which ran from early July to August 1991. The study was conducted in a field laboratory which had been fitted with 12 side stream loops, each receiving a continuous flow of Lake Erie water. Each loop contained a rack of glass slides and included a fouling chamber fitted with a cement panel to examine the impact of these treatments on larval settling and accumulation. Survival of both size classes of mussels was greater than 85% in all treatments. Mussels in all treatments also exhibited positive growth over the 30 days. Larger mussels increased in length by an average of 4.4 mm, while smaller mussels

increased by 6.8 mm in length. Treatments of 0.5 mg/L chlorine and bromine did not have a significant effect upon weekly larval settling rates as compared to controls, with all groups ranging between 50 and 70 larvae/slide/week. Daily 2-hr treatments with 1.0 mg/L chlorine significantly reduced larval settling to 10 to 25 mussels/slide/week. Accumulations of larger possibly transient mussels were also observed in all treatment loops. A second study which repeats the daily 2-hr 0.5 mg/L and 1.0 mg/L TRO chlorine treatments, and also includes a 2-hr 0.5 mg/L and 1.0 mg/L bromine and 4-hr 0.5 mg/L chlorine was initiated in August to further examine the efficacy of these treatments in controlling zebra mussel fouling.

COMPARATIVE GROWTH AND MORTALITY RATES OF *DREISSENA POLYMORPHA* FROM TWO SITES IN LAKE ST. CLAIR. **Angela M. Bitterman,[1] Robert C. Haas,[2]** and **R. Douglas Hunter,[1]** [1]Department of Biological Sciences, Oakland University, 2463 Otter, Warren, MI 48092, [2]Michigan Department of Natural Resources.

We measured growth and mortality rates of zebra mussels, *Dreissena polymorpha,* at two sites in Lake St. Clair during June, 1990 to May, 1991, to look for factors which might influence their success in colonizing the Great Lakes. Mussels were held in individual compartments in Plexiglass cages which were suspended one meter above the bottom. Growth measurements were taken on half of the cages at biweekly intervals, whereas the other half were measured only at the beginning and end of the 11 month period. Length, width, height, and weight were determined for each mussel for each sampling period. Nutrient conditions were assessed at cage sites by measurement of temperature, secchi depth, suspended organic carbon, and density of planktonic algae.

There were not measurable growth rate differences initially at the two sites (t-test; $P > 0.05$). However, the relatively small compartment size (13 mm radius, 10 mm thick) restricted growth after 10 weeks (mean length 12.8 mm). Lengths of individuals of widely different size at the start converged by the 12th week. Growth in width, height, and weight were not as constrained by compartment size indicating that these zebra mussels had compensated for limited growth in length. After being transferred to larger-holed cages, the mussels at the Clinton site responded faster to the change than the mussels at the Thames site (t-test; $P < 0.05$).

Zebra mussels were well established in the vicinity of the Thames River site; however, they were essentially absent in the vicinity of the Clinton River site. Thames River cages were continually being invaded by post-veliger migration and veliger settlement with as many as 10 additional mussels present in one compartment. Almost all compartments had at least one additional mussel. The cage size did inhibit the length of the zebra mussel. The mussel transferred to the larger-holed cages grew significantly greater at both sites than the mussels kept in the small-holed cages (t-test; $P < 0.001$).

Mortality of caged zebra mussels was not related to site location or growth conditions but was increased by greater sampling frequency (Binomial Test; $P < 0.014$). Mortality of mussels sampled only twice was 21% at the Clinton site and 13% at the Thames site. Mortality for the frequently sampled mussels was 45% at the Clinton site and 31% at the Thames site.

Physical, chemical, and nutrient sampling indicated that growth conditions differed between the two sites. Phytoplankton samples at the Thames site had an average of 99 cells/ml for greens and 586 cells/ml for diatoms. The Clinton site had 97 cells/ml for greens and 337 cells/ml for diatoms. The greens were not significantly different between the two sites but the diatoms were. Temperature increased and decreased at the same time for both sites. Secchi depths were greater at the Clinton.

ZEBRA MUSSEL INVASION OF THE UPPER MISSISSIPPI RIVER SYSTEM. **K. Douglas Blodgett,[1] Pamella A. Thiel,[2] Andrew C. Miller,[3]** and **Richard E. Sparks,[1]** [1]Illinois Natural History Survey and Long Term Resource Monitoring Program, P.O. Box 546, Havana, IL 62644, [2]U.S. Fish and Wildlife Service, Environmental Management Technical Center, [3]U.S. Army Corps of Engineers, Waterways Experiment Station.

The rapid expansion of the recently introduced zebra mussel (*Dreissena polymorpha*) forewarns of significant negative impacts on both the biology and economy of the Upper Mississippi River System (UMRS)—a resource the United States Congress has declared to be nationally significant both as an ecosystem and a commercial navigation corridor.

Zebra mussel populations developing in southern Lake Michigan provide a probable source for recent expansion of the species into the UMRS. Natural dispersal of larvae may occur with water diverted from Lake Michigan down the Illinois River (via the Chicago River, the Chicago Sanitary and Ship Canal, and the Des Plaines River) and into the Mississippi River. Both settled larvae and adults may be transported by recreational craft and commercial navigation vessels (barges) emanating from infested areas of southern Lake Michigan and traversing both the UMRS and the lower Mississippi River. Another possible means of dispersal is trailered recreational boats that transport larvae and adults overland to both lotic and lentic habitats in the Mississippi River Basin.

The first confirmed collection of a zebra mussel from the UMRS occurred on 18 June 1991 in a side channel of the Illinois River near Bath, IL, approximately 217 river miles (350 km) downstream from Lake Michigan and 110 river miles (117 km) upstream from the confluence of the Illinois and Mississippi rivers. The adult specimen was attached to a native threeridge mussel (*Amblema plicata*) and was taken by a commercial sheller. Additional adult specimens have been collected from the Illinois River by shellers and others, and settled larvae are reported as far as 118 river miles (190 km) downstream from Lake Michigan. In September, adults were reported from the Mississippi as far upstream

as Pool 8 near La Crosse, WI, and as far downstream as the Melvin Price Locks and Dam at Alton, IL.

Negative biological impacts may result from the zebra mussel's competition for resources, especially food and space. These biological impacts may also manifest themselves economically by affecting commercial and recreational fishing and the harvest of native mussel shells for use in the cultured pearl industry. Other negative economic impacts may include mechanical interference at locks and dams, clogging of municipal and industrial water intakes and systems, and damage to engine cooling systems of recreational and commercial vessels as well as decreased fuel efficiencies due to increased drag.

In conjunction with six Long Term Resource Monitoring Program field stations on the UMRS, we have initiated a baseline monitoring effort for zebra mussels. Our major monitoring and research objectives are to 1) document the temporal and spatial distributions of zebra mussels in the UMRS; 2) understand their life history in midwestern, floodplain rivers; and 3) ascertain and interpret any shifts in community structure of native biota (plankton, macroinvertebrates, and fishes) resulting from the physical and biological alterations the invader may cause. A thorough understanding of zebra mussels and their impacts will provide information to facilitate the development of environmentally sound control strategies.

UPTAKE AND DEPURATION OF HEXACHLORBENZENE, 3,3′,4,4′-TETRACHLORO- AND 3,3′,4,4′,5,5′-HEXACHLOROBIPHENYL BY ZEBRA MUSSELS. Gottfried Brieger[1] and **R. Douglas Hunter,**[2] [1]Department of Chemistry, [2]Department of Biological Sciences, Oakland University, Rochester, MI 48309-4401.

Zebra mussels from two western Lake Erie sites, one near Monroe and one near Luna Pier, were analyzed for specific contaminants. Initial levels of PCBs representative of Aroclor 1242 were found at levels of 121 ng/g wet tissue and Aroclor 1256 at 33 ng/g at the Monroe site and 157 ng/g 1242 and 184 ng/g 1254 at the Luna Pier site. The water content of the test animals averaged 86%; lipids averaged 1.1%. Analysis of material from other Great Lakes field sites is in progress.

Zebra mussels were exposed in the laboratory to *Chlorella* food separately containing 500 ppm of hexachlorobenzene, 3,3′,4,4′-tetrachloro-, and 3,3′,4,4′,5,5′-hexachlorobiphenyl. Within 10–13 days, the concentration of HCB rose from an initial level of 1.3 μg/g wet tissue to 7.5 μg/g, then leveled off to approximately 2 μg/g. The hexachlorobiphenyl levels rose from undetectable initial levels to 5 μg/g, then gradually leveled off to approximately 1 μg/g. The tetrachlorobiphenyl concentration rose from undetectable initial levels to approximately 3.5 μg/g, then leveled off around 2 μg/g. These studies will be repeated at lower feed levels. Levels of selected other chlorinated hydrocarbons were also de-

BIOACCUMULATION OF HYDROPHOBIC CONTAMINANTS BY THE ZEBRA MUSSEL, *DREISSENA POLYMORPHA*. K. A. Bruner, Susan W. Fisher, and **P. F. Landrum,** Department of Zoology, The Ohio State University, 1735 Neil Avenue, Columbus, OH 43223.

The prodigious filtering capacity of the zebra mussels, its large population numbers and its preference for littoral areas and outfalls which may have high toxicant loads, makes it likely that the mussels will be exposed to hydrophobic xenobiotics such as PCBs and PAHs. In addition, the mussels have a relatively high lipid content which facilitates partitioning of dissolved contaminants and assimilation of sorbed materials into the zebra mussel. Storage of PCBs and PAHs in zebra mussel tissues may be one significant way in which mussels can alter contaminant cycling in the Great Lakes. In this study, the ability of zebra mussels to accumulate highly lipophilic compounds through several routes of exposure was assessed using toxicokinetic models. In addition, the effect of mussel size and lipid content on accumulation was measured. We found that adult zebra mussels can accumulate significant amounts of PCBs and PAHs from contaminated water and seston. The accumulation of compounds from water was rapid with significant uptake of the chemicals occurring during the uptake clearance phase of the experiments. In contrast, elimination of these materials was comparatively slow although the presence of an organic sorbent in the guts of the animals did facilitate elimination. BAF values determined in this study indicate that zebra mussels can be expected to accumulate significant levels of these contaminants. The implications of these findings for contaminants cycling in the Great Lakes will be discussed.

PUMPING RATE CAPACITIES OF JUVENILE GREAT LAKES *DREISSENA POLYMORPHA* (PALLAS). Christopher Bunt, Hugh J. MacIsaac, and **W. Gary Sprules,** Department of Zoology, University of Toronto, 3368 Victory Cr., Mississauga, Ontario, Canada L4T 1L9.

Zebra mussel (*Dreissena polymorpha*) filtering activities may have a profound impact on water clarity, phytoplankton density and food availability to young, commercially important fish (e.g. perch and walleye). In order to predict these impacts, it is important to know the amount of water that the mussel population is capable of processing, thus diverting the flow of energy from pelagic to benthic food webs. While clearance rates are known for individuals > 10 mm, these mussels only compose between 5 and 21% of settled populations in western Lake Erie. Pumping rates of individuals between 2 and 11 mm were determined by injecting an inert dye into the inhalant flow of filtering mussels and measuring the velocity of the exhalant flow with high resolution video. Exhalant siphon dimensions were measured in conjunction with flow velocities to determine pumping rates. Preliminary data indicate that individuals between 2 and 11 mm are able to pump between 0.06 and 19 ml/h. At these rates, the juvenile zebra mussel populations in western Lake Erie (mean depth = 7 m) are capable of

processing between 5.2 $m^3/m^2/d$ and 10.5 $m^3/m^2/d$ (i.e. the entire water column may be processed 1.5–3.6 times each day by these individuals). Comparisons with independently measured clearance rates using microspheres were used to estimate gill retention efficiency of entrained particles.

DISPERSAL MECHANISMS OF THE ZEBRA MUSSEL. James T. Carlton, Maritime Studies Program, Williams College, Mystic, CT 06355.

More than 20 dispersal mechanisms can transport zebra mussels overland, upstream, and downstream. Dispersal mechanisms may be natural (planktonic dispersal on currents; adult dispersal on other animals or on driftwood) and human-mediated (ranging from boats and bait buckets to amphibious planes and pet turtles). No quantitative data are available in Europe or North America for any dispersal mechanism. Control programs are thus based entirely upon certain assumptions and empirical understandings. The existence of these diverse mechanisms further renders difficult the estimation of the rate of dispersal based only upon current speeds. Significant jumps are predictable. Our two-year research program seeks to quantify selected dispersal mechanisms both through direct observation and through experimental studies specifically designed to test putative vectors.

EFFECT OF ULTRAVIOLET-B RADIATION (280–320 NM) ON SURVIVORSHIP OF ZEBRA MUSSEL LARVAE (*DREISSENA POLYMORPHA*): A POTENTIAL CONTROL STRATEGY. Linda Chalker-Scott, J. D. Scott, C. Dunning, and **K. Smith,** Buffalo State College, 1300 Elmwood Avenue, Buffalo, NY 14222.

Concern over depletion of the earth's ozone layer has resulted in a great deal of information being gathered on the effects of ultraviolet-B (UV-B, 280–320 nm) upon planktonic organisms. There is increasing evidence that naturally occurring UV-B is an important limiting ecological factor in zooplankton communities. In a natural habitat, *Dreissena polymorpha* larvae and other planktonic organisms could escape harmful radiation by changing their position in the water column. In a water intake pipe, however, the area is much restricted and escape from artificially-supplied ultraviolet radiation would be unlikely.

The critical point in controlling zebra mussel infestation is the settling of the planktonic larvae. When postveliger larvae settle, they secrete strong byssal threads which firmly attach the mussels to the substrate. Once settled, mussels are difficult and expensive to remove from intake pipes and other structures. Prevention of settling would be the easiest and most cost-effective mechanism to remediate zebra mussel fouling problems.

One possible control method, which apparently has not been tested, is exposure of the larvae to ultraviolet-B radiation. UV-B is a naturally occurring, water-transmissable radiation that can have profound effects on aquatic organisms. The use of ultraviolet-B radiation as a control for zebra mussel settlement would have several advantages over other methods. It is not a potential water pollutant as are chemical treatments such as chlorine, it is not labor intensive as are most mechanical methods, and it does not require costly cooling systems as does heat treatment. Additionally, this method retools existing technology and should therefore be much cheaper to implement than would a method developed "from scratch."

Veliger larvae of the zebra mussel, *Dreissena polymorpha*, were collected from Lake Erie and maintained in lake water under natural temperature (20°C) and day length (14 hr light/10 hr dark) conditions. Following acclimation, larvae were transferred to the experimental chamber and subjected to enhanced UV-B radiation. To control the wavelengths of exposure, Mylar and cellulose acetate filters were employed for experimental and control organisms, respectively. Larvae were maintained in lab and analyzed 24 hours post-exposure for mortality. Surviving larvae were defined as those whose velum was still functioning (i.e., are capable of swimming).

Recent experiments indicate that zebra mussel larvae are negatively affected by enhanced UV-B radiation. There is an increase in mortality above control levels after only 1 hour of exposure, which suggests that mid-range ultraviolet radiation may be effective in reducing larval populations.

DEPTH DISTRIBUTION OF COLONIZATION OF ARTIFICIAL SUBSTRATES IN HAMILTON HARBOUR AND LAKE ONTARIO. Murray N. Charlton, National Water Research Institute, Environment Canada, P.O. Box 5050, Burlington, Ontario, Canada L7R 4A6.

In stratified lakes, the vertical distribution of *Dreissena* should be related to depth and temperature due to the requirement for moderately warm water for spawning. If mussel colonization is strongly reduced in deep cold water, problems with water intakes may be avoided. To test this hypothesis artificial substrates were placed in Hamilton Harbour and Lake Ontario. Clear results have been obtained from Hamilton Harbour where there is a summer oxygen depletion in the hypolimnion. Results from both locations related to temperature, oxygen, and turbidity profiles will be presented.

ULTRASTRUCTURAL CHARACTERISTICS OF THE DEVELOPING AND OVULATED OOCYTES OF THE ZEBRA MUSSEL, *DREISSENA POLYMORPHA*. David Bruce Conn, Soo-Jin Lee, and **Kimberly A. Shoen,** Department of Biology, St. Lawrence University, Canton, NY 13617.

The explosive population growth and widespread dissemination of the zebra mussel, *Dreissena polymorpha*, in the Laurentian Great Lakes system is partly a result of its very high reproductive potential. Despite the obvious importance of this reproductive potential, virtually nothing is known about the cellular and subcellular aspects of reproduction in *D. polymorpha*. The present study was undertaken to elucidate the basic ultrastructural features of the oocytes in this species.

Adult female *D. polymorpha* were collected in July, 1991 from a well-established population in the St. Lawrence River near Massena, New York. Within 5 minutes of collection, each mussel was dissected and its visceral mass removed and processed for light and transmission electron microscopy.

Oocytes concurrently occupying each gravid ovarian acinus were divisible into two basic groups: developing (preovulated) oocytes which were closely attached to the acinar epithelium, and ovulated oocytes that were free in the acinar lumen. All oocytes contained numerous free ribosomes and mitochondria, heterogeneous membrane-limited vesicles, scant granular endoplasmic reticulum (GER), and a few myelin figures. Each possessed a single nucleus with a prominent nucleolus, abundant closely-spaced nuclear pores, and little heterochromatin. The plasma membrane of each oocyte was folded into a dense brush border consisting of numerous cylindrical microvilli of uniform length and diameter supporting a prominent glycocalyx. Homogeneous membrane-limited cortical granules occupied the cortex of each oocyte. Ovulated oocytes differed from developing oocytes in having cytoplasm of lower electron density, cortical granules of higher electron density, fewer free ribosomes, more dilated GER cisternae, and a more spherical nucleus. Between the oocytes, each acinus was completely filled by a somewhat granular, moderately electron-dense material.

The presence of a large nucleus with large nucleolus and abundant nuclear pores suggests a high level of maternal RNA transcription and ribosome production with export to the cytoplasm. Protein synthesis resulting in numerous cytoplasmic granules and vesicles may be associated with autosynthetic vitellogenesis and/or cortical granule production; the latter may be a provision for polyspermy prevention. The close physical association between developing oocytes and ovarian acinar cells may suggest a mechanism for heterosynthetic vitellogenesis as described previously for other mollusc species. The well-developed glycocalyx associated with oocyte microvilli may play an important role in gamete recognition and fertilization.

These data provide vital baseline information for understanding the process of oogenesis in *D. polymorpha*. This information will be crucial to our understanding of many aspects of reproduction in this species, and might prove useful in the development and evaluation of new technologies for control based on interference with reproductive output.

This study was supported by grants from the Pew Charitable Trusts and St. Lawrence University.

THE SPREAD OF THE ZEBRA MUSSEL, *DREISSENA POLYMORPHA*, IN THE ST. LAWRENCE RIVER, AND ITS POTENTIAL INTERACTIONS WITH NATIVE BENTHIC BIOTA. David Bruce Conn, Kimberly A. Shoen, and Soo-Jin Lee, Department of Biology, St. Lawrence University, Canton, NY 13617.

Beginning in October, 1989, zebra mussels, *Dreissena polymorpha*, were discovered attached to various substrates in and around the Snell Lock of the St. Lawrence Seaway at Massena, New York. Subsequent studies revealed no mussels on substrates examined throughout the approximately 160-kilometer stretch of the St. Lawrence River upstream from the locks at Massena, while substrates downstream from that site harbored many mussels. These results suggested that the zebra mussel colony near the locks at Massena did not become established from natural dispersal downstream from Lake Ontario, but more likely from ballast being dumped into the river by a ship going through the locks. The present study was designed to monitor the movement of zebra mussels into the section of the St. Lawrence River upstream from Massena, and to gather baseline data on benthic macroinvertebrates that might be affected by this invasion.

Between September, 1990 and August, 1991, benthic macroinvertebrates were collected from 60 sites along the upper 180 km of the St. Lawrence River. Collections were made by hand from both hard and soft bottoms and from aquatic macrophytes, mostly in shallow nearshore waters. Other collections were made from navigational buoys and their anchors in the river's channel, and by SCUBA dives in deeper waters. Plankton samples were taken from most of the sites using a 63 mm mesh plankton net.

A few newly settled zebra mussels were found attached to rocks at a site approximately 1 mile upstream from Massena. No newly settled or adult zebra mussels were found at any site farther upstream, despite the fact that other researchers reported isolated colonies at Cape Vincent, New York (at the river's outflow from Lake Ontario) and Prescott, Ontario (halfway between Lake Ontario and Massena). No veligers were found in plankton samples taken upstream from Massena.

Native molluscs collected included 26 snail species (Gastropoda), and numerous unionid and sphaeriid clams (Pelecypoda). Unionid clams near the Massena site were frequently covered on their posterior (siphonal) ends with zebra mussels; many heavily covered unionids were dead. Insects collected represented 9 orders and 27 families. The number of families of each order were: 7 Trichoptera (caddisflies), 5 Coleoptera (beetles), 4 Hemiptera (true bugs), 4 Ephemeroptera (mayflies), 3 Odonata (dragonflies & damselflies), 1 Megaloptera (dobsonflies), 1 Diptera (true flies), 1 Hymenoptera (wasps), and 1 Lepidoptera (moths). Amphipod crustaceans were abundant, but isopods, crayfishes, sponges, bryozoans, *Hydra* and flatworms generally occurred in smaller numbers. The most abundant animals were caddisflies of the families Brachycentridae and Hydropsychidae. These insects require clean flowing water for feeding, and hard substrates for attachment. Because these requirements closely parallel those of *D. polymorpha*, strong competition may result as the latter spreads through the river. The results of such competition might have a negative impact on sport fishes that feed heavily on the insects.

This study was supported by grants from the Pew Charitable Trusts and St. Lawrence University.

ZEBRA MUSSEL SPAWNING INDUCED BY SEROTONIN: VIDEO RECORDING OF SPERM AND EGG EJECTION AND SUBSEQUENT FERTILIZATION AND DEVELOPMENT. Gary W. Crawford, Jon J. Mojares, James U. Walker, and **Jeffrey L. Ram,** Department of Physiology, Wayne State University, 540 East Canfield, Detroit, MI 48201.

As reported elsewhere (Ram et al., this volume of abstracts) spawning of both male and female zebra mussels can be induced by external application of 10^{-3} M serotonin (5-HT). Video recording illustrates several features of the induced behavior and resultant gametes. Within 15 min of 5-HT application to males, sperm began to appear in a narrow "jet" from the excurrent siphon. This well-defined stream extended at least 15 mm from the spawning animal (the limit of the small vials used). Animals continued to expel sperm for an hour or more, in some cases producing enough sperm to obscure the spawning animal in the 10 ml total volume in which experiments were done. Similarly, eggs appeared in the excurrent stream of females within one and a half hr of 5-HT application. In some females, eggs were expelled with larger particulates, resembling gonadal fragments of ovarian tissue. Although valve closures and openings were repeatedly observed in spawning animals, this behavior does not appear to be directly involved in expulsion of gametes, since gametes were continuously ejected while valves were apart and siphons open. In the small vials in which experiments were done, a portion of the discharged eggs and sperm were drawn into the incurrent siphon, a phenomenon which may account for con-specific offspring suppression by zebra mussels, as described by MacIsaac & Sprules (1st Int. Zeb. Mus. Res. Conf., 1990). Projection of gametes to a distance from spawning animals in the observed jet streams may be a way of countering this tendency to offspring ingestion.

Eggs and sperm spawned in response to 5-HT were examined with videomicroscopy. Sperm swam actively. Within 2 hr of adding sperm to released eggs, many of the eggs began to show signs of fertilization: double nuclei, cleavage, and, in some instances, four divisions containing separate nuclei, etc. These observations indicate that viable gametes can be produced by 5-HT-induced spawning.

BENTHIC COMMUNITY ASSOCIATED WITH ZEBRA MUSSEL COLONIES. Ronald Dermott[1] and **David Barton,**[2] [1]Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, P.O. Box 5050, Burlington, Ontario, Canada L7R 4A6, [2]Department of Biology, University of Waterloo.

The establishment of mussel colonies along the shores of the Great Lakes is expected to increase the amount of algal production consumed by the benthic fauna. To examine any changes in the nearshore benthic community due to *Dreissena,* sampling was conducted in 1990 and 1991 on the rocky northeast shore of Lake Erie, and in sandy substrates in Lake Ontario off the Niagara River. Due to the additional interstices, mussel colonization of rocky shoals increases the habitat available to epibenthic invertebrates which normally inhabit the attached algae and crevasses among the rubble. Other than the detrimental competition for space and food that occurs between *Dreissena* and native clams on sandy substrates, no direct exclusion was observed between the invertebrates on rocky shoals. Like the Unionidae in soft sediments, exposed *Sphaerium* can be colonized by small mussels.

Although poorly studied, the composition of the major taxa along the north shore of Lake Erie has not changed in areas colonized by mussels. Depending on the season, the caddis *Hydropsyche, Helicopsyche;* the midges *Crictopus, Rheotanytarsus;* and naidid worms remain common after two years of colonization. However, total abundance of invertebrates has increased within the mussel colonies as well as an overall increase since the late 1970's. Species that are benefiting most from the association with *Dreissena* are the amphipod *Gammarus* (to 41,000 m^{-2}), with increases also occurring for the leeches, *Erpobdella, Dina* and *Helobdella* (460 m^{-2}). Abundance of invertebrates is greatest among the mussel beds, where biomass excluding the *Dreissena* attained 21 g m^{-2} (dry shell-free).

DEMOGRAPHY AND DISPERSAL OF THE ZEBRA MUSSEL (*DREISSENA POLYMORPHA*) IN UPSTATE NEW YORK WATERS. Donna L. Dustin, Edward L. Mills, Mindy M. Gardner, and **Thomas Greig,** Cornell Biological Field Station, Department of Natural Resources, Cornell University, Ithaca, NY 14853.

Zebra mussel (*Dreissena polymorpha*) collections have been made June through October in the Erie-Barge Canal, the Oswego River, and Oneida Lake in 1990 and 1991. At all sampling sites, secchi disc measurements and samples for zebra mussels (both veligers and adults) were taken as was water for chlorophyll, dissolved oxygen, and selected nutrient analyses. In 1990 veliger densities were highest at the western end of the canal and declined steadily eastward. By early fall adult densities were observed as far east as Palmyra. No mussels, including veligers, were observed in the Oswego River and Oneida Lake in 1990. Sites with abundant mussels showed a marked decrease in chlorophyll and an increase in water clarity and soluble reactive phosphorus compared to sites with few or no mussels. In May 1991, adult zebra mussels were first observed on native unionid clams in Oneida Lake. Rapid warming of canal waters in spring of 1991 allowed swift growth and early reproduction of adults surviving over winter. Veliger densities of over 70,000 m^3 occurred in mid-June at Palmyra, with veligers detected at all sites (including Oswego River and Oneida Lake) by mid-July. In contrast to 1990, early summer veliger densities in the eastern waters of the Erie Canal were higher than those in western portions. The Erie Canal system is a major vector through which zebra mussels will infest inland waters of New York State.

STRUCTURAL CHARACTERISTICS OF THE BYSSUS OF THE ZEBRA MUSSEL, *DREISSENA POLYMORPHA* (PALLAS), WITH COMPARISONS TO THE BYSSUS OF THE BLUE MUSSEL, *MYTILUS EDULIS* (LINNAEUS). **Larry R. Eckroat** and **Louise M. Steel,** The Pennsylvania State University at Erie, The Behrend College, Erie, PA 16563-0203.

To better understand the morphology of the byssus of the zebra mussel (*Dreissena polymorpha*), the surface and internal structures of the stem, threads, and plaques were examined with a scanning electron microscope. The information provided adds to the current understanding of the morphology of the byssus of *D. polymorpha* and shows that the byssuses of *D. polymorpha* and *Mytilus edulis* are markedly dissimilar.

Micrographs indicated that the byssus of *D. polymorpha* was a continuous structural unit that was attached to the inside of the mussel's shell by retractor muscles. In some specimens, cuffs were present on the stems at the bases of the threads. Because these cuffs seemed to be lost by specimens from which the byssus was manually pulled, the process by which the mussels voluntarily detach from a substratum may not involve pulling forces. The thread-branching pattern suggested that the stem remains the same length when new threads are formed. Information concerning the interior of the threads was obtained using the freeze-fracture method. Although the appearances of the cross-sectional fracture faces were variable and provided little detail, longitudinal fracture faces indicated that the thread had two parts: an interior cortex containing longitudinal fibers embedded in a matrix and an exterior sheath, which was a separate layer that is likely to form as a result of an enzyme-catalyzed tanning process. In addition, it was observed that the outer surfaces of the threads were smooth proximally and became increasingly ridged distally. This thread topography may result because the thread material is molded to the walls of the ventral groove of the mussel's foot. Plaques, which are likely to be filled with an adhesive, were attached to substrata in rows, which could increase the stability of the mussel's anchorage.

It has been reported that the byssus of *D. polymorpha* is similar to the byssal apparatus found in *M. edulis* and other marine bivalves. Although there are many similarities between *D. polymorpha* and *M. edulis*, differences in their byssuses were distinguished using the scanning electron microscope. For instance, in these two biofouling species, differences exist in the anchorage of the stem, the pattern in which the threads branch from the stem, the thread surface topography, and the morphology of the region of the thread that extends into the plaque. These differences in byssus morphology are likely to be related to how the byssus is formed.

Because byssal attachment is fundamental to the success of mussels that colonize hard substrata, morphological differences between the byssuses of various biofouling mussels should be recognized as researchers develop and adapt control mechanisms.

EFFECT OF LITHIUM/HYDRAZINE WATER CHEMISTRY ON SHORT-TERM SURVIVAL OF ZEBRA MUSSEL. **David W. Evans** and **Lisa M. Coughlin,** Chemical Research Department, Ontario Hydro Research Division, 500 Kipling Avenue, Toronto, Canada H8Z 5S4.

The emergency coolant injection (ECI) systems at Ontario Hydro's nuclear generating stations are part of the special safety systems used for cooling of the nuclear fuel in the event of a loss-of-coolant accident. The water reservoirs for the ECI systems basically consist of large volumes of demineralized water or lakewater, dosed with lithium hydroxide (pH 9 to 11) and hydrazine (50 to 200 mg/L) to control the corrosion rates of carbon steel components. Macrofouling by zebra mussels could potentially impair the availability of this safety system. A laboratory testing program was therefore undertaken to determine the effect of typical lithium/hydrazine chemistry on the survival of young (ca. 5 mm) zebra mussels, in the short term.

Batch tests were run for 96 hours in well-sealed, 1L glass vessels. Tests were conducted in both demineralized water and dechlorinated tap water. Initial hydrazine concentrations were 0, 20, 50, 100 and 200 mg/L. Lithium hydroxide was added to raise the initial pH to 10. All lithium/hydrazine dosed tests were maintained at 20°C, without aeration. Controls (no lithium or hydrazine) were run at 10°C and 20°C, both with and without aeration. About 20 young mussels, naturally attached to PVC substrates, were used in each treatment. All tests were duplicated. The mussels were not fed during the course of the testing. Dissolved oxygen, pH, conductivity and lithium and hydrazine concentrations were measured at the beginning and end of the test. Although an electro-shock method was available, the live/dead determinations were done readily by behaviourial observation.

After 96 hours complete mortality was achieved in all hydrazine-dosed treatments. No mortality was observed in any of the treatments in dechlorinated tap water where hydrazine was absent (i.e. no mortality in controls or in pH 10 Li-dosed, 0 mg/L hydrazine treatment). Elevated control mortality was found in demineralized water at 20°C, but no mortality was seen in corresponding 10°C controls. Observations made during the course of the experiment indicate that, at ≥20 mg/L hydrazine, 100% mortality could be achieved within 1 hour in demineralized water and within 24 hours in dechlorinated tap water.

The results indicate that hydrazine levels of 20 mg/L or greater should be rapidly toxic to zebra mussels in either dechlorinated tap water (which is similar to the lakewater used in the station ECI systems) or demineralized water. The precise mode of hydrazine toxicity is unknown, but the rapid mortality suggests that death is not caused by deoxygenation of the bulk water. Toxicity may result from some localized reductive mechanism affecting oxygen uptake at the respiratory surfaces. Not surprisingly, demineralized water alone also increased mussel mortality. Under the test conditions, pH, in the range 7.3 to 10, did not influence survival rates.

The present ECI system chemistry regime, established to minimize materials corrosion, should prevent macrofouling by zebra mussels.

TESTING OF CANDIDATE MOLLUSCICIDES ON THE ZEBRA MUSSEL. Susan W. Fisher and **H. Dabrowska,** Department of Entomology, The Ohio State University, 1735 Neil Avenue, Columbus, OH 43210.

Methods were developed for measuring the toxicity of molluscicides to the zebra mussel, *Dreissena polymorpha* (PALLAS) in conjunction with studies conducted at the U.S. National Fisheries Research Center, La Crosse, WI. Adult zebra mussels (20–25 mm in length) were randomly selected from a stock culture 24 hours prior to initiation of toxicity tests. Groups of 13 mussels were placed on the bottom half of 9 mm glass petri dishes and were allowed to secrete new byssal threads over a 24-hour period. Those mussels which did not reattach were discarded. An average of 10 mussels per dish did secrete new byssal threads and were considered suitable for use in toxicity tests.

Toxicity tests were run in triplicate in hard standard reference water. Mortality was assessed at 24 and 48 hours after which the test animals were placed in clean water for 96 hours to screen for recovery and survival. Toxicity data were analyzed by probit analysis at give LC_{50} values and 95% confidence limits.

Using this protocol, the toxicity of a series of 12 molluscicides was determined in adult zebra mussels. These toxicity data were then compared to the results of tests performed with the same chemicals on two bivalves related to the zebra mussel; the latter tests were performed at the National Fisheries Research Center at La Crosse. From these data, the reliability of the standard method was assessed. In addition, a body of toxicity data was developed for molluscicides proposed for use on zebra mussels from which it will be possible to assess the efficacy of these chemicals in zebra mussel control and the likelihood of hazard to closely related freshwater bivalves.

THE TOXICITY OF POTASSIUM CHLORIDE TO ZEBRA MUSSEL VELIGERS AND SELECT NONTARGET ORGANISMS. Susan W. Fisher,[1] K. R. Polizotto,[2] and **Beth Schneider,[3]** [1]Department of Entomology, The Ohio State University, 1735 Neil Avenue, Columbus, OH 43210, [2]PCS Sales, [3]Environmental Sciences Program, The Ohio State University.

The toxicity of potassium chloride was measured in static tests to zebra mussel veligers. In addition, the toxicity of continuous, low-level (25–50 ppm) exposure to KCl was measured in flow-through tests. The latter was accomplished using a customized testing apparatus consisting of a series of 9 pipes which were 4 inches in diameter and 1M in length. The pipes were suspended above the water at Stone Laboratory, Put-in-Bay, Ohio and Lake Erie water was pumped continuously through each pipe at a rate of 0.5 M/sec. Inside each pipe, a second pipe with a slightly smaller diameter was placed. Each of the inner pipes were transected by a series of 6 glass slides to serve as setting plates. The inner pipe and glass slides were removed daily for examination of veliger settling and survival. Three of the pipes received a continuous application of 50 ppm KCl and three pipes received 25 ppm KCl. The remaining three pipes served as untreated controls. The experimental application of KCl took place for a period of two weeks from August 1, 1991 to August 15, 1991.

In addition to assessing veliger settling and survival in each pipe, the number and condition of the veligers being pumped into the pipes were analyzed daily. The concentration of potassium in the effluent of each pipe was assessed daily using an ion specific potassium electrode and verified weekly with atomic absorption spectrophotometry. Veliger abundance in the lake water was also monitored on a daily basis.

The toxicity of KCl was tested in several benthic invertebrates. These included juvenile *Anodonta imbecillus* with and without sediment, adult *Corbicula fluminea* and fourth instar larvae of the midge, *Chironomus riparius*.

ZEBRA MUSSELS AND THEIR EFFECTS ON FISH SPAWNING IN LAKE ERIE. John D. Fitzsimons,[1] Joseph Leach,[2] S. Nepzy,[2] and **V. W. Cairns,[1]** [1]Fisheries and Oceans, 867 Lakeshore Road, Burlington, Ontario, Canada L7R 4A6, [2]Ontario Ministry of Natural Resources.

The rapid colonization by zebra mussels of shallow rocks substrates in Lake Erie has dramatically altered their physical appearance. This colonization, along with associated chemical and biological changes, may reduce the potential for successful reproduction on these substrates. We are examining the effects of mussels on spawning by three lithophils—walleye, white bass and lake trout.

Both walleye and white bass continue to spawn on historic spawning shoals despite high numbers of mussels averaging 150,000 m^2. Spawning by walleye appears to be random, with no apparent selection for areas rendered devoid of mussels by ice scour. Viability of walleye eggs and the dissolved oxygen levels under which they incubate were unaffected by the presence of mussels.

Work underway at potential lake trout spawning areas indicates that mussels are primarily associated with the top layer of multilayered cobble substrates where they don't appear to affect interstitial dissolved oxygen. Observations on lake trout spawning this fall will be discussed.

FRESHWATER DRUM (*APLODINOTUS GRUNNIENS*), A PREDATOR OF THE ZEBRA MUSSEL IN LAKE ERIE. John R. P. French and **Michael T. Bur,** U.S. Fish and Wildlife Service, National Fisheries Research Center—Great Lakes, 1451 Green Road, Ann Arbor, MI 48105.

Freshwater drum (*Aplodinotus grunniens*) are considered major mollusk predators because they possess molariform pharyngeal teeth used to crush shells. However, mollusks were not found to be an important food of Lake Erie freshwater drum in the 1960–1980

period when they comprised less than 7% of the benthic communities in the western part of the lake. The European zebra mussel (*Dreissena polymorpha*) established a population in the late 1980s and became one of most abundant macroinvertebrates in western Lake Erie. We conducted a study to determine if these mollusks had become a major food of freshwater drum. We collected drum from 22 stations in the western basin of the lake and examined their gut contents in May, July, and September 1990. Freshwater drum did not feed significantly on zebra mussels until they were at least 250 mm long. Predation on zebra mussels increased as drum size increased. Medium-sized drum (250 to 374 mm long) fed mainly on dipterans in May and shifted their diets to zebra mussels in July and small fish in September. Large drum (over 375 mm long) fed almost exclusively on zebra mussels throughout the collection season. Since freshwater drum over 375 mm long feed heavily on zebra mussels, they should be considered as one component of a zebra mussel control strategy. We recommend that management agencies consider regulatory and public education options for increasing the population of large freshwater drum in Lake Erie to improve the likelihood of greater predation pressure on zebra mussels.

GENOTYPE-DEPENDENT METABOLISM AT THE PHOSPHOGLUCOSE ISOMERASE LUCUS AT AMBIENT AND ELEVATED TEMPERATURES. David W. Garton and **Ann M. Stoeckmann,** Department of Zoology, The Ohio State University, 1735 Neil Avenue, Columbus, OH 43218-1293.

Recent electrophoretic studies have shown that *Dreissena* populations in North America possess remarkably high levels of genetic variation at many enzyme loci. The locus coding for the glycolytic enzyme phosphoglucose isomerase (PGI, EC 5.3.1.9) is the most variable, with seven alleles present in the *Dreissena* population at our study site in western Lake Erie. Studies on variable loci in other invertebrate species have identified fluctuating environmental factors as primary agents responsible for maintaining genetic variation. In several marine and terrestrial species, environmental gradients of temperature result in corresponding clinal variation in PGI allele frequencies. Accordingly, we performed a series of experiments during the summers of 1990 and 1991 to determine if metabolic rate was genotype-dependent at ambient and elevated temperature in *Dreissena*.

Oxygen consumption of individual mussels was determined in a Gilson differential respirometer and expressed as ul h^{-1} std $mussel^{-1}$ at STP. Following oxygen consumption measurements, PGI genotypes were determined for each mussel using cellulose acetate electrophoresis. In 1990, metabolic rate was determined at ambient temperature (23.8°C) and 2 and 5 days following transfer to 31.2°C (N = 120 for ambient and Day 2, N = 80 for Day 5). In 1991, experiments were performed four times (May, July, August and October), with metabolic rate measured at ambient lake temperature, and 3–4, 8–11 and 15–16 days following transfer to 31.2°C (N = 40 for each sample date; total N = 480). All experiments were performed at Ohio State University's F.T. Stone Laboratory in western Lake Erie. Analysis of the 1991 series of experiments is currently in progress.

There were three common and four rare alleles at the PGI locus, numbered 1–7 in reverse order of electrophoretic mobility. Although nine genotypes were detected, the six genotypes of three common alleles (homozygous genotypes 33, 44 and 55; heterozygous genotypes 34, 35 and 45) comprised 87.5% of the population. Data analysis was restricted to common genotypes in order to maintain a balanced experimental design. Average oxygen consumption of heterozygotes was significantly less than homozygotes (21.8 vs 25.0 ul hr^{-1}, respectively, ANCOVA $p < 0.025$). Heterozygous genotypes 34 and 35 had much lower average metabolic rates than all other genotypes, whereas the rarest homozygous genotype (55) had significantly higher average metabolic rate. Average metabolic rates of genotypes 33, 44 and 45 were intermediate between genotypes 34 and 35, and 55. Genotype-dependent differences in metabolic rate were temperature sensitive, with significant differences between heterozygotes and homozygotes occurring at elevated temperature (2 and 5 days post-transfer to 31.2°C) and not at ambient lake temperature (23.8°C).

Increasing water temperature has been hypothesized as a factor limiting southern expansion of *Dreissena* in North America. Our results provide evidence that *Dreissena* possesses considerable genetic variation, and that variation at PGI can respond to selection via elevated temperature. This implies that zebra mussels have the ability to adapt to local temperature regimes, and that thermal tolerance measured on "northern" populations may not accurately predict ultimate thermal tolerances of "southern" populations of *Dreissena*.

EFFECTS OF ZEBRA MUSSELS ON THE DIETS AND GROWTH OF JUVENILE YELLOW (*PERCA FLAVESCENS*) AND WHITE PERCH (*MORONE AMERICANA*) IN LAKE ERIE. D. M. Graham,[1] W. Gary Sprules,[1] and **S. J. Nepzy,[2]** [1]Department of Zoology, University of Toronto, Erindale College, 3359 Mississauga Road, Mississauga, Ontario, Canada, [2]Ontario Ministry of Natural Resources.

Since their appearance in 1987 in western Lake Erie, zebra mussels have depleted algal resources and likely altered the zooplankton community. Since young fish rely on certain species and sizes of zooplankton, we hypothesize that these changes in community structure will have a negative effect on their condition and growth. Our results indicate a reduction in growth rate for young-of-the-year yellow perch. Weight increased by a factor of 1.04 per day for YOY yellow perch in 1988, when zebra mussel densities were low, and only by a factor of 1.013 per day in 1990 when zebra mussel densities were high. No difference in growth rate were evident for YOY white perch. The diet of 1990 yellow perch may explain this apparent reduction in growth. Early in the season they consumed mostly *Daphnia* but as the summer continued *Daphnia* were no longer consumed and diet diversified as the fish

consumed a greater number of taxa. White perch have a broader diet and are therefore less likely to be adversely affected by the reduction of a single prey taxon. Diet and growth rates of 1991 yellow and white perch will be compared to that of 1988 and 1990 to determine whether trends in growth reduction continue.

ZEBRA MUSSEL VELIGER DENSITIES AND WATER QUALITY PARAMETERS IN LAKE ERIE AT THE CLEVELAND WATER INTAKES. **Alan Greenberg,[1] Gerald Matisoff,[2] Gerald Gubanich,[1]** and **Julius Ciaccia,[1]** [1]City of Cleveland Division of Water, 1201 Lakeside, Cleveland, OH 44114, [2]Department of Geological Sciences, Case Western Reserve University.

Zebra mussel (*Dreissena polymorpha*) veliger densities were measured in the open waters of Lake Erie near the Kirtland water intake in Cleveland in order to identify times and depths of veliger settling to optimize chemical and mechanical control procedures. A small number of veligers first appeared in the water in late May when the surface water temperature was about 22°C. A small density maximum (27 L) was recorded on May 29, 1991. There was a second, larger density maximum (316 L) which occurred on July 30, 1991. There has been more than an order of magnitude increase in peak veliger densities in the Cleveland area since 1988. Maximum veliger densities increased each year from just a few per liter in 1988 to well over 300 L in 1991. Maximum veliger densities in the Cleveland area are lower than those reported from the western basin of Lake Erie in previous years. The highest veliger density is usually found in the epilimnion just above the thermocline. Somewhat lower densities are found at the surface and almost no veligers are found in the hypolimnion. The water intakes receive water from a depth of about 35 feet, within the zone of high veliger densities. However, almost no veligers survive transport to the Kirtland water plant at the other end of the intake pipe. Mechanical pumping was employed on August 13–14, 1991 to dislodge zebra mussels from the intake pipes. This resulted in 35 yd^3 of zebra mussel debris collected at the Kirkland shore shaft and well screen. Monthly average summer turbidity data and Secchi disc measurements from 1985 to present support the premise that zebra mussels are "cleaning up the lake" and lowering turbidity in the central basin near Cleveland. In fact, the Cleveland Division of Water is now investigating coagulating agents other than alum because of alum's inefficiency in low turbidity water.

MONITORING THE INTRODUCTION AND SPREAD OF ZEBRA MUSSELS IN THE OHIO RIVER: PROTOCOLS AND PROBLEMS. **Kim H. Haag, James H. Thorpe,** and **Fang Wei,** University of Louisville, Water Resources Laboratory, Louisville, KY 40292.

Monitoring the invasion and dispersal of zebra mussels in rivers poses a unique set of problems for investigators. Current velocity and the amount of suspended sediment are the most obvious parameters distinguishing lakes from rivers, and they can severely limit methods for sample collection of veligers, post-veligers, and adults. Commonly used devices will not close properly, cannot withstand the stress exerted by even moderate currents, or clog rapidly. Even when heavily weighted gear is employed, it is difficult to position at specified depths under high current conditions. In addition, fluctuating water levels due to artificial regulation and precipitation can stand or inundate samplers left tethered in place. Alternative approaches to sampling rivers for zebra mussels are proposed.

We initiated a zebra mussel monitoring study on the Ohio River in January 1991. The study encompasses 280 river miles including the Cannelton, McAlpine, and Markland Pools. We have devoted our efforts this first year to monitoring the water column for the presence of veligers. Monthly vertical plankton tows are stratified by river reach within each pool. One set of samples is taken at a site in the lower reach near the downstream dam. In the upper reach of each pool, a major tributary has been selected (Salt, Kentucky, and Miami Rivers, respectively), and one set of samples is collected in each tributary two miles upstream of its confluence with the Ohio. Additional sets of samples are gathered at sites in the Ohio River two miles upstream and two miles downstream of the mouth of the tributary. In addition to vertical tows, replicate quantitative plankton samples are collected at the surface both in the channel and near the bank. Ten to 20 liters of water are filtered through a 63 um mesh net, preserved on our boat, and returned to the laboratory for later enumeration and identification of zooplankton and phytoplankton. To date, no zebra mussel veligers have been found. Routine examination of channel buoys in the three pools have also failed to reveal the presence of adult *Dreissena* in this portion of the Ohio River.

USE OF AN OXIDIZING CHEMICAL PROGRAM FOR ERADICATION/PREVENTION OF ZEBRA MUSSELS. **Fred L. Harlan** and **Robert E. McCarthy,** Nalco Chemical Company, 1 Watco Center, Waperville, IL 60563.

Chemical control for zebra mussel eradication has centered on oxidizing and nonoxidizing biocides. The purpose of this paper will be to highlight the differences between these two approaches, and to show the efficacy of the former in actual operating conditions.

The program of an oxidizing biocide and biodispersant to control zebra mussels under operating conditions will be discussed. The use of both continuous and intermittent applications will be covered, as well as their effect on the plant operations.

The use at a customer location indicates that the continuous application can be used as a kill (remediative) mode while the intermittent method can be used in the preventative mode.

Levels of free bromine at 0.3–0.5 ppm FRO are necessary to obtain complete kill. Time, temperature, and system parameters are important variables to be considered when the type of treatment is to be chosen.

FROM THE FRONT LINE OF THE INVASION: EARLY-WARNING DETECTION OF ZEBRA MUSSELS. Clifford Kraft,[1] Mary Balcer,[2] Art Brooks,[3] Jory Jonas,[1] Allen H. Miller,[1] and **Hans Pearson,[4]** [1]University of Wisconsin Sea Grant Institute, University of Wisconsin, Green Bay, WI 54311, [2]University of Wisconsin—Superior: Center for Lake Superior Studies, [3]University of Wisconsin—Milwaukee: Center for Great Lakes Studies, [4]Silver Lake College.

Since late 1989 it has been obvious that zebra mussels would eventually colonize Wisconsin waters of Lakes Michigan and Superior. Key questions were when this invasion would materialize, and whether a coordinated sampling effort could provide more useful information about the arrival of zebra mussels than unplanned observations. For two summers (1990 and 1991) we mounted a substrate and plankton sampling program in harbors and water intakes. In concert with this sampling effort, we conducted an intensive educational effort for utility personnel, divers and the general public concerning the impending arrival of zebra mussels.

At most sample sites the presence of zebra mussels was first reported from unplanned observations that were not part of our coordinated sampling effort. However, in most instances such reports were soon followed by quantified abundance estimates from our sampling program, providing information on the rate of colonization. These results suggest that: (1) at their earliest stage of infestation, zebra mussels are not likely to be detected by standard sampling protocols; (2) educated observers can provide useful clues regarding potential infestation by zebra mussels; and (3) a standard sampling program can provide useful information once a local reproductive population of zebra mussels has become established.

ORGANIC CONTAMINANT AND HEAVY METAL CONCENTRATIONS IN ZEBRA MUSSEL TISSUE FROM WESTERN LAKE ERIE. Russell G. Kreis, Jr., Michael D. Mullin, Ronald Rossmann, and **Laurie L. Wallace,** U.S.E.P.A., Large Lakes Research Station, ERL—Duluth, Grosse Ile, MI 48138.

Zebra mussels were collected from rock substrates in a nearshore zone of western Lake Erie at Sterling State Park, Monroe, Michigan. Mussel soft tissues were removed from shells and four samples were analyzed for organic contaminants, heavy metals, and other standard parameters.

Total lipids and moisture averaged 1.5% and 87%, respectively. Total PCB concentrations averaged 520 ug/kg wet weight, where the prevalent homolog groups were tetra-, penta-, and hexachlorobiphenyls. The distributions of PCB homologs and congeners were similar in all tissue samples. PCB congener #52 was the dominant, comprising 5.6% of the total PCB concentration. Several congeners quantified in the analyses have MFO- and AHH-inducer potential. Hexachlorobenzene, the sums of DDT [illegible]logs, and chlordane components averaged 0.83, 22, and 14 [illegible]et weight, respectively.

[illegible]ntal analysis indicated that carbon, hydrogen, and nitrogen averaged 42%, 6%, and 6.5% respectively. Heavy metal analyses were conducted using ICP-AES and AA; results indicated good agreement between the two methods. Cadmium, chromium, lead, and mercury concentrations were either below 5.3 mg/kg dry weight or the limit of detection. Copper, nickel, and zinc averaged 15, 19, and 160 mg/kg, respectively.

A comparative examination of organic contaminants and heavy metal concentrations in zebra mussels and the native unionid, *Lampsilis radiata,* was conducted for the Huron-Erie corridor. Based on limited data, total PCB concentrations in zebra mussels varied by an order of magnitude and in some cases, were an order of magnitude greater than concentrations in *Lampsilis*. Of particular interest was the observation that lipids were 3 to 10 times greater in zebra mussel than in *Lampsilis*. Generally, PCB concentrations increased in all bivalve samples in a southward direction through the corridor. Heavy metal concentrations did not exhibit a spatial distribution or a difference between bivalve types. Lead and cadmium concentrations were below 10 mg/kg dry weight in all bivalve samples and exhibited moderate variation.

Results of this study indicate that zebra mussels can accumulate organic contaminants and heavy metals. Limited data suggest that PCB concentrations are considerably greater than concentrations in native unionids, exhibit a spatial distribution in the Huron-Erie corridor, and have potential for contaminant transfer to higher trophic levels. Conversely, the very limited heavy metal and other organic contaminant data do not suggest concentration differences in bivalves or spatial distributions.

THE RANGE EXTENSION OF THE ZEBRA MUSSEL (*DREISSENA POLYMORPHA*) IN THE INLAND WATERS OF NEW YORK STATE. Cameron L. Lange and **Roberta K. Cap,** Acres International Corporation, 148 John James Audubon Parkway, Amherst, NY 14228.

Through 1990, the zebra mussel (*Dreissena polymorpha*) extended its range into the inland waters of New York State via the State Barge Canal System from the Niagara River near Buffalo, New York to Palmyra, New York, located about 120 miles inland. This rapid range extension exceeded the expectations of many investigators. In 1991, the Empire State Electric Energy Research Corporation (ESEERCO) sponsored a continuation and expansion of a monitoring program initiated in 1990 to follow the continued spread of the mussel through New York State. Monthly sampling commenced in March 1991 at eight locations on the Barge Canal, four on the Cayuga/Seneca Canal/Finger Lakes System, seven along the Hudson River and three in the Susquehanna River drainage. A total of six other locations were sampled on the Genesee, Oswego, Schoharie and Delaware Rivers.

In 1991, zebra mussel veligers were found for the first time in May. Veligers were present at the Palmyra and Baldwinsville locations only. The Baldwinsville sighting increased the zebra mussel's known range by 65 canal miles. In May 1991, adult mussels were independently reported to be in Oneida Lake, 25 miles further inland from Baldwinsville. In June, zebra mussels had extended their range into the Oswego River at Fulton. Zebra mussel

veligers had infiltrated all of the Barge Canal and associated river systems where water flows downstream from the Niagara River source waters. To further extend its range, they would need to be lifted to a higher water elevation through the locks. Whether this would impede the mussel's progress was not known.

In July, veligers were found on the Cayuga Lake side of Lock C-1 on the Cayuga/Seneca Canal. Zebra mussel veligers were also identified in samples collected on the Susquehanna River near Binghamton, New York. Additional sampling in early August verified their presence. This was the first report of zebra mussels in a drainage system not connected to the Great Lakes system by a canal. Adult zebra mussels were also reported in discrete areas along the Mohawk and Hudson Rivers, but through July no veligers were collected during this program. Results indicate that two methods of transport are occurring in the inland waters: veliger drift and active transport of mussels by boats or other means.

Through July, the density of veligers ranged to 58,000 m^3 at Palmyra (June collections). The maximum density of settled mussels during the same period was 8,400 m^2 also at Palmyra. Sampling continued through November.

TOWARDS MITIGATION OF ZEBRA MUSSELS AND ASIATIC CLAMS: THE USE OF ENDOD, *PHYTOLACCA DODECANDRA*. **Harold H. Lee**[1] and **Aklilu Lemma,**[2] [1]Department of Biology, The University of Toledo, [2]International Child Development Centre, Toledo, OH 43606.

Experiments using a static bioassay system as a basis to develop a focal control method for *Dreissena* and *Corbicula* illustrate the potential usefulness of plant molluscicides, Lemmatoxins, from *Phytolacca dodecandra* or Endod. Endod at a dose higher than 15 mg/L is lethal to adult zebra mussels and Asiatic clams, while at lower doses prevent adhesion and aggregation of the mussels. In addition to being noncarcinogenic and nonmutagenic, Endod is biodegradable. Since Endod plants have been successfully grown as monoculture, demands on large quantities of Endod usage in water intakes should stimulate further agricultivation. Since infestations of zebra mussels and Asiatic clams are long-term problems and waterworks vary in design and environment, a conceptual methodology for mitigation is suggested using Endod as the primary agent in combination with mechanical and chemical means to remove adult mussels and Asiatic clams from and to prevent aggregation in water intake pipes. (Funded by a DeArce Research Award of the University of Toledo and an Ohio Sea Grant).

DEVELOPMENT AND TESTING OF AN AUTOMATIC PLANKTON SAMPLER FOR USE IN ZEBRA MUSSEL MONITORING. **D. R. Lowther**[1] and **D. Barker,**[2] [1]Ontario Hydro, [2]J. J. Downs Industrial Plastics Inc., 700 University Avenue, A7-C5, Toronto, Ontario, Canada M5G 1X6.

Ontario Hydro has seventeen facilities on the Great Lakes and connecting waterways as well as sixty seven inland hydraulic facilities. There is a need to accurately establish the seasonal presence of zebra mussel veligers at these diverse locations, to optimize the individual zebra mussel control regimes. Hand sampling was considered to be too labour-intensive based on the distance between facilities. As an alternative, an automated plankton sampler was commissioned by Ontario Hydro from a local supplier.

The sampler has the capability of collecting seven discrete samples at intervals determined by the researcher. Samples are strained through a mesh size of choice and the final sample is preserved.

This paper outlines the physical features of the plankton sampler, method and flexibility of operation as well as some practical considerations for installation and use.

SOVIET EXPERIENCE ON ZEBRA MUSSEL RESEARCH AND CONTROL. **Michael Ludyanskiy, John F. Garey,** and **Derek M. McDonald,** Marine Biocontrol Corp., P.O. Box 636, Sandwich, MA 02563.

Recently, in the Great Lakes region in general, and portions of Lake Ontario and Lake Erie in particular, populations of the freshwater zebra mussel, *Dreissena polymorpha,* have increased dramatically. The economic impact of this rapid change, and the resistance of *D. polymorpha* to established control methods, have had a profound effect on the power utilities.

D. polymorpha originated in the North Kaspiy region of the European USSR, and for over 2 decades has been considered the main macrofouling organism throughout the Soviet Union. A considerable body of untranslated Russian information concerning the biology, economic effect(s), control and regulation of the zebra mussel remains unavailable for study in North America—more than 600 scientific papers on these subjects have been published in the Soviet Union. In an effort to mesh this information with current studies in this country, the authors present a comprehensive review of this literature, focusing briefly on *D. polymorpha* biology, and primarily on Soviet methods for the (1) prevention of settlement, (2) inhibition of growth, and (3) methods of removal from already infested areas.

Active investigation of *D. polymorpha* biology in the Soviet Union began during the 1950's. Initial studies focused on structural and functional descriptions of *D. polymorpha* populations in different habitats—abundance, mass and size structure, and degree of settlement. Since that time, numerous reports investigating the peculiarities of fertilization, ontogenesis, phenology and growth of larval stages have been published. Of significance are investigations on the developmental patterns of *D. polymorpha* which are influenced by water quality, temperature and velocity.

A sizeable amount of study has been devoted to the prevention and/or control of zebra mussel populations. Studies in collaboration with Soviet utilities report abundances of *D. polymorpha* in sections of industrial water piping up to 0.5 million individuals/$meter^2$, fouling layers from 1–15 cm in thickness, and biomass levels up to 30 $kg/meter^2$. Buildup in some reservoirs of the Ukraine have reached 2000 tons, and annual estimates of zebra mussel soft tissue in the Volgograd Reservoir exceed 1 million

tons. In these industries, and currently in power generation utilities in the northern United States, this fouling causes reduction in process water, leading to plant shutdowns, equipment failures, and reduced operating efficiency. The Soviet experience with methods for control and regulation of *D. polymorpha* demonstrates the need for *site-specific* evaluation of fouling potential and underscores the need for preventative measures, rather than

EFFECTS OF SALINITY ON GROWTH AND SURVIVAL OF ZEBRA MUSSELS (*DREISSENA POLYMORPHA*). Gerry L. Mackie and **Bruce W. Kilgour,** Mackie & Associates Water Systems Analysts, 381 Elnira Road, Guelph, Ontario, Canada M1K 1H3.

Previous studies that describe the tolerance of zebra mussels to saline waters indicate ranges in the LC_{50} between 1.84 and 12.3 ppt. The variability may be due to differences in salts used. There have been no studies conducted to describe the influence of salinity on growth of zebra mussels. The objectives of this study were: (1) to describe the salinity tolerance of zebra mussels to different salts (Instant Ocean, road salt, potassium chloride); (2) to describe the growth of zebra mussels in different salinities (with Instant Ocean); and, (3) to explore the possible interactions between temperature and salinity on survival and growth. The growth experiments were conducted at three different temperatures (3–4, 10–12, and 19–22°C). The 96 h bioassays were conducted at 4 and 19°C. Experiments to determine the salinity tolerance to different salts are in progress.

The 96 h LC_{50} for Instant Ocean at 19°C was 7.6 ppt. During the six week growth test at 4 and 10°C, mortality in the treatments with 8.0 ppt did not exceed 15%. Differences in mortality between the 96 h test and the six week growth experiment may be due to differences in the acclimation time of the mussels. In the 96 h test, mussels were not acclimated to the different salinities whereas in the six week growth test, mussels were acclimated to test concentrations (0 to 8.0 ppt) by increasing salinities by 1.0 ppt per day. Zebra mussels appear to be able to adapt to slowly changing saline concentrations.

During the six week growth tests, there were no significant effects of salinity on maximum shell length or ash free dry weight (AFDW) of mussels at 4°C. There was a statistically significant, but not substantial, effect of salinity on survival at that temperature. Mortality ranged between 0.8% at 0 ppt to 4.1% at 8.0 ppt. At 10°C there was no significant effect of salinity on maximum shell length, but there was a significant effect on AFDW. A linear contrast showed that the change in AFDW was linearly related to salt concentrations. The effect of salinity on mortality at 10°C was not as large as at 4°C. At 10°C, mortality ranged between 0.6% at 0 ppt to 1.4% at 8.0 ppt. Results are not available for tests conducted at 19°C due to high mortalities observed in control treat-

ults to date indicate that above 6.0 ppt there are significant alinity on zebra mussel growth and survival. Survival was reduced at 8.0 ppt at all temperatures, whereas growth (i.e., changes in AFDW) was also impaired at 8.0 ppt at 10°C.

FILTERING IMPACTS OF LARVAL AND ADULT ZEBRA MUSSELS IN WESTERN LAKE ERIE. Hugh J. MacIsaac and **W. Gary Sprules,** Department of Zoology, University of Toronto, Erindale College, Mississauga, Ontario, Canada L5L 1C6.

Filtering rates of larval and adult zebra mussels (*Dreissena polymorpha*) must be known in order to predict the impact of this exotic species on Lake Erie phytoplankton stocks. We determined filtration rates of *Dreissena* veliger larvae using solutions of fluorescent 2.87 μm polystyrene beads, and compared them to rates derived from the literature (Kryger and Riisgård 1988) for settled mussels. Mean filtration rates of veligers ranged between 10.2 and 17.4 μL individual^{-1} hour^{-1} between bead concentrations of 10^3 and 10^5 beads mL^{-1}; at very high bead densities (>2×10^5 beads mL^{-1}) filtration rates were very low (<2 μL individual^{-1} hour^{-1}). Large veligers have higher filtration rates than small individuals, and some are capable of ingesting particles as large as 11 μm. Filtration rates of settled mussels (2–29 mm) are between 10 and 13446 times greater than those of veligers on an individual basis, and 440 times greater on a population basis for the Hen Island reef population in western Lake Erie. Settled zebra mussel filtering impacts also greatly exceed those of other zooplankton species in western Lake Erie. Based on 1990 Hen Island reef population densities, settled mussels filter up to 132 m^3 m^{-2} day^{-1} (i.e. they potentially filter the water column > 19 times per day).

PHYSIOLOGICAL AND MORPHOLOGICAL COMPARISONS OF *DREISSENA POLYMORPHA* AND *MYTILOPSIS LEUCOPHAETA* (BIVALVIA: DREISSENIDAE). David B. MacNeill, New York Sea Grant, 248 Nartwell Hall, SUNY College at Brockport, Brockport, NY 14420-2928.

The introduction of the zebra mussel, *Dreissena polymorpha,* into North America may have serious economic and ecological ramifications. Within the next ten years, *Dreissena* is predicted to spread into several estuarine areas along the eastern seaboard of the U.S., potentially resulting in a range overlap with the dark false mussel, *Mytilopsis leucophaeta,* a euryhaline dreissenid native to North America. Like other dreissends, *Mytilopsis leucophaeta* has biofouling tendencies, although believed to be of lesser consequence than *Dreissena*. Results of a literature review indicate partially overlapping salinity tolerances and habits of *Dreissena* and *Mytilopsis* in Europe. Because of their related phylogenies, these two bivalve species display close morphological similarities, particularly as juveniles, leading to probable field misidentification as their populations become sympatric. This presentation reviews physiological and morphological descriptions of *Dreissena polymorpha* and *Mytilopsis leucophaeta* and provides an abbreviated guideline for their definitive identification.

THE RELATIVE IMPORTANCE OF *DREISSENA* FILTRATION AND *DAPHNIA* GRAZING ON PHYTOPLANKTON ABUNDANCE AND WATER CLARITY. Eileen Malloy Desormeaux and **Joseph C. Makarewicz,** Department of Biological Sciences, State University of New York College at Brockport, Lennon Hall, Brockport, NY 14420.

Microcosm experiments evaluated the relative impact of *Dreissena* filtration and *Daphnia* grazing on phytoplankton abundance and water quality. Experimental vessels contained levels similar to abundance in Lake Erie:

Phytoplankton only (80,000 organisms/ml)
Phytoplankton and *Daphnia* (30,000 m^3)
Phytoplankton and *Dreissena* (30,000 m^3)
Phytoplankton, *Daphnia* and *Dreissena*

Data from four experiments in which temperature was maintained at a uniform 20°C indicated a 30–50 percent reduction in phytoplankton abundance over a 72 hour period for both *Daphnia* and *Dreissena*. Turbidity levels decreased in vessels containing *Dreissena* and increased in vessels with *Daphnia*. SRP values increased significantly in *Daphnia* vessels but not in *Dreissena* vessels.

Although both *Daphnia* and *Dreissena* reduced phytoplankton abundance, *Daphnia* released phosphorus thus providing a continuous nutrient source for phytoplankton. SRP levels decreased in vessels containing *Dreissena* consequently reducing the availability of a necessary nutrient for phytoplankton growth and reproduction.

GENETIC POLYMORPHISM AMONG DISJUNCT POPULATIONS OF ZEBRA MUSSELS IN THE GREAT LAKES. J. Ellen Marsden[1] and **Bernie May,[2]** [1]Illinois Natural History Survey, Lake Michigan Biological Station, P.O. Box 634, [2]Cornell Laboratory for Ecological and Evolutionary Genetics, Department of Natural Resources, Cornell University, Zion, IL 60099.

Information about the population structure of zebra mussels is critical for the development of effective, long-term management and control. High juvenile mortality in combination with the broad dispersal of larvae could result in a small group of zebra mussels producing the majority of the subsequent year class in a given area. The potential therefore exists for genetic drift to separate zebra mussels into genetically distinct sub-populations with divergent life history characteristics, and possibly different responses to control methods. Even a cursory examination of zebra mussel morphology reveals wide variation in body shape and color pattern. Zebra mussels also have very high levels of genetic variability as detected by starch-gel protein electrophoresis. We examined the genetic structure of multiple populations of zebra mussels from geographically disjunct locations throughout the Great Lakes and inland waterways using protein electrophoresis. Fifty enzyme systems were examined; 21 polymorphic loci were detected which had bands that could be reliably interpreted. We have also initiated measurement of a set of morphological characters in each population. These data permit determination of (1) whether zebra mussels within the Great Lakes represent a single, genetically uniform population or multiple discrete sub-populations, and (2) whether disjunct populations of zebra mussels within the Great Lakes represent separate introductions from Europe. The results will be discussed in relation to the need for basic biological information on non-indigenous pest species before large-scale control strategies are implemented. We will also present data which suggest that there is a second species of *Dreissena* in the Great Lakes system.

STATUS OF ZEBRA MUSSELS IN LAKE MICHIGAN—TEMPORAL AND SPATIAL DISTRIBUTION OF VELIGERS AND ADULTS. J. Ellen Marsden,[1] Lidia Bardygula,[2] and **Jan Savitz,[2]** Illinois Natural History Survey, Lake Michigan Biological Station, P.O. Box 634, Zion, IL 60099.

Most of the information about zebra mussels in North America has come from Lakes Erie, Ontario, and St. Clair. Lake Michigan, in contrast to these lakes, is generally colder and has a north-south orientation. This latter feature has important consequences for the distribution of organisms which are affected by temperature or other latitude-dependent variables. Two simple hypotheses are proposed for zebra mussel populations in Lake Michigan: their density and growth rates should decline from south to north, and their reproduction and settling should be progressively delayed from south to north. The testing of these hypotheses will provide useful information for utilities who need to know how soon they will face "critical" densities of mussels in their intake systems. We report the results of veliger monitoring and adult sightings along the western Lake Michigan shoreline, and SCUBA assessments of adult densities in the near-shore waters of Lake Michigan.

OCCURRENCE OF POST-METAMORPHIC DRIFTING IN ZEBRA MUSSELS: IMPLICATIONS ON DISPERSAL AND RECRUITMENT. Andre Martel, Canadian Museum of Nature, P.O. Box 3443, Stn. O Ottawa, Ontario, Canada K1P 6P4.

Field experiments conducted along the north shore of the central basin of Lake Erie using off-bottom collectors have revealed that post-metamorphic drifting may play a significant role in the life history of the zebra mussel, *Dreissena polymorpha*. These collectors, made of rectangular pieces of fibrous material (11.7 × 12.0 cm, 0.8 cm thick), were deployed for short time periods, 24–48 hours, thus preventing colonizing zebra mussels from growing significantly while on the collectors. For each zebra mussel recruiting to the collectors, the examination of the larval (prodissoconch II) and juvenile shell (dissoconch), including the distinct demarcation between these two regions of the shell, made it possible to determine the stage at which each individual colonized the collectors: (1) as a free-swimming planktonic veliger (colonizers of 240–270 um shell length or less), or (2) as a drifting juvenile, namely individuals that had already metamorphosed (310–330 um shell length and above). The occurrence of early juvenile stages

drifting in the water column was also confirmed by their common presence in horizontal near-shore plankton samples taken near the collectors (10–100 m from the shore; water depth: 2–7 m). Moreover, much higher numbers of juveniles were drifting and recruiting to collectors during periods of high wave action (commonly over 30 juveniles/collector/24 hours).

Mechanisms involved in the drifting of newly-metamorphosed zebra mussels that would enable early juveniles (mostly 300–900 um shell length) to drift in the water column may include the secretion of drifting mucous threads or the adhesion of individuals to detritus particles. Such mechanisms would make small juvenile zebra mussels virtually neutrally buoyant during transport by water currents and are currently being investigated. The occurrence and the high numbers of drifting juveniles recruiting to off-bottom collectors suggest that post-metamorphic drifting has significant implications on the dispersal and recruitment processes of zebra mussels.

DESIGN OF PIPE-CRAWLING VEHICLES FOR ZEBRA MUSSEL CONTROL. Benjamin Martin and **Samuel E. Landsberger,** Cornell University, Department of Mechanical and Aerospace Engineering, Ithaca, NY 14863.

We are designing vehicles for operation in water pipes to control the growth of zebra mussel colonies. We address the problems involved in operating in the hostile environment present in water intake pipes; (1) operation in a confined space at a great distance from the control center, and (2) navigation and propulsion in high currents with varying geometry and obstacles. We then examine our designs for a pipe travelling vehicle in the light of the past history of autonomous and remotely controlled vehicles. These systems have had a varied record of success in the industrial world. There are examples of commercial success, such as the underwater use of Remotely Operated Vehicles (ROV's). On the other hand there are many cases of systems which worked wonders in the laboratory but whose field performance fell short of expectation. We examine these past robotic systems to determine what characterized the successful and the unsuccessful systems, and examine our proposed design solutions in this light.

INFESTATION AND IMPACT OF ZEBRA MUSSELS ON THE NATIVE UNIONID POPULATION AT PRESQUE ISLE STATE PARKE, ERIE, PA. Edwin C. Masteller[1] and **Donald W. Scholesser,**[2] [1]The Pennsylvania State University at Erie, The Behrend College, Station Road, Erie, PA 16563-0203, [2]U.S. Fish and Wildlife Service, National Fisheries Research Center—Great Lakes.

In June, July, and September 1990 and July 1991 a capture-
apture study of unionid mussels was initiated at four sites near
irved sandspit of eastern Lake Erie known as Presque Isle.
is a unique geological feature that has substantial num-
. onids with over 21 species, including eight species of endangered Pennsylvania unionids. Based on the evidence observed in western Lake Erie, it is believed that the unionid populations of Presque Isle are in danger of increased mortality or extinction due to infestations by the exotic zebra mussel, *Dreissena polymorpha*. To date, over 1,000 unionid have been collected by SCUBA divers, marked, and returned to their collection sites. Zebra mussels were counted and measured from a representative sample of unionids. Dead shells of unionids were also collected. The most abundant unionid species were *Lampsilis siliquoidea, Potamilus alatus, Anodonta grandis, Leptodea fragilis* and *Amblema plicata*. The capture-recapture methodology of Lincoln-Peterson indicates that the Presque Isle population of unionids is composed of approximately 6,000 individuals. The infestation of unionids by zebra mussels at Presque Isle is occurring at a rapid rate and parallels that observed in western Lake Erie with a time lag of about two years. In early 1990, one-year-old zebra mussels were present at only one of the sites at Presque Isle. By the end of 1990, young-of-the-year zebra mussels were found on unionids at all four of the study sites. In July 1991, zebra mussels were found on 484 of the 485 unionids collected. In addition, infestation rates increased from 121 zebra mussels per unionid in 1990 to 234 per unionid in 1991. Similar data of infestation of the unionids in western Lake Erie occurred in 1989 just prior to exponential increases in numbers of zebra mussels throughout the basin. Fortunately, observations indicate that as of late July, few young-of-the-year zebra mussels were settling in unionids near Presque Isle. However, dramatic increases in densities of zebra mussels is occurring in the area and it is believed that it is only a matter of time before the zebra mussels will begin to have a negative influence on the unionid populations near Presque Isle.

EFFECTS OF POTASSIUM, CLORAMINE, AND CHLORINE DIOXIDE ON CONTROL OF ADULT ZEBRA MUSSELS. Gerald Matisoff,[1] **Alan Greenberg,**[2] **Gerald Gubanich,**[2] and **Julius Ciaccia,**[2] [1]Department of Geological Sciences, Case Western Reserve University, Cleveland, OH 44106-7216, [2]Cleveland Division of Water.

Chemical dose/response studies of zebra mussel adults in flow-through aquaria were conducted to evaluate the effectiveness of potassium, chloramine, and chlorine dioxide on adult mortality for exposure periods of up to several days. Potassium permanganate was effective at concentrations greater than about 2 ppm. Potassium hydroxide concentrations less than 1 ppm did not induce mortality in the mussels, but complete mortality was observed at concentrations greater than 10 ppm. Chlorine dioxide treatment resulted in mortalities of about 10%–20% at the 0.2–0.3 ppm level with 100% mortality reached in 24 hours at concentrations greater than 0.5 ppm. This yields an LC_{50} of about 0.4 ppm. Chloramine treatment resulted in 100% mortality in 24 hours at total chlorine concentrations as low as 1.2 ppm. However, the chloramine concentrations in those experiments were as low as 0.1 ppm, indicating a chloramine production yield of only about 10%. Thus, it is unclear if the mortality-inducing agent is chloramine at 0.1 ppm or free chlorine at 1.1 ppm. The high effectiveness of chlorine diox-

ide at inducing zebra mussel mortality, coupled with the fact that the addition of chlorine dioxide forms less THM's than chlorine and chloramine, indicates that it may be preferred as an oxidizing agent for zebra mussel control at water treatment plant intakes even if slightly higher concentrations are needed.

EFFECTS OF TEMPERATURE AND RELATIVE HUMIDITY ON DESICCATION RESISTANCE IN ZEBRA MUSSELS (*DREISSENA POLYMORPHA*): IS AERIAL EXPOSURE A VIABLE CONTROL OPTION? **Robert F. McMahon**[1] and **Barry S. Payne,**[2] [1]Center for Biological Macrofouling Research, The University Texas at Arlington, P.O. Box 19498, Arlington, TX 76019, [2]U.S. Army Corps of Engineers, Waterways Experiment Station.

Wet weighed adult zebra mussels (*Dreissena polymorpha*) were emersed at 5°, 15° and 25°C in relative humidities (RH) of <5%, 33%, 53%, 75% and >95% (RH maintained in desiccators with silica gel, super-saturated solutions of $MgCl_2 \cdot 6H_2O$, $Mg(NO_3)_2 \cdot 6H_2O$, NaCl and water, respectively). Subsamples (n = 6) were removed periodically (frequency dependent on desiccation rate), wet weighed, tested for viability by 12 h reimmersion and dried at 90°C. Cumulative total water (TW = corporal + extracorporal water) loss was assumed to be the decrease in wet weight during emersion (initial wet weight − final wet weight) and was expressed as a percentage of TW weight (initial wet weight − dry weight). Increasing temperature and decreasing RH decreased emersion tolerance. At 25°C, LT_{50} ranged from 42.2 h (<5% RH) to 70.2 h (>95% RH). Corresponding LT_{100} range was 69.7 − 96.7 h. At 15°C, LT_{50} ranged from 67.7 h (<5% RH) to 266.2 h (>95% RH). Corresponding LT_{100} range was 153.1–537.1 h. At 5°C, LT_{50} ranged from 169.8 h (<5% RH) to 346.1 h (75% RH) (data for >95% RH was incomplete at abstract due date). Corresponding LT_{100} range was 362.5–482.7 h. At all temperatures, individuals continually shut valves at <5% RH, but periodically gaped and displayed open inhalant siphons at higher RH. Water loss rates generally increased with increased temperature and decreased RH. At 15° and 25°C over <5–75% RH, mean per cent TW loss for living individuals in samples just preceding those with 100% mortality was 59–71%, suggesting that mortality was due to desiccation. In contrast, mean TW loss prior to death at >95% RH was 25% at 15°C and 43% at 25°C, suggesting that death was due to other causes (i.e., acidosis, anaerobic endproduct or ammonia toxicity, or energy store depletion). A similar pattern occurred at 5°C. These data suggest that dewatering to kill zebra mussels would require 3–4 days at ≥25°C. At 15°C, it would require 7–9 days below 75% RH, but 23 days above 95% RH. At 5°C, minimal time for 100% kill would be >11 days and is likely to exceed 30 days above 95% RH. Thus, the most appropriate time for application of dewatering to control zebra mussels is mid-summer when elevated ambient temperatures would induce rapid kills. At lower ambient temperatures, application of dry and/or heated air to dewatered components may be required to produce acceptable kill rates.

SUBMERSED MACROPHYTES IN THE LITTORAL OF LAKE HURON'S SAGINAW BAY. **Cal D. McNabb, T. G. Coon,** and **T. R. Batterson,** Department of Fisheries and Wildlife, Michigan State University, Environmental Sciences Section, P.O. Box 25007, Denver, CO 80225-0007.

Five locations in the shorezone of Saginaw Bay were used in 1991 to establish baseline data on distribution and abundance of submersed macrophytes. Our intention is to document changes that may occur during the next few years if zebra mussels become abundant and clarify water in the bay. Sites were selected to obtain a range for principal determinants of macrophyte distribution; namely, light penetration and sediment type. Measurements of these parameters, turbidity, and macrophyte abundance were obtained on corridors (transects with 50 m width) at each location. Maximum depth boundaries for submersed macrophytes varied among corridors. Boundaries were located at depth contours 0.5, 0.6, 0.9 and 2.8 m at sites near Au Gres, Bay City State Park, Quanicassee and Pinconning respectively. No plants were observed along a corridor on Sand Point. Absence of plants at Sand Point, and shallow maximum depth boundaries at Au Gres and State Park, appear to be associated with sediment type rather than turbidity or light penetration. Boundaries at Quanicassee and Pinconning appeared to be related to turbidity and light penetration rather than sediment type. Abundance of submersed plants shoreward of maximum depth boundaries was calculated from frequency of occurrence of macrophytes ($\%F_M$) in random grab samples taken along corridors. Macrophyte beds were poorly developed at Au Gres and State Park; $\%F_M$ was 32 and 12 at these sites respectively. Plant abundances at Quanicassee and Pinconning were higher, reaching 82 and 52 $\%F_M$ respectively. Species abundance at sites was taken from $\%F_S$; percent frequency of individual species in grab samples. Most commonly occurring species were the charophyte, *Nitella flexilis,* the filamentous algae, *Cladophora glomerata,* and *Najas flexilis, Potamogeton pectinatus, Potamogeton richardsonii,* and *Vallisneria americana.* We expect in year-2 to relate annual production of common macrophyte species (maximum seasonal standing crop), and abundance of microfaunal food resources developed from common plants via detrital food webs, to potential standing crops of larvae (egg—25 mm) of littoral spawning fish (e.g. yellow perch, common carp). We intend to develop quantitative data regarding the hypothesis that shifts in water clarity caused by zebra mussels will increase macrophyte biomass and increase the capacity of the macrophyte-occupied littoral to support early life stages of fish that spawn there.

RESPONDING TO THE ZEBRA MUSSEL THREAT: A CASE HISTORY. **Thomas C. McTigh,**[1] **Ford J. Ritz,**[2] and **John R. Amend,**[2] [1]Monroe County Water Authority, 4799 Dewey Avenue, Rochester, NY 14612, [2]Malcom Pirnie, Inc.

Dreissena polymorpha, the zebra mussel, is a species of freshwater clam native to the Black and Caspian Seas of northeastern Europe. Since its accidental introduction into the Great Lakes in 1986, this non-native mollusk has caused tremendous problems for

water users and suppliers throughout the lower Great Lakes and adjacent waterbodies.

In light of the severe problems encountered by water users in Lake Erie during 1989 and 1990, the Monroe County Water Authority (MCWA) viewed the possibility of zebra mussel infestation of its sole water intake as a critical planning issue. MCWA's concerns were heightened by peak summer water demands which routinely required production approaching the nominal capacity of the intake. Knowing that it had little capacity to spare and cognizant of the speed at which these mollusks were migrating, MCWA decided that the zebra mussel threat required a proactive and aggressive response.

ZEBRA MUSSEL CONTROL USING ACOUSTIC ENERGY. John Menezes, Sonalysts, Inc., 215 Parkway North, Waterford, CT 06385.

The spread of the zebra mussel *Dreissena polymorpha* (Pallas) into the Great Lakes and adjoining waters is a great concern to utility, municipal, industrial, and environmental interests because of the propensity of the species to rapidly cover the area it colonizes. A practical and economical device or method that reduces zebra mussel colonization without detrimental side effects is highly desirable.

The research and technical programs in which Sonalysts has participated focused on affecting veliger and immediate post-veliger developmental stages using acoustic energy. In 1990, small-scale studies were performed under contract to Empire State Electric Energy Research Corporation (ESEERCO) by Sonalysts to survey the effect of underwater sound on the viability of larval, post-veliger, juvenile, and adult zebra mussels.

The acoustic energy was generated by several devices designed for underwater and laboratory applications using narrow frequency bands between 155 Hz and 1 MHz. The initial screening was conducted by evaluating gross morphological and behavioral effects in very small-scale (<1 liter) systems using short-term exposures at or near the maximum operating amplitude of each device. When an initial survey test showed promising results (e.g., veligers destroyed, mussels gaping, shells fractured or detached) the test was conducted on a larger scale with replicate and reference samples at different amplitudes, ranges, or durations.

Based on the 1990 results, ESEERCO sponsored Sonalysts to conduct additional research during the summer of 1991 (currently ongoing) which provided an opportunity to extend this work towards the optimization of acoustic parameters and an increase in scale (range, volume). A pilot-scale system will be developed and deployed in 1992 based on the information and results collected during the summer of '91 testing. The pilot-scale system is being designed to prevent the attachment of viable juveniles to intake walls and induce mortality as veligers pass through moving volumes of water.

From the surveys, we learned that high intensity acoustic can lethally disintegrate veligers and shatter the shells of juvenile zebra mussels. The effect on juveniles is size and time dependent. *In situ* tests also indicated that juveniles and adults longer than 10 mm could also be killed, even at lower amplitudes, with less than 18 hours of treatment. Sonalysts will discuss this acoustic research on zebra mussel control and their plan to demonstrate a full-scale installation of acoustic control devices at an operating intake.

BIOLOGICAL CONTROL OF ZEBRA MUSSELS: SCREENING FOR LETHAL MICROORGANISMS. Daniel P. Molloy and **Barbara Griffin,** New York State Museum Biological Survey, State Education Department, Albany, NY 12816.

Initiated in April 1991, this research project focuses on the development of a biological method for controlling zebra mussels. Microorganisms are being tested in the laboratory to identify those which are lethal to attached zebra mussel life stages. Over 260 different microorganisms are being screened over a two year period. These candidate control microorganisms will not be "natural" parasites of zebra mussels, but rather naturally occurring soil and water microbes, which just by chance happen to be lethal to zebra mussels when the mussels are exposed to artificially high densities of the microbe. A microorganism which at artificially high densities is poisonous to zebra mussels undoubtedly exists in nature, and the proposed research is designed to identify it. This type of research approach has a track record of success, since it has already produced a commercially available, environmentally safe, microbial control agent for another aquatic, filter-feeding, invertebrate pest—the black fly. Preliminary results of those microorganisms screened to date will be presented.

THERMAL BACKFLUSHING TO CONTROL ZEBRA MUSSELS AT STEAM STATION. E. F. Neuhauser,[1] M. A. Rhode,[1] J. J. Knowlton,[2] R. J. Wahanik,[2] M. Borden,[2] D. P. Lewis,[3] and **G. Mackie,[4]** [1]Niagara Mohawk Power Corporation, Syracuse, NY, [2]Gilbert/Commonwealth, Inc., Reading, PA, [3]Aquatic Sciences Inc., St. Catharines, Ontario, [4]Mackie and Associates Water Systems Analyst, Guelph, Ontario.

Other than thermal treatment, the use of various chemical treatments to control zebra mussels (*Dreissena polymorpha*) in both the U.S. and Canada is well documented and appears to be effective under a number of different protocols. However, concerns over the potential impact of these treatments to receiving water bodies has motivated industry to investigate alternative or complementary control approaches. The control of zebra mussels by thermal treatment is an alternative approach to chemical treatment which may be effective while producing minimal harm to the environment.

To remove the zebra mussel infestation in the circulating water system at Niagara Mohawk Power Corporation Dunkirk Steam Station, it is proposed to increase the temperature of the intake water used for steam condenser cooling from 55–65°F (13–18°C) to at least 95°F (35°C). The construction of the circulating water

system at the Dunkirk Station is such, that by adjusting some of the gates in the system, it is possible to redirect the main condenser heated discharge water back to the intake structure and to the screenhouses instead of discharging into the harbor. This redirecting of the condenser discharge water to the intake structure/screenhouse has resulted, during previous treatments, in an increase in circulating water intake temperatures to at least the anticipated temperatures required to kill zebra mussels. To prevent equipment damage, the maximum intake temperature will be limited to approximately 100°F (38°C).

Biological and temperature monitors were placed throughout the circulating water system. These monitors were used to first establish a control base of mussels present at Dunkirk Station prior to thermal treatment implementation and then to monitor mussel activity associated with temperature changes.

This Research and Development project to control zebra mussels by thermal treatment at Niagara Mohawk Power Corporation's Dunkirk Steam Station yields results which can be used in several ways. The main goal of this project is to follow up on the experience gained during previous thermal treatments and to establish a controlled study base using sound experimental technologies. The main benefits from the project are the applicability of the results to similarly designed power generating stations and the potential use for the design of new generating facilities.

DETERMINING THE SEX AND REPRODUCTIVE STATUS OF ZEBRA MUSSELS. Susan J. Nichols, U.S. Fish and Wildlife Service, National Fisheries Research Center—Great Lakes, 1451 Green Road, Ann Arbor, MI 48105.

Determining sex ratio and reproductive status of zebra mussels can provide useful information on when veliger production will begin and a general estimate of overall numbers. The reproductive status of a mussel is determined by removing a portion of the combined digestive-reproductive tract, and examining this material under a microscope. Zebra mussels fall into four categories—male, female, hermaphrodite, or immature (unidentifiable). The ratio of male-to-female, as well as the degree of hermaphrodism, varies considerably from colony to colony. Immature is a term used to describe mussels whose gametes are not differentiated at this time, regardless of shell size or previous spawning record. The general appearance and size of the gametes found during dissection indicates when spawning will occur. Mature females contain eggs showing both nuclei and germinal vesicles. However, although eggs can show both nuclei and germinal vesicles at a size of 0.030 mm, these eggs must reach over 0.060 mm before spawning occurs. Mature males contain sperm that are triangular in shape, less than 0.01 mm in size, with multiple tails. Sperm are not released until they are triangular in shape as well as tailed. In general, it takes about 8 to 10 weeks at 20°C for an immature mussel, with no distinguishable gametes, to develop fully ripe gametes.

REPRODUCTIVE CYCLE OF ZEBRA MUSSELS (*DREISSENA POLYMORPHA*) IN WESTERN LAKE ERIE AT MONROE, MICHIGAN. Susan Jerrine Nichols and **B. Kollar,** U.S. Fish and Wildlife Service, National Fisheries Research Center—Great Lakes, 1451 Green Road, Ann Arbor, MI 48105.

The reproductive cycle of zebra mussels was investigated to determine length of veliger production, length of ripe gamete production, percentage of females present, and size of sexual maturity. This study was conducted weekly since May 1990 at the Detroit Edison plant in Monroe, Michigan. Basically, veligers are present in the water column for 6 months. In 1990, veligers first appeared May 30 at densities of 75 L, peaked July 26 at 187 L, and were last found October 3 at 4 L. In 1991, veligers appeared on May 12 at 30 L, and by June 18 were at 179 L. Although veligers were present in the water column for only 6 months of the year, zebra mussels carried ripe gametes every month of the year. In May, June, and July 1990, over 85% of the mussels were carrying ripe gametes. This percentage dropped to 8% by January 1991, increased to 19% by February and was at 92% by May 1. Zebra mussels showed seasonal variation in sex ratios and size at sexual maturity. In samples collected from May 1990 to May 1991, the proportion of females increased from 52% to 74%. The mussels also showed a decrease in size at sexual maturity, from 13 mm shell length in May 1990 to 5 mm by May 1991. These seasonal changes indicate that the reproductive cycle of zebra mussels is readily affected by local environmental conditions, and therefore may vary considerably from site-to-site.

BIOLOGY OF ZEBRA MUSSEL SPERM. Tim Otter, Department of Zoology, University of Vermont, Burlington, VT 05405.

I have sought to establish some baseline information on the reproductive effort of male zebra mussels and the viability and motility of zebra mussel sperm. These data should be of interest in two ways: 1) to comprehend the physiology of normal sperm and the process of external fertilization in zebra mussels, and 2) to identify potential targets for the control of zebra mussel reproduction.

Spawning was induced by immersing each animal in Millipore-filtered lakewater (FLW) containing 0.1–1.0mM 5-hydroxytryptamine (5HT) buffered at pH 8.4 (4–6mM Tris-HCl). Lower doses of 5HT were ineffective, but all doses of 5HT ≥0.1mM induced spawning in ca. 20% of the mussels. In some cases spawning could be seen directly as a cloudy plume being discharged from the excurrent siphon, as soon as 4 min. after exposure to 5HT. In other cases no plume was visible and the water became noticeably cloudy with sperm some 30–60 min. after addition of 5HT. Because females appear relatively unresponsive to 5HT (Ram & Nichols, '90) and the sex ratio is ~1:1 (Garton & Haag, '91), I conclude that the non-spawners were immature mussels, females, or males that had recently spawned in nature. Shell length varied from 13mm–27mm, and sperm count (hemocytometer) ranged from 7.3 million to 350 million, with larger mussels releasing

proportionately more sperm, assuming that a constant percentage of body volume is gonad. Roughly half of the mussels in this population are 5–15mm long and capable of producing gametes, whereas less than 10% of mussels are larger than 15mm. Taken together, these data suggest that during an episode of mass spawning, over half of the sperm in the water would have been released by small (5–15mm, probably 1 yr.-old) mussels.

Sperm swimming paths and sperm morphology were recorded on videotape using darkfield or phase-contrast microscopy and then analyzed by computer-assisted methods. The average sperm length is 54.7 ± 3.1 μm (50μm flagellum; 4.5μm long bullet-shaped head). In some sperm, phase-dense regions were observed at the base of the tail (swollen mitochondria?) and near the tip of the head (acrosome?). A more detailed analysis of swimming path parameters is in progress, but sperm movement can be grouped into five basic categories: immotile; erratic twitching; intermittent swimming; slow smooth swimming in circular paths; fast smooth circular swimming. In the best preparations, the initial percent motility approached 90%. Sperm became progressively less motile within minutes to hours after spawning, with the time course of loss of motility highly dependent on temperature (range 15°C–27°C). These results imply that at the peak summer temperatures in Lake Erie, fertilization must occur within minutes after spawning while in cooler waters fertilization might be delayed considerably. One major question that needs to be investigated is whether 5HT has any direct (stimulatory) effects on sperm motility, as it does in certain other bivalve molluscs.

Support: Lintilhac Foundation, Univ. of Vermont.

Location: F. T. Stone Laboratory, Put-in-Bay, OH.

ZEBRA MUSSEL SPAWNING: RELEASE OF EGGS AND SPERM IN RESPONSE TO EXTERNAL APPLICATION OF SEROTONIN. Jeffrey L. Ram, Gary W. Crawford, and **James U. Walker,** Department of Physiology, Wayne State University, Detroit, MI 48201.

One approach to controlling zebra mussels (ZMs) is to find weak points in their life cycle that can be exploited in developing a species specific control method. We have been focussing on reproductive mechanisms. Previously, this laboratory (Ram and Nichols, 1st Int. Zeb. Muss. Res. Conf., 1990) reported that injection of serotonin (5-HT) induced ripe males (but not females) to spawn. We now report that 5-HT can stimulate spawning in both males and females and can be applied either externally or by injection. ZMs, maintained in a closed system, responded to injection of 10^{-3} M 5-HT initially (May, 1991) with only male spawning, as previously reported. However, after maintenance in our system for 2 weeks, identical experiments elicited spawning from both males and females. Furthermore, 5-HT could be applied either by injection or external application (no significant difference between injecting 0.1 ml 10^{-3} M 5-HT or immersing animals in 1 l of 10^{-3} M 5-HT). Spawning responses with 10^{-3} M 5-HT were elicited from ZMs maintained in this system for more than 3 months (and continuing), with on average 43 ± 2% producing sperm and 28 ± 4% spawning eggs (n = 8 expts., total 440 animals; females are significantly different from males, $p < 0.025$). Of 120 controls, given identical election and handling, but no 5-HT, none spawned. The shortest latency observed for spawning in response to external 5-HT application was 15 min for males and 1.5 hr for females at ambient temperature (about 22°C). In response to a range of [5-HT], the lowest concentration to produce spawning by external application was 10^{-4} M, which elicited spawning at approximately half the frequency as 10^{-3} M ($p < 0.05$). Upon retesting previously spawned animals, 70% of both males and females responded again when tested one day later. 5-HT can, thus, be used as a non-invasive means for identifying ripe males and females. Furthermore, ZMs appear to give environmental chemicals access to their reproductive system, a property that may be exploited for purposes of control. (We gratefully acknowledge the assistance of S. J. Nichols in obtaining animal and doing initial experiments, and J. J. Mojares in analyzing spawn.)

MATHEMATICAL MODELS TO PREDICT THE DISTRIBUTION AND ABUNDANCE OF *DREISSENA POLYMORPHA* IN NORTH AMERICAN LAKES. Charles Ramcharan, Dianna K. Padilla, and **Stanley T. Dodson,** Department of Biology, Mount Allison University, Sackville, Nova Scotia, Canada E0A 3C0.

Useful estimates of the ecological and economic impacts of *Dreissena polymorpha* rely on accurate predictions of its potential distribution (where it will or will not be present) and abundance (mussel density). We constructed mathematical models that predict the distribution and abundance of *Dreissena* based on correlations between mussel abundance and limnological characteristics of the lakes they inhabit. Data on *Dreissena* populations and limnological variables were collected from published studies of European lakes. Over the last 200 years, *Dreissena* has colonized many types of European lakes that are found in a wide range of latitudes, providing the best dataset in which to search for environmental constraints on mussel growth. Our analyses show that physical and morphological characteristics of lakes do not affect their suitability for *Dreissena*. On the other hand, *Dreissena* is often absent from lakes that have low levels of calcium and low pH. A model developed by Discriminant Function Analysis is 90% successful at predicting presence or absence of *Dreissena* based only on calcium and pH levels in a lake. *Dreissena* reaches higher population densities in lakes that have low levels of the algal nutrients PO_4 and NO_3. Our study indicates that hard-water, mesotrophic lakes with rocky substrates are ideal habitats for *Dreissena*.

GENETIC COMPARISON AND CHARACTERIZATION OF FIVE ZEBRA MUSSEL POPULATIONS IN THE GREAT LAKES. Jennifer L. Rose and **Larry Eckroat,** The Pennsylvania State University at Erie, The Behrend College, Station Road, Erie, PA 16563-0203.

Dreissena polymorpha (Pallas), first observed in the Great Lakes in Lake St. Clair, is spreading rapidly throughout the Great

Lakes Basin. The original of the various Great Lakes populations is still open to speculation, as they may be colonists from Lake St. Clair or stem from separate ship ballast water discharges. Analysis of the allozyme polymorphisms present in several Great Lake populations provides information regarding the genetic relationship of these populations.

Approximately one hundred zebra mussels were sampled from each of five populations in Lakes Erie, St. Clair, and Ontario and their shell length recorded. Individual mussels were then genetically characterized by examining allozyme variation of whole body extracts using starch gel electrophoresis. Eight enzymes (G-6-PDH, EST, IDH, LAP, LDH, MDH, ME and PGI) revealed thirteen interpretable loci.

The five populations sampled had variation similar to that found in other organisms with respect to polymorphic loci (100%), average heterogeneity/locus (30.7–34.4%), and alleles/locus (3.9–4.2). All populations demonstrated heterozygote deficiency; three of the populations exhibited this deficiency at all 13 loci whereas the other two populations exhibited heterozygote deficiency at 11 and 12 loci, respectively, as is characteristic of previously analyzed mussel populations. This heterozygote deficiency is reflected by non-conformity to Hardy Weinberg expectations; however, one locus (EST-6) was in Hardy-Weinberg equilibrium in all five populations. While there are numerous potential explanations for the widespread occurrence of heterozygote deficiency in molluscs, the most plausible suggestion may be selection due to reduced fitness. As shell size is proportional to the age of the mussel, a correlation of shell size with heterozygosity provided information regarding the relationship of mussel fitness and genetic heterozygosity.

The use of F statistics to allocate genetic variability to the population, subpopulation, or individual demonstrated genetic differentiation between subpopulations for some of the 13 loci analyzed. Tests for interpopulational heterogeneity indicated a significant difference at one locus (PGI); the differences at the other loci were not significant.

The genetic identity and genetic distance values between four of the five populations suggested uniformity and therefore supports the idea that colonization occurred from one original population. This uniformity is presumably due to the pelagic dispersal of zebra mussel larva. However, one population (Lampe Marina, Lake Erie) was significantly different from each of the other four, and may be a result of restricted larva dispersal in the sheltered marina environment.

CASE STUDIES FOR THE ENGINEERING OF MUSSEL CONTROL FACILITIES IN RAW WATER INTAKE SYSTEMS. Richard San Giacomo and **Mona Cavalcoli,** R & D Engineering, 600 R&D Centre, 268 Main Street, Buffalo, NY 14202.

The threat of potential capacity reduction, head loss, deterioration of pipes, and taste and odor problems caused by zebra mussel infestation prompted municipalities and water suppliers in the United States and Canada, including the Niagara County Water District, Erie County Water Authority, Regional Municipality of Hamilton-Wentworth, Township of Kingston, Town of Deseronto, and City of Buffalo, to seek assistance in implementing effective yet cost-efficient methods to eliminate existing zebra mussels and prohibit future infestation. Case studies were done to evaluate, design, and install zebra mussel control facilities for raw water intake systems. R&D Engineering, P.C. provided the necessary engineering services.

The first step undertaken was to conduct underwater investigations of intake structures to determine present mussel growth and assess structural integrity of the raw water facilities. Chemical control alternatives were then evaluated, including chlorine and its various derivatives, potassium permanganate, and ozone. Present facilities were also evaluated (size, location, capacity, storage capability) for compatibility with the various chemical control methods. Recommendation was then made as to which type of control system to implement to effectively kill zebra mussels but minimize costly additions and/or alterations to the existing facilities. Design components included layout of the proposed solution line and diffuser assembly, to effectively dose the chosen chemical. In addition, weighting systems were designed to aid installation of the application line.

The Niagara County Water District's zebra mussel control system is installed and on-line. Other projects are currently under construction or scheduled to begin construction during the summer of 1991.

CONTROL OF ZEBRA MUSSEL FOULING BY COATINGS. R. D. Smithee and **William P. Kovalak,** Technical and Engineering Services, Detroit Edison Company, 2000 Second Avenue, Detroit, MI 48226.

The effectiveness of 14 different coatings, most of which were applied to steel plates, in controlling zebra mussel fouling was tested in the intake canal at Detroit Edison's Monroe Power Plant (western Lake Erie) between June–October 1991. Preliminary results based on visual inspections indicated most of the coatings affected some reduction in fouling. The most effective products were those that were silicone based and those that contained copper or other toxic metals. Also promising were thermal plastic coatings, although these were not exposed to colonization for the entire study period. Plates will be quantitatively sampled in mid-October. This will allow a better evaluation of the cost-effectiveness of the various products.

APPLICATION OF LOW VOLTAGE ELECTRIC FIELDS TO DETER ATTACHMENT OF ZEBRA MUSSEL TO STRUCTURES. A. Gary Smythe,[1] Cameron L. Lange,[1] J. F. Doyle,[1] and **Paul M. Sawyko,[2]** [1]Acres International Corporation, 140 Audubon Parkway, Amherst, NY 14228, [2]Rochester Gas and Electric Corporation.

The objective of this study is to determine if electric fields could be useful in reducing the attachment rate of zebra mussels

(*Dreissena polymorpha*) to submerged structures located within these fields. Conceptually we are assuming that if an electric field is of sufficient intensity the settling stage veligers will not attach while under the influence of, and for a short time after, exposure to a field. Any suitable flow of water will then carry the settler away from the "protected" structure.

The study site is at the Rochester Gas and Electric Russell Station in Rochester, New York, adjacent to Lake Ontario. A series of test flumes were set up and a continuous flow of power plant forebay water channeled through the flumes to provide a source of larvae. Bar rack arrays were placed in each of the test flumes. Flows in each flume were adjusted to about 0.5 fps (0.15 m/sec), a flow which could be expected to exist at some intakes. The bar rack arrays were constructed of steel plates 4 inch (or 6 inch) by 7 inch (5 plate/array) spaced to approximate that of a typical trashrack.

Low voltage electric fields of various intensities are being tested for 60Hz Alternating Current (AC), continuous Direct Current (DC) and pulsed DC. Analytically we are comparing the densities of attached mussels on control arrays to attached densities on arrays subjected to test electric fields. Spacial distribution of attached mussels for each plate is also being noted.

Laboratory analysis for several arrays has been completed. To date the data analysis has not been completed. However, in general, we can state that there has been zebra mussel attachment at some level on all arrays tested to August 28, indicating we are not attaining 100 percent success for the configurations tested so far.

Data analysis for tests already completed, for tests under way, and tests to be conducted over the next two weeks, will be finalized prior to presentation at the November conference.

APPLICATION OF CENTRIFUGAL SEPARATORS FOR CONTROL OF ZEBRA MUSSELS IN RAW WATER SYSTEMS. A. Garry Smythe,[1] Cameron L. Lange,[1] T. M. Short,[1] and **L. Ray Tuttle,[2]** [1]Acres International Corporation, 140 Audubon Parkway, Amherst, NY 14228, [2]New York State Electric and Gas Corporation.

Two centrifugal separators are being tested to determine their effectiveness in the control of zebra mussel (*Dreissena polymorpha*). The objective of the study is to assess the feasibility of using these separators to remove presettling and settling stage larvae from raw water supplies (Study Phase I), and to determine the effectiveness of such devices in filtering these larvae from the water (Phase II). An experimental set-up, including a Krebs desander and a Lakos Super-Separator, was installed at the New York State Electric and Gas Kintigh Station, Somerset, New York, adjacent to Lake Ontario. Testing utilizes Lake Ontario raw water from NYSEG's fire protection system. The Krebs desander (separator) is a hydrocyclone with a closed apex, used primarily in high pressure applications. The Lakos Super-Separator relies upon the same basic operating principle with some modifications to hydrocyclone design. The Lakos design offers some operational advantages relative to wear potential and equipment maintenance.

Both separators utilized centrifugal force to remove particles. The desander, due to its inlet design, will accelerate particles to a higher angular velocity in comparison with Lakos separator. The desander, therefore, may have a slight "edge" in separation efficiency.

Phase I sample collections began in July, 1991. The source water was water obtained through connection to the fire protection service water system in the circulating water pump house bay at the power station. Flows through the separators varied from approximately 0.4 to 0.8 M^3/min (100 to 220 gpm), system pressures varied from 2.8 to 9.8 kg/cm^2 (40 to 140 lbs/in^2) depending on valve settings for a given test.

Although the test apparatus and procedures are somewhat complex, the analytical approach is quite simple. The total number of larvae (differentiating veliger from post-veliger) was determined for the supernatant samples. The number obtained from each of these samples was added together and then divided into the number in the filtrate. This provided a separation efficiency factor for the veliger (D-forms) and post-veliger (umbonal forms).

Volumetric measurements have also been made using flow meters and pressure gauges. Accurate volume measurement, however, is quite difficult considering the high flows/volumes encountered for this study. As a result the direct measure and comparison of the numbers of larvae in the supernatant/filtrate are therefore appropriate.

The mean size of the umbonal forms was determined for a subsample of most of the supernatant/filtrate samples. Through the last lab analysis, the mean size of the umbonal forms in the filtrate was always greater than that in the supernatant sample for any sample pair (Krebs or Lakos). This would indicate that centrifugal force is selectively separating umbonal form larvae by size and/or specific gravity as was anticipated.

The separator efficiency for umbonal forms has ranged from 4.8 percent in early samples to 78.4 percent in later samples. It would appear that as the umbonal population ages, and the population mean size increases, the separator efficiency would increase as was anticipated. It is possible, however, that the maximum separation efficiencies obtained to date are being underestimated relative to settling stage larvae, since we are not differentiating the smaller umbonal forms. Larger umbonal forms are more susceptible to separation and are of more concern since they are at or close to settlement stage. It is anticipated that separation efficiencies will increase later in the season. Details of Phase I data, as well as any data analyzed from the Phase II study effort, will be presented.

EVALUATION OF POTASSIUM CHLORIDE FOR REMOVAL OF ZEBRA MUSSEL VELIGERS FROM COMMERCIAL FISH SHIPMENTS. Fred L. Snyder,[1] Susan W. Fisher,[2] and **Beth Schneider,[3]** [1]Ohio Sea Grant College Program, Camp Perry, Building 3, Port Clinton, OH 43452, [2]Department of Entomology, The Ohio State University, [3]Environmental Sciences Program, The Ohio State University.

Shipments of live fish are potential vectors for the spread of

zebra mussels (*Dreissena polymorpha*) into watersheds outside of the Great Lakes region. Currently, species such as emerald shiner (*Notropis atherinoides*), white bass (*Morone chrysops*) and carp (*Cyprinus carpio*) are being shipped in water from the Lake Erie region to other watersheds, allowing the possibility that zebra mussel veligers might also be transported. As the range of the mussel expands, shipments of species such as channel catfish (*Ictalurus punctatus*), golden shiner (*Notemigonus crysoleucas*) and fathead minnow (*Pimephales promelas*) could represent additional pathways for invasion.

A potential chemical treatment for selectively removing zebra mussel veligers from the water in fish shipments is under evaluation. Potassium chloride solutions of 0 ppm (control), 500 ppm and 1000 ppm are being used to determine 24 hr mortality rates for emerald shiner, fathead minnow, golden shiner, white bass and carp. Zebra mussel veligers are scheduled for testing in potassium chloride solutions of 0 ppm (control), 25 ppm and 50 ppm to determine 24 hour mortality rates.

Initial results from testing on these five commercial fish species suggest that 24 hr exposure to potassium chloride concentrations as high as 1,000 ppm did not cause mortality in excess of that experienced by fish held in the 0 ppm control tanks.

Extremely low densities of zebra mussel veligers in western Lake Erie during the study period precluded testing of the lethality of potassium chloride to veligers. This portion of the investigation will be continued in 1992. Previous research has produced LC_{50} values for potassium chloride to adult zebra mussels as low as 138 ppm. Veligers are frequently seen to be more sensitive to toxicants than are adult mussels.

Identification of potassium chloride levels lethal to veligers but harmless to commercially shipped fish species could lead to recommendations for commercial fish haulers on the prophylactic use of this chemical in retarding the spread of zebra mussels by their activities.

METABOLIC RESPONSES OF ZEBRA MUSSELS TO INCREASED FOOD SUPPLY AND INDUCED SPAWNING. **Ann M. Stoeckmann** and **David W. Garton,** Department of Zoology, The Ohio State University, 1735 Neil Avenue, Columbus, OH 43210-1293.

Metabolic responses of *Dreissena polymorpha* to increased food supply and induced spawning were measured during July and August, 1991. Zebra mussels were maintained under ambient conditions of food and temperature in running lakewater aquaria at F. T. Stone Laboratory located on South Bass Island in western Lake Erie. Mussel diet was supplemented from 20 May to 28 August by adding 5×10^9 cells of a preserved algal mixture twice a day to a 40 l aquarium. The supplemented diet consisted of a concentrated mixture of cultured marine algae (*Tetraselmis* and *Thalassiosira,* Coast Oyster Co., Diet A). Ingestion of the artificial diet was confirmed by histological analysis and increased fecal production. Recently collected mussels were induced to spawn by adding serotonin (10^3 M 5-hydroxytryptophan) to filtered lakewater in the respirometer flasks. Spawning was detected by visual inspection. All mussels were preserved for histological analysis to determine stage of gametic maturation (''readiness'' for spawning). Oxygen consumption of mussels under increased food and induced spawning conditions, measured using a Gilson Differential Respirometer and expressed as ul h^{-1} std $mussel^{-1}$ at STP, were compared to oxygen consumption of mussels maintained under ambient conditions. All metabolic rates were standardized to a dry weight of 15.5 mg.

Preliminary results show metabolic rate increased significantly following increased food and induced spawning. Average oxygen consumption of food supplemented mussels was 1.3 times that of ambient mussels (42.68 vs. 33.51 ul O_2 h^{-1}, respectively). Similarly, average adjusted dry weight of food supplemented mussels was nearly twice the average dry weight of mussels under ambient conditions (24 vs 14 mg, respectively). Average oxygen consumption of mussels exposed to serotonin, but not spawning, was significantly greater than for ambient mussels (43.20 vs 33.51 ul O_2 h^{-1}, respectively). After correcting for the effects of serotonin, mussels induced to spawn increased average oxygen consumption 1.7 times that of ambient mussels (56.45 vs 33.51 ul O_2 h^{-1} respectively).

Significant increases in metabolic rate and length-standardized dry weight of mussels in the food supplemented experiment are evidence that *Dreissena* is food-limited in western Lake Erie. This observation supports the conclusion that growth and reproductive output of *Dreissena* will be proportional to the availability of phytoplankton. Elevated metabolic rate indicates spawning is a physiologically stressful event. Reduced resistance to additional stressors during active spawning may have application for mitigation and control strategies for *Dreissena*.

USE OF A RIVERINE MESOCOSM TO STUDY THE ENVIRONMENTAL TOLERANCES OF ZEBRA MUSSELS IN LOTIC ECOSYSTEMS. **James H. Thorp** and **Kim H. Haag,** University of Louisville, Water Resources Laboratory, Louisville, KY 40292.

There is a paucity of information on the biology and impact of zebra mussels in rivers. In Europe, where *Dreissena* is well established, the polluted condition of many lotic habitats has presumably limited riverine populations. In the United States, the zebra mussel has only recently spread from lacustrine ecosystems to rivers draining into the Gulf of Mexico and Atlantic Ocean. Significant differences in environmental conditions existing in rivers versus lakes make it inadvisable to rely solely on available data from lakes when predicting impacts of zebra mussels in rivers.

To overcome this lack of applicable data we have designed an outdoor, flow-through riverine mesocosm to study the environmental tolerances of zebra mussels in lotic ecosystems. The initial phase consists of forty PVC troughs with a maximum depth of 4 inches and a length of 10 feet. After entering a head tank, untreated water from the nearby Ohio River is pumped through the individual troughs at an ''average'' current velocity of 0.1 m/sec.

From 50 to 75% of the water is recirculated, to reduce the required pump capacity. Waste water from the system is pumped through a mixed-media filter, treated with high concentrations of chlorine, and then sent to a holding lagoon. Channels are colonized with adult zebra mussels attached to removable, unglazed ceramic tiles at various densities (4000 m^2 in an initial study).

We are conducting studies to determine effects of temperature on the biology of *Dreissena* and the effects of zebra mussels on native mussels. For the thermal experiments, ten replicates of 3 different temperature regimes are being run: ambient (corresponding to the average weekly temperature at the intake pipe on the Ohio River); 25% lower than ambient; and 25% higher than ambient. Dependent variables in this 18-month temperature experiment are percent survival, growth rate, and patterns of reproduction.

We are also building an indoor, 100% recirculating mesocosm which will use untreated water from the Ohio River. Initial studies will examine the effect of current velocity on settling of postveligers. A headtank will be colonized by adults and temperatures will be maintained at 18–20°C. Veligers will be circulated through the channels at current velocities of 1.0–2.0 m/sec. Glass slides and other substrates will be placed in the channels and examined semiweekly to determine settling frequency.

ZEBRA MUSSELS: ENEMIES OR FRIENDS? Abraham Bijde Vaate, Institute for Inland Water Management and Waste Water Treatment, Rijkswater-staat, The Netherlands.

The zebra mussel has been a commonly occurring inhabitant in The Netherlands since 1827, when the first record was made. From the view of industrial activity it should be noted that the extension of the zebra mussel into Western Europe started before the period of industrial development in the 19th century. When the use of freshwater became more important for different purposes, the designers of intake systems already had to take the presence of zebra mussels into account, and antifouling measures were developed. Therefore the presence of zebra mussels in Western Europe did not have the same impact on industrial activity as they have nowadays in North America. Recently, studies have been started to use zebra mussels in water quality management: to reduce the amount of phytoplankton in eutrophic shallow lakes (a measure to increase the density of submerged waterplants), and to reduce the amount of polluted silt in the water supply of a manmade lake. Filtration rate and pseudofaeces production have been investigated for both employments respectively. They have been related to some important abiotic parameters. In addition, possibilities for obtaining sufficient densities of zebra mussels in these types of water management have been investigated as well. Removal of polluted silt calls for the whole water column to be filtered by the zebra mussels. Therefore an application of zebra mussels in hang-cultures (biological filter) was studied. A summary of the of all mentioned investigations is presented, and the role of sels in river and lake ecosystems is discussed.

CONTROL OF ADULT ZEBRA MUSSELS BY CHLORINE: COMPARISON OF LABORATORY AND FIELD STUDIES. John E. Van Benschoten, James N. Jensen, and **Daniel DeGirolamo,** Department of Civil Engineering, State University of New York at Buffalo, Buffalo, NY 14222.

Chlorine has been shown effective for the control of adult zebra mussels. In previous laboratory studies, zebra mussel mortality has been shown to be a function of chlorine dose, contact time, and water temperature. For example, in continuous flow laboratory studies (DeGirolamo *et al.*, presentation at the 1991 American Water Works Association Annual Meeting), 100% mortality was observed at 23, 27, and >36 days at chlorine doses of 3.0 mg/L (0–5°C), 1.0 mg/L (9–15°C), and 1.0 mg/L (0–5°C). Chlorination conditions on the field scale may differ from laboratory conditions. For example, field scale chlorinations may not be continuous for several weeks without interruptions in chlorine dosing due to equipment failure or a change in treatment goals.

The objectives of the present study are threefold. First, zebra mussel mortality was determined during full scale prechlorination at an operating drinking water treatment facility. Second, mussel mortality during full scale and laboratory scale chlorination was compared. Third, the results from intermittent chlorination studies will be used to determine if noncontinuous chlorination can explain differences in mussel mortality between the laboratory and full scale.

A full scale chlorination study was conducted at the Erie County Water Authority's Van de Water Water Treatment Plant (Tonawanda, NY) between April 8 and May 28, 1991. The plant treats water from the Niagara River. The nominal chlorine dose was 1.0 mg/L and water temperatures during the study period ranged from 6 to 12°C. A portion of the chlorinated raw water was diverted to flow through reactors where zebra mussel mortality was determined. Mussels were considered dead if they did not show any activity or respond to gentle probing after a 24 hour recovery period in unchlorinated water. Intermittent studies were conducted by pumping a chlorine stock solution into unchlorinated raw water prior to introduction into the reactors.

Mussel mortality during full scale chlorination exhibited an "S" shape observed in previous laboratory studies. Fifty percent mortality was observed after 30 days and 100% mortality after 50 days. Laboratory experiments under similar conditions (1.0 mg/L chlorine residual, 9–15°C) showed 50% and 100% mortality at about 13 and 27 days, respectively.

Hourly chlorine readings during full scale chlorination revealed that chlorine addition was not continuous. To investigate the effects of intermittent treatment, laboratory studies were conducted with three chlorine addition regimes: 1.0 mg/L chlorine dosed continuously, 1.0 mg/L chlorine dose with no chlorine added for four hours every 24 hours, and 1.0 mg/L chlorine does with no chlorine added for eight hours every 24 hours. The time to 50% mortality was 4.5, 8 and 18 days for continuous, 20 hour/24 hour and 16 hour/24 hour chlorinations.

Mortality was slower during intermittent chlorination even when the data were corrected for exposure time. This suggests that mussels recover and can withstand chlorination longer during intermittent treatment. Even intermittent treatment showed much faster mortality than the full scale chlorination. Thus, maintenance of a continual chlorine does appears to be critical for minimization of the time necessary for zebra mussel death.

EVALUATION OF THE EFFECTS OF CANDIDATE MOLLUSCICIDES ON TWO NONTARGET BIVALVES. D. L. Waller, L. L. Marking, and **J. J. Rach,** National Fisheries Research Center, U.S. Fish and Wildlife Service, P.O. Box 818, Le Crosse, WI 54602-0818.

A variety of molluscicides have been proposed for use in control of zebra mussels, but their effect on nontarget aquatic organisms has not been evaluated. Standard methods were adapted for assessing the toxicity of candidate molluscicides to two nontarget bivalves. Fingernail clams, *Musculium transversum,* and the fawnfoot mussel, *Truncilla donaciformis,* were selected to represent the two families of native bivalves. Both are similar in size to the zebra mussel and are commonly found in the Upper Mississippi River. Test organisms were collected from pools 6 to 9 of the Upper Mississippi River near La Crosse, WI. Handling, holding, and acclimation procedures were adapted to minimize the stress to both species of bivalves. Tests were run in triplicate with groups of 10 organisms in each test vessel. Static acute toxicity tests were conducted for 48 hours followed by a 96-hour monitoring period in untreated water to more fully assess survival and mortality. Toxicity data were analyzed by probit analysis to give LC_{50} values and 95% confidence limits. We evaluated the same chemicals as those tested at Ohio State University against zebra mussels. Results from our studies and those conducted at Ohio State University will be used to evaluate the effectiveness of chemicals in zebra mussel control and their potential hazard to nontarget organisms.

NEW INVASIONS, INCREASE, AND ECOLOGICAL EQUILIBRIUM OF *DREISSENA POLYMORPHA* POPULATIONS IN CENTRAL AND SOUTHERN EUROPE LAKES AND RIVERS. Norbert Walz, Zoologisches Institut der Universität München, Seidlstr. 25, O-8000 München, Germany.

Dreissena invaded Central Europe in two steps, first in the beginning of the 19th century, second after 1960. The River Rhine, for instance, was invaded in the first period. Stocks declined with saprobic pollution between 1950–70 and recovered by river sanitation measures after 1980. In the Northern Alps (e.g. Austria, Germany, Switzerland, France) many prealpine and alpine lakes were invaded in the middle of the 1960s. In the 1970s prealpine lakes in Southern Europe followed. Spreading to other than prealpine lakes in Yugoslavia, Italy and Spain was very rare. Stocks in the prealpine Lake Constance increased up to >10000 macroscopic visible individuals m^{-2} after about 5 years of colonization. *Dreissena* populations decreased further after 2 years because of predation by overwintering diving ducks (*Aythya ferina* and *A. fuligula*) and coots (*Fulica atra*). Mean consumption by waterfowl every winter is 97%. Young mussels (0^+) are protected by their small size until the next autumn, when they gain high biomasses again. Regular counts of larvae show fluctuations without a special trend on a low level, elucidating a predator controlled equilibrium. The same scenario takes place also in other lakes.

INFLUENCE OF POPULATION AGE STRUCTURE ON THE TOXICOKINETICS OF THE ZEBRA MUSSEL, *DREISSENA POLYMORPHA*. Tamara Lynn Yankcovich and **G. D. Haffner,** Department of Biological Sciences, Great Lakes Institute, University of Windsor, Windsor, Ontario, Canada N9B 3P4.

Field and laboratory observations indicate that *Dreissena polymorpha* colonization strategies, both on natural substrates and artificially-placed substrates, such as cement blocks, are dependent on population densities and age structure. Size frequency distributions of populations illustrate that habitat selectivity declines with increasing population density. It is likely that selective preferences are related to *Dreissena*'s filtering mechanism, where a "better" position equates to more food. Filtration represents a critical mechanism by which chemicals are bioaccumulated in organisms such as zebra mussels. Additional investigations are currently underway in order to determine the filtering rate and calibrate the chemical dynamics of *Dreissena polymorpha* in the laboratory and in the field. Once calibrated, this information will be used to index the toxicokinetics of *Dreissena polymorpha* with reference to population age structure and mussel orientation on substrate.

Journal of Shellfish Research, Vol. 11, No. 1, 243–244, 1992.

ERRATUM

APPROACHES TO ZEBRA MUSSEL CONTROL THROUGH INTERVENTION IN REPRODUCTION. Jeffrey L. Ram, Wayne State University, Department of Physiology, Detroit, MI 48201; **Susan J. Nichols,** U.S. Fish and Wildlife Service, 1451 Green Road, Ann Arbor, MI 48105.

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 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.

***POTAMOCORBULA AMURENSIS*, A RECENTLY INTRODUCED ASIAN CLAM, HAS HAD DRAMATIC EFFECTS ON THE PHYTOPLANKTON BIOMASS AND PRODUCTION IN NORTHERN SAN FRANCISCO BAY. Andrea Alpine** and **James Cloern,** U.S. Geological Survey, MS 496, 345 Middlefield Road, Menlo Park, CA 94025.

Potamocorbula amurensis, a benthic suspension feeding bivalve, accidently 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^3 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^3 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^2 compared with average annual production of 110 g C/m^2 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, F. H. Nichols, and **L. E. Schemel,** U.S. Geological Survey, MS 496, 345 Middlefield Road, Menlo Park, CA 94036.

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^2) 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.

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COVER PHOTO: Waved whelk, *Buccinum undatum* Linnaeus, 1758. Photo courtesy of François Hazel (see pp. 1–6)

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JOURNAL OF SHELLFISH RESEARCH

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REPRODUCTION AND GROWTH OF GREEN URCHINS *STRONGYLOCENTROTUS DROEBACHIENSIS* (MÜLLER) NEAR KODIAK, ALASKA

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ABSTRACT The factors affecting gonad recovery and quality of green urchins (*Strongylocentrotus droebachiensis*) were investigated near Kodiak, Alaska, to aid a developing fishery. Seasonal monitoring of a gonad index and ripeness indicated a single spring spawning with variable timing and duration. Mean index values were highest from January through the spawning period with index values for females significantly higher (45%) than males during this period.

The effect urchin size had upon the gonad index and sexual maturity was determined from a single prespawning sample. Index values were very low for urchins less than 17 mm test diameter but increased dramatically between 18 and 49 mm and leveled off at greater sizes. Maximum recovery rates per individual could be obtained by restricting harvest to urchins 50 mm and larger. Sexual maturity was reached at either age 2 or 3 and size at 50% maturity was 25.2 mm.

Growth was studied by tracking the progressive increase in size of modal groups observed in test diameter frequency distributions. Percent increase in test diameter between collections indicated that the majority of annual growth took place between March and September. Von Bertalanffy estimates of mean size at ages 1.0 through 4.0 were 9.9, 29.3, 44.0, and 55.1 mm. This growth rate is one of the highest reported for green urchins. The majority of a year class should reach fishable size (≥50 mm) at age 3.5.

KEY WORDS: reproduction, growth, *Strongylocentrotus droebachiensis*, green urchin

INTRODUCTION

Landings of the green sea urchin (*Strongylocentrotus droebachiensis* Müller) around Kodiak Island, Alaska, increased annually from 6 metric tons (t) live weight in 1985 (ADFG 1988) to 86 t in 1988 and have subsequently declined to 38 t (ADFG 1991). Information needed for managing this developing urchin "roe" fishery includes basic knowledge about factors affecting gonad recovery and quality as well as an estimate of growth. Despite a wealth of literature concerning sea urchin biology, area-specific information regarding reproduction and growth is not generally available.

Green urchins have a circumpolar distribution and are found over a considerable range of latitudes. Reports of spawning time vary from March through July, and the spring phytoplankton bloom has been suggested as a factor controlling the onset of spawning (Himmelman 1975, 1978). Spawning time is indicated (Giese 1959) by an initial gradual increase and then subsequent rapid decrease in the gonad index (GI). While the GI is a useful indicator of product recovery, actual recovery and suitability of green urchins for various markets also depend on additional knowledge concerning seasonal changes in gonad ripeness and color.

The primary reason for using a GI is to remove the effect of body weight on gonad weight and therefore allow comparison of reproductive condition or effort between samples from different times or places (Gonor 1972). While examining the limitations of gonad ratio methods, Gonor found that *S. purpuratus* Stimpson of differing body size did not have similar gonad ratios. Changes in gonad growth rates with increasing test diameter have also been shown for *S. intermedius* Agassiz (Fuji 1967) and *Lytechinus variegatus* Lamarck (Moore et al. 1963) and were suspected for *S. droebachiensis*.

Urchin growth rates exhibit a substantial spatial variability that is generally attributed to food availability (Fuji 1967, Ebert 1968, Himmelman 1986, Swan 1961). Much of the work concerning green urchin growth deals with "rocky-barren" populations where lack of food limits both growth rates and the ultimate size of adults (Raymond and Scheibling 1987, Keats et al. 1985, Lang and Mann 1976). Little work has been done on green urchin populations in other environments where food is abundant. Reliable age information is lacking for large size classes (Himmelman 1986) due partially to reservations about test-plate ageing (Propp 1977, Breen and Adkins 1976) and the limitations of size-frequency analysis.

This study of Kodiak green urchins was conducted to estimate size at age and to define the relationship between GI and season, sex, and size.

MATERIALS AND METHODS

Season and the Gonad Index

Urchins used for studying the effects of season on the GI were collected during dives from 1 to 5 m mean lower low water (MLLW) at a single location in Womens Bay, Kodiak, Alaska (57°43.3′N, 152°31.5′W). The sampling site was protected from wave action and was characterized by a broken rock slope (20–40%) down to approximately 4 m in depth where the grade became flat with substrate a mixture of cobble, silt, and shell. Brown, red, and green macroalgae were abundant and there was periodic input of drift algae, particularly in the fall and winter. Sampling intervals varied from 63 to 10 days and were reduced as spawning time neared. For each sample, 35 to 45 urchins were collected and returned to the lab where they were weighed, measured, opened, and the gonads removed and weighed. Water temperature was

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service (NMFS), National Oceanic and Atmospheric Administration.

continuously monitored by a Ryan Tempmentor[1] recording thermograph at approximately 8 m in depth. Gonad index was measured as,

$$GI = \frac{GWW}{LWW} * 100 \quad (1)$$

where GWW is gonad wet weight and LWW live wet weight in grams. For the first 10 months of the study (30 September 1985 to 29 July 1986), a single mean GI was calculated collectively from a predetermined sample of 4.54 kg (±0.02 kg). Beginning 29 August 1986, GI was determined for each urchin in order to obtain a measure of sample variance and to assess other factors which could affect gonad quality.

Individual urchins were allowed to drip dry on a paper towel for 1 minute and weighed live. Gonads were removed, blotted to remove adhering perivisceral fluid and weighed wet to the nearest 0.01 g. Sex, gonad ripeness, and color were also recorded. When the sex was not apparent due to absence of mature gametes, a fresh squash or preserved section was examined. Ripeness was coded as unripe, slightly ripe, or very ripe depending on the absence or relative amount of mature gametes which oozed from skeins at processing time. Some postspawning animals with very small amounts of residual gametes, in addition to having a very low GI, were also coded as unripe. Test diameter (TD) was measured to the nearest millimeter from the middle of one ambulacral area to the opposite interambulacral area with knife-edge calipers. Seasonal collections were limited to urchins measuring 60–80 mm TD. Statistical comparison of GI data was performed using a two-sample t test for unequal sample sizes on arcsine-transformed values and assuming equal variances between samples (Snedecor and Cochran 1967).

Sex and the Gonad Index

While processing prespawning seasonal samples, it became apparent that differences between male and female GI values were causing increased variance around sample means when sexes were combined. Therefore, GI was plotted separately by sex for the period over which complete sexing was available to determine when and to what extent means differed. Mean GI of male vs female was compared only within each sampling date with a t test.

Size and the Gonad Index

To determine the effect of TD on the GI, a size-stratified sample was collected over the entire observed size range. Initially, attempts were made to take this sample at the same location as the seasonal samples, but urchins smaller than 40 mm were difficult to find at this site and the few found between 30 and 40 mm already appeared mature. An alternate site was located nearby, in Chiniak Bay (57°37.5′N, 152°20.6′W), which was also shallow (1–2 m MLLW), had a variety of attached macroalgae, periodically experienced influxes of drift algae, and had adequate amounts of small urchins. Fifteen urchins were sampled per 5-mm interval except over the expected size range of maturation (15–39 mm) where sample size was increased. This collection was taken before spawning (3 March 1987) to maximize differences between mature and immature animals. Urchins >17 mm were processed, and the GI calculated in the same manner as that used for the seasonal study. An approximate GI value for very small urchins (4–11 mm) was obtained based on representative measurements of skein length and diameter using an ocular micrometer and an assumed specific gravity of 1.0.

Gonad index values from urchins >17 mm were fit to a logistic growth function,

$$GI = \frac{C}{1 + Ae^{B(TD)}} \quad (2)$$

where GI is the predicted GI at TD and A, B, and C are constants based on Marquardt's algorithm for nonlinear least-squares parameter estimation (Saila et al. 1988). These urchins were also coded for maturity based on the presence or absence of free gametes at processing time. These data were stratified by 5 mm and fit to a logistic function,

$$P_m = \frac{1}{1 + Ae^{B(TD)}} * 100 \quad (3)$$

where P_m is the percent mature at TD and A and B are constants by the method of Somerton (1980) using weighted nonlinear regression and the Levenberg-Marquardt algorithm (ZXSSQ, IMSL 1984).

The relationship between GI and TD, specifically for larger urchins, was additionally examined by two different methods. Gonad index values from only those urchins ≥50 mm in the Chiniak size-stratified sample were regressed on TD. Secondly, GI values from the Womens Bay seasonal data (n = 1115; range 60 to 80 mm TD) were first standardized to Z values to remove seasonal variation and then regressed on TD. Slopes were judged significantly different from zero if their 95% confidence intervals did not encompass zero.

Growth

Urchins used for growth studies were collected from the Chiniak sampling site. This sampling site is protected from wave action and is characterized by sand-cobble flats punctuated by patches of eelgrass and small depressions (approximately 1–2 m in diameter and 10–20 cm deep) containing empty clam shells. Urchins were found on the flats among cobble and in the depressions covered with shells. Each of these microhabitats was sampled equally every 3 months from March 1987 through December 1988 and all urchins were measured to the nearest millimeter.

Test diameter frequency histograms display conspicuous modes that appear to represent year classes of recruiting urchins. The mean size of each modal group was obtained using Macdonald and Pitcher's (1979) method of maximum likelihood estimation for grouped data (Program MIX 2.3, Ichthus Data Systems). Growth was described by subsequently fitting to these data a von Bertalanffy growth curve,

$$L = L_\infty (1 - e^{-K(T - T_0)}), \quad (4)$$

where L is the predicted test diameter, L_∞ the asymptotic diameter, K the Brody growth coefficient, T the age in years, and T_0 the hypothetical age at size zero (Saila et al. 1988). Relative growth for individual age groups was obtained from the increase in modal size between collections.

RESULTS

Season and the Gonad Index

Following spawning, the GI of Womens Bay green urchins increased steadily and reached a prespawning maximum by mid- to late winter (Fig. 1). Spawning was evidenced by both a significant reduction in mean GI over a relatively short time and a

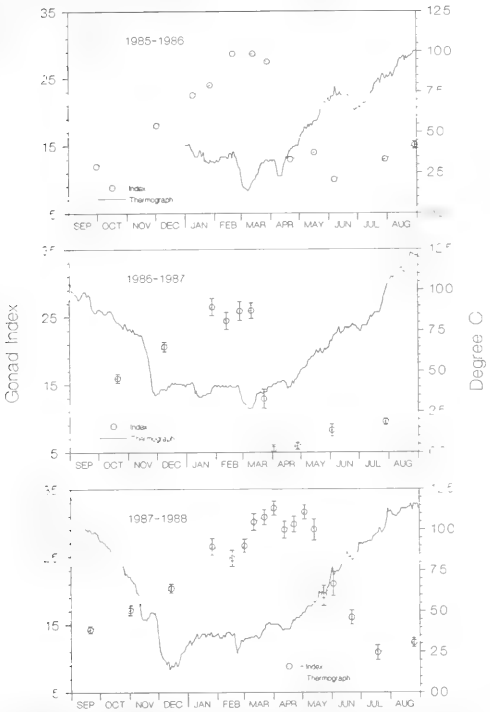

Figure 1. Seasonal gonad index (mean ± SE) for Womens Bay green urchins. "Thermograph" is mean daily water temperature at 8 m.

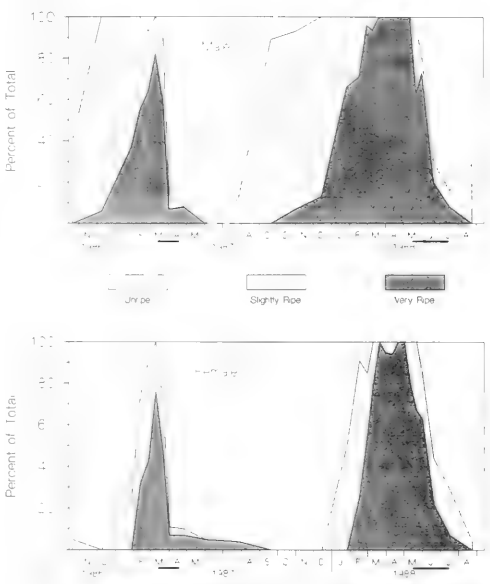

Figure 2. Percent of total by ripeness code for male and female green urchins from October 1986 through August 1988. Sample sizes per collection averaged 16.9 and 21.5 for males and females. Spawning interval (as indicated by drop in gonad index) noted on calendar axis.

concurrent change in the apparent ripeness of gonads (Fig. 2). Although this population appears to have a single annual spawning period, the timing and duration of the period varied considerably between years.

In 1986 and 1987, spawning was coincident with increasing water temperatures soon after the late-winter minimum (Fig. 1). Urchins spawned approximately 2 weeks earlier in 1987 than in 1986 and, while mean winter water temperatures were warmer in 1987, spawning occurred over roughly the same temperature range (2–5°C). Average water temperatures in 1988 were also warmer than 1986; however, the 1988 spawning took place much later at temperatures from 5 to 10°C. The resolution of GI sampling (10–14 d) precluded determining the actual date(s) or duration of spawning and therefore the analysis of temperature gradients as a possible cue.

The ripening process for male urchins was gradual and began in late summer; most were "very ripe" by late January (Fig. 2). Ripening in females occurred later than in males and was more dramatic; the majority were not coded very ripe until late February. Both sexes exhibited no marked differences between years, although once categorized very ripe in 1988 they remained in this condition longer, presumably due to the later spawning. Following the completion of spawning in each year, samples through the summer (approximately 40 urchins) typically contained one or two (usually female) ripe, unspawned urchins.

The observation of GI and ripeness by individual during the 1987 and 1988 spawning suggested that individuals do not completely spawn over the course of several days. Even during the most rapid of three spawnings (1987) the midspawning sample (Fig. 3) contained urchins characterized as having shed all ($GI < 8$), only a portion ($8 < GI < 20$), or none ($GI > 20$) of their gametes. Midspawning collections taken during the protracted (2 month) 1988 spawning also contained a similar mix of urchins in various stages of spawn-out.

Gonad color varied considerably between individuals but was typically straw yellow or orange. The most significant effect on gonad color was caused by ripening of the gonads. Males became paler and females a deeper orange as the accumulation of ripe gametes seasonally changed the overall color of the skein.

Sex and the Gonad Index

Mean GI values for the sexes remained similar (Fig. 4) and did not differ significantly (t test, $p > 0.001$) from postspawning through December. At this time mean GI for female urchins increased dramatically to a level considerably greater than males. In the four 1987 prespawning samples mean GI for females averaged 54% higher than males; in all four the sexes differed significantly (t test, $p < 0.001$). In nine 1988 prespawning samples, females averaged 35% higher and in all but one sample (12 April 1988) the sexes differed significantly (t test, $p < 0.001$).

Size, Age, and the Gonad Index

Gonad index values from the 3 March 1987 Chiniak size-stratified collection increased rapidly at sizes >25 mm TD, then

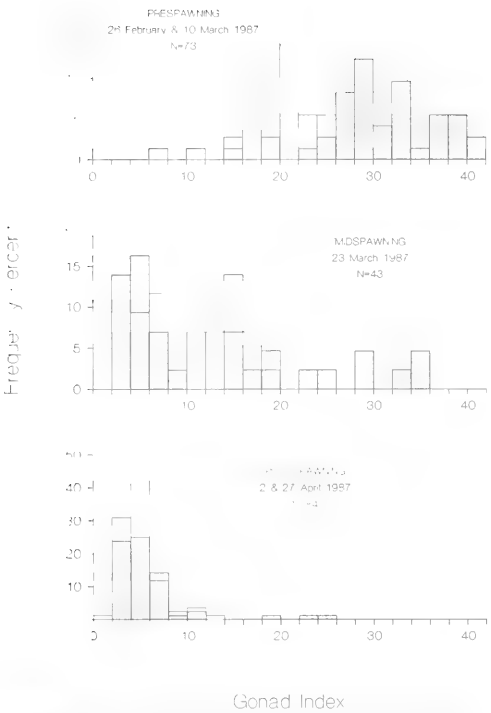

Figure 3. Frequency of gonad index values for pre-, mid-, and post-spawning seasonal samples of green urchins in 1987. Females with shading, males without.

Figure 5. A.) Gonad index and B.) percent maturity by test diameter for the 3 March 1987 Chiniak collection. A.) Diamonds show approximate GI for the 4–11 mm size class. Vertical lines mark divisions between age classes. B.) Points are data stratified by 1 mm and shown as sample size over the critical range. The logistic curve was fit to data stratified by 5 mm.

leveled off at about 50 mm (Fig. 5A). Because of the significant difference between sexes for prespawning urchins separate logistic growth curves were fit to these data. Since immature urchins could not be sexed but needed to be included in the logistic fitting procedure, Equation 2 was fit to data including immature urchins >17 mm and mature females ($A = 3311$, $B = -0.23$, $C = 26.5$, $r^2 = 0.78$) and then to immatures and mature males ($A = 779$, $B = -0.19$, $C = 23.4$, $r^2 = 0.86$). This resulted in higher r^2 values for the separate fits than for a single curve fit to all data. The approximation of GI (0.3%) for 4–11 mm urchins was not included in data used to fit these curves but was shown in Figure 5A for reference.

Figure 4. Seasonal gonad index differences by sex.

Each of the 22 urchins between 4 and 11 mm had translucent, thread-like gonads and was coded immature. Sexual maturity (Fig. 5B) was obtained between 18 and 33 mm and had a considerable effect on GI values. Urchins measuring 18 mm were all immature and had a mean GI of 0.86% (n = 7, SD = 0.23, range 0.7–1.3%). The smallest urchin to possess gonads with mature gametes was 19 mm. Over the 18–33 mm size range the mean GI of immature urchins (1.67%, n = 49, SD = 1.43, range 0.28–5.9%) was significantly less (t test, $p < 0.001$) than that of mature urchins (7.17%, n = 41, SD = 4.24, range 1.6–18.0%). Equation 3 ($A = 3463.2$, $B = -0.3229$) was fit to the percent maturity data stratified by 5 mm and the resulting estimate of 50% maturity was 25.2 mm TD (SD = 0.58).

Concurrent work on growth (discussed in next section) for this population allowed restratifying GI and maturity data by age. Divisions between ages 0.9, 1.9, and 2.9 were reasonably distinct (Fig. 7; 13 March 1987); however, there was overlap between age-2.9 urchins and those presumed to be age 3.9 and older. Nominal 1-year-old urchins (4–11 mm) were all immature and had a very low GI of approximately 0.3%. Approximately half the

2-year-old urchins (18–33 mm) were sexually mature with a mean GI of 4.2% for the entire age group irrespective of maturity. At 3 years of age (34–49 mm) all urchins were sexually mature with a mean GI of 18.6%. Urchins ≥50 mm were grouped together and considered age 4 and older; their mean GI (23.6%) was significantly greater (t test, $p < 0.001$) than that of nominal 3-year-old urchins.

Although results were mixed, the two additional analyses concerning the relationship between GI and TD for larger urchins provided no strong evidence for a change in GI over the size range examined. Regression of GI values on test diameter (Fig. 6A) for urchins ≥50 mm from the 3 March 1987 Chiniak size-stratified sample produced slopes of 0.12 for males and −0.05 for females; however, neither was significantly ($p > 0.05$) different from zero. The Womens Bay seasonal collections included many more ($n = 1115$) large (60–80 mm) urchins than did the single Chiniak collection. The standardized GI values for males (Fig. 6B) were significantly correlated ($n = 491$, $r = 0.10$, $p < 0.05$) with test diameter and the slope of the regression line (−0.02) was significantly ($p < 0.05$) different from zero. Females, however, showed no such correlation ($n = 624$, $r = 0.046$, $p > 0.05$), nor was the slope of the regression line (−0.009) significantly ($p > 0.05$) different from zero (Fig. 6C).

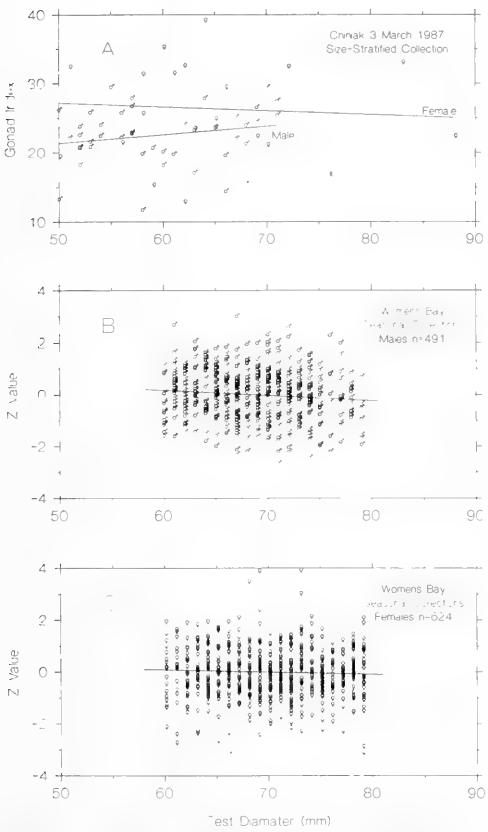

Figure 6. A. Regression of gonad index values on test diameter for male and female green urchins ≥50 mm from the 3 March 1987 Chiniak collection. B. and C. Regressions of standardized seasonal gonad index values (Z values) on test diameter for male and female Womens Bay green urchins.

Through the course of this study a substantial number ($n = 1620$) of TD and weight observations were made over a wide range of sizes (4–89 mm). Regression of natural log-transformed data produced the following relationship for Kodiak green urchins,

$$\ln(\text{LWW}) = 2.83 \cdot \ln(\text{TD}) - 7.18 \quad (7)$$

where LWW is live wet weight in grams ($r^2 = 0.998$).

Growth

Modes in TD frequency distributions from Chiniak samples (Fig. 7) were assumed to represent year classes in the smaller sizes. However, modes became obscure after age 3 as growth slowed and they merged with a multi-age group above 50 mm. A von Bertalanffy growth curve was fit to mean TD (Table 1) of the first 3 or 4 age groups (Fig. 8) and the parameters estimated were $L_\infty = 89.4$, $K = 0.28$, and $T_0 = 0.58$ ($r^2 = 0.99$, $F = 5402$, $p < 0.001$).

Relative growth rates (percent increase in TD between quarterly collections) fluctuated seasonally and decreased with increasing age (Fig. 9). Spring (March–June) and summer (June–September) growth, together, accounted for a majority of the annual increase in test diameter (75, 71, and 62% for the 1+, 2+, and 3+ age groups).

Initial diameter-frequency collections indicated a greater proportion of the smaller size classes were found in the "depression-shell" habitat than on the "sand-cobble flats." Subsequent subsamples were measured separately to verify that no gross difference in growth rates existed between these habitats which could account for the modes in a combined sample. This didn't appear to be the case, as modes existed at similar sizes (Fig. 10) in each subsample. Additionally, urchins were observed occasionally moving into and out of the depressions.

DISCUSSION

Season and the Gonad Index

The range in green urchin spawning times evident from 3 years of sampling at Kodiak was considerable and similar to the range generally attributed to the indirect effects of latitude on spawning time. Himmelman (1978) reviewed accounts of reproductive activity for *S. droebachiensis* and found no good evidence for a correlation of spawning with ambient water temperature. Water temperature observed during the Kodiak spawnings ranged from 2 to 10°C and also did not suggest temperature dependence. Himmelman (1975, 1978) also reported evidence that an increase in phytoplankton abundance was the probable cue for spawning in green urchins. Chlorophyl-α levels (National Marine Fisheries Service, unpublished data, P.O. Box 1638, Kodiak, AK 99615) indicate that locally, the spring phytoplankton bloom typically takes place from late March through April. At this time there is also a substantial reduction in water visibility observed while diving. Both the 1986 and 1987 spawning began during this time period; however, spawning in 1988 began 6–8 weeks after such a bloom. Spawnings considered anomalous by their observers have

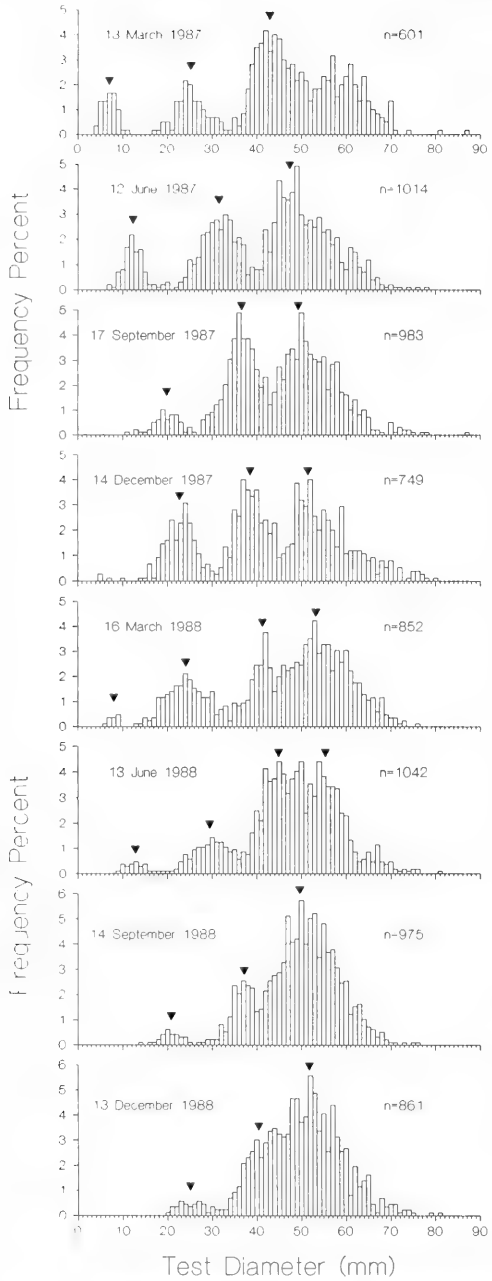

Figure 7. Test diameter frequencies from quarterly green urchin collections at Chiniak. Triangles mark estimated mean of modes used to fit von Bertalanffy growth equation.

TABLE 1.

Sample dates and estimated mean test diameter (mm) for probable year classes of Chiniak green urchins.

	Year Class			
Sample Date	**1987**	**1986**	**1985**	**1984**
13 Mar 1987		7.0	25.2	42.9
12 Jun 1987		12.3	31.5	47.4
17 Sep 1987		19.8	36.6	49.3
14 Dec 1987		22.7	38.5	51.4
16 Mar 1988	8.0	24.1	41.3	53.2
13 Jun 1988	12.8	29.5	44.9	55.4
14 Sep 1988	20.8	37.1	49.6	
13 Dec 1988	25.1	40.4	51.8	

also been reported for *S. purpuratus* (Bennet and Giese 1955, Gonor 1973a). Apparent irregularities in reproductive cycles are frequently attributed to changes in sampling location, small sample size, or variability in the numbers of different-sized animals used. Since I did not encounter these problems, a reason for the relatively late and prolonged 1988 spawning remains unknown.

The maximum GI levels reached prespawning in Womens Bay varied between years with higher peak values associated with later spawnings. Gonor (1973a) also noticed peak index differences between years for *S. purpuratus* and attributed them to a difference in the time when gonads stopped growing before spawning and not to a difference in rates of gonad growth throughout the growing period. Mean GI values for green urchins were noticeably uniform for 3 years in both magnitude and rate of increase from October through February. This contrasted with the highly variable levels outside that time period caused by changes in the timing and duration of spawning.

Accumulation of storage products, gametogenesis, and subsequent loss of gametes through spawning was the single largest factor affecting the GI of mature urchins. The collective reduction observed in the mean GI from pre- to postspawning was approximately 70%. After spawning began, sample means reflected the relative proportions having shed all, some, or none of their ga-

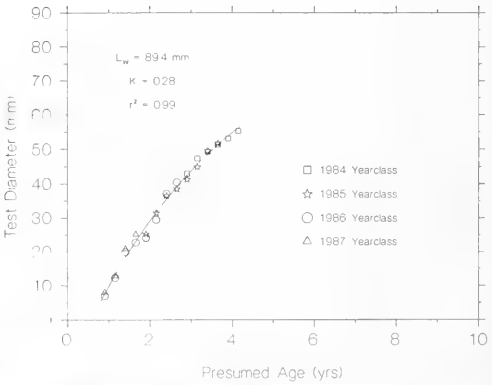

Figure 8. Composite size at age for Chiniak green urchins with fitted von Bertalanffy growth curve.

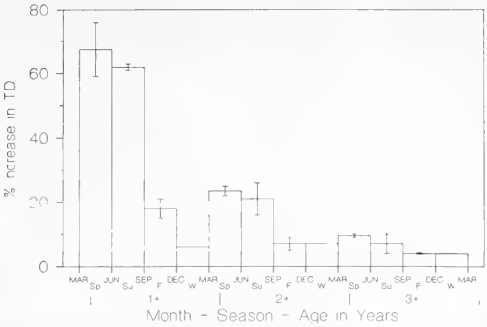

Figure 9. Mean seasonal percent increase in test diameter between quarterly collections for Chiniak green urchins by age group. Vertical lines on bars show the range of the two observations. Seasonal growth for winter (December–March) was only observed once.

metes. Protracted spawning adds this source of variability and should be accounted for when interpreting changes in the mean GI with time (Bennet and Giese 1955). For example, minor mid-spawning increases in 1986 and 1988 can easily be attributed to this and should not imply a real increase in the population's GI through gonad growth.

Complete spawning in Kodiak varied from a rapid 3 weeks in 1987 to almost 10 weeks in 1986 and 1988. During the 2 years when spawning was prolonged, its beginning was still characterized by an initial dramatic decrease in the index. Himmelman (1978) characterized spawning as a rapid event for British Columbia green urchins and observed it to take from 2 to 4 weeks to complete. However, he also found unspawned animals in post-spawning samples and suggested that spawning may be delayed in some areas.

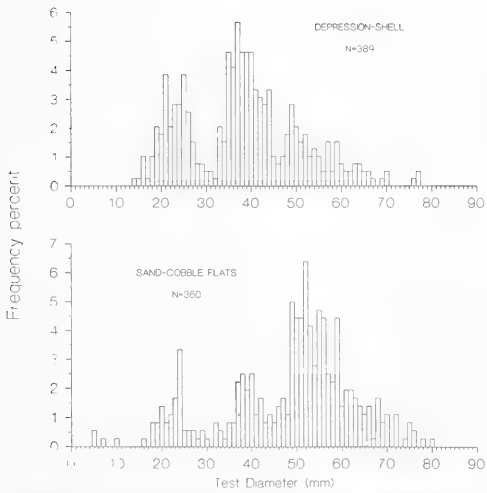

Figure 10. Frequency distributions from the two microhabitat types comprising the 14 December 1987 Chiniak collection.

The differences in gonadal ripening observed between sexes were distinct and involved both the time when free gametes first appeared and the subsequent changes in amount. Detailed work on developmental stages in *S. intermedius* and *S. nudus* Agassiz gonads by Fuji (1960a, 1960b) suggests these differences result from differing processes of gametogenesis. He found males produced germ cells continuously, causing a gradual change in both gonad size and the amount of free gametes. Females, however, characteristically showed ovarian eggs becoming free from the follicle wall and moving to the lumen at one distinct stage of gonadal development. Stephens (1972) also found Cape Cod green urchin females to ripen after males and indicated mature ova could be obtained from only 10% of the females in mid-December, 50% in late January, and 90% by mid-February.

Sex and the Gonad Index

Kodiak green urchin females displayed a significant December–January increase in GI which immediately precedes a dramatic increase in the amount of mature ova that ooze from roe during processing. Advanced oocytes from *S. intermedius* and *S. nudus* (Fuji 1960a) in the "pre-mature" stage show a large size increase as they free from the follicle wall and collect in the lumen as ripe ova. Late December is proposed as the time when local green urchin females enter this same developmental stage.

Stephens (1972) showed that *S. droebachiensis* had both a larger egg size and heavier jelly coat than *S. purpuratus* and *Arbacia puntulata* Gray. Such a larger egg size may be why *S. droebachiensis* display a sexual difference in GI but *S. purpuratus* (Bennet and Giese 1955), *S. franciscanus* Agassiz (Bernard 1977) or *S. intermedius* and *S. nudus* (Fuji 1960b) do not. However, neither Himmelman (1978) nor Cocanour and Allen (1967) reported similar sexual differences in their studies of *S. droebachiensis*. Significant differences in GI between sexes have been reported for a sand dollar, *Echinaracnius parma* Lamarck (Cocanour and Allen 1967) and a heart urchin, *Echinocardium cordatum* Pennant (Moore 1934). Differences noticed in *E. parma* were also limited to several months preceding spawning.

The pronounced effect of sex upon GI values for several months prior to spawning is an added source of variability and potential bias which should be considered in studies employing GI methods. Sex ratios differed from 1:1 at both Kodiak sites and were also reported to differ for other *Strongylocentrotus* spp. (Gonor 1973b; Bernard 1977) between sampling locations and times. A t test of proportions (Snedecor and Cochran 1967) showed that the Chiniak ratio differed significantly ($p < 0.05$, $n = 170$) from 1:1 with the proportion male being 0.59. The ratio from Womens Bay urchins also differed significantly ($p < 0.05$, $n = 1115$) from 1:1 with the proportion male averaging 0.44 and ranging from 0.29 to 0.56 for individual samples. Gonad index means for seasonal samples were computed as simple averages andnot weighted by sex. During the prespawning period this produced spurious fluctuations in sample means ranging from -0.45 to 1.19%.

Size, Age, and the Gonad Index

The relationship between the GI and test diameter for green urchins appears to exhibit an increase similar to that of related species and, as expected, this increase took place across the size range where maturity was achieved. The leveling of gonad growth at approximately 50 mm indicates that Womens Bay seasonal sam-

ples (60–80 mm) were well above a size needed to avoid the bias associated with using small urchins (Gonor 1972).

Reports of size or age at maturity for *S. droebachiensis* are scarce. Ripe animals as small as 36 mm were found (Paul and Paul 1984) in Homer, Alaska; however, they were also the smallest urchins taken in samples. Raymond and Scheibling (1987) proposed that Nova Scotia *S. droebachiensis* mature and spawn for the first time at an age of about 3 years and at sizes >18 mm. At least half the Chiniak urchins attain maturity and presumably spawn over this same size range, but they do so a year earlier. The estimate of size at 50% maturity (Fig. 5B) was identical to the estimated mode (25.2 mm) for the nominal 2 year age group (Fig. 7; 13 March 1987).

Interpretation of the increase in GI with TD is enhanced by the estimates of age. The addition of maturity information taken prior to annual spawning allows speculation as to why the GI does not reach a maximum at the same age all individuals are mature. The Chiniak prespawning GI values increased from near zero at age 0.9 to approximately 25% for adult urchins (age 3.9 and older). Most of this increase occurred across the 1.9 and 2.9 age groups. Because smaller 2.9-year-old urchins had lower values of GI than larger 2.9-year-old urchins (Fig. 5A), it may be reasonable to hypothesize that the smaller and less fecund of this age group will spawn for the first time, and had been the smaller of the age-1.9 cohort which were immature the year before. One (primiparous) spawning may occur before an urchin reaches the maximum prespawning GI. Gonor (1972) similarly concluded that gonad production for *S. purpuratus* was acquired gradually during the second and third year of life.

A logistic growth model (Fig. 5A) generally describes the increasing proportions of body weight that maturing urchins dedicate to reproduction. Relative proportions of immatures and primiparous spawners at age 2 and primiparous and second time spawners at age 3 are probably responsible for apparent sigmoidal increases in the GI to TD relationship. Beyond a size of 50 mm the Womens Bay standardized data suggest the relationship for green urchins, if not "flat" (i.e., asymptotic), declines only slightly with increasing size. Gonor (1972) and Moore et al. (1963) found a decrease in gonad ratios at larger sizes for two other species of urchins.

Growth

Ages for Chiniak green urchins were assigned by arbitrarily using mid-April as an estimate of spawning time. Therefore, modes observed in mid-March samples would be approximately 0.9, 1.9, and 2.9 years old. The size and more-or-less known age of juveniles settling in off-bottom larval collectors (Table 2) supports conclusions reached concerning the age of young modes in the Chiniak diameter-frequency collections.

Larval development for green urchins reportedly ranges between 51 and 152 days (Strathman 1978) and takes approximately 63 days (Turner 1965) at temperatures (4–7°) similar to that observed in Kodiak from April through June. Chiniak young-of-the-year were encountered as early as December but were more typically recruited to diver samples in March at 5–9 mm and presumed to be 11 months old. Settlement in the Gulf of Maine apparently occurs in June (Harris et al. 1985) and considerable numbers of juveniles (0.5–1.5 mm TD) were reported (Swan 1961) on 29 June at York, Maine. New recruits at St. Margarets Bay, Nova Scotia (Raymond and Scheibling 1987), were approximately 1–2 mm from September through November over the course of several years.

Growth rates and size-at-age for Chiniak urchins were both slightly higher than that observed by Swan (1958, 1961; Maine) and Propp (1977; Barents Sea) and substantially greater than that observed by Himmelman (1986; Newfoundland), Himmelman et al. (1983; St. Lawrence estuary), or Raymond and Scheibling (1987; Nova Scotia). Propps' (1977) work with Barents Sea green urchins is one of the few studies which assigns ages to animals over 50 mm although his methods were not apparent. The present study was only able to reliably track modes to 3 or 4 years of age and 50 to 55 mm. Between-year differences in growth were small and variation around the fitted growth curve was due primarily to quarterly sampling and changing seasonal growth. Predicted L_∞ and size-at-age for larger (>55 mm) urchins are likely overestimated (Paloheimo and Dickie 1965, Knight 1968) because data were limited to smaller, faster growing urchins.

Himmelman (1986) observed low growth for eastern Newfoundland juveniles with rates increasing as animals grew larger. He hypothesized that as food-limited juveniles shifted their cryptic behavior to a mobile existence they were able to obtain more food. While Chiniak juveniles are indeed cryptic, they apparently either receive enough of the periodically abundant drift algae or have adequate supplies of benthic microalgae to maintain high growth rates. It is unlikely that food abundance limited growth at the Womens Bay or Chiniak Bay site. "Rocky-barren" areas do, however, exist in the Kodiak archipelago and have been observed by the author and urchin divers to have smaller urchins.

During underwater collections, small urchins (<10 mm) were not often seen; most were found in the umbo of an empty clam shell as larger urchins were sorted from the attached shells and measured. Many studies underestimate the abundance of small, cryptic age group(s) to some degree, and this work is no exception.

Juvenile urchins at Chiniak showed a shift in the type of habitat they occupied at approximately age 3 when they were found more commonly on the sand-cobble flats. Whether this apparent behav-

TABLE 2.

Size, location, and age of juvenile green urchins from off-bottom collectors with known soak times in the vicinity of Kodiak Island.

Set	Pulled	Location	Test Diameter (mm)		n	Age in Months
			Mean	Range		
May 1986	Sep 1986	S. Kodiak Is.[1]	1.8	1.5–2.3	8	5
Jul 1987	Jul 1988	S. Kodiak Is.[1]	13.6	11–17	22	15
Apr 1990	Oct 1990	Middle Bay[2]	2.5	1.8–4.9	34	6

[1] Osborne, Kodiak Area Native Assn., Kodiak, Alaska, pers. comm.
[2] F. Blau, Alaska Department of Fish and Game, Kodiak, Alaska, pers. comm.

ior change was due to their outgrowing a physical niche provided by empty clam shells or because they reached a size less prone to predation is not known. Differential use of habitat by age groups has also been reported by Himmelman (1986) and Propp (1977), who found that small green urchins dwell predominantly under a crust of calcareous algae or in rock crevices for the first 2 years.

Recruitment of *S. droebachiensis* is reported to be quite variable between years and areas (Ebert 1983) and the evidence from the Kodiak sampling supports this. Recruitment at Chiniak appeared to occur annually from at least 1984 through 1987, although the 1987 year class was clearly not as strong as the previous three had been. Many local green urchin beds exhibit diameter-frequency distributions characterized by a unimodal (and presumably multi-age) group of animals between 40 and 70 mm. Thorough, yet unproductive searches of these areas for cryptic juveniles suggests sporadic recruitment may be a reason. The accumulation of fast-growing juveniles from successive years of lower settlement-survival is also a possibility. The initial multimodal size structure at Chiniak was quite different from that seen in Womens Bay and other unimodal sites. Harris et al. (1985) discussed differential survival for post-settlement urchins and its potential significance in determining their subsequent presence in samples. The depression-shell niche at Chiniak probably provides good habitat for both post-settlement and juvenile urchins.

Conclusions and Aspects Relevant to the Fishery

Determining the timing and degree to which season, sex, and size affect product recovery in the local fishery were primary objectives of this study. Of these factors, the annual reproductive cycle had the single most important and largest effect in determining a fishing season. The GI typically increased from roughly 14% in early October, at a rate of approximately 3% per month, to about 28% by late February.

The effect of sex upon recovery became an important factor from late December through spawning. During this interval, recovery from males averaged 20–25% and from females 30–35%. This dramatic increase in recovery from females also takes place at a time when ovary color is at its best.

Application of these results initially suggested that October through December might be the best time to harvest green urchins in Kodiak assuming a fresh product were desired. During this period an increasing GI has not yet been offset by probable reductions in recovery due to the softening texture of prespawning gonads in males. Some fishing, however, continues through late February, and it has been suggested (pers. commun., S. Guild, Northstar Seafoods, P.O. Box 3087, Kodiak, AK 99615, November 1989) that males may be used for some other product, as advanced ripeness precludes their use for the fresh market. This loss in value may then be compensated for by continued increases in the GI of females, whose later ripening does not preclude processing until closer to spawning.

Presuming a relative difference in the value of prespawning males and females exists, and given the observed departures from 1:1 sex ratios of different urchin beds it may be worthwhile to test beds and harvest those with higher ratios of males earlier in the fishing season and those with higher ratios of females later in the season.

The relationship between the GI and urchin size increased dramatically between 20 and 50 mm and suggests best recovery rates will be achieved by harvesting Kodiak green urchins >50 mm. While results were not conclusive regarding a trend in recovery rates beyond this size, any decline was small and probably <1% per 10 mm increase in TD through 80 mm.

The distinct multi-modal distribution encountered at Chiniak established younger modes as year classes and showed that most of these urchins recruit to the fall-winter fishery at 3.5 years of age. No size limit exists for green urchins delivered in Kodiak, however, buyers generally discourage divers from landing urchins less than 2 inches TD (50.8 mm). At this size and age all have likely spawned once and approximately half have had occasion to spawn a second time. These spawnings contribute few gametes though, due to a proposed low GI in first time spawners and also the exponential relationship between TD and body weight.

An unreliable L_∞ estimate precluded calculating a mortality rate at Chiniak and subsequently any meaningful analysis of yield per recruit. Age and growth information for larger size classes is still needed before questions of size limits and harvest levels can be addressed.

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WINTER MORTALITIES AND HISTOPATHOLOGY IN JAPANESE LITTLENECKS [*TAPES PHILIPPINARUM* (A. ADAMS AND REEVE, 1850)] IN BRITISH COLUMBIA DUE TO FREEZING TEMPERATURES

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ABSTRACT High mortalities of Japanese littleneck clams were investigated on 16 beaches in southern British Columbia during March and April of 1989 (9 beaches) and 1991 (7 beaches) following winters with periods of colder than normal temperatures. Histological examination was conducted on 159 tightly closed Japanese littlenecks collected from the beach surface (an unusual location) and on 99 apparently healthy Japanese littlenecks from the substrate. No infectious agents that might account for the mortalities were observed. However, gill damage consisting of necrosis (i.e., severe epithelial sloughing accompanied by disruption of the connective tissue) sloughing of ciliated epithelium without disruption of the connective tissue, and adhesion (i.e., clumping of the gill filaments and/or congestion of the connective tissues thereby occluding the internal water channel) was observed. A higher prevalence of gill damage occurred in Japanese littlenecks collected from the beach surface than in those dug from the substrate (84% and 27%, respectively). All other tissues appeared normal except for patches of necrosis in the digestive gland of 14% of the clams with necrotic gills. Histopathology of the gill tissues of Japanese littlenecks frozen in the laboratory (to -6.4°C and -7.2°C after 7.5 to 8.2 hr) was similar to that of Japanese littlenecks collected from affected beaches. The occurrence of adhesions in the gills of four clams by day 20 post freezing, probably represented an attempt by the clams to repair damaged gills. Surprisingly, 28 of the 238 Japanese littlenecks frozen during the three experiments survived the treatment unscathed. It was concluded that winter mortalities of Japanese littlenecks in British Columbia could be attributed to freezing that occurred during periods of low tides at least one month before the problem became evident.

KEY WORDS: *Tapes philippinarum*, mortalities, freezing, histopathology

INTRODUCTION

The Japanese littleneck also known as the Manila clam (*Tapes philippinarum* (A. Adams and Reeve, 1850)) (=*Tapes japonica*, =*Tapes semidecussata*, =*Venerupis japonica*, =*Venerupis philippinarum*, =*Venerupis semidecussata*, =*Ruditapes philippinarum*), accidentally introduced into British Columbia with the Pacific Oyster (*Crassostrea gigas*) from Japan, was first observed in Ladysmith Harbour, Vancouver Island in 1936 (Quayle 1964). The Japanese littleneck has since spread throughout the Strait of Georgia and along the entire western coast of Vancouver Island to become one of the major intertidal bivalves (Bourne 1982). The highest Japanese littleneck landings on record occurred in 1988 when 87% of intertidal clam landings in British Columbia were Japanese littlenecks (3908 tonnes) with a landed value exceeding $7 million (Fisheries and Oceans Canada, Annual B.C. Catch Statistics, Fisheries Management Pacific Region). Despite the success of this unintentional introduction, Japanese littleneck populations periodically have high mortalities during the early spring following severe winters (Quayle 1960, Bower et al. 1986).

From mid February through April 1985 (Bower et al. 1986), and again from 20 March to 11 April 1989 high mortalities of Japanese littlenecks were reported from various beaches in the Strait of Georgia. Less severe mortalities were observed in a few localities in the spring of 1991. Affected beaches were marked by an abundance of empty shells of recently dead Japanese littlenecks (a few containing decomposing tissue) as well as varying numbers of tightly closed and moribund Japanese littlenecks scattered over the surface of intertidal beaches. On these beaches, other species of clams and other molluscs did not appear to be affected.

No infectious disease agents have been found in Japanese littlenecks in British Columbia (Bower et al. 1992). However, a varying amount of damage to the gill tissue was observed in Japanese littlenecks from areas experiencing high mortalities. It was proposed that environmental factors were responsible for the Japanese littleneck mortalities (Bower et al. 1986). In order to substantiate this proposal, Japanese littlenecks from affected beaches were held in the laboratory and were examined histologically to confirm the absence of infectious agents. Also, attempts were made to reproduce the disease in the laboratory by exposing Japanese littlenecks to freezing. To compensate for the lack of information on the affect of freezing temperatures on bivalve tissues (Sparks 1972), the histopathology in Japanese littlenecks exposed to freezing was investigated.

MATERIALS AND METHODS

At the time of the mortalities in 1989 and 1991, Japanese littlenecks were sampled from 16 beaches (Table 1, Fig. 1). Within 6 hr after collection, 10 tightly closed clams from the beach surface and/or 10 apparently healthy clams dug from the substrate were fixed in Davidson's solution with acetic acid for histological examination. Prior to fixation, clams were shucked and the shell length and soft tissue wet weight determined. After at least 24 hr in the fixative two cross-sections, one through the region of the stomach and digestive gland and the other through the region of the heart and kidney were made. In most clams two additional sections, one through the adductor muscle and the other through the siphon, were taken. These pieces of tissue were prepared using routine histological techniques, and thin sections (5 μm) were stained with Harris's modified haematoxylin and 0.5% alcoholic eosin. Stained sections were examined under a compound microscope (100 to 1000× magnification).

At the time of sampling the nine affected beaches in 1989, additional Japanese littlenecks (between 30 and 57 tightly closed

TABLE 1.

Results of histological examination of gills of 10 Japanese littlenecks[a] (*Tapes philippinarum*) from the surface and/or substrate of various beaches sampled during investigations into mortalities in British Columbia during the early spring of 1989 and 1991.

Location Name (Identity Code)[b]	Date Sampled D/M/Y	Region of Beach	Shell Length (mm)	Number of Clams with Gill Damage Classified into Category[c]: 1	2	3	4	5	6
Fillongley Park,	21/03/89	surface	38.6 ± 4.1	7	2	1	0	0	0
Denman Island (A)		substrate	40.3 ± 5.7	0	0	1	0	4	5
Piper's Lagoon,	23/03/89	surface	38.3 ± 2.7	1	3	2	2	1	1
Nanaimo (B)		substrate	38.3 ± 2.7	0	0	4	0	0	6
Boulder Point,	28/03/89	surface	36.9 ± 2.8	2	1	2	0	2	3
Ladysmith (C)		substrate	37.7 ± 5.0	0	1	4	0	0	5
"Rosewall Creek",	29/03/89	surface	34.8 ± 2.8	4	1	0	1	1	3
Bayness Sound (D)		substrate	33.4 ± 2.5	0	0	2	0	0	8
"Hindoo Creek",	29/03/89	surface	41.6 ± 6.3	6	1	0	1	1	1
Baynes Sound (E)		substrate	37.0 ± 2.9	0	0	2	0	0	8
Qualicum Beach	04/04/89	surface	33.2 ± 2.9	7	0	0	2	1	0
(F)		substrate	34.0 ± 2.8	0	1	0	0	0	9
Parksville Beach	04/04/89	surface	32.7 ± 2.8	5	3	0	1	1	0
(G)		substrate	32.8 ± 2.6	0	2	1	0	0	7
Sibell Bay,	06/04/89	surface	40.2 ± 5.1	4	3	0	1	2	0
Ladysmith (H)		substrate	38.2 ± 3.5	1	0	2	0	0	7
Yellow Point	11/04/89	surface	32.8 ± 3.3	2	2	0	4	2	0
(I)		substrate	28.1 ± 3.0	0	0	0	0	2	8
Useless Inlet (J)	17/03/91	substrate[a]	46.0 ± 6.8	0	0	0	0	0	9
Malaspina Inlet (K)	18/03/91	surface	43.0 ± 5.6	1	2	0	2	5	0
Fanny Bay,	19/03/91	surface	35.3 ± 2.8	2	2	1	2	2	1
Bayness Sound (L)	16/04/91	surface	40.6 ± 4.8	3	0	0	1	1	5
Gorge Harbour (M)	21/03/91	surface	40.7 ± 5.5	4	3	0	1	0	2
Nanoose Bay (N)	21/03/91	surface[a]	37.0 ± 4.6	0	1	1	0	0	7
Departure Bay (O)	14/04/91	surface	40.4 ± 2.9	4	1	1	4	0	0
Mud Bay,	16/04/91	surface	37.9 ± 7.0	4	1	0	2	1	2
Baynes Sound (P)									

[a] Samples from which only 9 Japanese littlenecks were examined histologically.

[b] Letters in parenthesis correspond to locations marked on Figure 1.

[c] Categories of gill damage were classified according the following criteria:

Category 1—necrosis—clams with severe necrosis of both the epithelium and connective tissue in greater than ½ of the gill tissue in the histological sections (Figs. 6 and 7);

Category 2—sloughing extreme—clams with multifocal or diffuse patches of sloughed ciliated epithelium (Fig. 3) affecting greater than ⅓ of the gill surface and possibly with focal patches of necrosis (Fig. 4) affecting less than ½ of the gills;

Category 3—sloughing minimal—clams with focal to multifocal patches of sloughed epithelium affecting less than ⅓ of the gill surface and possibly with focal patches of necrosis and/or adhesion as a minor component of the overall histopathology;

Category 4—adhesion extreme—clams with multifocal patches of adhesion of the gill filaments and/or extensive haemocytes infiltration with congestion of the connective tissues thereby occluding the water channels within the gills (Fig. 5) affecting greater than ⅓ of the gills and possibly with patches of sloughed epithelial cells as a minor component of the overall histopathology;

Category 5—adhesion minimal—clams with multifocal patches of adhesion of the gill filaments and/or extensive congestion of the internal connective tissues affecting less than ⅓ of the gills and possibly with sloughed gill epithelium as a minor component of the overall histopathology;

Category 6—normal—clams with normal looking gills and none of the above conditions affecting greater than ⅛ of the gill tissues in the histological sections.

clams from the surface of the beach and between 40 and 98 apparently healthy clams dug from the substrate) were collected. Each group of clams was placed in its own 50 L pot tank at the Pacific Biological Station. The tanks were supplied with flowing ambient sea water (10 to 12°C and 30‰ salinity) and were monitored for mortalities every second or third day.

Three freezing experiments were conducted on Japanese littlenecks in early 1991. On 22 February 1991, about 300 Japanese littlenecks (mean shell length 37.8 ± 3.7 mm, n = 200) were collected from the substrate in Piper's Lagoon (location B, Fig. 1), a location not affected by mortalities in 1991. The clams were held in a 57 L flat bottom tank (10 cm depth of water) supplied with flow-through chilled sea water (about 3 L/min at 4 to 7°C and 30‰ salinity). Ten of the clams were used as controls and were preserved for histological examination on 25 February.

The first two freezing experiments were conducted on 5 March and 7 March. In both experiments, 80 Japanese littlenecks were placed in a chilled pyrex glass tray (35 cm by 22 cm) and totally submerged in 1.3 L sea water at about 5°C and 30‰ salinity. The clams were frozen in sea water to avoid complicating problems of desiccation, to make the experiments easily reproducible, and to circumvent difficulties inherent in describing and monitoring sub-

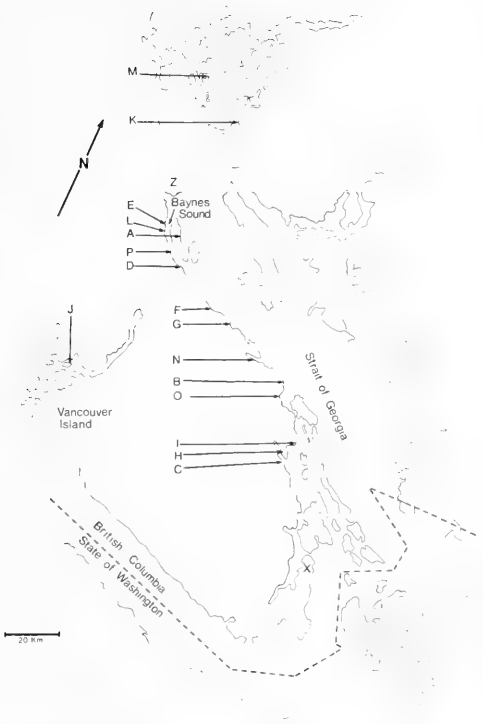


Figure 1. Localities (A to P) in British Columbia from which *Tapes philippinarum* were sampled between 21 March and 11 April 1989 and between 17 March and 16 April 1991 (see Table 1 for locality names and specific dates of sampling). Location X indicates Victoria International Airport and location Z indicates Comox Airport where meterological conditions were monitored.

strate conditions. The tray with clams in sea water was transferred to a freezer (set at −20°C). Water and air temperatures were monitored every 15 to 75 min without opening the freezer by using two fixed, thermistor probes. After 7.5 and 8.35 hr (roughly equivalent to the mean number of hours of exposure per day for Japanese littlenecks that are located at the 3 ± 1 m tide level in the Strait of Georgia), respectively, the tray with clams was removed from the freezer and submerged in a tank with flowing sea water similar to that for the original stock. During holding the clams were maintained in four groups: 1) dead clams (shell valves widely agape), 2) moribund clams (valves in the normal position but not tightly closed), 3) weak clams (valves as per the moribund group but with the clams closing tightly on handling, and 4) normal clams (the siphons out when undisturbed but siphons quickly withdrawn and valves tightly closed when disturbed). The day after freezing, 10 clams (three from each of the first two groups (1 and 2) and two from each of the healthier groups) were preserved for histological examination as described above. Japanese littlenecks from the "dead" group were discarded when the soft tissues poured from the shell. At the termination of the experiment on days 22 (experiment 1) and 20 (experiment 2), up to 10 clams from each of the surviving groups (groups 2 to 4) were preserved for histological examination.

The third freezing experiment was conducted as per the first two experiments except that 78 clams were exposed to freezing for about 7.5 hr on two consecutive days (March 14 and 15). Prior to the second freezing, 10 clams were removed for histological examination as per the first two experiments. To compensate for this loss of biomass, an additional 10 marked clams were added prior to, and then discarded after, the second freezing. After the second freezing, 10 additional clams were removed for histological examination. All remaining 58 clams were examined as per the first two experiments and the experiment was terminated on day 17.

RESULTS

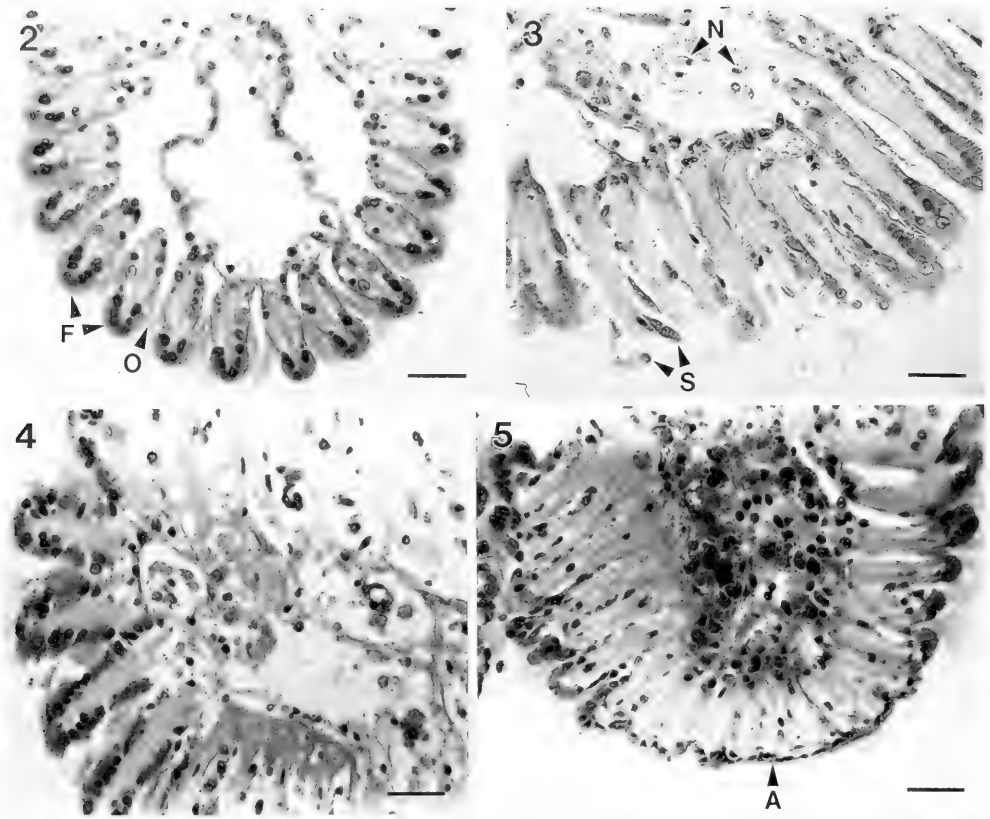
Histological Examinations for Disease

The size frequency distribution of moribund and dead clams was similar to that of the survivors, indicating that all sizes (and thus ages) were equally affected. The abundance of moribund clams on the surface of the affected beaches was usually low and correlated with population densities. Also, with each low tide cycle, a few additional moribund Japanese littlenecks could be found on the beach surface indicating that the mortalities continued to occur for about 3 to 4 weeks.

None of the 258 Japanese littlenecks from affected beaches that were examined histologically were infected with a disease agent that could have caused the mortalities. Most of the organs and tissues appeared normal. However, varying degrees of damage were observed in the gill tissues (Table 1). Japanese littlenecks with damaged gills were more prevalent in the group collected from the surface of the beach than those dug from the substrate (84% and 27%, respectively). In comparison with normal gill tissue (Fig. 2), the type and extent of gill damage in each clam was classified into one of six categories (Table 1).

Category 1 consisted of clams with necrotic gills (i.e., severe epithelial sloughing accompanied by disruption of the connective tissue in over half of the gill tissue present in the histological sections, Figs. 6 and 7). One percent of the clams dug from the substrate and 35% of the clams from the surface had necrotic gills. Category 2 involved extreme sloughing; it was evident as multifocal or diffuse patches of sloughed ciliated epithelium (Fig. 3) affecting more than a third of the gill surface and possibly with focal patches of necrosis (Fig. 4) affecting less than half of the gills. Extreme sloughing was observed in 4% of the clams from the substrate and 16% of the clams from the surface. Clams with minimal sloughing (Category 3) had focal to multifocal patches of sloughed epithelium affecting less than a third of the gill surface and possibly with small focal patches of necrosis and/or adhesion (see below) as a minor component of the overall histopathology. Sloughing was considered minimal in 16% of the clams from the substrate and 5% of the clams from the surface.

Gills of clams placed in Categories 4 and 5 all had obvious gill adhesions (i.e., clumping of the gill filaments and/or infiltration of haemocytes into the connective tissues causing congestion and occlusion of the internal water channels, Fig. 5). In some of these clams, focal sloughing of ciliated epithelial cells was observed but it only accounted for a minor component of the overall histopathology. Clams with extreme adhesions (Category 4) had multifocal patches of adhesions affecting greater than a third of the gills. None of the clams dug from the substrate but 15% of the



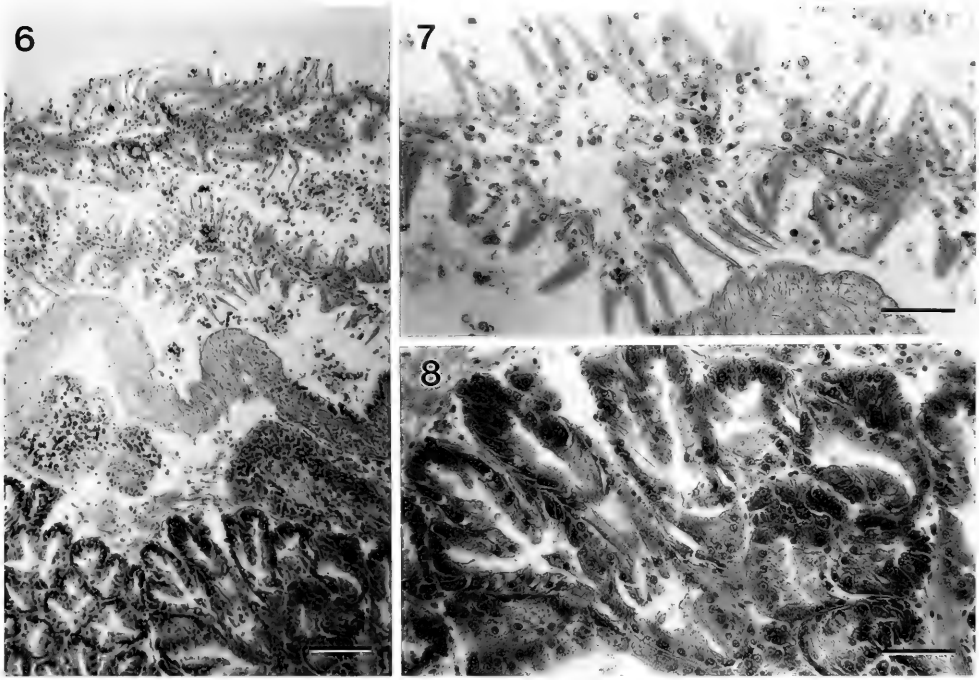
Figures 2 to 5. Histological conditions of gills from Japanese littlenecks (*Tapes philippinarum*) from British Columbia observed during the investigation into the cause of mortalities in the spring of 1989 and 1991 (haematoxylin and eosin stain, scale bars are 25 μm).
Figure 2. Structure of a normal eulamellibranch gill plica (fold) outlined by filaments (F, containing ciliated epithelium) which are separated by ostia (O) that allow water to pass from the mantle cavity into the water channels within the gill.
Figure 3. Sloughing of epithelial cells (S) from filaments with some necrosis (N) of connective tissue below the filaments.
Figure 4. Extensive necrosis and disruption of normal architecture within the gill but with filaments still supporting the ciliated epithelium.
Figure 5. Adhesion of gill filaments (A) and massive infiltration of haemocytes into the connective tissue thereby closing the water channel within the gill plica.

clams from the surface had extreme adhesions. Minimal adhesions (Category 5) were identified in clams with multifocal patches of adhesions affecting less than a third of the gills. Six percent of the clams dug from the substrate and 13% of the clams from the surface had minimal adhesions.

Category 6 included all clams that were considered to have normal gills (Fig. 2). Due to minimal damage to gill tissues that usually occurs during processing of clams for histological examination, clams with up to one eighth of the gill tissue damaged were placed in this category. All tissues of the clams in Category 6 were similar to those of normal Japanese littlenecks examined during an extensive survey of parasites and symbionts (Bower et al. 1992). Seventy three percent of the clams dug from the substrate but only 16% of the clams from the surface were identified normal.

At all beaches, Japanese littlenecks picked from the surface had a higher prevalence of gill damage than those dug from the substrate. However, the extent of gill damage and the number of clams affected, was variable for various beaches (Table 1). Also, the extent of the mortalities was more severe in 1989 than in 1991. This observation is reflected in the higher prevalence of Japanese littlenecks with gill damage picked from the beach surfaces in 1989 in comparison to equivalent clams collected in 1991 (91% and 73%, respectively).

In order to evaluate the morbidity of Japanese littlenecks with extensive gill damage, the digestive gland of all 57 clams classified as having necrotic gills (Category 1) was examined histologically for damage. Apart from the four clams in which the digestive gland was not present in the histological sections, the digestive glands of 36 (68%) of the Japanese littlenecks was normal



Figures 6 to 8. Histological sections of gills and adjacent body of a Japanese littleneck (*Tapes philippinarum*) with severe necrosis of the gill tissue but normal digestive gland morphology. This clam is representative of most clams in Category 1 (with necrotic gills) that were collected from the surface of beaches in British Columbia during the investigation into the cause of mortalities in the springs of 1989 and 1991 (haematoxylin and eosin stain).

Figure 6. Overview of necrotic gills and undamaged tissues of adjacent body (scale bar is 100 μm).

Figure 7. Magnification of eulamellibranch showing severe damage of sloughing and necrosis such that only a few necrotic cells and dense connective tissue remains (scale bar is 25 μm).

Figure 8. Magnification of normal-looking digestive gland and adjacent connective tissue (scale bar is 25 μm).

(Figs. 6 and 8). Nine clams (17%) had digestive glands in which some of the epithelial cells showed early evidence of necrosis (i.e., nuclear and/or cytoplasmic changes). Seven clams (13%) had focal patches of necrosis often encompassing only the epithelial cells within a few adjacent tubules. In only one clam (2%) did the digestive gland show advanced stages of degeneration. The digestive gland of all other Japanese littlenecks (Category 2 through 6) appeared normal.

Survival of Affected Clams in the Laboratory

Japanese littlenecks collected from the surface of the nine affected beaches sampled in 1989 had high mortalities in laboratory holding tanks (>82% after 32 days, Table 2) and most of the mortalities occurred within the first 10 days (Fig. 9). The accumulative mortality of Japanese littlenecks dug from the substrate of each beach varied between 4% and 78% over the 32 day holding period (Table 2). Unlike the clams collected from the beach surface, most of the mortalities in clams dug from the substrate occurred after the initial 10 days in the holding tanks (Fig. 9).

Clam Freezing Experiments

The subsample of 10 Japanese littlenecks from the group that was to be used in the freezing experiment were all normal by histological examination (Table 3). This signified that there was no indication of gill damage prior to experimentally freezing the clams.

The freezing regimen could not be exactly reproduced in each experiment using the same methodology due to mechanical limitations of the freezer. Air temperatures within the freezer fluctuated between $-4°$ and $-16.7°C$ and thus, the water temperature cooled at different rates (Fig. 10). At the termination of each session in the freezer, the clams were embedded in a solid block of ice. However, 28 Japanese littlenecks survived (six of them having been frozen twice) with no subsequent evidence of histopathology (Table 3).

It was difficult to determine the health of most clams after freezing and some clams that appeared normal had histological evidence of gill damage. The gill damage observed in all clams was similar to that observed in clams from the field and could be classified using the same six categories. However, two major dif-

TABLE 2.

Percentage of Japanese littlenecks (*Tapes philippinarum*) with severely damaged gills and corresponding accumulative mortalities during 32 days of holding in laboratory tanks.

Location Name (Identity Code)[a]	Date Sampled D/M/Y	Region of Beach	Percentage with Damaged Gills[b]	Mortalities in Holding Tanks[c]
Fillongley Park,	21/03/89	surface	90%	40/40 (100%)
Denman Island (A)		substrate	0%	18/49 (37%)
Piper's Lagoon,	23/03/89	surface	60%	39/47 (83%)
Nanaimo (B)		substrate	10%	2/45 (4%)
Boulder Point,	28/03/89	surface	30%	38/41 (93%)
Ladysmith (C)		substrate	10%	5/40 (13%)
"Rosewall Creek",	29/03/89	surface	60%	41/44 (93%)
Baynes Sound (D)		substrate	0%	16/98 (16%)
"Hindoo Creek",	29/03/89	surface	80%	29/30 (97%)
Baynes Sound (E)		substrate	0%	31/40 (78%)
Qualicum Beach (F)	04/04/89	surface	90%	45/45 (100%)
		substrate	10%	12/55 (22%)
Parksville Beach (G)	04/04/89	surface	90%	56/57 (98%)
		substrate	20%	12/90 (13%)
Sibell Bay,	06/04/89	surface	80%	53/57 (93%)
Ladysmith (H)		substrate	10%	6/60 (10%)
Yellow Point (I)	11/04/89	surface	80%	44/44 (100%)
		substrate	0%	9/40 (23%)

[a] Letters in parenthesis correspond to locations marked on Fig. 1.

[b] Calculated from results of histological examination and includes only clams with gill necrosis, extreme sloughing, or extreme adhesion (Categories 1, 2 and 4) as presented in Table 1.

[c] Recorded as accumulative number dead/initial number of clams in tank with percentage of clams that died in parenthesis.

ferences were noted: 1) presence of numerous sloughed ciliated epithelial cells adjacent to the gills of clams with severe gill damage even at the termination of the experiments (up to at least 22 days after freezing) and 2) a higher prevalence of extensive damage to the digestive gland in clams with necrotic gills (42% versus 2% observed in clams from the field). In addition, 1 of the 24 clams (4%) with necrotic gills from the freezing experiment had focal patches of necrosis in the digestive gland and three (13%) had evidence of early stages of cell necrosis. Eight of the 18 clams (44%) with extreme sloughing of the gill epithelium had extensive necrosis of the digestive gland. Also, the digestive gland of most dead and moribund clams examined on the first day after freezing was severely damaged.

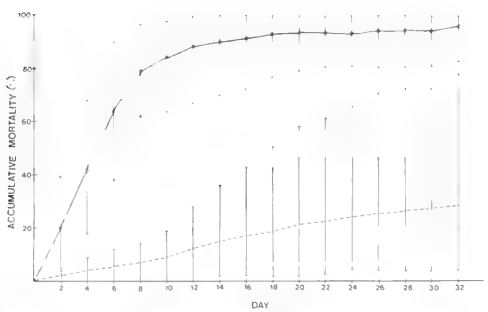


Figure 9. Mean accumulative mortality of tightly closed Japanese littlenecks (*Tapes philippinarum*) collected from the surface (solid line) and dug from the substrate (dotted line) of 9 beaches sampled in 1989 (Table 2) while being held in flow-through seawater tanks in the laboratory for 32 days. The bars indicate the range.

DISCUSSION

No aetiological agent was found that could have caused the Japanese littleneck mortalities and the laboratory holding studies did not indicate that an infectious agent was present. A variety of parasites and symbionts, including intracellular bacteria (Rickettsia or Chlamydia), protozoa (Apicomplexa and ciliates), and metazoa (Turbellaria and Trematoda metacercaria) were observed in the Japanese littlenecks. However, these organisms occur in similar prevalences and intensities in healthy Japanese littleneck populations (Bower et al. 1992). Also, parasites that have been associated with other clams of the Family Veneridae on the Pacific Coast of North America that were stranded on the substrate surface (specifically larval cestodes (*Echeneibothrium* sp.) in connective tissues of *Protothaca staminea* and *Protothaca laciniata* from California (Sparks and Chew 1966, Katkansky and Warner 1969, Warner and Katkansky 1969), and a coccidian in the kidney of *P. staminea* from Washington (Morado et al. 1984)) were not found.

The only pathology observed in the Japanese littlenecks from affected beaches was gill damage (Table 1). A higher prevalence of gill damage occurred in Japanese littlenecks from the abnormal location on the beach surface than in those dug from the substrate (84% and 27%, respectively). Also, there was a correlation between the prevalence of clams with gill damage and the mortalities of clams in laboratory tanks (Table 2) suggesting that the damage observed was sufficient to account for the high mortalities. Thus, it was concluded that the cause of the mortalities coincided with the cause of the gill damage. One explanation for the gill damage

TABLE 3.

Survival and results of histological examination of Japanese littlenecks (*Tapes philippinarum*, 37.8 ± 3.7 mm in mean shell length) following freezing in the laboratory.

Freezing Regimen[a]	Days After Freezing	Condition of Clams	Number of Clams (%)	Number Examined	Number of Clams with Gill Damage Classified into Category[b]: 1	2	3	4	5	6
Control	none	normal	248 (100%)	10	0	0	0	0	0	10
A	1	dead	3 (4%)	3	0	3	0	0	0	0
		moribund	35 (44%)	3	0	2	0	0	0	1
		weak	14 (17%)	2	0	0	1	0	0	1
		normal	28 (35%)	2	0	0	0	0	0	2
	22	dead	43 (61%)	0	—	—	—	—	—	—
		moribund	6 (9%)	6	4	1	0	1	0	0
		weak	7 (10%)	7	3	0	1	0	1	2
		normal	14 (20%)	10	1	0	1	0	1	7
B	1	dead	11 (14%)	3	0	3	0	0	0	0
		moribund	33 (41%)	3	0	0	3	0	0	0
		weak	17 (21%)	2	0	0	2	0	0	0
		normal	19 (24%)	2	0	0	2	0	0	0
	20	dead	46 (66%)	0	—	—	—	—	—	—
		moribund	9 (13%)	9	7	2	0	0	0	0
		weak	10 (14%)	10	3	1	2	1	0	3
		normal	5 (7%)	5	0	1	2	0	0	2
C1	1	dead	4 (5%)	3	1	0	2	0	0	0
		moribund	27 (35%)	3	0	1	2	0	0	0
		weak	27 (35%)	2	0	0	0	0	0	2
		normal	20 (25%)	2	0	0	0	0	0	2
C2	1	dead	7 (10%)	3	1	2	0	0	0	0
		moribund	34 (50%)	3	0	0	3	0	0	0
		weak	14 (21%)	2	0	0	1	0	0	1
		normal	13 (19%)	2	0	0	0	0	0	2
	17	dead	44 (76%)	0	—	—	—	—	—	—
		moribund	4 (7%)	4	4	0	0	0	0	0
		weak	6 (10%)	6	2	2	2	0	0	0
		normal	4 (7%)	4	0	0	1	0	0	3

[a] For groups A and B, 80 clams were submerged in sea water (1.3 L at about 5°C and 30‰ salinity) in a pyrex glass tray (35 cm by 22 cm) and placed into a freezer (set at −20°C) for 7.5 to 8.35 hr. In group C, 78 clams were exposed to freezing as for groups A and B and the procedure was repeated on the following day (C2). For a complete description of the methodology see text and for details on water and air temperatures during freezing for each group see Fig. 10. Controls were not exposed to freezing.

[b] Categories of gill damage were classified as in Table 1.

that is consistent with experimental observations is exposure to freezing.

Meteorological records indicate that the coldest period in the winter of 1988–1989 occurred between February 1 to 3 when mean daily air temperature ranged between −5.8 and −10.4°C at Departure Bay (location O on Fig. 1) and Victoria International Airport (location X on Fig. 1), respectively, with an extreme minimum of −12.0°C at Comox Airport (location Z on Fig. 1). These cold temperatures occurred in conjunction with winds from the north that peaked at 59 and 80 km/h at the airports in Comox and Victoria, respectively. Another cold period occurred between March 1 to 3 with daily mean temperatures at the two airports ranging between −0.7 and −3.2°C and an extreme minimum of −10.0°C at Victoria. The temperatures during the rest of the 1988–1989 winter were above these temperatures with the majority of the daily minimal temperatures above freezing. The winter temperatures of 1990–1991 when mortalities and histological damage to gill tissues was less severe were also less cold. Coldest periods occurred between December 19 to 22 and December 28 to 29, 1990. Mean daily air temperatures at the two airports ranged between −3.5 and −8.0°C with an extreme minimum of −11.7°C. However, these low temperatures were not accompanied by strong winds from the north. Minimum extreme temperatures dropped to about −6.0°C in late January and to about −4.0°C in early March but the remainder of the winter temperatures were moderate (Monthly record meterological observations in Canada, Atmospheric Environment Service, Department of the Environment).

Although temperature data of the beach substrate adjacent to the clams is not available, the meterological data suggest that the clams could have been exposed to freezing temperatures during the low tide cycles (from 1.3 to 0.1 m) that occurred during the nights (from 20:05 to 02:00) on the dates of the colder than normal weather reported above (Canada Tide and Current Tables, Volume 5 Juan de Fuca Strait and Strait of Georgia, published each year by the Department of Fisheries and Oceans, Hydrographic Service). The only exception was in early March 1991 when the low tide (1.5 m) occurred in the afternoon (14:50).

In addition to examining the histological effects of freezing temperatures in Japanese littlenecks, the laboratory freezing ex-

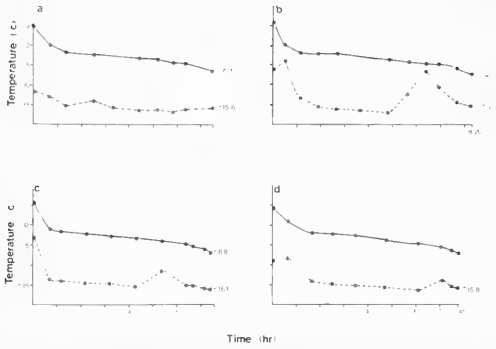


Figure 10. Thermographs of air (dotted lines) and water (solid lines) temperatures during sublethal freezing of Japanese littlenecks (*Tapes philippinarum*) in a laboratory freezer set at −15°C. Figures a and b correspond to the first two experiments, respectively, and figures c and d represent the two sequential freezings of experiment three (see text for details).

periments were an attempt to reproduce the field observations. Damage to the gills by freezing in the laboratory was similar to that of Japanese littlenecks collected from affected beaches. Although there were some differences (see last paragraph of Results), the laboratory freezing experiments demonstrated the ability of Japanese littlenecks to survive for prolonged periods after being frozen despite severe damage to the gills. Also, gill damage was then the only sign of injury in some of the survivors.

The differences observed between clams frozen in the laboratory and clams from affected beaches could be explained by the altered conditions experienced in the laboratory (i.e., in the field, clams are frozen in the substrate and not water with air temperatures to an extreme minimum of −12.0°C in comparison to between −15 and −16°C in the laboratory. Thus, freezing was probably more severe and holding conditions post freezing probably more benevolent in the laboratory). Histopathology in the digestive gland was more prevalent in clams frozen in the laboratory. It is likely that clams with such severe tissue damage in the field soon died and accounted for the accumulation of empty shells on affected beaches. The elapse of at least a month from the date of freezing to the time of examination of clams from the field also may explain the lower number of sloughed epithelial cells in the vicinity of the gills in comparison to the high numbers of sloughed epithelial cells observed in clams frozen in the laboratory.

Adhesions (i.e., clumping of gill filaments and/or congestion of connective tissue thereby occluding internal water channels (Fig. 5)), were observed in 19% of the Japanese littlenecks from the affected beaches and in 9% of the clams by day 20 post freezing in the laboratory. The adhesions possibly represent an attempt by the clam to repair damage incurred during freezing. Thus, some Japanese littlenecks seem capable of repairing damaged gill tissue.

The lack of microbial infections in damaged gill tissues of almost all Japanese littlenecks from both the laboratory experiments and from the field was unexpected. The mechanism(s) that prohibited microbes from flourishing in the severely compromised sues, which are exposed to the environment by virtue of their in the mantle cavity, requires further investigation.

f the laboratory experiments confirmed that some intertidal bivalves are tolerant of freezing temperatures as observed by Murphy and Pierce (1975) and Williams (1970). These workers and Kanwisher (1959) indicated that mortality due to freezing resulted from cell dehydration as a critical amount of tissue water is lost to extracellular ice formation. Murphy and Pierce (1975) and Murphy (1977a and b) concluded that increased freezing tolerance with low temperature acclimation results from an increased tolerance of cells to a greater level of dehydration by changes in cell metabolism and calcium dependent mechanisms.

The freezing experienced by the Japanese littlenecks in our laboratory experiments was probably more severe than in Japanese littlenecks exposed to freezing in the field during the winters of 1988–1989 and 1990–1991. Nevertheless, 28 of the 238 Japanese littlenecks frozen in the laboratory survived the procedure apparently unscathed. Possibly ice crystal formation (both in the mantle cavity and within the various tissues) was variable for individual clams or some clams may have had greater cellular tolerance to dehydration caused by ice formation.

In the Strait of Georgia, freezing temperatures coincided with periods of low night tides during December to mid February. However, exposed moribund clams were not observed until mid March or early April. The on-set of seasonal low tides during daylight hours (Canada Tide and Current Tables, Volume 5) may, in part, account for increased awareness of the mortalities. It is also possible that moribund clams are displaced to the beach surface through the movement of beach substrate via wave action during storms and receding tides (Zenkovich 1967). These moribund clams may be too weak to anchor themselves in the substrate with the foot and they thus gradually work their way to the surface. Once exposed on the surface, they may not be capable of digging back into the substrate. Another possibility is that clams with severe gill damage have a greatly reduced capacity to obtain oxygen. Also, Japanese littleneck activity probably increases in conjunction with the onset of spring which would increase the oxygen demand. Thus, damaged Japanese littlenecks may actively locate themselves on the surface where the oxygen is more readily available. As the tide recedes, the still living but severely damaged and weak clams are left stranded on the surface of the intertidal zone.

One marked feature of clam mortalities to date is that none of the native species of intertidal bivalves seem to be affected (Bower et al. 1986). The Japanese littleneck is relatively new to British Columbia and has colonized the mid-to-upper portion of the intertidal beach, an ecological niche that was not dominated by any other bivalve species prior to its introduction (Bourne 1982). The longer period of exposure during low tides and increased exposure to wind chill factors predispose this part of the beach to freezing during winter low tides. Thus, native species, which occur lower in the intertidal zone and deeper in the substrate, are probably not exposed to the same degree of freezing as Japanese littlenecks.

The extent of the mortalities on affected beaches could not be assessed accurately. Clam population estimates are available for only a few beaches. However, population estimates were only determined every two or more years. In the interim, beaches have undergone heavy commercial harvesting. Thus, the records could not be reliably compared with population estimates of survivors to determine the extent of the mortalities. Nevertheless, the accumulation of empty shells on affected beaches was excessive leaving little doubt that mortalities, particularly in 1989, were heavy.

Japanese littleneck mortalities in British Columbia that are attributable to freezing may be in addition to, or separate from, the cause of spring mortalities reported among Japanese littlenecks in

France. Goulletquer (1988) concluded that the cause of the Japanese littleneck mortalities in France was physiological, and that it resulted from increased energy consumption through anaerobic respiration at a time when energy reserves were low, food was scarce and the animal was resuming gametogenesis. It would be worthwhile to determine the metabolic reserves of Japanese littlenecks at the time of the mortalities in British Columbia.

Paillard (1992) described histological gill damage in moribund Japanese littlenecks in France and speculated that the cause could be attributed to the seasonal sulphureous conditions of the substrate or anoxia. None of the affected beaches in British Columbia were degraded or organically fouled. Thus, neither anoxia nor sulphureous accumulations were a problem. Nevertheless, it is interesting to note that at least two physical factors can cause similar histopathology. It would be interesting to identify the differences and possibly determine the processes induced by each of the physical insults by examining the ultrastructure of affected tissues.

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THE REPRODUCTIVE BIOLOGY OF THE JAPANESE LITTLENECK, *TAPES PHILIPPINARUM* (A. ADAMS AND REEVE, 1850) (BIVALVIA: VENERIDAE)

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ABSTRACT A study was undertaken of natural populations of Manila clams (Japanese littleneck clams), *Tapes philippinarum*, from five bays and inlets in the northwestern part of the Sea of Japan. Seasonal gonadal changes were observed histologically in samples of Manila clams collected from Vostok Bay between January and December 1985. Five stages of gonadal development were identified. The first stage was the Active Stage of growth and maturation. At the beginning of this stage the gonad was poorly developed. In April, gametogenic activity increased and a spreading system of follicles was observed in the gonad. Along with developing gametes in the follicles there were blood cells and reserve tissue. This stage ended in June. In the Ripe Stage, the gametes matured. The female gonad was filled with mature oocytes that measured 64 μm in diameter. The male follicles were filled with spermatozoa that formed strands. Spawning usually began in mid to late June when water temperatures reached 15–16°C. Most individuals spawned in July and August during which time the Partially Spent Stage of the gonad was observed. Spawning was nearly completed by October when water temperatures declined to 15°C. The Spent Stage was characterized by spent follicles that sometimes contained residual gametes. Individuals in this stage were seen until October. The final stage was the Resting Stage which was seen in November and December. During this stage resorption of residual gametes occurred.

Manila clams became sexually mature during the first year of life at a minimum shell length of 7–8 mm, however, this varied among populations. The sex ratio of most populations sampled was close to 1:1. Hermaphroditism and parasitic castration are described.

KEY WORDS: clams, *Tapes philipinarum*, development, populations, hermaphroditism, Sea of Japan, South Primorye

INTRODUCTION

The Manila clam (Japanese littleneck), *Tapes philippinarum* (Adams and Reeve, 1850) is a subtropical to low boreal species of the western Pacific. It is of commercial importance in Canada, China, France, Japan, Korea, and U.S.A. (Mann 1979a, Doumenge 1984, Shi et al. 1984, Bourne 1989, Chew 1989, Manzi & Castagna 1989). The reproductive biology of this species in the Far East of Russia has not been thoroughly studied (Kulikova 1979; Rakov 1986, 1988). In this paper we present data on the sex ratio of Manila clam populations and on the seasonal changes in gonads of *T. philippinarum* in the northwestern part of the Sea of Japan.

REVIEW OF THE LITERATURE

Synonymy

There are difficulties in studying the biology of *T. philippinarum* because of the many scientific names for this species in the literature: *Amygdala ducalis, A. semidecussata, A. philippinarum, Paphia bifurcata, P. philippinarum, P. (Venerupis) philippinarum, Protothaca philippinarum, Tapes decussatta, T. decussatus, T. denticulata, T. indica, T. japonica, T. philippinarum, T. semidecussata, T. variegata, T. violascens, T. (Amygdala) japonica, T. (Amygdala) philippinarum, Venerupis japonica, V. philippinarum, V. semidecussata, V. (Amygdala) philippinarum, V. (Ruditapes) philippinarum, Venus decussata, V. japonica, V. philippinarum, V. tesselata, V. (Tapes) decussata, Ruditapes philippinarum* (Partridge 1977, Scarlato 1981, Cesari & Pellizzato 1985, Chew 1989, Malouf & Briceli 1989).

A revision of species belonging to the subfamily Tapetinae and their synonymy has been given by Fischer-Piette and Métivier (1971).

Distribution

Natural populations of Manila clams occur in the Philippines, the South China and East China Seas, Yellow Sea, Sea of Japan, the Sea of Okhotsk and in shallow waters around the South Kurile Islands (Scarlato 1981) (Fig. 1).

Since the beginning of the 20th century Manila clams have been introduced to various parts of the world (Fig. 1). Manila clams were imported into the Hawaiian Islands from Japan (Bryan 1919, Thaanum 1921, Brock 1960) where populations exist at present (Yap 1977). In the 1930's the species was accidentally introduced to the Pacific coast of North America from Japan along with importations of Pacific oyster, *Crassostrea gigas*, seed (Quayle 1938, 1941) and it spread quickly along the west coast of the U.S.A. and Canada as far north as northern British Columbia (Nosho & Chew 1972, Bourne 1982).

During the late 1960's, Manila clams were imported into France where they are cultivated at present on both the Mediterranean and Atlantic coasts (Chevallier et al. 1975, 1976, Doumenge 1984, Rakov 1986, 1988). In the late 1970's, Manila clams along with Pacific oysters were introduced into the United Kingdom from Oregon and artificially propogated at Menai Bridge, North Wales. From the United Kingdom, Manila clam seed was exported to Ireland. Juvenile Manila clams from both English and French hatcheries were imported into Spain (Mann 1983). During the same years the species was introduced to Tahiti (French Polynesia) (Coeroli et al. 1984). Since 1983, Manila

Figure 1. Map showing the distribution of Manila clams, *Tapes philippinarum*, throughout the world. Arrows indicate the source of importations. ? indicates the source of the imports is unknown.

clams have been cultured in a lagoon near Venice, Italy (Cesari & Pellizzato 1985). Together with Pacific oyster seed, Manila clam seed was introduced into Flensburg Fjord in Kiel Bay, Germany from Scotland (Neudecker 1984). In recent years, experimental culture of this species has been carried out in artificial upwelling systems in St. Croix (U.S. Virgin Islands) (Rodde et al. 1976), in suspended culture in Tunisia (Gimazane & Medhioub 1979), in Belgian hatcheries (Claus et al. 1983), and in the effluent of marine fish culture ponds in Israel (Shpigel & Fridman 1990).

Along the far eastern coast of Russia, Manila clams occur in Primorye (Maritime Territory), near the western coast of Sakhalin Island, in Aniva Bay, Busse Lagoon and in the shallow waters of the South Kurile Islands (Kunashir and Shikotan Islands) (Golikov & Scarlato 1967, Scarlato 1981). In Primorye this species is distributed from Possjet Bay to the Amur estuary (Bazikalova 1931, Rasin 1934).

Reproduction

Tapes philippinarum is a dioecious animal (Bardach et al. 1972, Chew 1989, Eversole 1989, Devauchelle 1990). Only two instances of hermaphroditism (0.1% of the population) were reported in Manila clam populations from Big Beef Harbour and Misery Point in the Hood Canal, Washington (Holland & Chew 1974).

In natural populations, *T. philippinarum* becomes sexually mature in the first to the third year of age. In Akkeshi Lake (the northeast coast of Hokkaido Island) males mature at an age of two years and shell length of 22–27 mm, females at an age of 3 years and shell length of 30–35 mm (Yamamoto & Iwata 1956). Ko (1957 cit. in Holland & Chew 1974) observed earlier maturation of this species in Sasebo Bay, Japan. According to this author, mature gonads developed in Manila clams at a shell length of 12 mm and at shell length about 15 mm many individuals spawned. In ponds where juvenile Manila clams are raised in China (Dong Shi, Jinjiang District) 70% of Manila clams attained sexual maturity at the age of 1 year at which time they ranged in size from 12–15 mm (Qi & Yang 1988).

In Hood Canal (State of Washington, U.S.A.), Manila clams ...ped mature gametes at a shell length of 5–10 mm. However, they did not spawn at this size. A small proportion of the population spawned at a shell length of 15–20 mm and all individuals over 20 mm spawned which was in their first year (Holland & Chew 1974).

In Hawaii, the minimum size of male and female Manila clams with mature gametes was 15.1 mm and 15.3 mm, respectively. However, the major contribution to reproduction was made by individuals over 20 mm (about 1 year old), because the gonad was poorly developed in smaller individuals (Yap 1977). In French waters, Manila clams attained sexual maturity at an age of one year and a shell length of 15–20 mm (Devauchelle 1990).

In south Primorye, in Possjet and Slavyansky Bays, Manila clams began spawning at an age of 2 years, although the largest 1 year olds also had ripe gametes (Rakov 1986). In Vostok Bay, Manila clams became sexually mature at an age of 1 to 2 years (Denisenko 1978).

Spawning of *T. philippinarum*, can occur either once or twice each year depending on location and environmental conditions such as temperature (Table 1). In northern populations, spawning occurred once each year and was usually limited to one or two months that varied from April to October at various localities. Further south, spawning in some population extended for the duration of this period such as in Hood Canal, Washington, U.S.A. (Holland & Chew 1974). In other populations, such as those in the central and southern parts of Japan, Manila clams spawned twice each year, in late spring to early summer and again throughout the autumn (Obha 1959, Bardach et al. 1972, Kikuchi 1984). Populations located close to the equator spawn year round with peak spawning periods during December and January in Hawaii (Yap 1977) and again in May to July in Israel (Shpigel & Fridman 1990).

The fecundity of *T. philippinarum* as reported in the literature ranges from 4.32×10^5 eggs in individuals that are 20 mm in shell length to 2.35×10^6 eggs in individuals that are 40 mm in shell length (Yap 1977). Lower values of fecundity were reported in work from China, 1.88×10^5 and 1.503×10^6 eggs for Manila clams with shell lengths of 19 and 42 mm respectively (Shi et al. 1984). The diameter of ripe eggs has been reported as 63–66 Ùm (Miyazaki 1934), 60–75 Ùm (Loosanoff & Davis 1963) and 71–80 Ùm (Qi 1987).

MATERIALS AND METHODS

In the present study, *T. philippinarum* were sampled from the upper subtidal zone in five bays and inlets in the northwestern part of the Sea of Japan during 1984 to 1988 (Figs. 2 and 3). A square metal frame ($1/16$ m^2 in area) was used to collect clams randomly. All substrate was removed to a depth of 10 cm and then sifted through a 1 mm mesh screen and all adult clams removed. The clams were measured anteroposteriorly (shell length) to the nearest 0.1 mm. Age of clams was estimated by counting the number of annual growth rings (Zolotarev 1976, Silina & Popov 1989). A description of the sampling sites is given in Table 2.

For histological analysis of the gonads, 15 adult clams with a shell length over 20 mm were sampled monthly in Vostok Bay during 1985. Sections of gonads were fixed in Bouin's solution and embedded in paraffin. Histological sections 5–7 Ùm in thickness were stained with haematoxylin after the method of Heiden-

TABLE 1.

Spawning periods of the Japanese littleneck clam, *Ruditapes philippinarum*.

Location	J F M A M J J A S O N D	Temperature (°C)	Authority
Sakhalin Is.			
Busse Lagoon		18–20	Kulikova, 1979
South Primorye			
Vostok Bay, Litovka estuary		18–20	Denisenko, 1978
Vostok Bay, Srednyaya Bay		17–19	Denisenko, 1978
Vostok Bay, Tikhaya Zavod		15–24	This study
Slavyansky and Possjet Bays		18–22	Rakov, 1986
Japan			
Hokkaido is.		20–23	Cahn, 1951, cit. after Yap, 1977
Hokkaido is.			Yamamoto & Iwata, 1956
Tokyo Bay			Naito, 1931 cit. in Ohba, 1959
Tokyo Bay			Kikuchi, 1984
Hiroshima Bay			Hiroshima Fish. Exp. St., 1952 cit. in Ohba, 1959
Ariake Bay			Tanaka, 1954, cit. in Ohba, 1959
Kasaoka Bay			Yasuda et al., 1954, cit. in Ohba, 1959
Mucasa-shoal, the Bisan Seto		14–21,	Ohba, 1959
Channel, the Inland Sea		25–18	
Ariakenuomi			Kikuchi, 1984
Korea			
Inchon Bay			Choi, 1965
China			
Fujian Prov.		18–27	Qui et al., 1983
Fujian Prov.		18–27	Shi et al., 1984
Jinjiang Distr.		23–19	Qi & Yang, 1988
USA			
Oahu, Hawaiian Islands		21–28*	Yap, 1977
St. Croix, Virgin Islands		22–29	Rodde et al., 1976
St. Croix		22–26	Rodde et al., 1976
St. Croix		24–29**	Rodde et al., 1976
Big Beef Harbor, Hood Canal, Washington			Nosho & Chew, 1972
Big Beef Harbor, Hood Canal		13–20	Holland & Chew, 1974
Misery Point, Hood Canal		13–26	Holland & Chew, 1974
Canada			
Str. of Georgia, British Columbia		≥15	Bourne, 1982
France			
Thau Lagoon (Hérault)		20–25	Maitre-Allain, 1985
Normandy			Medhioub & Lubet, 1988
Israel			
Eilat		27–31	Shpigel & Fridman, 1990

Remarks: Thick line shows the period of intensive spawning, break—the spawning is obscure.
* Vermeij, 1978.
** Clams were induced to spawn by heat shock and addition of gonad suspension.

hain. The state of sexual maturation in the populations during the prespawning periods was done using smear preparations of the gonads. Statistical analysis was done according to the method of Urbakh (1984).

RESULTS AND DISCUSSION

Sexual Characteristics

The age at which Manila clams become sexually mature in the northwestern part of the Sea of Japan varied with geographic distribution (Fig. 4). In Possjet Bay and most sampling stations in Vostok Bay from about 3 to 60% of Manila clams were sexually mature in their first year. In most cases, the majority of clams that matured during their first year were males. In the second year over 80% of the clams from these two Bays were sexually mature. At this time in Melkovodnaya, Vladimir, and Olga Bays, less than 30% of Manila clams were sexually mature. In the third year, from 60 to 80% of the clams in Vladimir and Olga Bays were mature. At all locations, most of Manila clams over three years of age were sexually mature, and in most locations numbers of males and

Figure 2. Map of the northwestern part of the Sea of Japan showing the location of Peter the Great Bay and the location of study areas. 1. Vladimir Bay; 2. Olga Bay; 3. Melkovodnaya Bay; 4. Vostok Bay; 5. Possjet Bay.

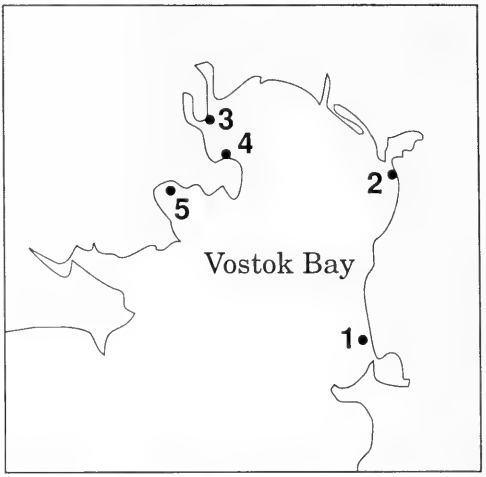


Figure 3. Map of Vostok Bay showing the location of sampling sites.

females were equal. The majority of the oldest clams were females.

Attainment of sexual maturity also depended on habitat. In Possjet Bay and in some localities of Vostok Bay (Stns. 2B and 3), all Manila clams were sexually mature in the second year and in most populations in Vostok and Melkovodnaya bays all were sexually mature at age three. In Olga Bay, sexual maturation of Manila clams was completed only in the fourth year and in Vladimir Bay in the fifth year.

The size (shell length) at which Manila clams become sexually mature varied among populations. In Possjet Bay and in most populations in Vostok Bay (Fig. 4) males matured at a shell length of 10–15 mm and females at 15–20 mm. In other populations that were sampled, sexual maturity occurred at a larger size. Most Manila clams in Vostok Bay became mature at a shell length of 20–30 mm. In Melkovodnaya and Olga Bays all Manila clams attained sexual maturity when their shell lengths were 30–35 mm while in Vladimir Bay some individuals as large as 40–45 mm did not have ripe gametes (Fig. 4). It should be noted that in many Manila clam populations there were individuals in the oldest (largest) age (size) groups whose sex was unidentifiable (Table 3). Occasionally smaller individuals were sexually mature. For example, in 1985, the minimum shell length of males in two populations in Vostok Bay (Stns. 2B and 4) was 8.0 mm and 8.6 mm respectively. At Station 2B, the minimum shell length of a sexually mature females was 7.5 mm.

Despite differences in minimum size at which sexual maturity is attained in males and females, a significant prevalence of males was observed only in some size groups (Fig. 4). Generally a sex ratio close to 1:1 was characteristic for most populations in the northwestern part of the Sea of Japan. An exception was the population in Vostok Bay where the number of females was higher than males, 1.5:1 (Stns. 2A and B) and 1.2:1 (Stn. 4, 1985) (Table 3).

Hermaphrodites were found in populations in Vostok and Vladimir bays but they did not exceed 2% of the population (Fig. 4, Table 3). Parasitic castration was observed in all populations except those in Possjet Bay (Table 3). The percentage of Manila clams infested with parasitic trematodes in Vostok Bay ranged from 0.3% (Stn. 4, 1984) to 4.1% (Stn. 2A). In the

TABLE 2.

The main characteristics of sampling sites in the northwestern part of the Sea of Japan.

Sampling Sites	Depth, m	Sediment	Degree of wave activity
Vladimir Bay	0.5–2.0	Boulder, coarse pebble with sand and shell	I–II
Olga Bay	1.0–1.5	Sand	II–III
Melkovodnaya Bay	0–0.5	Gravel, pebble	II–III
Vostok Bay			
St. 1	0.5–1.0	Boulder	II
St. 2A	0–0.5	Fine and medium sand	III
St. 2B	1.0–1.5	Gravel, fine sand	III
St. 3	0.5–1.0	Silty sand	I
St. 4	0–1.0	Gravel, pebble	I
St. 5A	0–0.5	Gravel, pebble	II
St. 5B	2.0	Medium sand	II
Bay	0.5–1.0	Sand	I–II

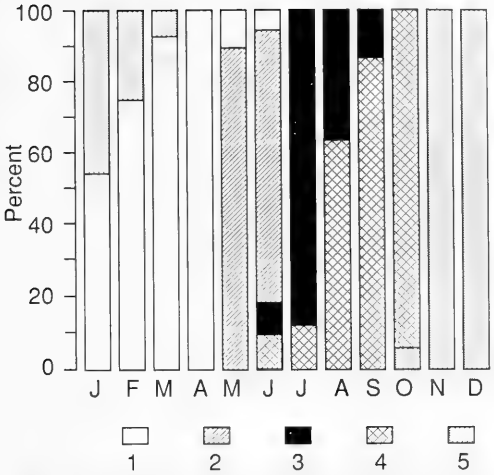


Figure 4. Distribution of male and female, hermaphrodite and castrated Manila clams, *Tapes philippinarum*, at sampling locations in the northwest part of the Sea of Japan in relation to age (A) and size (B). 1. sex unidentifiable; 2. male; 3. female; 4. hermaphrodites; 5. castrated animals.

other bays, the percentage of castrated Manila clams did not exceed 1.1%.

Reproductive Cycle

Seasonal changes in the gonads of Manila clams can be divided into several developmental stages.

Active Stage

This is the longest developmental stage and includes much of the basic gametogenic processes. At the beginning of this stage, the gonad was weakly developed. Small acini were loosely scattered in the visceral mass of the molluscs. In the female, oogonia and small developing oocytes occurred along the acinus walls. Gametogenic activity and enlargement of the system of acini was observed in April. The gonad was composed of small, near-wall oocytes (Fig. 5a). In the male, this stage was characterized by the presence of a fairly pronounced spermatogenous layer. Spermatogonia were visible along the wall of the acini. They had a lightly-coloured, oval-shaped nucleus, often with two nucleoli (Fig. 6a). Proliferation of spermatogonia proceeded in some regions of the acini. Rarely mitoses of spermatogonia was observed (Fig. 6b). Some individuals had numerous spermatocytes I and II in the gonad. There were few spermatozoa located in the centre of the lumen of the acini (Fig. 6c).

In both males and females, there were hemocytes (blood cells) and cells of reserve tissue along with developing gametes (Medhioub & Lubet 1988).

This stage ended during June in the study area.

Ripe Stage

In this stage, gametes located along the wall of the acini became fully mature. The amount of connective tissue and reserve cells in the acini decreased considerably and the acinus attained maximum size.

The female gonad was mainly composed of ripe oocytes measuring 64 Um in diameter (nonfixed cells). Many of the oocytes occurred near the wall of the acinus (Fig. 5b). In males a narrow spermatogenic layer was still present along the wall of the acinus. However, most of the gonad was occupied by spermatozoa arranged in strands (Fig. 6a). The head of *T. philippinarum* spermatozoa is shaped like a thickened comma.

Partially Spent Stage

In Vostok Bay, Manila clams began to spawn in the second half of June when water temperatures reached 15–16°C but most

TABLE 3.

Population sex structure of the Japanese littleneck clam, *Ruditapes philippinarum*, in the north-western part of the Sea of Japan.

Sampling Location	Year	No. of Animals	Male, %	Female, %	Juvenile, %	Hermaphrodites, %	Castrated %	Sex Unidentifiable, %
Vladimir Bay	1988	355	20.3	18.0	17.7	1.1	1.1	41.8
Olga Bay	1988	93	14.0	12.9	65.6	0	1.1	6.4
Melkovodnaya Bay	1985	97	30.9	42.3	25.8	0	1.0	0
Vostok Bay								
St. 1	1986	97	18.6	19.6	59.8	0	2.0	0
St. 2B	1985	390	14.1	21.5	63.3	0.3	0.8	0
St. 2A	1986	343	35.6	54.2	5.0	1.2	4.0	0
St. 3	1985	283	40.4	48.8	5.6	0.9	3.3	1.0
St. 4	1984	742	12.8	12.5	72.1	2.0	0.3	0.3
St. 4	1985	551	35.0	43.2	18.7	0.9	1.1	1.1
St. 5A	1985	349	16.0	19.5	57.3	0	0.6	6.6
St. 5B	1984	276	25.0	22.1	52.2	0	0.7	0
Possjet Bay	1988	148	14.2	12.2	73.6	0	0	0

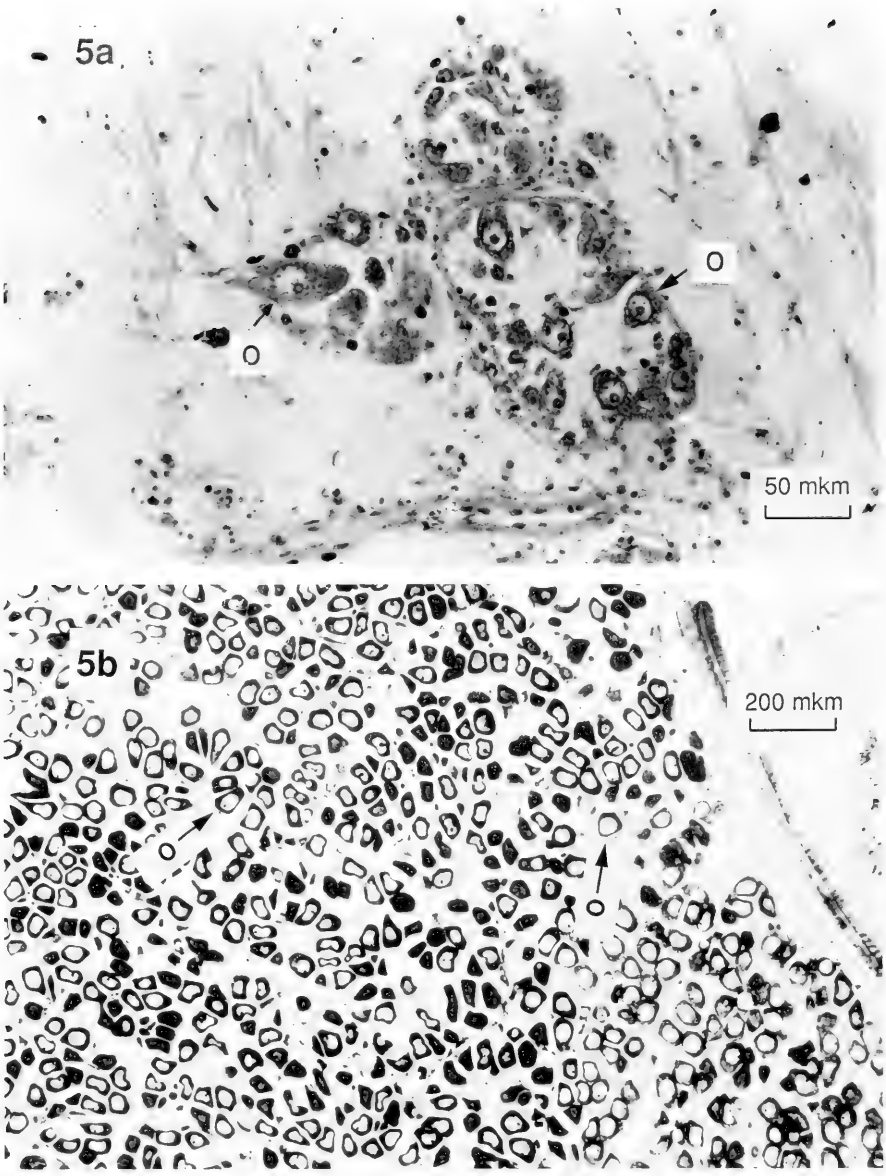


Figure 5. Photomicrographs of gonadal developmental stages of female Manila clams, *Tapes philippinarum*. A. Active Stage, developing oocytes along acini wall, scale – 50 μm; B. Ripe Stage, scale 200 μm. C. Spent Stage, scale 200 μm. (O = oocyte).

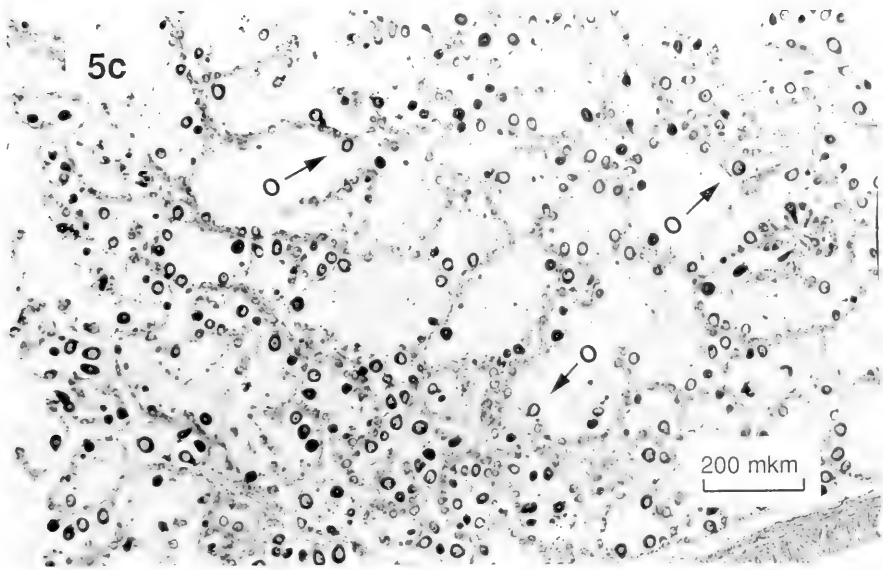


Figure 5. Continued.

individuals spawned in July and August (Fig. 7). There was one gametogenic cycle each year. In Vostok Bay, spawning was continuous during the reproductive period. However, there were peaks and lows in spawning activity. Spawning ceased at the end of September when water temperatures declined below 15°C.

Spent Stage

This stage was characterized by empty, shrunken acini. Residual gametes were present in some regions of the gonad (Figs. 5c and 6e). This stage lasted until November.

In October, resorption of residual gametes was completed. The connective tissue grew between the acini and their number and size decreased. Growth of reserve connective tissue inside the acini was characteristic for Manila clams in this stage (Fig. 6f).

Inactive Stage

In this stage it was difficult to determine the sex of animals even by histological examination of the gonad. Reduction of the gonad was most pronounced in November and December.

From January to March some gametogenic activity occured in gonads of some individuals which allowed sex identification. However, generally the gonad was undeveloped.

The percentage of each gonadal development stage in a population by month is shown in Figure 8.

The reproductive cycle of Manila clams in Vostok Bay was similar to findings for this species in other temperate waters. Spawning temperature and the single spawning per year indicated that spawning is primarily induced by water temperature, which is peculiar to this particular region, along with other, but none the less important and interrelated, environmental factors. Under experimental conditions, maturation of the gonad in *T. philippinarum* occurred at a temperature of 12°C and spawning occurred when water temperatures exceeded 15°C (Mann 1979b). As indicated above, in southern regions of Manila clam distribution, spawning is arrested at high water temperatures (Obha 1959, Maitre-Allain 1985). As with mussels in Primorye (Yakovlev 1986) low autumn temperatures arrest gametogenic activity and gonad maturation and prevent a second spawning.

Reproductive cycles in the same species can vary significantly with geographic location. This involves the timing of the gonadal cycle, the timing and duration of spawning and the number of spawnings per year. The phenomenon of a molluscan species spawning twice a year in the southern part of its range and only once in the northern part of its range has been reported for different species of mollusc (Ropes & Stickney 1968, Lubet 1984, Maximovich 1985, Yakovlev 1986).

A comparison of data on size and age at sexual maturity of Manila clams suggest that some individuals in populations at south Primorye became sexually mature at a smaller size than those in other parts of the world (Yamamoto & Iwata 1956, Yap 1977, Qi & Yang 1988). The situation observed in our study in south Primorye is similar to that in Manila clams in the State of Washington (U.S.A.) where males and females were sexually mature in the first year of their life at a shell length of 5–10 mm (Holland & Chew 1974). In most other areas the minimum size and age of sexual maturity is approximately the same, about 1 year of age and

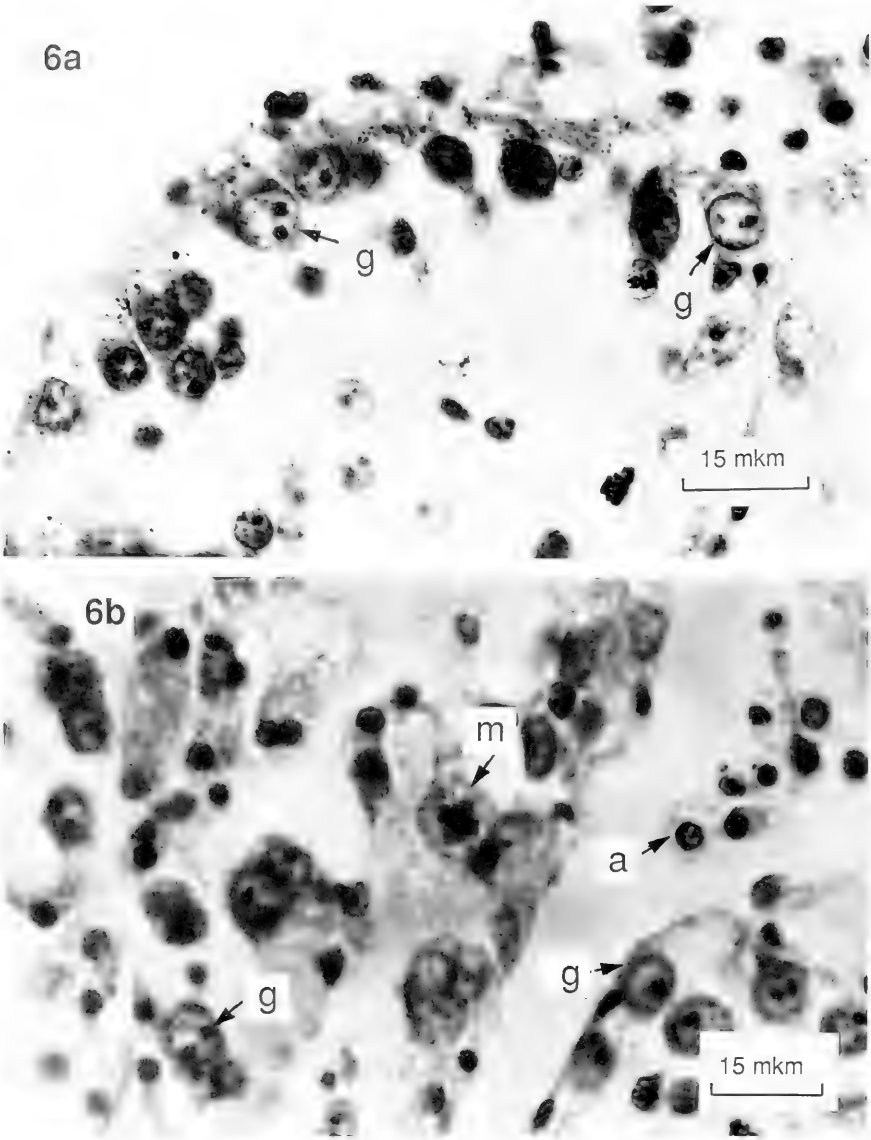
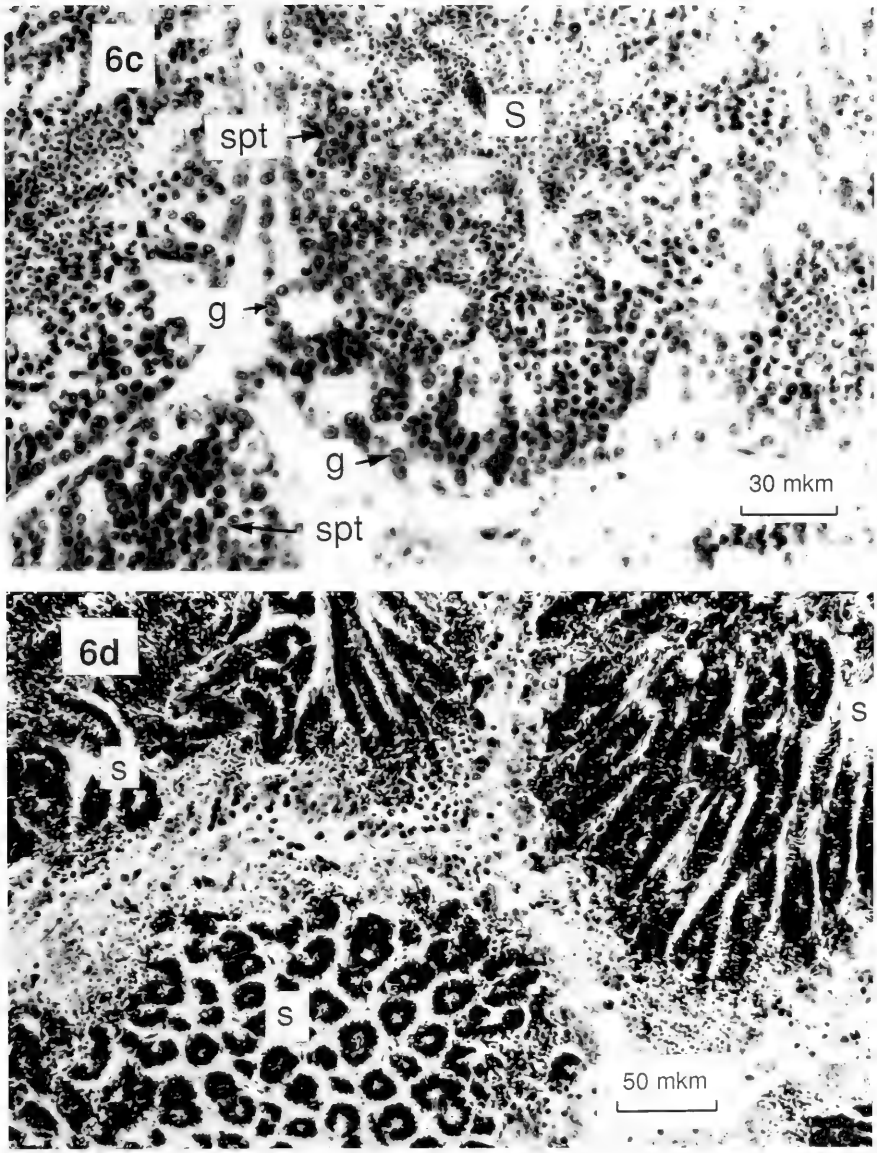


Figure 6. Photomicrographs of gonadal developmental stages of male Manila clams, *Tapes philippinarum*. A. Active Stage: spermatogonia with two nucleoli, scale 15 μm; B. mitosis of spermatogonia, scale 15 μm; C. developing male, scale 30 μm; D. Ripe Stage, scale 50 μm; E. Spent Stage, scale 30 μm; F. Resting Stage, residual spermatozoans and connective tissues in the lumen of acini, scale 50 μm. (a = amebocyte, g = spermatogonia, m = mitosis, s = spermatozoa; spt = spermatocyte; t = connective tissue).



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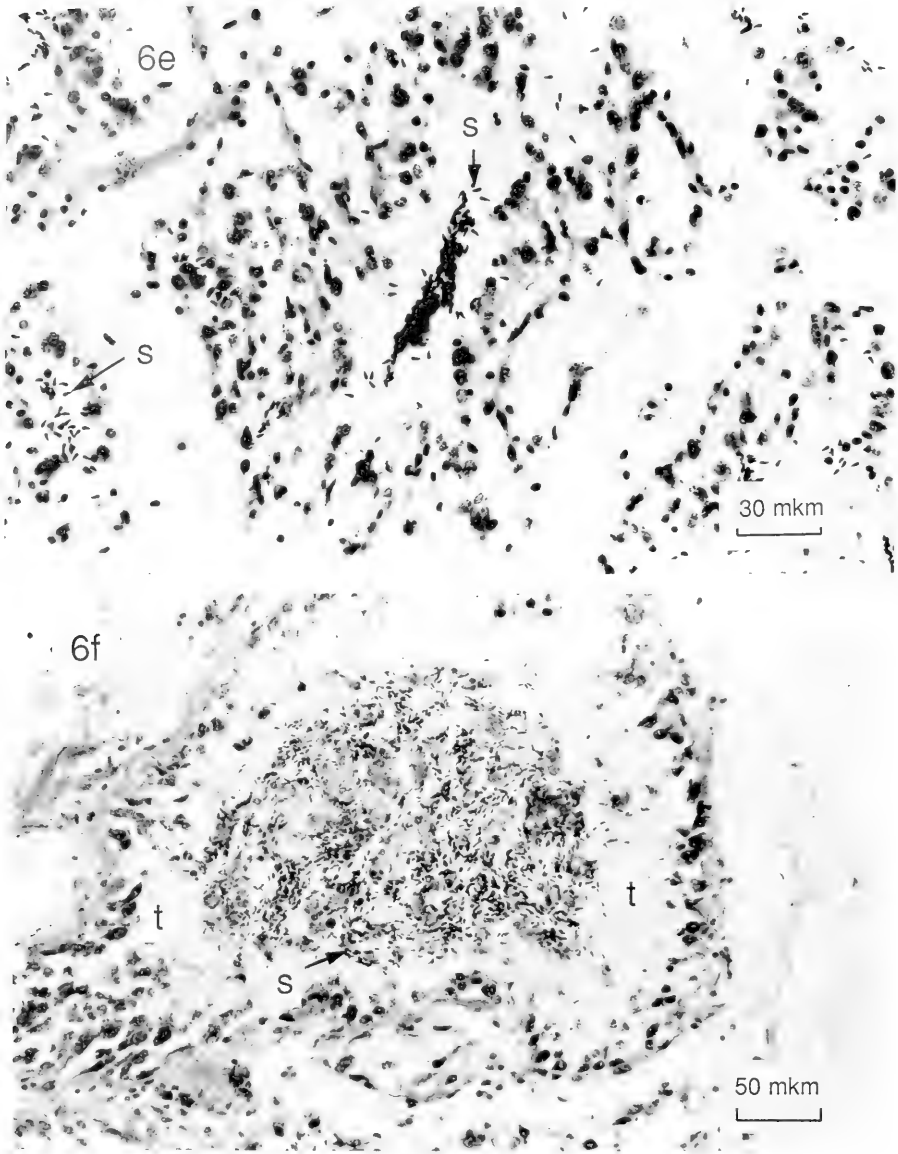

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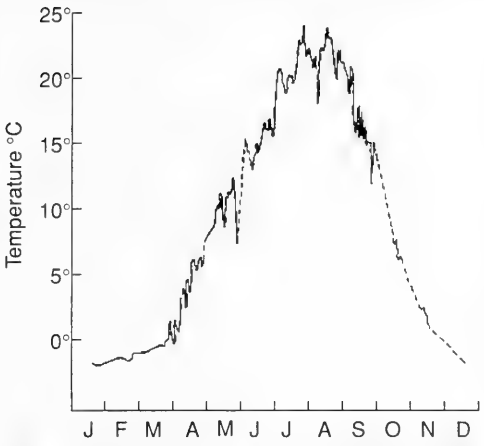

Figure 7. Seasonal changes in surface water temperatures (depth 0–0.5 m) in Vostok Bay during 1985.

a shell length of 10–15 mm (Ko 1957 cit. in Holland & Chew 1974, Yap 1977, Qi & Yang 1988).

Previous studies of the reproductive biology of *T. philippinarum* have shown that this species is dioecious (Bardach et al. 1972, Chew 1989, Eversole 1989, Devauchelle 1990). The number of hermaphrodites found in the present study and by other workers (Holland & Chew 1974) shows that this is an unusual phenomenon. Such unusual phenomena have been reported in other dioecious molluscan species (Ropes 1968, Naidu 1970, Bredge 1981, Mann 1982). However, Manila clams in some localities in the Sea of Japan show peculiar reproductive strategies. In Vostok (Stns. 3 and 4) and Possjet Bays all small (less than 15 mm shell length) individuals produced sperm during their first breeding season but in succeeding spawnings the number of males and females were equal but there was an excess of females in older (6–8 years) clams in some populations (Vladimir Bay and Stn. 4 samples in 1985 and Stn. 5A in Vostok Bay). Based on these statistically significant results we can assume that *T. philippinarum* is an alternative hermaphrodite in this region, according to Coe's classification (Coe 1943). The phenomenon of sex reversal may be connected with adaptation of this species to low boreal conditions in south Primorye.

Castration of Manila clams that is caused by the parasitic trematodes *Cercaria tapidis* and *C. pectinata* has been reported elsewhere in the literature (Rybakow 1983a and b).

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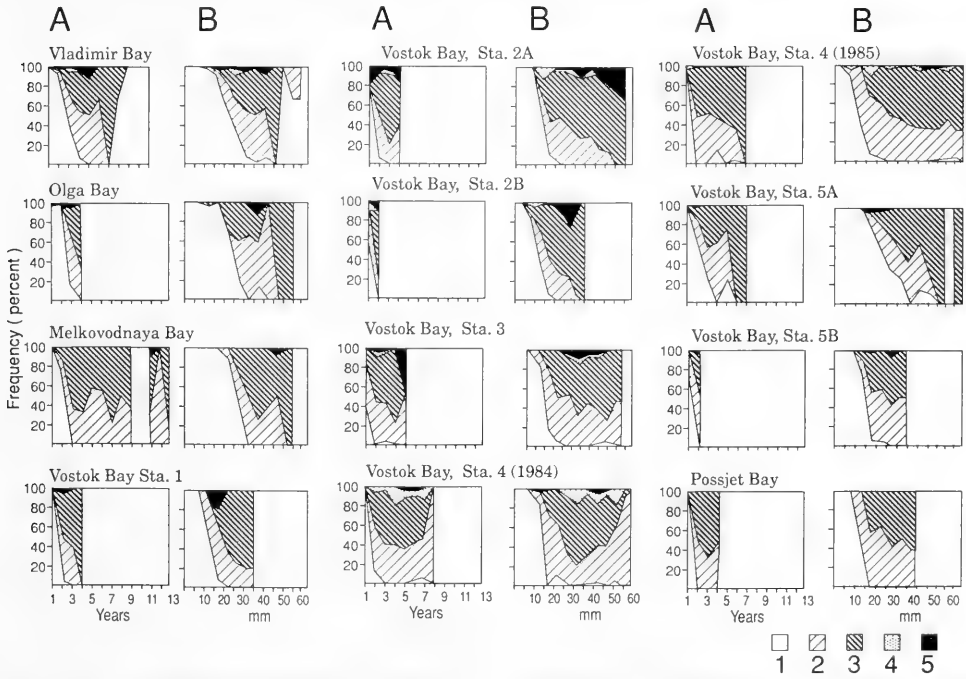

Figure 8. Stages of development in the gonadal cycle of manila clams, *Tapes philippinarum*, in Vostok Bay, 1985. 1. Active 2. Ripe 3. Partially Spent 4. Spent 5. Resting.

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A REVIEW OF THE FACTORS INFLUENCING THE GROWTH OF THE NORTHERN QUAHOG, *MERCENARIA MERCENARIA* (LINNAEUS, 1758)*

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ABSTRACT Factors affecting the growth of larval, juvenile, and adult northern quahogs, *Mercenaria mercenaria*, are reviewed. Larval growth is affected by temperature, salinity, current speed, dissolved oxygen concentration, and the amount of suspended sediments in the water, along with such nutritional factors as food quantity and quality. Growth of post-set juvenile and adult quahogs is similarly affected by the same physical and nutritional factors. Recent work suggests that there is a strong genetic contribution to quahog growth rate and that selective breeding programs may be useful for producing rapid growth strains. A growing body of evidence suggests that larval growth rates are poor predictors of post-set juvenile growth rates. Indeed, preliminary evidence suggests that shorter larval development periods correspond with higher rates of growth in post-set animals. Further research into this aspect of the developmental biology of quahogs is recommended.

KEY WORDS: quahog, *Mercenaria*, growth, development, larvae, juveniles

INTRODUCTION

With the long-standing interest in fisheries management of the quahog, *Mercenaria mercenaria*, there has been considerable work aimed at elucidating the factors affecting recruitment into the fishery and the subsequent growth rates of recruited juveniles, especially at the population level. The biology of larval and juvenile stages tends to be studied separately. A number of workers have examined the role of temperature, diet, and other environmental factors on larval survival and growth (reviewed by Pechenik, 1987). Traditionally, studies on juveniles have focused on the role of such physical factors as temperature, salinity, current speed, and substrate composition on quahog growth. With the increasing interest in commercial quahog culture, nutritional factors such as food quantity and quality have been a major focus. More recently, a number of culturists have begun focusing on the heritability of juvenile growth rates. The possible contribution of larval biology to juvenile growth has been much less studied.

Few studies have been undertaken to determine the possible interaction between rates of larval and juvenile development, and the extent to which larval biology and larval culture conditions may influence juvenile growth rates. It is generally recognized that larvae accumulate nutrients to fuel successful metamorphosis, but pre- and post-metamorphic quahogs feed using very different mechanisms. There is no *a priori* reason to believe that fast growing larvae will necessarily give rise to fast growing juveniles. Additionally, larval growth rate may not be the best indicator of overall development rate. In this paper, we review the factors known to influence the growth of both larval and juvenile quahogs, and identify some areas in which additional studies may be warranted.

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Environmental Influences on Larval Growth Rates

Effects of Temperature

Temperature is one of the influences on larval growth rate. Quahogs have been reared from egg to metamorphosis at constant temperatures between 18° to 30°C (Loosanoff et al. 1951). At 15°C or below, very few of the embryos reached the straight-hinge veliger stage. Above 33°C, abnormal larval development occurred and there was high mortality. There is strong evidence that larval growth is accelerated by warmer temperatures within the 18 to 33°C range. At 30°C, larvae can reach metamorphosis in 5 to 7 days, but at 18°C, it takes them 16–24 days to set and metamorphose (Loosanoff 1959). Davis and Calabrese (1964) and Davis (1969) reported that there is little interaction between salinity and temperature on the rates of growth and development, but decreased salinities reduced the range of temperature tolerance. Maximum growth in 27 ppt salinity occurred between 25 and 30°C. Lough (1975) reported larvae growing at maximal rates between 22.5 and 36.5°C.

Effects of Salinity

Changes in salinity have a minor effect on larval growth rates, compared with the effects of temperature changes. Quahog embryos and larvae grow at comparable, maximal rates between 20 and 32 ppt salinity (Davis 1958). The optimum salinity for embryo development was reported to be 27.5 ppt (Castagna and Chanley 1973), and the optimum salinity for larval growth was between 20 and 27 ppt salinity (Carriker 1961). Quahogs will settle and metamorphose only at salinities above 17.5 to 20 ppt (Castagna and Chanley 1973).

Effects of Dissolved Oxygen

Survival, development, and growth of quahog embryos are affected by dissolved oxygen concentration. In Morrison's (1971) study, embryological development was normal at dissolved oxygen concentrations of 0.5 mg O_2/L and above. At 0.34 mg O_2/L,

larvae did not develop beyond the trochophore stage. Quahog larvae stopped growing when dissolved oxygen was reduced to 2.4 mg O_2/L or below, but resumed growth once normal oxygen concentrations were restored. Larvae grew most rapidly when dissolved oxygen concentrations were at least 4.2 mg O_2/L.

Effects of Particulate Inorganic Matter

A number of experiments performed at the Milford Laboratory have shown that suspended sediments may inhibit larval growth, although effects varied markedly with the type of material in suspension (Davis 1960, Davis and Hidu 1969). Larval growth rate was inhibited at concentrations of 250 mg/L chalk and 500 mg/L clay and Fuller's earth. In contrast, quahog embryos and larvae grew and developed normally in concentrations of natural silt up to 750 mg/L. Larval growth rate was reduced in 1 g/L silt, and larvae did not grow at all at concentrations above 2 mg/L silt. Although quahog larvae can reject particulate inorganic matter (PIM) at low concentrations of suspended material, they lose this ability at higher PIM concentrations. This leads to the ingestion of PIM and subsequent deleterious effects on growth.

Effects of Nutrition

From the earliest days of bivalve mollusk hatcheries, phytoplankton has been cultured as food for larvae and for broodstock in their conditioning for spawning (Wells 1920; Loosanoff and Davis 1950, Loosanoff 1951). With the desire to optimize hatchery production of quahogs, a number of studies have been undertaken to quantify filtration rates on particular diets (e.g. Riisgard 1988) and to assess the nutritive value of various phytoplankton species for quahogs. Davis and Guillard (1958) and Walne (1970) compared the food value of several species of phytoplankton by monitoring larval growth (μm shell growth/day). Phytoplankton which were able to sustain rapid growth in quahogs included: *Isochrysis galbana, Dicateria inornata, Pavlova* (*Monochrysis*) *lutheri, Tetraselmis suecica, Thalassiosira pseudonana, Skeletonema costatum,* and *Chaetoceros calcitrans*. There is considerable evidence that mixtures of two or more good quality algal species will allow much faster growth of quahogs than any single phytoplankton species alone (Walne 1974, Epifanio 1976, Epifanio 1979a). One drawback with the use of cultured phytoplankton is that their nutritional value may vary with culture conditions (Wickfors 1986, Thompson et al. 1990), and may subsequently affect larval or juvenile growth (Wickfors et al. 1984, Whyte et al. 1990). A secondary drawback with algal diets is that in commercial aquaculture applications, production of algal foods for larval and juvenile bivalves can represent a major fraction of the total production costs. Walsh et al. (1987) estimated that the production of algae at the Aquacultural Research Corporation hatchery in Dennis, Massachusetts to be $250/kg dry weight. To lower production costs and standardize food quality, efforts have been taken to develop artificial (non-algal) diets for bivalve larvae and juveniles. Although some non-algal food supplements have been used for rearing bivalves (e.g. Haven 1965), there has been very little progress toward a nutritionally complete artificial diet for bivalve larvae (Epifanio 1979b, 1982, Urban and Langdon 1984, Coutteau and Sorgeloos 1992). Langdon (1982) used the technique of microencapsulation of nutrients as the basis of his artificial diet, but the growth of larvae was only about 20% of his algal-fed controls. The reasons for this slow progress in developing an artificial diet include the difficulty of determining the specific nutritional requirements of bivalve larvae, the digestibility of potential food items, and the leaching of water soluble nutrients from particles in the optimum 3 to 35 μm/size range (Webb and Chu 1982, Langdon and Siegfried 1984).

Environmental Influences on Juvenile and Adult Growth Rates

Effects of Temperature

Growth of post-metamorphic juvenile and adult quahogs is greatly affected by temperature. Ansell (1968) reviewed the growth of quahogs in various locations along the eastern coast of the United States, and concluded that the optimum temperature for shell growth was about 20°C and that shell growth ceased below 9°C or above 31°C. There was little evidence that the relationship between temperature and quahog growth rate differed throughout its geographical range.

In his studies of the effects of temperature on quahog growth, Ansell (1968) compared growth of animals of roughly the same age. Comparisons of growth rate between juvenile quahogs and older, larger individuals can be problematic. This is because most organisms, including bivalves, experience an ontogenetic decline in growth rate (e.g. Reiss 1989). Typically, growth rates of biological organisms are described by a negative exponential function that reaches an asymptotic value. By using a technique of fitting empirical growth data to various negative exponential functions (Kaufman 1981), Jones et al. (1989) concluded that quahog growth is best described by the von Bertalanffy (1938) growth function. Of course if one were simply making short term measurements of juvenile growth, simple measurements of size attained per unit time are adequate in most instances. However, for long term growth studies or analysis of field populations, the von Bertalanffy growth function is most appropriate. With adequate care (Knight 1968; Appledoorn 1983), the Bertalanffy growth model can be used to compare differences in growth between quahog populations and to deduce effects of temperature and other physical and biological factors on growth.

Ansell (1968) used shell growth as the key criterion for growth, but Peterson and Fegley (1986) suggested that shell growth is not the only criterion by which growth can be assessed. Growth of soft tissues is not necessarily coupled to shell growth at all times of the year. Peterson and Fegley showed that even after correction for ontogenetic growth rate differences, adult quahogs in North Carolina have anomalously lower growth rates during winter months in relation to juveniles. They interpreted this as differences in energy and nutrient reserve partitioning prior to the spring burst of gametogenic activity (Loosanoff 1937). Although there may be these differences in resource partitioning between soft tissues and the shell on a month to month basis, average annual growth is reflected in shell growth patterns. Using this as the basis of their study, Jones et al. (1989) showed that there is a high degree of correlation ($r = 0.88$) between mean annual growth of quahogs in Narragansett Bay and mean annual water temperature.

Effects of Salinity

In comparison to temperature, changes in salinity do not have a major influence on growth rates. In most instances quahog juveniles and adults grow fastest when salinities exceed 20 ppt (Castagna and Kraeuter 1981), and growth is reported to be optimal between about 26 and 27 ppt (Davis, n.d.).

Effects of Substrate

A number of studies have shown that shell growth of juvenile or adult quahogs is 19% to 30% greater in predominantly sandy sediments as compared to muds (silt/clay sediments) (Pratt 1953, Pratt and Campbell 1956, Rhoads and Panella 1970). More recent work (Grizzle and Morin 1989, Grizzle and Lutz 1989) employed an experimental protocol to compare the relative effects of sediment type, current speed and seston concentration. The results suggested that although there was an influence of sediment type on quahog growth, they are quite minor in comparison to the effects of current velocity and seston concentration. The increased growth associated with sandier sediments in the earlier studies has been reinterpreted to be a secondary result of sandier sediments being associated with higher current regimes.

Effects of Food Quantity and Current Speed

The rates at which quahogs feed on various phytoplankton species have been determined in static culture systems (e.g. Rice and Smith 1958, Walne 1972). In Walne's (1972) study, quahogs with valve lengths of 4 to 5 mm had maximum filtration rates of 3.4×10^5 cells/hr for *Isochrysis galbana* and 9.8×10^5 cells/hr for *Phaeodactylum tricornutum*. Efforts have been made to relate rates of filter feeding with quahog growth (e.g. Goldstein and Roels 1980). Food filtration rate and growth determinations of this type are useful only in the specialized case of rearing animals static systems. In most modern nursery and growout systems, as well as in the wild, food is delivered to quahogs via water currents and phytoplankton concentrations are typically three to four orders of magnitude lower in concentration than in the static rearing systems. Recognizing this recent studies have sought to determine quahog filtration rates at environmentally realistic phytoplankton concentrations and current regimes (e.g. Hibbert 1977, Doering and Oviatt 1986, Judge et al. 1992b).

Since the pioneering work of Kellogg (1903), it has been recognized that current speed has a major stimulatory effect on the growth of quahogs. Subsequent researchers (Kerswill 1949, Haskin 1952, Hadley and Manzi 1984, Manzi et al. 1986) have also reported this relationship and have explained it as a result of increased food (seston) supply rate in higher current regimes. The works by Grizzle and Morin (1989) and Grizzle and Lutz (1989) strongly suggest that quahog growth is primarily determined by horizontal seston flux (the product of seston concentration and current speed) past the animals. Quahogs in very dense assemblages (>100 adults/m^2) grow more slowly than do individuals in less dense assemblages, suggesting that food-limited stunting can occur in nature (Peterson and Beal 1989, Rice et al. 1989) as well as in grow out culture systems (Eldridge et al. 1979, Eversole et al. 1990). Recently, Judge et al. (1992a) performed a field experiment in which quahogs were placed in a series of artificial channels designed to vary current speeds, yet allow all quahogs to be exposed to the same ambient seston concentrations. This protocol allowed for discriminating between growth effects due to water flow and effects due to food availability. Under conditions of adequate food supply (food-limited growth not a factor), a doubling of current speed (up to 27 cm/sec) had no effect on growth rate. So current speed alone in the 10 to 27 cm/sec range does not in itself affect growth, but only serves to replenish food locally depleted by the actively filtering bivalves.

Although most researchers have concluded that quahog growth and current speed are positively correlated, there are some circumstances in which this general rule does not hold true. One study suggests that quahog growth can be inhibited in sandy sediments with very high current speeds and in silty areas in which storm surges may resuspend sediments, leading to decreased nutritive value of available particulates (Murphy 1985). In other studies, increased growth was noted in quahogs in seagrass beds (Peterson et al. 1984, Irlandi and Peterson 1991), yet overall current speeds were much less in the seagrass than in adjacent areas without seagrass. These surprising results are explainable by the observation that seagrass beds contain much higher food concentrations than in adjacent non-seagrass areas (Judge et al. 1992b). Although current speeds are lower, quahog growth remains high because of the localized productivity within the seagrass, and possibly because of "hydrodynamic trapping" of particulates by the low flow rates in the sea grass (e.g. Eckman 1990).

Effects of Food Quality

Unlike the example of quahogs in artificial culture situations, quahogs in the wild are frequently faced with less than optimum food quality. For example, Walne (1970) noted that some species of phytoplankton such as *Nannochloris atomus* and *Phaeodactylum tricornutum* were very poor food organisms for quahogs. A series of experiments outlined by Epifanio (1982) showed that it is unlikely that slow growth of quahogs fed *Phaeodactylum tricornutum* could be attributed to a toxic algal metabolite, but the amino acid profile and other nutrient levels appeared adequate to support growth of quahogs. The conclusion was that for some reason, quahogs have difficulty digesting *P. tricornutum*. *Nannochloris atomus* is often found during the warm summer months in embayments which receive nitrogen and phosphate enriched effluents (Mitchell-Innes 1973). Bass (1983) and Bass et al. (1990) demonstrated that cells of this phytoplankton species pass through the gut of quahogs almost completely undigested, which may explain the reduced quahog growth observed in areas characterized by persistent summer blooms of *N. atomus*.

In addition to the question of food quality and digestibility, there is some evidence that various phytoplankton species produce toxins that adversely affect bivalve feeding, and ultimately growth. For example, due to toxins associated with the brown tide organism *Aureococcus anophageferens*, blue mussels *Mytilus edulis* ceased feeding, resulting in reduced growth and elevated mortality (Tracey et al. 1988). It seems likely that *A. anaphageferens* can similarly interfere with suspension feeding by quahogs (Draper et al. 1990). The dinoflagellate *Alexandrum* (*Protogonyaulax*) *tamarens* causes quahogs to exhibit pronounced valve closure and a reduction of feeding rate (Shumway and Cucci 1987). Recent work by Wickfors and Smolowitz (1992) similarly suggests that toxins associated with blooms of the dinoflagellate *Prorocentrum minimum* can interfere with quahog growth.

Effects of Particulate Inorganic Matter on Feeding and Growth

Since the work of Rice and Smith (1958), it has been known that substantial concentrations of silt can lower the filtration rates of juvenile and adult quahogs. More recently, Bricelj and Malouf (1984) showed that although silt concentration below 5 mg/L had no effect on filtration rate, concentrations between 20 mg/L and 40 mg/L reduced particle filtration rate by 31% and 52% respectively. This reduction in filtration rate may impair food acquisition and overall nutritional state of quahogs. Bricelj et al. (1984) found no effect on shell growth of juvenile quahogs in silt levels up to 44

mg/L. Soft tissue growth was not affected at 25 mg/L silt, but was reduced by 16% in 44 mg/L. Murphy (1985) noted decreased shell growth in quahogs from areas of high suspended silt. In a recent study, Turner and Miller (1991) found that filtration rates and shell growth of quahogs were depressed in simulated storm events in which suspended sediment levels reached 193 mg/L.

Although silt or particulate inorganic matter (PIM) is known to reduce juvenile and adult filtration rates, the mechanisms of particle sorting and pseudofeces formation can compensate for the reduction of total filtered particles and allow for ingestion of high quality food particles. Bricelj and Malouf (1984) found that the threshold for pseudofeces production was 10 mg/L total seston (particulates). Sorting of particulates by the labial palps resulted in 78% retention of algae and >70% rejection of PIM. In circumstances when there are mixtures of phytoplankton and PIM, some of the PIM is ingested. In low to moderate suspended silt concentrations, there may actually be an increase in growth rate. This phenomenon has been attributed to a possible abrasive effect of the silt in disrupting algal cells as they pass through the digestive tract, particularly the style sac (Reid 1982).

Influence of Genetic Factors on Quahog Growth

With the growing interest in quahog aquaculture, there has been an interest in selective breeding programs to enhance growth potential and other commercially desirable characters of hatchery stocks (see reviews by Newkirk 1980, 1983). The rate at which juvenile bivalve mollusks grow can be considered one component of fitness, since the primary mechanism by which juvenile quahogs escape size-selective predation and smothering by siltation is through growth (MacKenzie 1977, Blundon and Kennedy 1982, Boulding and Hay 1984, Rawson and Hilbish, 1990). Additionally, the age at which quahogs become sexually mature and the total reproductive output correlate strongly with size (Bricelj and Malouf 1980, Bayne and Newell 1983, Peterson 1986). Thus individuals showing the most rapid juvenile growth are likely to have greater lifetime reproductive success. Because of these selective pressures one would expect that there would be a chronic selection for high growth rates and low levels of heritable variation (Mousseau and Roff 1987, Rawson and Hilbish 1990). Yet many studies of juvenile quahog growth under optimum environmental conditions show very high levels of growth variability (e.g. Eldridge et al. 1979, Eversole et al. 1986, Malinowski 1986, Littlefield 1991). A study by Rawson and Hilbish (1990) suggests that genetic variability of quahog growth may be maintained despite selective pressures because its planktotrophic larval dispersal will distribute siblings over a wide range of environments where genetic variation in growth may be differentially expressed. Thus genotype-environmental interaction may act to maintain high levels of genetic variation in any particular locale (see Via and Lande 1987, Gillespie and Turelli 1989 for discussions of genotype-environment interactions). Rawson and Hilbish (1990) concluded that there is sufficient genetic variability among quahogs so that selective breeding programs can be useful for producing high-growth strains.

A number of studies have been undertaken to explain the genetic component of growth rate variability among individual bivalves. Hilbish et al. (1992) have demonstrated that most of the variation in larval growth rate is heritable in the quahog *M. mercenaria*. Also, significant correlations between multiple locus heterozygosity at various enzyme coding genes and growth rate have been found in a number of bivalves in field populations, including the mussel *Mytilus edulis* (Koehn and Gaggney 1984), the oyster *Crassostrea virginica* (Zouros et al. 1980), and the coot clam *Mulina lateralis* (Garton et al. 1984). Such correlations suggest that dominance interactions at some gene loci may be responsible for bivalve growth variation, but this relationship has been much less clear in hatchery and nursery studies. Hatchery studies of oysters (Foltz and Chatry, 1986) and quahogs (Adamkewicz et al. 1984) have failed to show a significant correlation between growth and isozyme heterozygosity. Recent evidence suggests that quahogs from stable natural environments can exhibit a lack of correlation between growth and degree of heterozygosity (Slattery et al. 1991). Citing several other similar studies as well as their own data, Gaffney and Scott (1984) showed that there may be a linkage disequilibrium between enzyme marker loci and the loci affecting growth in hatchery stock and expressed strong skepticism about enzyme heterozygosity being an adequate tool for molluscan breeding programs aimed at growth enhancement. Dillon and Manzi (1988) provide evidence that the phenomenon of linkage disequilibrium may occur in hatchery/nursery stocks of quahogs. This linkage disequilibrium between growth and heterozygosity has been recently exploited by Manzi et al. (1991) who showed that reciprocal crosses between growth selected hatchery stocks can be useful for increasing or maintaining genetic variability without loss of rapid growth traits.

Relationships Between Rates of Larval and Juvenile Growth

Although a substantial fraction of the variation in the growth rate of bivalves is genetically determined, there is a growing body of evidence that the correlation between larval growth rates and the growth rates of juveniles may be very poor. This is in spite of the fact that it is common hatchery practice to cull slower growing larvae (e.g. Dupuy et al. 1977, Castagna and Kraeuter 1981). Poor correlations were found between larval and juvenile growth rates for the oysters *Crassostrea gigas* (Lannan, 1972), *Crassostrea virginica* (Losee 1979), and *Ostrea edulis* (Newkirk and Haley 1982) and for the mussel *Mytilus edulis* (Strömgren and Nielsen 1989). Similar studies with the gastropod *Crepidula fornicata* also suggest that individual larval growth rates may be poor predictors of individual post-metamorphic growth rates (Pechenik et al. 1987 and unpubl. data). More recently, Heffernan et al. (1991) found that quahog larvae produced by adults selected for rapid post-metamorphic growth grew significantly ($p < 0.01$) more slowly than did those produced by control populations. The implication is that slower-growing larvae may give rise to faster-growing adults, although that possibility has not yet been assessed directly. These data suggest that the routine culling of small larvae in standard hatchery practice may be removing those individuals that are likely to grow to market size most quickly following their metamorphosis (Heffernan et al. 1991, 1992). As emphasized recently by Hilbish et al. (in press), this apparent lack of correspondence between larval and adult growth rate probably reflects shifts in the expression of those genes associated with feeding and digestive processes during development.

Growth of juvenile bivalves may be better predicted by aspects of larval development other than larval shell growth rate. Growth is but one component of development, typically reflecting increases in cell numbers; differentiation is another component of development—a less commonly studied component—that reflects coordinated shifts in gene expression (discussed by Pechenik,

1987, p. 569; Pechenik et al., 1990). Rates of molluscan growth and differentiation are not necessarily well coupled. For example, Pechenik et al. (1990) found that rearing mussel larvae (*Mytilus edulis*) at different temperatures dramatically altered the relationship between individual shell growth rate and the amount of time required for larvae to develop "eyespots."

Two little-studied components of development that may influence juvenile growth are 1) the time required for individual larvae to become competent to metamorphose and 2) the amount of time that larvae delay their metamorphosis before they finally metamorphose, as discussed in the next section.

Competence, Delayed, Metamorphosis, and Correlations with Growth

Bivalve larvae typically must develop for a time in the plankton before they become capable of metamorphosing (reviewed by Pechenik 1985, 1990). We presently have a poor understanding of what makes an individual competent to metamorphose. Likely candidates include the construction and activation of certain epithelial sensory receptors, the completion of specific neurosecretory systems, the activation of chemical receptors on target tissues, or the development or completion of key neural pathways (Hadfield 1978, Trapido-Rosenthal and Morse 1986, Yool et al. 1986, Pechenik and Heyman 1987, Todd et al. 1991, Pechenik and Gee, in press). None of these mechanisms are directly related to larval growth rate. Indeed, Zimmerman and Pechenik (1991) have demonstrated for the marine gastropod *Crepidula plana* that the relationship between larval growth rate and time required for larvae to become metamorphically competent can be altered dramatically by shifts in ambient temperature and salinity; time to competence was poorly predicted by relative larval growth rate in their experiments.

Whatever the mechanism, the possibility that individuals that become competent to metamorphose sooner give rise to juveniles with higher fitness has not been examined. However, preliminary data presented by Newkirk et al. (1977) and by Losee (1979) suggest that for the oyster *C. virginica,* shorter time to metamorphosis correlates with higher juvenile growth rates. Additional studies examining the relationships between larval growth rate, time to competence, and post-metamorphic growth rate seem warranted.

Once a larva becomes competent—but not before—metamorphosis can generally be triggered by contact with certain chemical or physical environmental cues (reviewed by Thorson 1950, Crisp 1974, Sheltema 1974, Burke 1983, Hadfield 1984). Larvae of the oysters *Crassostrea virginica* and *C. gigas,* for example, are induced to metamorphose by certain bacterial films (and supernatants of those films) (Fitt et al. 1989, Weiner et al. 1989) and mussels *Mytilus chilensis* are induced to metamorphose by the presence of byssal adhesive disks (Padilla 1989). In the absence of such cues, larvae delay their metamorphosis and continue to swim (reviewed by Pechenik 1990). As metamorphosis is delayed, the threshold stimulus required to induce metamorphosis typically declines, so that larvae become less discriminating with time and will eventually attach and metamorphose on substrates that were unattractive to newly competent individuals (Scheltema 1961, Knight-Jones 1953, Crisp 1988, Coon et al. 1990, Fitt and Coon 1992).

Larvae that metamorphose on the plastic or resin walls of larviculture tanks, or on other artificial substrates provided in bivalve hatcheries may have become competent to metamorphose much earlier in development. There is evidence from several invertebrate phyla that fitness may decline during delayed metamorphosis, depressing juvenile growth and/or survival (Morse et al. 1979, Highsmith and Emlet 1986, Woollacott et al. 1989, Pechenik and Cerulli 1991, Pechenik et al. in press). Few workers have investigated this phenomenon in bivalves, but a gradual physical degeneration of mussel (*Mytilus edulis*) larvae delaying metamorphosis has been described by Bayne 1965, suggesting that post-metamorphic survival and growth may be compromised. The potential impact of delayed metamorphosis on the growth and survivorship of juvenile quahogs seems worthy of study.

SUMMARY AND CONCLUSIONS

The physical and nutritional factors affecting the growth of larval, juvenile and adult quahogs have been studied since the turn of the 20th century. Key environmental factors affecting quahog growth include temperature, salinity, current regime, and concentration of suspended sediments. Nutritional factors affecting quahog growth include the quantity and quality of particulate foods, especially phytoplankton. Considerable work has been aimed at developing nutritionally complete, non-algal diets for larvae and juveniles but is progressing slowly and may deserve further attention. Recent efforts to improve hatchery stocks of quahogs has led to a growing understanding of the genetic factors determining growth. Evidence suggests that there is sufficient heritable variability among quahogs to permit selective breeding programs to produce high-growth strains.

However, much of the work involving environmental, nutritional and genetic determinants of quahog growth have focussed on either larvae or post-set animals. Recent work with various bivalve species in which both larval and post-set growth are monitored in the same experimental protocol has shown that larval and juvenile growth rates are often poorly correlated. Some preliminary evidence suggests that juvenile growth rates may be better correlated with brevity of the larval developmental period than with rapidity of larval growth. Moreover, there is a reason to suspect that post-metamorphic growth rate or survival may be enhanced by promoting metamorphosis shortly after larvae become metamorphically competent. These aspects of quahog developmental biology and their correlation with quahog growth deserves further attention because of both practical and academic implications.

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A COMPARISON OF GROWTH RATES IN *ARCTICA ISLANDICA* (LINNAEUS, 1767) BETWEEN FIELD AND LABORATORY POPULATIONS

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ABSTRACT Fifty juveniles of the ocean quahog, *Arctica islandica* (L.), (estimated between 2 and 5 years old) were collected from a commercial bed (depth = 50 m) in eastern Maine in August of 1987. These quahogs were kept in the laboratory at ambient seawater temperatures in sediment until December, 1987, when they were individually marked, measured and placed in a sand-filled tray (61 cm × 76 cm × 13 cm deep) at the Darling Marine Center in Walpole, Maine. During the next three years, they received only ambient seawater from the Damariscotta River at a constant flow of 6 l/min. Individuals were remeasured after one year (December, 1988) and again in March, June, September and December of 1989 and finally in December, 1990. After two years in the laboratory, individuals had grown from a mean shell length (SL) of 9.6 mm ± 0.29 SE to a commercial size with a mean SL of 46.6 mm ± 0.50 SE. In three years, the mean SL was 53.9 mm ± 0.58 SE. This growth rate is similar to that reported by Ropes and Pyoas (1982) who examined *Arctica* from southern Georges Bank, but significantly faster than reported rates from populations located in the Mid-Atlantic Bight, southwestern Nova Scotia, and the western Baltic. Our results indicate that this species has the potential of being cultured in shallow-water sites protected from predators.

KEY WORDS: *Arctica islandica*, culture, growth, Maine

INTRODUCTION

The zoogeographic range of the ocean or mahogony quahog, *Arctica islandica* (Linnaeus), extends subtidally from Newfoundland to Cape Hatteras in the Northwest Atlantic inhabiting a range of muddy bottoms to depths of 250 m (Abbott 1974). Ocean quahogs supported an extensive war-time fishery beginning in 1943 off the coasts of Rhode Island and Massachusetts (Loosanoff 1953). Since the mid-1970's, *A. islandica* has been heavily exploited in the Mid-Atlantic Bight (Murawski et al. 1982, 1989) and more recently along the coast of eastern Maine (Beal and Kraus 1989).

To answer basic questions concerning the dynamics of numerous quahog populations throughout the species' eastern range, fisheries biologists have investigated population and/or individual growth rates using a variety of techniques such as mark-recapture, length-frequency time series and internal and external banding patterns (Thompson et al. 1980a,b, Jones 1980, Ropes and Murawski 1980, Murawski et al. 1982, Ropes and Pyoas 1982, Ropes et al. 1984 a,b, Beal and Kraus 1989). One theme is common to most of these studies: *A. islandica* is an unusually slow-growing and long-lived, infaunal bivalve, which reaches sexual maturity later in life than most other commercially-harvested bivalves. Ages approaching and exceeding 100 years are common. Ropes and Murawski (1983) reported this species has a potential life span of nearly 225 years. In the Mid-Atlantic Bight, individuals with a shell length (SL) of 50 mm were estimated to be between nine and seventeen years old (Thompson et al. 1980a, Murawski et al. 1982). A wider range of ages (7 to 20 years) were reported for 50 mm individuals from southwestern Nova Scotia (Rowell et al., 1990). Samples from Sable Island Bank (Ropes and Pyoas 1982) and the western Baltic (Brey et al. 1990) indicate similar growth rates. Conversely, *Arctica* from southern Georges Bank had unusually fast growth rates compared with populations elsewhere. For example, an individual attained a length of 53.9 mm in four years from a site on Georges Bank. Using an age-length curve constructed from the internal bands of 82 individuals, Ropes and Pyoas (1982) estimate that individuals with a SL of 50 mm are, on average, 2.3 yr. Although no statistical tests were performed on the data, Ropes (1984) observed that the golden brown or mahogony coloration of the periostracum of these fast-growing individuals, which is usually seen only in small individuals from most locations, persisted in large quahogs (SL = 72 mm) from Georges Bank, suggesting that the age and growth characteristics of this population were different from those of other geographic areas.

The majority of quahogs commercially harvested in eastern Maine lack the golden color mentioned by Ropes, probably indicating slow growth. Beal and Kraus (1989) examined the fate of 2,235 individually marked and measured commercial-size *A. islandica* (SL $\bar{X}$ = 48.7 mm ± 0.07 SE) inside field enclosures located at a depth of 20 m in eastern Maine during a one-year period (September 1985–1986). Average annual growth of 1,842 survivors at densities of 130/m^2 and 323/m^2 (which reflected nat-

[1]Order of first two authors decided by a coin toss.

ural densities in local, commercial beds) was 1.03 mm ± 0.01 SE. This rate was similar to that observed by Murawski et al. (1982) in the Mid-Atlantic Bight for large quahogs (SL $\bar{X}$ = 76.8 mm) at a depth of 53 m. These individuals grew an average of 1.17 mm over a year.

Other studies conducted in the Mid-Atlantic Bight by Thompson et al. (1980a) demonstrate mean size at sexual maturity was 42.0 mm ($\bar{X}$ age = 11 yr). Ropes et al. (1984b) found that male and female quahogs taken from the same area differed in their mean size and age at sexual maturity (males = 47.1 mm, $\bar{X}$ age = 9.8 yr; females = 55.0 mm, X age = 13.2 yr). Conversely, Rowell et al. (1990) report that male and female quahogs from nearshore populations in south-western Nova Scotia attained sexual maturity at a mean size of 47.1 mm ($\bar{X}$ age = 13.1 yr) and 49.2 mm ($\bar{X}$ age = 12.5 yr), respectively, although some individuals of both sexes matured as small as 40.0 mm (age seven). In all these studies males appear to mature sexually at a significantly earlier age than females.

Similar slow growth rates have been reported for the larval and early post-larval stages of this species by Lutz et al. (1981, 1982). For example, it took between 32 and 56 days at culture temperatures between 8°C and 14°C for metamorphosis to occur. After 7.5 months, laboratory-reared individuals had attained a shell length of only 1.0 to 6.5 mm. In another experiment, Lutz et al. (1983) tested the growth potential of these cultured juveniles inside sediment-free containers suspended from pilings near Boothbay Harbor, Maine. Although these animals grew 1.8 to 2.3 mm/month from June 1981 to October 1982), Lutz et al. (1983) concluded that their growout system was not well-suited for commercial applications primarily because they were unable to obtain a market-size individual in less than three years after fertilization.

Based on the wide ranging growth rates reported for this species, we designed a laboratory study to investigate growth rates of wild, juvenile quahogs held in a predator-free, sediment-filled container, which received only ambient seawater from the Damariscotta River during a three-year period (December 1987–1990). The growth rates that were observed under these artificial conditions suggest that ocean quahogs may be a reasonable candidate for culture in shallow water embayments.

MATERIAL AND METHODS

Collection and Aging of Quahogs from Commercial Beds in Eastern Maine

We collected (dredged) 663 quahogs over a four-year period (Table 1) from commercial beds with sandy-mud bottoms at depths between 30 and 50 m in Machias Bay (Lat. 40°35′N; Long. 67°26′W). Each quahog was measured to the nearest 0.1 mm using vernier calipers. Quahogs were aged according to Ropes (1984), in which shells were sectioned, embedded in epoxy, polished and briefly etched with 1M HCl to expose the internal banding patterns. From this prepared surface, acetate peels were produced and then examined microscopically to determine age. Murawski et al. (1982) demonstrated that each internal band is analogous to one year's growth for quahogs ≤ 60 mm.

Age-length data for our 663 quahogs were fit to six growth curves: linear, quadratic, power exponential, negative exponential, von Bertalanffy and Gompertz. The FISHPARM software developed by Prager et al. (1989) was used for all growth models except the linear and negative exponential. The NLIN procedure, Marquardt method (SAS 1985), was used to create the negative exponential curve.

Collection and Culture of Juvenile Arctica islandica

Fifty juvenile ocean quahogs were collected by Smith-MacIntyre grab sampler on 20 August 1987 from commercially harvested beds near Bucks Harbor in eastern Maine. Quahogs were kept in a shallow (ca. 5 cm deep) tray of sand in a flow-through system at the Beals Island Regional Shellfish Hatchery at Beals, Maine, until 26 November 1987. On the following day, quahogs were individually marked using colored paint dots in the umbo region as described by Peterson (1982). Standard length, height and thickness measurements of each individual were made to the nearest 0.1 mm. The quahogs were then maintained without food in chilled seawater (4°C) inside a walk-in refrigerator at the University of Maine at Machias for the next two weeks. On 8 December 1987 these 50 quahogs (SL range = 6.3 mm to 15.2 mm; $\bar{X}$ = 9.6 mm ± SE 0.29) were transferred to a shallow (61 cm × 76 cm × 13 cm deep), sand-filled tray in a constant-flow (6 l/min) seawater system at the Darling Marine Center in Walpole, Maine. This flow rate was sufficient to exceed filtration rate of all fifty animals based on estimates provided in Winter (1969). (The seawater intake for this flow-through system is located approximately 2 m from the bottom of the Damariscotta River and is 3 m from the surface at low tides.) Seawater temperatures were recorded for the first two years of the experiment (Fig. 1). Periodically, quahogs were excavated from the tray (December 1988, March, June, September and December 1989, December 1990), remeasured and returned to the same tray filled with clean sand. Growth was again modeled using the same six growth equations described above.

We prepared acetate peels from five randomly selected individuals reared at the Darling Marine Center. Each time we handled (examined, measured and/or repainted) the quahogs a distinct internal band was produced which confounds any unambiguous assessment of an annual mark.

TABLE 1.

Wild quahogs collected for age-length curve.

Quahog Size	Number	Mean Shell Length (mm) ±1 SE	Size (mm) Range	Age (yr) Range	Date(s) Collected
Small	70	11.8 ± 0.48	5.5 to 24.3	2 to 9	Sept. 1987
[illegible]um	31	23.1 ± 1.06	12.4 to 32.8	5 to 13	July 1989
	562	48.2 ± 0.21	36.8 to 65.5	21 to 66	Sept.–Oct. 1985
					April–May 1986

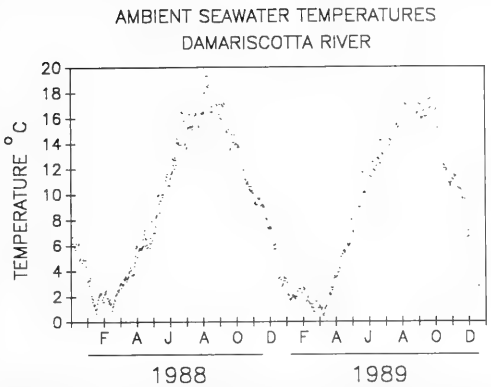

Figure 1. Seawater temperatures for the Damariscotta River taken periodically from December 1987 to December 1989 at the Darling Marine Center.

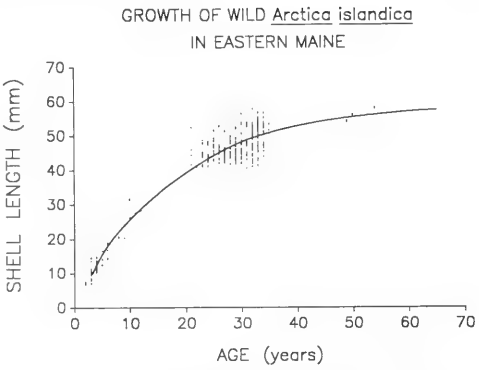

Figure 2. Growth of *Arctica islandica* from the Machias Bay region, n = 663. (See Table 2 for equations for the curve.)

RESULTS

In situ *Quahog Growth from Commercial Beds in Eastern Maine*

Figure 2 shows the relationship between age and length for all 663 quahogs. Of six growth curves fit to these data, the power exponential model explained the greatest amount of variability (Table 2). Small quahogs showed less variability in length at a given age than larger individuals. For example, a linear model fit to the 70 small specimens resulted in an r^2 of 0.68. The same model applied to the 31 medium and 562 large individuals resulted in an r^2 of 0.57 and 0.32, respectively. Applying 95% confidence intervals for the β_0 and β_1 parameters of the power exponential equation (Table 2), we estimate that between 27.2 and 34.7 years (with a mean of 30.6 years) are required for *A. islandica* to reach a commercial size of 48.2 mm (Table 1).

Growth of Laboratory-reared Quahogs

Using the power exponential equation in Table 2, we estimate the average initial age of the fifty juvenile quahogs used in the laboratory study was 3.5 years (range = 2 to 5 yr). Of the original quahogs placed in a sediment-filled tray at the Darling Marine Center, six died during the three-year experiment. These six plus another individual which grew approximately 40% slower than the remaining 43 individuals, were removed from all growth analyses.

Significant ($p < .01$) increases in mean SL occurred between each sampling date except from September to December 1989. After 24 months the SL of the 47 live quahogs averaged 46.6 mm ± 0.50 SE (Fig. 3). This size was not significantly different ($F = 3.35$; $df = 1,93$; $p = .07$) from the length attained by these same quahogs after 21 months (SL $\overline{X}$ = 45.3 mm ± 0.51 SE, n = 48). An average of only 7.3 mm of growth occurred in the third year of the experiment (SL $\overline{X}$ = 53.9 mm ± 0.58 SE, n = 43). This apparent slowing of growth during the last three months of 1989, which continued through 1990, may be attributed to the onset of sexual maturity, since individuals were observed spawning both in late September 1989 and 1990 (S. Chapman, pers. obs). Coinciding with this decrease in growth rate, the periostracum of the quahogs changed from golden to dark brown.

We constructed an age-length curve for these laboratory-reared quahogs (Table 3). Time (or age), the independent variable, was assigned values denoting months (for example, 1 = December, 1987, and 37 = December, 1990). Again, the power exponential equation explained more of the variability around the curve than the other five models. In the first two years at the Darling Marine Center, quahogs had grown an average 37.0 mm ± 0.48 SE (n = 47) from a mean SL of 9.6 mm to 46.6 mm. From the other power exponential growth curve generated for the quahogs harvested from commercial beds in eastern Maine (Table 2), we estimate that this 37 mm increase in SL would have taken between 25.8 and 32.9 years in the wild.

DISCUSSION

Slow growth combined with a prolonged time to reach sexual maturity are characteristics of a K-selected strategy adopted by other bivalves such as *Mytilus californianus* (Paine 1976) and *Modiolus modiolus* (Seed and Brown 1978) that experience intense juvenile mortality but may reach a size refuge from predation. Typically, these bivalves delay reproduction until a refuge size has been attained. For example, in *M. modiolus*, this size is between 35–45 mm SL and occurs after four years (Seed and Brown 1978). For populations of *A. islandica*, these growth/reproductive characteristics combined with high fishing pressures have fisheries managers concerned about stock sustainability and depletion (S. Murawski, pers. comm.).

Ocean quahogs are among the slowest growing and long-lived commercially valuable marine bivalves (Thompson et al. 1980a, Ropes 1984, Murawski et al. 1982). Growth rates for this species are variable with respect to location. For example, Thompson et al. (1980a,b) and Murawski et al. (1982) used internal banding patterns to determine that quahogs from the Mid-Atlantic Bight reached a commercial size (ca. 50 mm) between nine and seventeen years. In addition, commercial-size animals (SL = 59 to 104 mm) which were marked and recaptured in that same area grew approximately one millimeter in a year (Murawski et al. 1982). In eastern Maine, quahogs take an average of 30.6 years (Fig. 2, Table 2) to reach a commercially harvestable size. This rate is among the slowest reported for this species. Beal and Kraus (1989) demonstrated a significant inverse relationship between growth

TABLE 2.

Growth models and parameter estimates for wild-grown quahogs.

Model	r^2	Equation	Parameters with 95% Confidence Intervals
Linear	0.805	$L_{(t)} = \beta_0 + \beta_1(t)$	$\beta_0 = 14.130 \pm 1.176$ $\beta_1 = 1.080 \pm 0.041$
Power Exponential	0.937	$L_{(t)} = \beta_0(t)^{\beta 1}$	$\beta_0 = 5.008 \pm 0.183$ $\beta_1 = 0.662 \pm 0.013$
Negative Exponential	0.909	$L_{(t)} = \beta_0(1 - \exp[-\beta 1\{t\}])$	$\beta_0 = 58.791 \pm 1.586$ $\beta_1 = 0.057 \pm 0.004$
Von Bertalanffy	0.909	$L_{(t)} = L_\infty(1 - \exp[-K\{t - to\}])$	$L_\infty = 59.470 \pm 2.089$ $K = 0.055 \pm 0.006$ $t_0 = -0.235 \pm 0.483$
Gompertz	0.905	$L_{(t)} = L_0 \exp(G[1 - \exp\{-gt\}])$	$L_0 = 6.621 \pm 0.851$ $G = 2.124 \pm 0.117$ $g = 0.092 \pm 0.007$
Quadratic	0.901	$L_{(t)} = \beta_0 + \beta_1 t + \beta_2 t^2$	$\beta_0 = 5.251 \pm 1.083$ $\beta_1 = 2.054 \pm 0.081$ $\beta_2 = -0.021 \pm 0.002$

$n = 663$, $L = \text{length}_{(t)}$, $t = \text{time}$.

rate and intraspecific density. This may have been the result of competition for food or space.

Ropes and Pyoas (1982) reported extraordinarily rapid growth rates for ocean quahogs in the southern Georges Bank region where quahogs attained a SL of 50 mm in an average of 2.3 years. These unusually fast growth rates indicate the potential of the ocean quahog as a candidate for mariculture. Lutz et al. (1981, 1983) spawned adult *A. islandica*, reared their larvae and raised the juveniles in sediment-free, predator-exclusion containers suspended from a dock near Boothbay Harbor, Maine. Starting with individuals having a mean SL of 2.5 mm, Lutz et al. (1983) reported a final mean size of 26.0 mm (an average increase of 23.5 mm) after sixteen months. (In the present study, quahogs grew an average of 27.2 mm during the first sixteen months of culture.) They hypothesized that such fast growth rates were the result of the interaction between increased food availability in surface waters along with higher average water temperatures compared with conditions experienced by benthic populations located in relatively deep water (>20 m). However, many of the quahogs in that sediment-free system were misshapen, apparently from almost constant movement within each container due to tide, wind and wave action (J. Ropes, pers. comm., 1987). In our study, quahogs grown in sediment not only had a characteristic shape, but appeared golden brown (a quality that Ropes and Pyoas [1982] associated with rapid growth) during their period of fastest growth (December 1987 to September 1989).

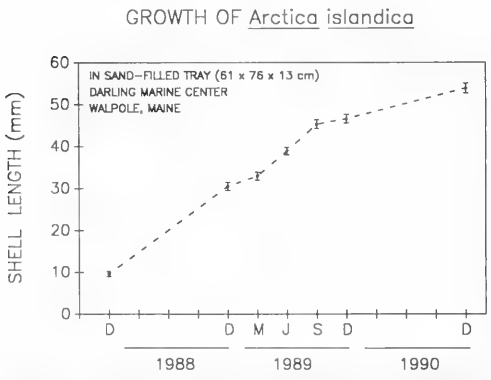

Figure 3. Growth of juveniles of *Arctica islandica* at the Darling Marine Center, December 1987, n = 49; December 1988, n = 49; March, June, September 1989, n = 48; December 1989, n = 47; December 1990, n = 43. (See Table 3 for equations for the curve.)

In this study, quahogs received only the naturally occurring phytoplankton from the Damariscotta River through an intake system that was frequently fouled by other suspension feeders. In addition, on three of six occasions (December 1988, September 1989 and December 1990), when the quahogs were removed from the sediment-filled tray for measuring, large numbers of juvenile *Ensis directus* Conrad, *Mya arenaria* L. and other filtering organisms were found. These were subsequently removed and the quahogs returned to "clean" sediments. The removal of quahogs from the sediments for repetitive measurements produced a distinct internal shell band and may also have reduced their growth rates. We have found that this species is extremely sensitive to handling with respect to both growth and survivorship. (We conducted a field experiment (depth = 20 m) in Machias Bay in which adult quahogs were marked and measured once, twice and three times and then transplanted to field enclosures containing sediments. After one year, relative growth rates were significantly [$p < 0.01$] less [20.8%] for those quahogs handled more than once.) Given the influence of disturbance and intra- and interspecific competition for limited amounts of phytoplankton at the Darling Marine Center, we believe the growth we observed in the laboratory is a conservative estimate of their growth potential. This rapid growth rate is particularly surprising, since these juveniles are presumably from the same genetic population as those in the commercial beds in eastern Maine, which grew significantly slower than populations at other geographic locations. Therefore the growth potential of these "slow growing" quahogs in eastern

TABLE 3.

Growth models and parameter estimates for laboratory-reared quahogs, December, 1987 to December, 1990.

Model	r^2	Equation	Parameters with 95% Confidence Intervals
Linear	0.931	$L_{(t)} = \beta_0 + \beta_1(t)$	$\beta_0 = 8.524 \pm 0.894$ $\beta_1 = 1.592 \pm 0.050$
Power Exponential	0.945	$L_{(t)} = \beta_0(t)^{\beta 1}$	$\beta_0 = 9.200 \pm 0.104$ $\beta_1 = 0.489 \pm 0.014$
Negative Exponential	0.865	$L_{(t)} = \beta_0(1 - \exp[-\beta 1\{t\}])$	$\beta_0 = 64.524 \pm 3.912$ $\beta_1 = 0.052 \pm 0.006$
Von Bertalanffy	0.933	$L_{(t)} = L_\infty(1 - \exp[-K\{t - to\}])$	$L_\infty = 73.500 \pm 5.474$ $K = 0.035 \pm 0.005$ $t_0 = -2.829 \pm 0.063$
Gompertz	0.938	$L_{(t)} = L_0 \exp(G[1 - \exp\{-gt\}])$	$L_0 = 7.890 \pm 0.913$ $G = 2.029 \pm 0.105$ $g = 0.083 \pm 0.006$
Quadratic	0.935	$L_{(t)} = \beta_0 + \beta_1 t + \beta_2 t^2$	$\beta_0 = 6.961 \pm 1.047$ $\beta_1 = 2.137 \pm 0.110$ $\beta_2 = -0.023 \pm 0.003$

n = 332, L = length$_{(t)}$, t = time measured in months.

Maine is similar to the observed growth of individuals on Georges Bank (Ropes and Pyoas 1982).

This growth potential suggests that *A. islandica* may be amenable to culture. The fast growth rates observed here suggest that the growout phase would probably not create a production "bottleneck" in the culture of this animal. Oyster (*Crassostrea virginica* [Gmelin]) and hard clam (*Mercenaria mercenaria* L.) aquaculturists strive to produce a marketable product in two years from nursery-sized individuals (12 mm to 25 mm) (W. Mook, Mook Sea Farms, Inc. pers. comm.). Lutz et al. (1981) demonstrated that rearing these animals in the hatchery is feasible. Further development of culture conditions to produce ca. 15 mm individuals is essential to initiating a private mariculture program.

Besides biological criteria, cost effectiveness is critical to the establishment of any culture program. Fisherman in eastern Maine currently receive about $40.00 a bushel. At this price, mariculture of this species would not be profitable. One reason for this price is poor product quality. After quahogs are landed, they are often stored in floating pens until they are marketed. During storage, many quahogs die and decay resulting in low product value. Secondly, most commercially harvested quahogs from eastern Maine have a dark, unappealing shell, while individuals produced in culture or occurring naturally in areas where growth is rapid, have a rich, golden-brown color that might increase their value for the half-shell market.

We are encouraged by the potential for cutluring *A. islandica*. As is the case with *M. mercenaria*, small, 43 mm to 48 mm "cherrystone" individuals are usually the most lucrative size. Field studies in North Carolina (Beal 1983, Peterson and Beal 1989), South Carolina (Eldridge et al. 1976) and Georgia (Walker 1983) indicate that a minimum of two years are required for individuals of *M. mercenaria* less than 15 mm to attain a marketable size. Although *A. islandica* attained an average size of 46.6 mm in two years in the laboratory (which is several mm smaller than the average commercial size of quahogs harvested in eastern Maine—48.2 mm) future investigations should focus on field growth of juveniles in a shallow, subtidal, predator-free system.

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MOVEMENTS OF JUVENILE SEA SCALLOPS *PLACOPECTEN MAGELLANICUS* (GMELIN, 1791) IN PASSAMAQUODDY BAY, NEW BRUNSWICK

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ABSTRACT Movements of naturally occurring juvenile sea scallops, *Placopecten magellanicus* (initial size = 15 to 25 mm) were observed by SCUBA using a 144 m^2 underwater grid. Scallops were grouped into three classes (tagged, handled, and control) in order to assess the effects of handling and tagging on movement. A χ^2 test showed no significant difference in scallop movement among the three classes ($P > 0.05$). Pooled data of juvenile scallops over the period of August to November, 1987 showed that 76% of the scallops were mobile and distance travelled within and nearby the grid averaged 3.3 ± 3.9 m. Juvenile scallops observed underwater displayed three different behaviors; either swimming, byssal attachment or unattached and non-mobile. Further, individual sea scallops were observed to alternate between swimming activity and byssal attachment.

KEY WORDS: swimming, scallops, behavior, bottom culture, byssal attachment

INTRODUCTION

Grow-out strategies for culturing scallops are varied and existing technology involves either suspending animals in the water column or culturing them on bottom (Ventilla 1982). Bottom culturing, whereby juvenile scallops are seeded on to the bottom, is a low cost culturing method (Wildish et al. 1988). This technique is also the basis for restocking programs (Brand et al. 1991). A potential disadvantage of such a strategy to acquaculturists is the swimming behavior of many species of scallops (Caddy 1968, Peterson et al. 1982, Joll 1989).

Tagging studies of the sea scallop, *Placopecten magellanicus* (Gmelin, 1791) on Georges Bank showed that adults move at an average rate of 9.9 km · yr^{-1} (Melvin et al. 1985). In a field experiment, in which adult sea scallops were initially stocked at high densities, Caddy (1989) found scallops were either consumed by predators or rapidly dispersed.

Except for laboratory studies on the byssal attachment of juveniles by Caddy (1972), preliminary tagging studies by Kenchington et al. (1991) and hydrodynamics of juvenile scallops by Manuel and Dadswell (1991), the importance of swimming activity and movement in the early life-history of the sea scallop is largely undocumented. The aim of this field study was to examine the extent and variation of movement in the early juvenile phase of naturally occurring sea scallops.

MATERIALS AND METHODS

In a natural sea scallop bed which contained juveniles, an underwater grid was established and used to monitor movement over the period July to November, 1987. The study site was located on the northern side of Tongue Shoal, Passamaquoddy Bay, New Brunswick, Canada (Lat. 45°05′ Long. 67°00′) at a depth of 10 m at low tide. The substrate was homogeneous over the grid and consisted of gravel and a few small rocks with a thin over-layer of silt. Bottom water temperatures during the study declined from 11.6 to 7.8°C. Maximum tidal currents were about 25 cm · s^{-1} (Brooks 1992).

Movement of scallops was determined by using tagged juveniles (n = 62 individuals tagged and 200 observations). In order to assess the effect of handling and tagging on the movement of the tagged scallops, two groups of controls were used, one with scallops that were handled but not tagged (n = 74 individuals handled and 100 observations) and the second group consisting of scallops that were left, *in situ*, without any handling, except for measuring them without breaking the byssal attachment (n = 19 individual controls and 19 observations).

A rope grid (12 × 12 m), divided into 9 squares (each measuring 16 m^2) was deployed at the study site. Six of the nine squares were randomly selected (3 squares for tagged and 3 for handled scallops) and all juvenile scallops present were removed and returned to the lab. Scallops in the remaining 3 squares were undisturbed controls. The removed scallops were measured for shell height (to the nearest 0.1 mm) and were either tagged or handled as if they had been tagged. Scallops were tagged with an orange plastic disk (8 mm in diameter), which contained a 3 digit identifying number. The disks were glued to the upper valve of the shell. Tagged and handled scallops were returned, within 24 hr, to the previously randomly selected squares and the location of all scallops were individually marked with a color coded flag (one color for each class and an identification number corresponding to each of the tagged scallops). Flags consisted of a 2 × 4 cm piece of hard plastic affixed to a piece of wire, 15 cm long.

The grid was surveyed, by SCUBA, at two to three week

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intervals from mid-August to mid-November. The presence or absence (i.e. not adjacent to the flag) of a scallop from a previously flagged position was recorded as 'not moved' and 'moved', respectively. A scallop was defined as 'not moved' if it was within 0.5 m of its original flagged position. A record of bysally attached scallops was also kept. If a tagged scallop moved and could be located within a 2 m perimeter of the grid, the point to point distance travelled was estimated with a calibrated rope (to the nearest 0.5 m). All scallops that moved or remained on the grid and new juvenile scallops which immigrated onto the grid were newly flagged for the next observation period. At the conclusion of the experiment, tagged scallops were collected, measured, and growth rate determined.

RESULTS

Movement of juvenile scallops among the three classes (tagged, handled, and control) was tested for independence by the $\chi^2_{0.05}$ (data pooled over all observation periods). Movement was found to be independent of the classes (χ^2, $P > 0.05$) indicating tagging and handling had no effect on swimming activity (Fig. 1). Movement data from the three classes were pooled and movement among the different observation periods was tested for independence and was found to be significantly different (χ^2, $P < 0.01$) (Fig. 2.) There was a consistently and significantly (χ^2 goodness of fit, $P < 0.01$) higher percentage of scallops which moved (overall mean = 76%) versus those which did not move, with the mean percent movement over the five sampling occasions varying from 66 to 90%.

The distance travelled was only estimated for recovered tagged scallops collected within the grid or within 2 m of the periphery of the grid and represented point to point distances, thus the maximum distance that could be resolved was 19 m. Fifty-eight percent of the tagged scallops were recovered, of which 11% were multiple recaptures. The overall mean distance travelled of 'moved' scallops was 3.3 ± 3.9 m, with the majority (61%) travelling ≤ 2 m (Fig. 3).

The number of scallops byssally attached varied among the three classes and over the sampling period (Fig. 4). The overall mean percent attachment was 30.4 ± 21.1%. There were no significant differences in the number of byssally attached scallops between the tagged and handled classes (χ^2, $P < 0.05$) but the differences were significant among the five sampling occasions (χ^2, $P < 0.01$, handled and tagged data pooled). There was not a sufficient number of unhandled control individuals in all cases to be included in the above analyzes. Scallops were observed to be attached to the sides of rocks, on gravel, and on shell fragments but were unattached when on soft substrate.

Initial mean shell height of scallops was 18.7 ± 1.7 mm (SD). The shell height of all recovered individually tagged scallops increased over the duration of this study to 33.2 ± 2.4 mm and their mean growth rate was 0.15 ± 0.03 mm · d^{-1}.

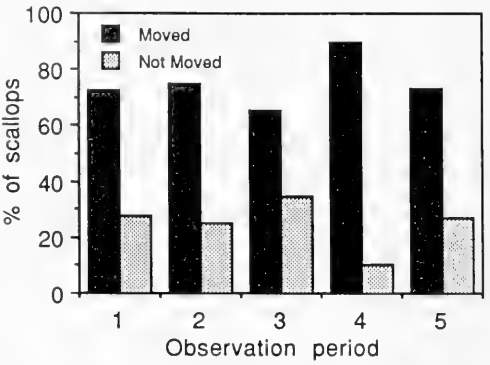

Figure 2. Percent frequency histogram of 'moved' and 'not moved' juvenile sea scallops for the 5 observation periods between August and November, 1987 (all data pooled for the 3 classes).

DISCUSSION

Adult sea scallops are capable of swimming (Caddy 1968, Dadswell & Weihs 1990) and at times, move over considerable distances (Melvin et al. 1985). Only recently, has the movement and swimming ability of juvenile sea scallops (<30 mm) been reported (Kenchington et al. 1991, Manuel & Dadswell 1991). Our findings indicate that juvenile sea scallops were capable of frequent movement. The mean net movement of 3.3 m that we observed during a four month period, however, was an underestimate of the actual movement since no attempt was made to recover scallops outside a 2 m periphery of the grid. In fact one

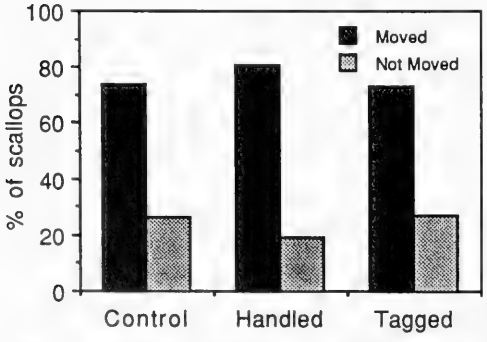

Figure 1. Percent frequency histogram of 'moved' and 'not moved' juvenile sea scallops for the three different classes (tagged, handled, and control). All data were pooled for the 5 observation periods.

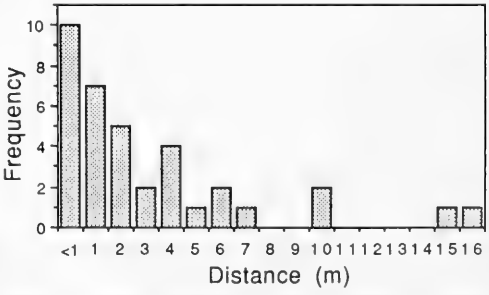

Figure 3. Frequency histogram of distance travelled for 'moved' tagged juvenile sea scallops found within a 2 m periphery of the underwater grid.

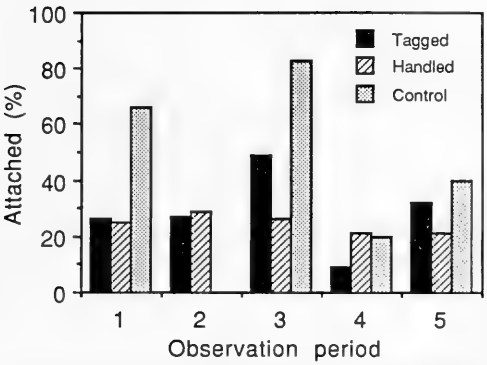

Figure 4. Percent frequency histogram of byssally attached juvenile sea scallops for each observation period and class.

tagged individual was recovered incidentally a year later (July, 1988) 0.5 km from the experimental site. Kenchington et al. (1991) reported juvenile movements of <10 to 100 m within a 12 d period.

Most other scallop species are known to move but juveniles have seldom been used in experiments (Peterson et al. 1982). Joll (1989) reported that individual *Amusium balloti*, <30 mm, did not move. However, these observations were made on animals that had been dredged and moved to an experimental site, and Joll (1989) suggests they may have been stressed by their capture method.

Swimming and byssal attachment have been described as antagonistic behavior in sea scallops (Caddy 1972). However, byssal attachment does not preclude being able to swim, as sea scallops can instantly detach (pers. obs., GJP). Because a large and variable number of juveniles were found attached (mean = 30%) and a high percentage were observed to move between sampling periods (76%), we suggest that sea scallops frequently detach, move, and re-attach. Caddy (1972) observed that sea scallops could alternate between being attached and unattached. In laboratory experiments, Caddy (1972) reported a higher rate of attachment (up to 75% to scallops < 10 mm) and Parsons and Dadswell (unpubl. data) observed 50% of 25 mm wild sea scallops to be attached. High rates of byssus attachment are known for other species of scallops, especially of *Chlamys* while species of *Pecten* seldom attach after leaving their primary settlement sites (Caddy 1972, Gruffydd 1978).

If potential bottom culturing or restocking programs are planned for sea scallops, their movement and swimming activity must be considered. The release of juveniles on the bottom will result in movement and dispersal and this will be especially crucial if release areas are limited and not enclosed. The Japanese seed *Patinopecten yessoensis* into the center of a lease site or in patches within a site so as to allow for outward expansion (Caddy 1989) of up to 1 km in 2 yr (Ventilla 1982, Caddy 1989). We suspect that movement of sea scallops will be more extensive than this.

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EXPLOITING LIFE-HISTORY CHARACTERISTICS OF THE SEA SCALLOP, *PLACOPECTEN MAGELLANICUS* (GMELIN, 1791), FROM DIFFERENT GEOGRAPHICAL LOCATIONS IN THE CANADIAN MARITIMES TO ENHANCE SUSPENDED CULTURE GROW-OUT

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ABSTRACT Sea scallops on the Atlantic coast of Canada exhibit both annual and semi-annual spawning cycles depending on their geographical location. Populations with annual cycles normally spawn during late summer (Aug. to Sept.) and collected spat can be grown to commercial size (90 mm shell height, 15 g meat) in suspended culture in 33 to 36 mo. Populations with semi-annual reproduction usually spawn both during early summer (June to July) and fall (September to October). Spat collected from the early cohort can be grown to market-size in 25 to 27 mo, while the late set requires 36 mo for grow-out. Use of early spawning population for spat collection or hatchery production will significantly reduce the grow-out period for this scallop. Strategies exploiting both population types would yield a steady supply of market size scallops for the farmer, especially if an assortment of end products were desired (e.g. 50 to 70 mm scallops for live markets and larger scallops for roe-on meat).

KEY WORDS: gonadosomatic index, reproduction, spat settlement, growth, harvesting

INTRODUCTION

One of the serious challenges to the development of aquaculture for any molluscan species, especially when in competition with an established wild fishery, is to develop a culture strategy that ensures continuity of product for harvest as well as meets the fluctuating demands of the market place throughout the year. The problem becomes more acute when suspended culture techniques increase growth rate which in turn often decreases the size variation among individuals of a cohort. For many aquaculture species, these problems can be overcome by extending the reproductive period over the entire year using artificial propagation and by fine-tuning the product (harvest-size, processing, etc.) for specific markets (Pillay 1990). However, for species such as sea scallops, whose reproductive output is confined to a specific annual period and whose juveniles, presently, must be collected from the wild, aquaculture development is difficult.

The sea scallop, *Placopecten magellanicus* (Gmelin 1791), is recognized as a good candidate for aquaculture (Naidu and Cahill 1986, Aiken 1987). While the culturing of this species has been under investigation since the 1970's (Naidu and Cahill 1986), its development has been slow. A major problem hampering progress is the general view that sea scallops are slow growing, which leads to its poor assessment in economic models (Frishman et al. 1980). Additional problems include: limited availability of natural spat, an unreliable and inadequate supply of spat, and dominations of the commercial fishery in the marketplace.

Recent findings, however, may change the perception concerning the economic viability of sea scallops. Trials using naturally produced spat indicate this species can easily be grown in suspended culture to a market size of 90 mm shell height (S_h) in 36 mo (Wildish et al. 1988, Dadswell and Parsons 1991) and the development of hatchery techniques for propagation are progressing (Dabinett 1989). We report further developments in the grow-out potential for *Placopecten magellanicus* using wild caught spat from selected geographical locations, and discuss how the availability of different cohorts may be exploited to develop suspended culture grow-out strategies for sea scallops.

BACKGROUND

Sea scallops occur on the Atlantic coast of North America from Newfoundland to Virginia. Commercial exploitation of large concentrations (beds) takes place throughout this range and annual landings average about 22,000 t (meat weight) (Naidu 1991). Most landings are of adductor muscle only (the scallops are shucked at sea) but recently a roe-on fishery has developed on Georges Bank when concentrations of paralytic shellfish toxins in the tissues permits (Shumway et al. 1988). The North American market demands a 35 to 40 meat count (≈15 g meat). The European market, dominated by France, demands a roe-on product (De Franssu 1990). Generally they desire a large scallop (121 mm S_h for *Pecten maximus* Linnaeus, 1758). Although the European market is difficult to access from North America, doing so, would increase the yield per scallop by 50%, since a ripe gonad is about half the weight of the adductor muscle or "meat" (Bourne and Read 1965). The whole animal market is only in the developmental stage in North America and is now dominated by the bay scallop (*Argopecten irradians* Lamarck 1819).

Ideal conditions for suspended culture of sea scallops exist in coastal regions ranging from Newfoundland to Cape Cod. Growing sea scallops in suspension usually results in faster growth (Wildish et al. 1988, Dadswell and Parsons 1991) and greater partitioning of energy towards somatic tissue growth (MacDonald 1986, Parsons and Dadswell 1992). Sea scallops typically reproduce annually (Beninger 1987, Parsons et al. 1992), but some populations have two reproductive periods each year (Naidu 1970, Dupaul et al. 1989, Dibacco 1991).

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MATERIALS AND METHODS

Studies were conducted at aquaculture sites in Passamaquoddy Bay (PB) on the lower New Brunswick coast of the Bay of Fundy (45°04′N, 67°01′W) and in Mahone Bay (MB) on the Atlantic coast of Nova Scotia (44°30′N, 64°15′W) (Fig. 1). Passamaquoddy Bay has a 7 m mean tidal range which results in a well mixed water column and cool annual (7°C) and summer (12°C) mean temperatures (Trites and Garrett 1983). The aquaculture site was located off Tongue Shoal (Fig. 1). Mahone Bay has a 2 m mean tide and is thermally stratified most of the summer (Dadswell and Crawford-Kellock 1989). Water temperatures in the upper water column (0 to 5 m) reach 20°C and bottom temperatures (15 m) range from 3 to 16°C during the growing season. The aquaculture site was situated off Graves Shoal (Fig. 1). Salinities in both bays ranged from 29 to 31‰ during normal conditions.

In PB, we have monitored the reproductive cycle and spat settlement of *Placopecten magellanicus* since 1983, and in MB, since 1988. The reproductive cycle of scallops was monitored in order to determine the spawning time of the local population. Since scallop larvae remain planktotrophic for ≈35 days (Culliney 1974), estimates of their settlement dates can then be predicted. In PB, wild adult scallops were collected on bottom about monthly at depths of 10 and 60 m and examined for Gonadosomatic Index (GSI). From 1985 to 1990, scallops were collected at weekly or biweekly intervals from the beginning of July until the end of the spawning period (late August). In MB, wild adult scallops were collected from shallow (5 to 10 m) and deep (15 to 25 m) sites at bi-weekly or monthly intervals from April until November during 1989 to 1990. GSI's were determined after drying tissues for 24 to 48 h at 80°C where, GSI = (gonad weight/total soft body weight) (Thompson 1977, Barber et al. 1988), after the foot and crystalline style had been separated from the gonad and added to the viscera. In MB, macroscopic examination of the gonads, based on the staging of Davidson and Worms (1989), was made to determine the proportion of spent scallops. After a decline in the GSI indicated that spawning had occurred, monitoring collectors were deployed for determining settlement time of both sea star (*Asterias* spp.) and sea scallop (*Placopecten magellanicus*). Sea stars are predators of scallop spat and both were monitored for aquaculture purposes solely to determine when sea star settlement was complete and when scallop spat settlement started, thus determining the optimal time for deployment of the commercial spat collectors (Parsons et al. 1990).

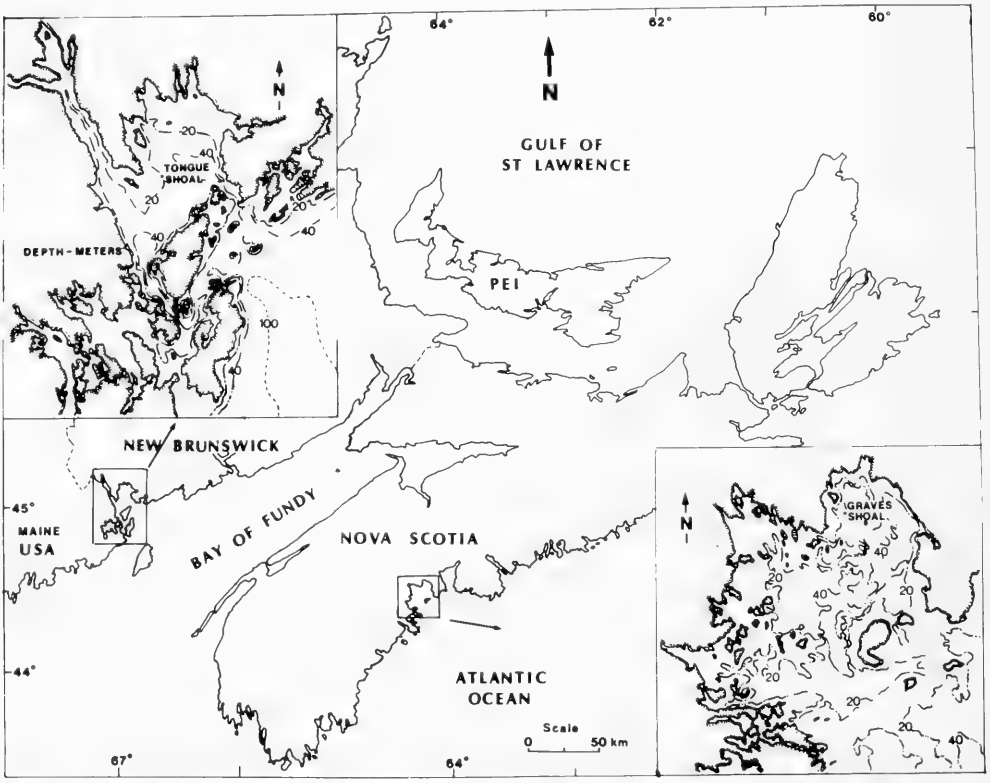

Figure 1. Canadian Maritimes indicating the location of Passamaquoddy Bay, NB (top, left), and Mahone Bay, NS (bottom, right) and the aquaculture sites used (Tongue Shoal, Graves Shoal).

Collectors consisted of Japanese "onion" bags (1.5 mm mesh) filled with monofilament gillnet (Naidu and Cahill 1986). The monitoring collectors were deployed 1 to 6 m off-bottom on suspension arrays (Aoyama 1989) weekly during July and October in PB and bi-weekly between May to October in MB. Commercial collectors, deployed after sea star settlement, were left until mean spat size was about 10 mm S_h then the spat bags were retrieved (in PB, June to July at ≈10 mo, summer set in MB, November to December at ≈5 mo, and fall set in MB, August to September at ≈10 mo) and sorted. For intermediate culture, juveniles were placed in pearl nets (6 mm mesh) with a floor area of 0.12 m^2 at a stocking density of 30/net (Parsons and Dadswell 1992) and deployed at sea on suspension arrays. When mean S_h reached 50 mm (in PB at 21 to 23 mo, in MB summer set at 15 to 16 mo, and in MB fall set at 20 to 22 mo), the pearl nets were retrieved and the scallops transferred to a final grow-out system. Final grow-out was either in lantern nets (24 mm mesh) or by using ear-hanging (Ventilla 1982, Aoyama 1989). Scallops were stocked at either 10/floor in the lantern nets (floor area = 0.2 m^2/level) or at a density of 50/5 m of ear-hanging line. Scallops were then redeployed on the suspension array and left to grow until commercial size (mean S_h 90 mm). Scallops in suspended culture were held at depths of 4 to 10 m at low tide at PB and 5 to 15 at low tide at MB. Nets were always deployed at >1 m off-bottom.

Throughout the grow-out period, scallops were sampled intermittently for shell height and adductor muscle weight. Shell height was measured to the nearest 0.1 mm, using calipers, and meat weight (W_m) was determined to the nearest 0.1 g. Scallops were left undisturbed in each culture phase until examined, to reduce the effect of handling on growth (Parsons and Dadswell 1992). Each sample consisted of 30 scallops.

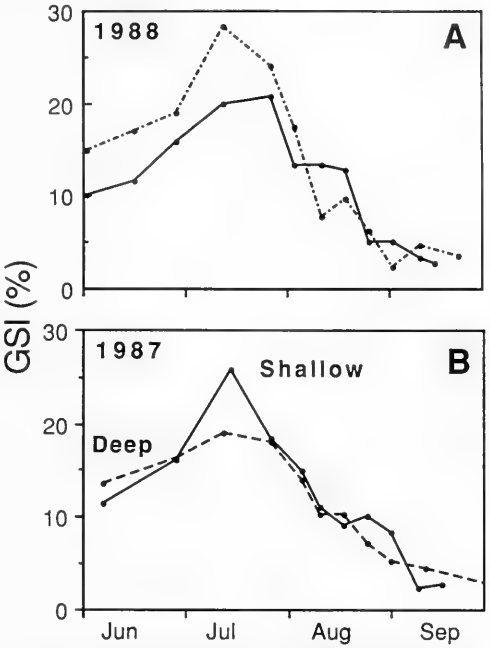

Figure 2. Gonadosomatic Indices of sea scallops collected on bottom at weekly to bi-weekly intervals from (a) two shallow sites (10 m) and (b) a deep (60 m) and shallow (10 m) site for representative years in Passamaquoddy Bay, NB (after Parsons et al. 1992).

RESULTS

In PB, reproduction was an annual, episodic event occurring from late July to early September at both deep-water and shallow sites (Fig. 2). Spat set occurred from late August until late September (Table 1). Scallops from this location grew to 90 mm S_h after 34 to 36 mo (Fig. 3). The usual protocol was to leave spat in collectors for 10 to 12 mo, transfer juveniles to pearl nets for 10 to 12 mo, then place individuals in final grow-out for 12 to 14 mo (Dadswell and Parsons 1991). We successfully grew three cohorts (year-classes) of the PB population (1986, 1987 and 1988) and have a fourth (1989) at commercial size.

In MB, reproduction took place semi-annually or annually depending on the depth at which the scallops lived and was extended in duration. Monitoring of GSI (Figs. 4 & 5), histological examination of the gonads (Keizer 1991), and observations of spat settlement (Table 1) and size-frequency distribution (Fig. 6), indicated that there were two reproductive periods. Scallops in shallow depths spawned from late June until late July and again in September through October. Scallops at greater depths usually spawned once a year during September and October (Figs. 4 & 5). After 6 to 12 mo of growth in the spat collectors, spat size allowed differentiation of two distinct cohorts (t-test, $P < 0.01$, Fig. 6). The summer cohort ranged from 12 to 42 mm S_h (mean = 23.3 ± 5.2 mm [SD], n = 315) and the fall cohort from 2 to 14 mm S_h (mean = 6.0 ± 2.1 mm [SD], n = 220). The summer cohort (age 1+ yr) grew rapidly between August and November (0.33 mm · d^{-1}) and was ready for transfer to final grow-out at 14 to 16 mo of age. It attained 90 mm S_h in 25 to 27 mo (Fig. 7). The fall cohort (age 6+ mo) had a growth pattern similar to the PB scallops (0.12 mm · d^{-1}). It was ready for transfer to final grow-out systems at about 21 mo and attained market size at 33 to 36 mo (Fig. 7). We have successfully grown two fall cohorts (1988 and 1989) and one summer cohort (1989) to market size.

Mean wet adductor muscle weight of a 90 mm summer set and fall set scallop from MB was 15 g (Fig. 8). A 90 mm scallop grown in suspension in PB was 15.3 g and did not differ appreciably from that of the MB populations (Table 2). At both grow-out sites (PB and MB), wet meat weights of scallops grown in suspension exceeded that of similar-sized wild, bottom grown scallops by 25 to 30% (Table 2).

DISCUSSION

Most growth studies of sea scallops have used "annual" ring counting as a means of ageing (Fig. 3) (Stevenson and Dickie 1954, Merrill et al. 1966), often without empirical verification. In addition, studies have often been concerned with deep-living, and/or slow-growing populations at the northern end of the sea scallop range (Schick et al. 1988, MacDonald 1986). Also, when growth of sea scallops in suspended culture was assessed for the first time, the experiments were conducted at the northern extreme of the species range and information on stocking densities, which can

TABLE 1.

Intensity of sea scallop and sea star settlement using monitoring collectors at bi-weekly intervals in Mahone Bay, NS, 1989 (mean of 10 collectors) and weekly in Passamaquoddy Bay, NB, 1988.

Mahone Bay—1989			Passamaquoddy Bay—1988		
Date Deployed	Number Scallops	Number Sea Stars	Date Deployed	Number Scallops	Number Sea Stars
Summer set			July 19	0	0
May 23	0.0	190.5	July 28	0	20
June 6	0.0	286.5	August 3	2	310
June 14	0.5	148.5	August 9	10	604
July 6	7.0	13.7	August 16	23	203
July 19	22.5	14.5	August 23	28	195
July 31	1.5	3.5	August 30	75	126
Fall set			September 5	88	217
August 11	4.2	3.5	September 14	302	98
August 30	1.0	5.3	September 19	542	44
September 11	3.3	9.3	September 28	431	5
September 31	6.0	1.3	October 4	414	0
October 17	7.5	1.5	October 13	283	0
October 31	0.0	0.2	October 21	283	0
November 21	0.0	0.0	October 26	27	0

affect growth rate, were unreported (MacDonald 1986). These studies established and reinforced the concept the *Placopecten magellanicus* is slow growing, i.e. about 4.5 to 6 yr to 90 mm S_h (Stevenson and Dickie 1954, Merrill et al. 1966). Evidence to the contrary, obtained from southern populations and using new techniques has been largely ignored (Krantz et al. 1984). It is not surprising then, that when economic assessments were done, they reflected poorly on sea scallops as an aquaculture candidate. Our study, however, confirms that sea scallops grow rapidly in suspended culture and a commercial size of 90 mm can be attained in

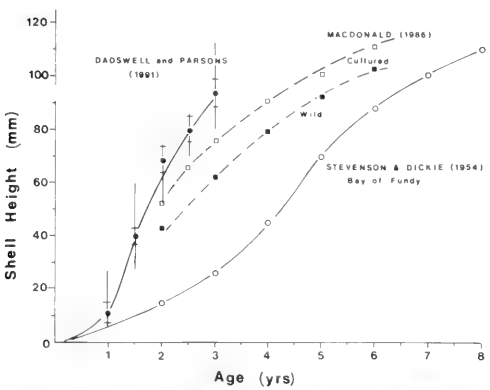

Figure 3. Growth (shell height vs age in years) of sea scallops from Passamaquoddy Bay, NB and various other geographical locations in the Northwest Atlantic: Stevenson and Dickie (1954), Bay of Fundy on bottom; MacDonald (1986), Newfoundland suspended culture and on bottom; Dadswell and Parsons (1991) in suspension. Closed circles are means, vertical lines are ranges, and horizontal lines are ±SD.

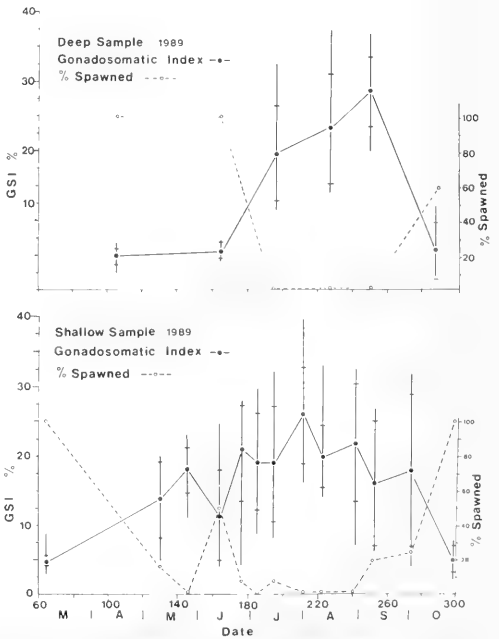

Figure 4. Gonadosomatic Indices of sea scallops collected on bottom at monthly or bimonthly intervals in Mahone Bay, NS during 1989. Top: deep sample (15 to 20 m); Bottom: shallow sample (5 to 10 m). Closed circles are means (n = 12 for each sample), vertical lines are ranges, and horizontal lines are ±SD.

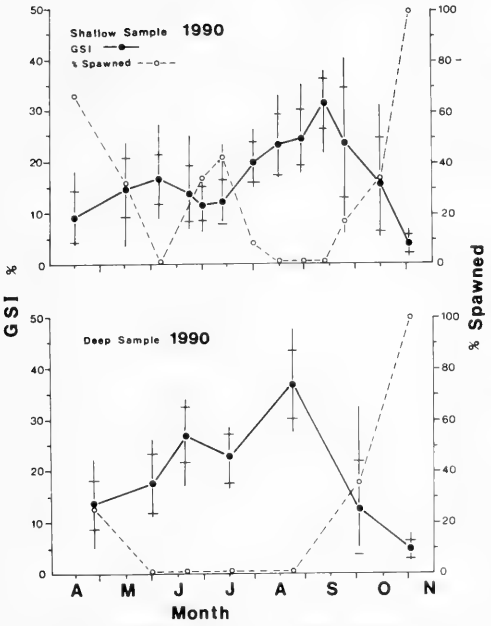

Figure 5. Gonadosomatic Indices of sea scallops collected on bottom at monthly or bimonthly intervals in Mahone Bay, NS during 1990. Top: shallow sample (5 to 10 m); Bottom: deep sample (15 to 20 m). Closed circles are means (n = 12 for each sample), vertical lines are ranges, and horizontal lines are ±SD.

25 to 36 mo depending on the source of the natural spat set and the market niche desired. For the live market, a 50 mm S_h size can be attained in 16 mo for Mahone Bay summer set and 21 to 24 mo for Mahone Bay fall set and Passamaquoddy Bay. It is probable that as long as stocking density guidelines are adhered to (Ventilla 1982) and culture protocol strictly followed, rapid growth could be obtained anywhere from Newfoundland to Cape Cod.

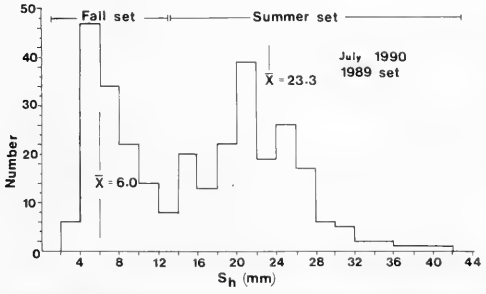

Figure 6. Shell height frequency distribution for sea scallop spat from the summer and fall cohorts of 1989 in Mahone Bay, NS after sorting in July, 1990.

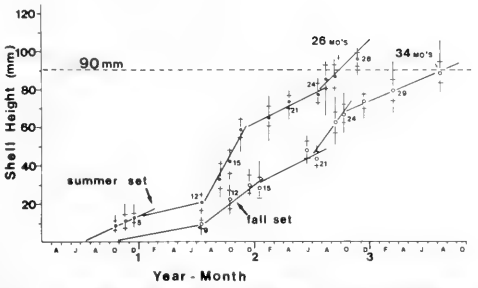

Figure 7. Shell height growth of sea scallops from the 1989 Mahone Bay, NS summer (closed circles) and fall cohorts (open circles) grown in suspended culture. Closed circles are means, vertical lines are ranges, and horizontal lines are ±SD. Number beside mean represents months of growth since spat settlement.

It may also be possible using hatchery techniques (Dabinett 1989) to acquire sea scallop spat as early as April. The Japanese scallop (*Patinopecten yessoensis* Jay 1857) sets naturally in April or May and grows to 90 mm S_h in suspension culture in 18 mo or less (Ventilla 1982, Aoyama 1989). Grow-out of early settled sea scallops might then require as little as 18 to 20 mo to achieve the 90 mm S_h market size. Further economic analyses on the feasibility of sea scallop aquaculture should be done using our recent growth findings and taking into account the potential of hatchery spat.

An additional benefit for the aquaculturist, which results from the possibility of obtaining two or more sea scallop cohorts annually, is the availability of a wide range of sizes throughout the year, particularly the 50 to 70 mm S_h, whole market product. It is quite probable that if detailed investigations were carried out on coastal sea scallop populations from other geographic locations along the Atlantic the incidence of semi-annual spawnings might

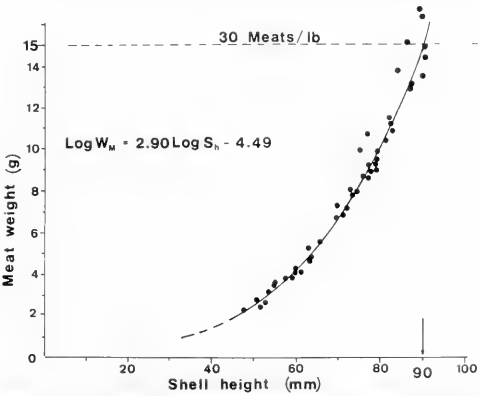

Figure 8. Combined weight-length relationship for summer and fall cohorts of sea scallops from Mahone Bay, NS grown in suspended culture. Each point represents the mean of 30 scallops grown to size without prior handling during the final grow-out period.

TABLE 2.

Weight-length relationships (wet meat weight, W_m vs shell height, S_h) and wet adductor muscle weights (90 mm S_h) for sea scallops grown on bottom and in suspension at two geographical locations in the Canadian Maritimes.

	Location	
	Passamaquoddy Bay, NB	**Mahone Bay, NS**
Weight-Length		
on bottom	$\text{Log } W_m = 3.02 \text{ Log } S_h - 4.86$	$\text{Log } W_m = 2.57 \text{ Log } S_h - 3.92$
in suspension	$\text{Log } W_m = 3.42 \text{ Log } S_h - 5.50$	$\text{Log } W_m = 2.90 \text{ Log } S_h - 4.49$
Wet Muscle Weight (90 mm S_h)		
on bottom	11.1 g	12.6 g
in suspension	15.3 g	15.0 g

be found to be common. In most scallop aquaculture regimes, for the whole, live market (bay scallop for instance), the entire cohort reaches market size over a short time period (Walker et al. 1991). During the remainder of the year, unless there is access to other hatchery produced cohorts or growth rates can be adjusted by manipulating stocking densities, the aquaculturist will lack yield from this crop. Having available a number of cohorts and optimizing the contribution of each cohort to a marketing strategy should greatly improve the potential for sea scallop aquaculture.

ACKNOWLEDGMENTS

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BIOCHEMICAL COMPOSITION OF BROODSTOCK AND EARLY JUVENILE CHILEAN SCALLOPS, *ARGOPECTEN PURPURATUS* LAMARCK, HELD IN TWO DIFFERENT ENVIRONMENTS

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ABSTRACT Early juvenile *Argopecten purpuratus* (post metamorphic larvae of approximately 400 micrometers shell height) from the same brood, were divided into two groups: one group was held in hatchery conditions and fed a mixture of *Chaetoceros calcitrans*, *Chaetoceros gracilis* and *Isochrysis galbana* (T-iso), the other group was placed in pearl nets which were suspended in the sea. After 40 days, shell height of juveniles from both groups was measured and their biochemical composition analyzed. Early juvenile scallops reared in the hatchery had a slower growth rate and contained more carbohydrate and lipid, but less protein and RNA and had a lower RNA:DNA index than those held in the ocean. Analysis of fatty acids showed that juveniles held in the hatchery had a higher content of C16:1n7 and C18:1n9, but much less C22:6n3 than those held in the ocean. Adult scallops were induced to spawn and then divided into two groups which were held in the same manner as the early juveniles. Every twelve days, samples were taken to determine gonadal index and biochemical composition of the gonad. Gonads of spawned adults held in the laboratory for 48 days did not become fully ripe but those adults held in the ocean spawned spontaneously during this period. Adults that had been held in the ocean for 36 days and were transferred to the hatchery for twelve days, showed a decrease in their gonadal index and the RNA:DNA ratio. Results of biochemical studies on adults were similar to those observed in early juveniles.

KEY WORDS: scallop conditioning, scallop physiology, *Argopecten purpuratus*, juvenile growth

INTRODUCTION

Culture of the Chilean northern scallop, *Argopecten purpuratus* Lamarck (1819), has developed extensively since the initial studies of DiSalvo et al (1984). This has led to biological research to determine optimum conditions for development and growth. The Chilean scallop is a functional hermaphrodite and exhibits a continuous gametogenic activity with two spawning peaks: one in late summer and another in autumn (Brown and Guerra 1982, Wolff 1988). Martínez (1991a) found a seasonal biochemical cycle in adult *A. purpuratus* that was related to the gametogenic cycle, but this differed from that of juvenile scallops (under 25 mm shell height, not sexually mature).

A successful molluscan aquaculture industry depends on the availability of high quality juveniles which will grow rapidly to commercial size. Many factors affect the performance of larval stages and subsequent early juvenile (post larval stages) and later juvenile stages. First, factors affecting gametogenesis and broodstock conditioning must be considered (Sastry 1979, Lannan 1980). Survival of larvae is partly dependent on stored energy reserves and this is determined by parentally derived endogenous reserves in the eggs. The quantity and quality of food, temperature and other environmental factors will also affect the success of rearing larvae and ultimate juvenile bivalves (Pechenik 1987).

One of the major factors preventing development of the *A. purpuratus* culture industry in Chile is a sufficient supply of juveniles (seeds). This is due to poor catches of natural sets which occur cyclically in Chile (Illanes 1988). It has been necessary to develop a hatchery system to provide a consistent supply of juveniles under controlled conditions. This has led to studies of optimal diets for scallop larvae (Martínez 1991b); however, Uribe (personal communication) observed that early juvenile scallops held in pearl nets in the ocean for two months after metamorphosis, grew and survived better than those cultured in the hatchery.

The aim of this study was to compare the biochemical condition of two groups of scallop broodstock, one maintained in the hatchery and the other held in the ocean. A similar comparison is made between early juvenile scallops (defined here as post metamorphic larvae of approximately 400 micrometers) held in both environments.

MATERIALS AND METHODS

Broodstock Experiments

Mature adult scallops (8–10 cm shell height) were obtained from Herradura Bay, Coquimbo-Chile, and induced to spawn. They were then divided into two groups: one group was held in the laboratory (hatchery) and fed with a mixture of *Isochrysis galbana* (T-iso), *Chaetoceros gracilis* and *Chaetoceros calcitrans;* and the other group was placed in pearl nets and suspended in the sea at 5–8 m depth. After being held in the ocean for 36 days, two animals from this group were taken from pearl nets and held for an additional 12 days in the hatchery. Considering that gonadal recovering time of this scallop is usually between 40 to 60 days (unpublished data), every 12 days two individuals from each group were taken for analysis of the gonadal index and gross biochemical composition of the gonad. For this, each gonad was homogenized, dried to constant weight at 70°C (Davies et al. 1990, Beninger et al. 1984, Thompson 1977) and kept until analysis. For fatty acid analysis, fresh, wet samples were used.

Gonad index was determined as the percentage of the total tissue weight of the animal that consisted of gonad.

Survival of individuals was 100% for both groups.

Post-Larval Experiments

A. purpuratus early juveniles (post-larvae) were taken from a single batch of larvae cultured after the method of Disalvo et al.

(1984). When the juveniles attained a size of 400 microns they were divided into two groups: one group was kept in the hatchery and fed a mixture of *C. calcitrans*, *C. gracilis* and *I. galbana* (T-iso), the other group was placed in pearl nets and suspended in the sea. After 40 days the shell height of both groups was measured (to the nearest 0.1 mm) and an analysis was undertaken to determine their biochemical composition. For protein, carbohydrate, lipid and nucleic acids analysis, 50 individuals (the same that were measured) from each group were washed with 3.4% ammonium formate and dried to constant weight at 70°C. The dried individuals were ground with a tissue grinder and two subsamples were taken for each analysis.

Although not exactly measured, the mortality of early juveniles held in the ocean was approximately twice that of early juveniles held in the hatchery.

Water temperature and salinity in the ocean and laboratory were similar (15 ± 1°C, 33–34‰).

Experiments were done between May and July 1991. This period corresponds to the winter season when the phytoplankton is most stable but rather poor (Uribe 1989).

Analytical Methods

Protein, total carbohydrates and lipids were analysed following the method of Martínez (1991a).

Analysis for RNA and DNA was done after removing lipids by extraction with chloroform:methanol (1:2) by the method of Wright and Hetzel (1985). RNA:DNA ratios provided a good measure of condition index.

Ash-free dry weight (organic matter) was determined by ashing animals or tissue for 5 hours at 500°C after drying the samples to constant weight at 70°C. The concentration of each biochemical constituent of early juveniles was expressed as μg per mg of ash-free dry weight because relative organic matter (dry ash-free weight:total dry weight) has been noted as the best quantitative condition index for larvae and post-larvae of bivalves (Lucas and Beninger 1985, Lucas et al. 1986, Mann and Gallager 1985). This renders the biochemical composition of both groups of post-larvae more comparable.

For fatty acids analysis, lipids were extracted from fresh, wet samples with chloroform:methanol containing 0.01% butylated hydroxytoluene as an antioxidant. Methyl esters were prepared by esterification with BF_3 in methanol following the method of Morrison and Smith (1964) and analyzed with a gas chromatograph (Carlo Erba 6000) equipped with a silica capillary column SP-P 2330, 30 m, 0.50 mm i.d.

Although the fatty acid composition of microalgae used in this study has been reported previously (Bourne et al. 1989) it was analyzed here to determine the composition under our experimental conditions. Results are shown in Table 1.

Data Analysis

Barlett's test was used to determine homogeneity of variances, one-way and two-way factorial analysis of variance was used to test for differences in the biochemical composition among treatments and experimental times (Steel and Torrie 1980). A Tukey's or Student's test was applied to evaluate the significance of the possible differences found in the study (Steel and Torrie 1980).

TABLE 1.

Fatty acid composition of lipids in microalgae used to feed broodstock and early juvenile *Argopecten purpuratus*. Values shown are percentages of the total fatty acids. Results of two analysis are shown.

Fatty Acid	*Isochrysis galbana*		*Chaetoceros calcitrans*		*Chaetoceros gracilis*	
SATURATED						
C14:0, myristic	14.6	13.2	10.2	10.3	ND	ND
C16:0, palmitic	8.3	8.2	22.6	22.1	17.3	18.0
C17:0, heptadecanoic	0.2	0.2	1.0	1.0	1.7	1.8
C18:0, stearic	0.4	0.4	5.1	5.4	6.3	6.3
C24:0, tetracosanoic	ND	ND	ND	ND	ND	ND
TOTAL	23.5	22.0	38.9	38.8	25.3	26.1
MONOENOICS						
C16:1n7, palmitoleic	3.6	3.4	34.7	35.1	31.3	31.7
C18:1n9, oleic	6.3	6.1	0.7	0.7	0.6	0.9
C20:1n9, eicosaenoic	22.1	21.5	0.3	0.2	ND	ND
TOTAL	32.0	31.0	35.7	36.0	31.9	32.6
POLYENOICS						
C18:2n6, linoleic	2.4	2.3	0.9	0.8	0.6	0.6
C18:3n6, g-linolenic	0.3	0.3	0.7	0.7	ND	ND
C18:3n3, a-linolenic	6.6	6.7	ND	ND	0.5	0.6
C20:2n6, eicosadienoic	ND	ND	ND	ND	ND	ND
C20:3n6, eicosatrienoic	ND	ND	ND	ND	ND	ND
C20:4n6, araquidonic	ND	ND	3.8	3.6	3.6	3.3
C20:5n3, eicosapentaenoic	1.2	1.2	8.2	7.9	10.5	9.8
C22:4n6, docosatetraenoic	ND	ND	ND	ND	ND	ND
C22:5n6, docosapentaenoic	ND	ND	0.3	0.1	ND	ND
C22:6n3, docosahexaenoic	13.9	13.6	1.0	1.0	1.3	1.2
TOTAL	24.4	24.1	14.9	14.1	16.5	15.5

ND: not detected.

RESULTS

Broodstock Conditioning

Gonadal Maturation

The gonadal index of scallops held in the hatchery showed a slower increase than those held in the ocean (Fig. 1). Animals held in the ocean had spawned before day 48 while those held in the hatchery for 48 days had a gonadal index of 8.7. Individuals held in the ocean for 36 days and then transferred to the hatchery showed a decrease in gonadal index after that transfer.

Biochemical Analysis of Gonads

Carbohydrates. A two-factorial analysis of variance showed that the level of carbohydrates in gonads changed with time in both groups of scallops although the pattern of change differed in each group. Those animals held in the hatchery showed a sustained increase in carbohydrate level until day 36. Carbohydrate contents in animals held in the ocean increased initially, declined abruptly on day 24 and then recovered (Table 2).

Lipids. Lipid content changed over the course of the study. Even if the differences observed were not significant statistically, a clear tendency of a higher lipid content in scallops held under hatchery conditions was observed (Table 2).

Proteins. In general, scallops held in the hatchery had significantly (Tukey, $p < 0.05$) lower levels of gonadal proteins than those maintained in the ocean. Scallops held in the ocean for 36 days and then held in the hatchery for 12 days showed a decline in protein level in the gonad (Table 2).

RNA and DNA. Gonadal RNA and DNA levels were always lower in scallops held in the hatchery compared to those scallops held in the ocean (Table 3). The greatest difference between the two groups of scallops was found on day 24 for RNA, and on day 12 for DNA, when levels for those scallops held in the ocean amounted to more than twice that in scallops held in the hatchery (Tukey, $p < 0.05$). A slight, non-significant decrease in RNA and DNA was detected in those animals held in the ocean for 36 days and then transferred to the hatchery for 12 days.

Biochemical condition index, RNA:DNA ratio. A two-way factorial analysis of variance showed that the RNA:DNA ratio changed with time, but this change was dependent upon the environment. It was significant ($p < 0.05$) only in those animals held in the hatchery which showed a decline in the ratio from day 12 until day 36 when this index was at the lowest value (Table 3).

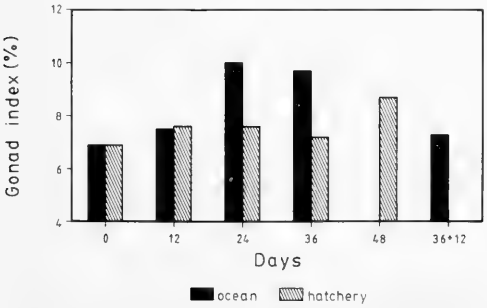

Figure 1. Mean gonadal index of *Argopecten purpuratus* at different times after spawning when held in the open environment (ocean) and in the hatchery.

Fatty acids. Fatty acid composition of gonadal lipids of *A. purpuratus* conditioned in the two different environments is shown in Table 4. No great variations were detected with time in saturated fatty acids except for a small increase in palmitic acid and a slight decrease in stearic (C18:0) and tetracosanoic (C24:0) acids in both groups of scallops; however, the percentage of palmitoleic acid (C16:1n7) showed a sharp decrease in those animals held in the ocean. The amount of this acid increased sharply on the first day of analysis in those animals held in the hatchery although it returned to initial values on day 48. The increase in percentage of this same acid in those individuals held in the ocean and returned to the hatchery on day 36 is noteworthy. The greatest changes in polyenoic fatty acids were detected in eicosapentaenoic (C20:5n3) and docosahexaenoic (C22:6n3) acids; the first one decreased over time in both groups of scallops, although more quickly in those animals held in the hatchery, and docosahexaenoic acid showed a large increase in animals held in the ocean, attaining the highest value on day 36. Contents of this fatty acid declined sharply in those animals held in the ocean for 36 days and then transferred to the hatchery.

Early Juvenile Experiments (Post Larval Experiments)

Growth Response and Biochemical Condition Index

Early juvenile of *A. purpuratus* held in the ocean for 40 days attained a higher shell height, ash-free dry weight and biochemical condition index (RNA:DNA ratio) than those scallops held in a hatchery and fed a mixture of *C. calcitrans*, *C. gracilis* and *I. galbana* (T-iso) (Table 5).

Biochemical Composition

Early juveniles raised in the hatchery had a slightly higher carbohydrate and DNA content and a much larger lipid content than those animals held in the ocean (Table 6); however, the observed tendencies were not statistically significant.

Fatty Acid Analysis

Results of two analysis of the fatty acid composition of lipids in early juvenile *A. purpuratus* held in the two environments are shown in Table 7. Within classes of fatty acids, the most significant difference between the two groups was the higher percentage of monoenoics and the lower percentage of polyenoics in animals held in the hatchery. Within the saturated fatty acids, early juveniles held in the hatchery had less palmitic (C16) and stearic (C18) acids, but more myristic (C14) acid than animals held in the ocean. The latter differences are consistent with the major percentage of palmitoleic (C16:1n7) and oleic (C18:1n9) acids. Within the identified polyenoic acids, the greatest difference found between the two groups was the minor content of docosahexaenoic (DHA, C22:6n3) acid found in animals held in the hatchery.

DISCUSSION

In bivalve hatcheries, much of the variation in larval performance is due to the state of gonadal development of the parents. Variation in this development has been shown to be due to both environmental and genetic components (Lannan 1980). The present study focused on investigating the gonadal condition of *A. purpuratus* when held in the hatchery and in the ocean. Results showed that gonads of animals held in the natural environment

TABLE 2.

A. Mean levels of carbohydrates, lipids and proteins in gonads of *Argopecten purpuratus* at different times when conditioned in two environments, the ocean and a hatchery.

Time (days)	Carbohydrates		Lipids		Proteins	
	Hatchery	Ocean	Hatchery	Ocean	Hatchery	Ocean
0	37.2 ± 1.2	37.2 ± 1.2	42.4 ± 14.2	42.4 ± 14.2	368.2 ± 19.1	368.2 ± 19.1
12	38.0 ± 7.6	47.5 ± 4.7	65.2 ± 12.4	49.1 ± 0.9	332.7 ± 2.4	384.8 ± 0.5
24	44.6 ± 0.5	41.1 ± 0.3	77.9 ± 9.5	64.5 ± 7.2	354.7 ± 27.2	403.6 ± 22.4
36	57.9 ± 4.9	54.4 ± 6.3	50.4 ± 2.8	61.8 ± 9.4	374.7 ± 13.9	406.2 ± 32.5
48	54.7 ± 7.6		72.6 ± 5.7		297.2 ± 1.9	
36 + 12		54.4 ± 2.7		57.1 ± 14.3		368.3 ± 19.2

B. Two-way factorial analysis of variance (to 36 days).

Source of Variation	d.f.	F	p
Carbohydrates			
Time	3	14.22	<0.05
Environment	1	0.09	N.S.
Interaction	3	2.06	N.S.
Lipids			
Time	3	3.59	<0.1
Environment	1	2.64	N.S.
Interaction	3	3.03	N.S.
Proteins			
Time	3	1.85	N.S.
Environment	1	10.85	<0.05
Interaction	3	1.41	N.S.

(36 + 12) were animals held for 36 days in the ocean and then transferred to the hatchery for an additional 12 days.
Values shown are expressed as μg per mg of dry weight.

TABLE 3.

A. Mean levels of nucleic acids in gonads of *Argopecten purpuratus* at different times when conditioned in two environments, the ocean and in a hatchery.

Time (days)	RNA		DNA		RNA/DNA	
	Hatchery	Ocean	Hatchery	Ocean	Hatchery	Ocean
0	13.1 ± 0.1	13.1 ± 0.1	14.2 ± 5.6	14.2 ± 5.6	0.83 ± 0.15	0.83 ± 0.15
12	14.0 ± 3.9	23.6 ± 3.7	14.4 ± 3.5	34.0 ± 2.5	0.97 ± 0.04	0.69 ± 0.06
24	21.0 ± 2.5	43.2 ± 0.7	30.9 ± 3.2	50.5 ± 13.5	0.68 ± 0.01	0.89 ± 0.22
36	13.9 ± 2.2	28.9 ± 1.7	31.1 ± 5.2	39.5 ± 1.3	0.45 ± 0.00	0.73 ± 0.01
48	32.8 ± 0.7		48.5 ± 0.7		0.67 ± 0.00	
36 + 12		26.6 ± 2.3		37.7 ± 1.7		0.71 ± 0.02

B. Two-factor analysis of variance (to 36 days).

Source of Variation	d.f.	F	p
RNA			
Time	3	46.27	<0.001
Environment	1	99.13	<0.001
Interaction	3	15.84	0.001
DNA			
Time	3	14.78	<0.005
Environment	1	15.02	<0.005
Interaction	3	2.41	N.S.
RNA/DNA			
Time	3	4.21	<0.05
Environment	1	0.86	N.S.
Interaction	3	5.15	<0.05

(36 + 12) were animals held for 36 days in the ocean and then transferred to the hatchery for an additional 12 days.
Values shown are expressed as μg per mg of dry weight.

TABLE 4.

Fatty acid composition of lipids in gonads of *Argopecten purpuratus* over time when conditioned in the ocean and in a hatchery.

			From the Ocean						From Hatchery								36 in Ocean + 12 in Hatchery	
Fatty Acid	Just Spawned		12 Days		24 Days		36 Days		12 Days		24 Days		36 Days		48 Days			
Saturated																		
C14:0	4.4	5.0	7.8	4.2	3.4	3.5	3.6	3.2	4.0	4.2	4.3	3.6	4.7	4.6	4.7	3.1	4.9	4.0
C15:0	ND	ND	ND	0.6	0.7	0.7	0.9	0.9	0.5	0.5	0.5	0.4	ND	ND	0.9	0.7	ND	0.6
C16:0	15.3	16.2	20.4	15.6	16.8	16.9	17.6	16.9	15.3	15.6	15.5	14.9	17.8	17.7	19.2	17.4	19.1	19.0
C17:0	0.9	ND	ND	0.8	1.1	1.0	1.1	1.0	0.7	ND	0.5	0.5	ND	ND	ND	0.1	ND	0.5
C18:0	5.5	6.2	4.9	4.5	4.9	4.5	5.5	7.2	4.7	4.9	3.3	3.4	4.2	4.5	4.5	4.9	4.3	4.2
C24:0	2.4	2.5	2.2	2.2	1.8	1.8	2.0	1.7	1.6	1.8	1.6	1.6	ND	1.4	1.6	1.9	ND	0.8
Total	28.2	29.9	35.3	27.9	28.7	28.9	30.7	30.9	26.0	27.0	25.7	24.4	26.7	28.2	30.9	28.1	28.3	29.1
Monoenoics																		
C16:1n7	7.8	8.0	9.6	7.5	3.9	4.0	4.2	4.1	12.0	12.7	13.7	12.8	14.0	13.7	7.7	6.0	16.1	16.2
C18:1n9	8.0	8.9	6.9	6.9	6.6	6.9	7.6	6.9	6.2	6.4	7.1	7.0	6.9	6.7	6.6	5.8	7.5	8.2
C20:1n9	2.6	ND	2.5	2.4	2.5	2.5	2.1	2.3	1.8	1.5	2.0	2.0	1.8	2.1	2.0	2.1	1.7	1.6
Total	18.9	19.5	19.0	16.8	13.0	13.4	13.9	13.3	20.0	20.6	22.8	21.8	22.7	22.5	16.3	13.9	25.3	26.0
Polyenoics																		
C18:2n6	1.3	1.3	1.9	1.8	2.3	2.3	2.8	2.7	1.7	1.7	2.1	2.0	2.7	2.6	2.7	2.3	2.4	2.5
C18:3n3	ND	ND	ND	0.9	1.6	1.7	1.3	1.3	2.0	1.7	2.5	2.3	2.3	2.4	1.5	1.6	2.5	2.9
C18:4n3	1.3	1.6	1.9	2.0	6.5	6.6	4.8	4.6	3.5	3.5	3.4	3.3	3.4	3.3	3.8	3.3	3.5	3.6
C20:2n6	1.0	ND	ND	0.8	1.1	1.2	ND	ND	0.9	0.9	1.1	1.1	ND	ND	1.0	ND	ND	ND
C20:3n3	ND	ND	ND	0.3	ND	ND	ND	ND	ND	ND	0.6	0.6	ND	ND	ND	ND	ND	ND
C20:4n6	2.7	2.8	2.0	1.9	1.5	1.5	2.5	2.3	3.1	3.1	3.3	3.4	3.8	4.1	2.0	2.2	4.7	4.1
C20:5n3	19.6	19.7	19.8	20.5	13.9	13.9	14.6	13.3	15.7	16.3	14.2	14.4	12.8	13.1	15.1	15.2	11.7	10.6
C22:4n6	0.7	ND	ND	0.2	ND	ND	ND	ND	ND	ND	ND	0.3	ND	ND	ND	ND	ND	ND
C22:6n3	15.8	16.2	14.6	15.0	18.9	18.9	24.0	21.1	15.1	15.7	12.4	12.9	13.8	14.8	16.8	18.9	13.8	10.6
Total	42.4	41.6	40.2	43.4	45.8	46.1	50.0	45.3	42.0	42.9	39.6	40.3	38.6	35.1	42.9	43.5	38.6	34.3

ND: Not detected.
Only those fatty acids that were detected in the analysis were included.
Values are percentages of total fatty acids.
Results of two analysis are shown.

recovered more quickly after spawning than those of animals held in the hatchery. Further, animals held in the ocean for 36 days and then returned to the hatchery showed a decrease in gonadal condition after transfer to the hatchery. This decline in gonadal index was probably due to nutritional stress that resulted in some resorption of ripe gametes. Bayne et al. (1978) showed that a simultaneous regression and resorption of previously formed gametes occurred in *Mytilus edulis* under temperature stress or a lack of food. It has been clearly established that gonadal development is an energy-demanding process (Sastry 1979). Sastry (1968) showed that postspawning adults of *Argopecten irradians* required an abundant food supply and exposure to minimum threshold temperatures for initiation of gonadal growth and gametogenesis. In the present work, temperature was not a variable because it was equal in the hatchery and in the ocean. It is possible that the food used in the hatchery was not sufficient in quantity or quality to supply all the nutrients required for the regeneration of the gonads. The sharp increase in gonadal DNA content observed on day 12 in *A. purpuratus* held in the ocean corresponds to a large production of oogonia which was confirmed histologically (unpublished data). An increase in DNA content was found indicative of an increase in number of cells in rock scallop embryos by Whyte et al. (1990). A similar increase in DNA content, although not as great as the previous one, detected later (day 24) in the animals held in the hatchery confirmed that gametogenesis is retarded. The decline in carbohydrate content on day 24 in animals held in the ocean may have been due to a greater synthetic activity (especially proteins) that was consistent with a rise in the RNA level. Carbohydrate has been shown to be the metabolic substrate used by *A. purpuratus* for maturation of gametes (Martínez 1991a).

TABLE 5.

Final shell height, ash-free weight and RNA:DNA ratio of early juvenile *Argopecten purpuratus* when raised in the Ocean and in a hatchery.

	Shell Height (mm)	Individual Ash-free Weight (mg)	RNA:DNA Ratio
Hatchery	2.92 ± 0.46	1.25 ± 0.05	1.03 ± 0.19
Ocean	3.99 ± 0.62	3.19 ± 0.01	1.63 ± 0.14

Values are means + SD.

Wright and Hetzel (1985) used the RNA:DNA ratio as an indicator of nutritional stress and Robbins et al. (1990) stated that it was a good indicator of the sexual state of development for at least the male gonad. In the present work a decline in the ratio was demonstrated for those animals held in the hatchery. The value of the ratio in the group of animals held for 48 days in the hatchery was the same as for animals held for only 36 days in the ocean. This result indicates that adults held in the hatchery were not supplied with sufficient nutrients.

TABLE 6.

Levels of carbohydrates, lipids, proteins and nucleic acids of early juvenile *Argopecten purpuratus* when raised in the ocean and in a hatchery.

	Carbohydrate (μg/mg)	Lipids (μg/mg)	Proteins (μg/mg)	RNA (μg/mg)	DNA (μg/mg)
Hatchery	119.6 ± 35.7	183.4 ± 85.5	288.3 ± 62.6	16.5 ± 3.1	15.9 ± 0.9
Ocean	101.2 ± 13.9	69.2 ± 15.4	394.1 ± 28.3	21.5 ± 1.9	13.2 ± 1.0

Values, expressed as a function of ash-free dry weight, are means + SD.

Results obtained with early juveniles were similar to those found for broodstock. Early juveniles held in the ocean grew faster and had a higher RNA:DNA ratio than those held in the hatchery. These results could be due to nutritional stress. Holland and Spencer (1973) exposed *Ostrea edulis* larvae and spat to starvation and showed that neutral lipid and protein accounted for 65.7–86.4% of the total organic matter that was lost. So, the lower protein content of animals held in the hatchery may be due to this factor and not necessarily to the nutritional deficiency of this specific component. Utting (1986) showed that after metamorphosis *Crassostrea gigas* grew better on low protein diets.

Analysis of the fatty acid composition helps explain the slower growth of early juveniles held in the hatchery. Early juveniles held in the ocean showed a lower percentage of monoenoics but a higher percentage of polyenoic acids than those held in the hatchery. The principal polyenoic acids detected were eicosapentaenoic (C20:5n3) and docosahexaenoic (C22:6n3). Eicosapentaenoic acid has been shown to be the most frequent acid of most species of invertebrates (Isay and Busarova 1984). In the present work a higher content of docosahexaenoic acid was found in individuals held in the ocean than in animals held in the hatchery. As shown in another work (Langdon and Waldock 1981), many marine animals have a limited ability to synthesize higher polyunsaturated fatty acids, so the differences found in fatty acid composition of the different animals could be due to the microalgae used as food. In this study, the diet used for rearing *A. purpuratus* in the hatchery consisted of a mixture of *I. galbana* (T-iso), *C. calcitrans* and *C. gracilis* and *Isochrysis galbana* is richer in docosahexaenoic acid and poorer in eicosapentaenoic than the two *Chaetoceros* spp. (Bourne et al. 1989, Table 1).

TABLE 7.

Fatty acid composition of juvenile *Argopecten purpuratus* reared in the Ocean and in a hatchery.

Fatty Acid	Hatchery		Ocean	
Saturated				
C14:0	7.7	6.8	4.4	4.2
C16:0	14.0	13.8	18.4	17.5
C18:0	6.8	7.0	9.3	9.5
C24:0	ND	2.2	ND	ND
Total	28.5	29.8	32.4	31.2
Monoenoics				
C16:1n7	9.6	9.2	3.2	2.6
C18:1n9	8.8	8.3	6.0	6.9
C20:1n9	ND	2.1	2.5	3.1
Total	18.4	19.6	9.2	12.0
Polyenoics				
C18:2n6	2.6	2.5	ND	ND
C18:3n3	2.2	2.7	ND	ND
C18:4n3	5.4	5.2	2.8	2.7
C20:4n6	ND	ND	2.2	2.2
C20:5n3	12.5	12.1	13.6	12.7
C22:6n3	16.5	15.7	29.0	28.3
Total	39.2	38.2	47.6	45.9

ND: Not detected.
Only those fatty acids that were detected in the analysis are included.
Results of two analysis are shown.

Several studies have clearly demonstrated the essential requirement of n-3 polyunsaturated fatty acids for growth of juvenile bivalves. Langdon and Waldock (1981) found poor growth in *C. gigas* spat when fed *Dunaliella tertiolecta,* a microalgae deficient in docosahexaenoic acid. Laing and Millican (1986) evaluated various algal diets for growth of *O. edulis* spat and found that *Tetraselmis suecica* had the poorest nutritional value which might be explained by the absence of 22-carbon polyunsaturated fatty acids.

In the present study the group of faster growing early juveniles (those held in the ocean) had a lower content of total monoenoic fatty acids and C18:3n3 and C18:2n6 than the group with the poorest growth. These results are in agreement with those of Whyte et al. (1989) using *Patinopecten yessoensis* larvae fed on different diets indicating a minor dietary requirement for the linoleic, n-6, family of fatty acids for development and growth in scallops. Results of the present work are also in agreement with work reported by Joseph (1982) in his review on lipids in invertebrates. All the scallop species discussed had high percentages of C20:5n3 and C22:6n3 with the notable exception of the rock scallop *Hinnites multirugosus,* which had a high percentage of C18:2n6 and C18:3n3 fatty acids.

We conclude from this study that the diet used for conditioning broodstock and rearing early juveniles of *Argopecten purpuratus* in the hatchery, was not sufficient to produce optimum yields and further research work is necessary to perfect diets. Until optimum diet is found it would be better to hold broodstock and early juveniles in the natural environment where they will obtain a more balanced diet.

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SCALLOP (*PECTEN FUMATUS*) SETTLEMENT IN BASS STRAIT, AUSTRALIA

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ABSTRACT Settlement patterns of *Pecten fumatus* in Bass Strait were investigated over two years to determine the time and location of spatfall. Although small numbers of spat settled throughout the sampling period, most settled during one or two short periods at each of six locations. Most settled about 10 m above the sea bed; size-frequency analysis showed that the same cohort of spat settled at all depths. The numbers of spat settling were not the same in all six locations examined, and were different in successive years at a locality. The numbers present on collectors set at different depths also changed during the year; however the changes were similar in all localities. By measuring the uniformity of size and numbers of spat settling on collector lines in one locality, it was found that a single cohort of larvae can settle over an area of at least 4 km^2. Larval advection paths were examined by modelling a particle track backwards from each collecting region at the times of the major settlement, using a depth-averaged, wind-sensitive numerical circulation model. The model suggested that larval advection may be influenced by the strength and direction of the wind during the larval period at the onset of summer stratification. In one year, the modelling suggested there was little net movement of water (or larvae); however in the following year, when winds were generally much stronger, there was a pronounced displacement to the east. It is postulated that during calm summers scallop beds can be self-recruiting but, during windy summers, settling larvae may derive from beds great distances away.

KEY WORDS: scallops, recruitment, settlement, advection, spat

INTRODUCTION

The commercial scallop *Pecten fumatus* (Reeve 1852; = *Notovola meridionalis, Pecten alba*) occurs in coastal waters of southern Australia. Its distribution extends from the central New South Wales coast in the east, around Tasmania, and westward beyond the border between South Australia and Western Australia. The western limits of its distribution are unclear. It occurs in a range of habitats from enclosed embayments to exposed oceanic conditions, in depths ranging from 7 to at least 60 m and on substrates varying from muddy to coarse sand.

Depletion of *Pecten fumatus* beds by fishing was first recorded near Hobart, Tasmania in 1908. Since then, depletion by fishing has been recorded in the D'Entrecasteaux Channel, Norfolk Bay, Oyster Bay and Ringarooma Bay in Tasmania, Coffin Bay in South Australia, and in Jervis Bay, New South Wales (Young and Martin 1989). Beds of this species were found in Bass Strait off the north coast of Tasmania and first exploited in 1973 (Fig. 1). The subsequent discovery of large new grounds off Flinders Island in eastern Bass Strait in the late 1970s brought a surge in fishing activity. In 1982–83 landings from Bass Strait reached a record high of 4136 tonnes of scallop meat (approx. 12,000 tonnes live weight). However, by 1985 landings were declining rapidly, the last major bed (Banks Strait) was fished out during the 1986 season (McLoughlin et al. 1991). Subsequent surveys have found little recruitment to the region (Zacharin 1987, McLoughlin et al. 1988, Martin et al. 1989, Zacharin 1990).

The large fluctuations in catches of *P. fumatus* in southern Tasmania were described by Fairbridge (1953) and Olsen (1955) as erratic and unrelated to stock size. Many other scallop fisheries throughout the world have shown similar large fluctuations in both recruitment and commercial catches from year to year. Similar fluctuations in *Pecten maximus* in British waters were described by Baird (1966). Citing these references, Hancock (1973) suggested that because recruitment is so irregular, any relationship between stock and recruitment is not apparent. He later proposed that fishing regulations for *Pecten* species should be designed to compare yields and economic returns from fishing to "extermination" with various fishing strategies (Hancock, 1979).

The present management, which followed this approach, did not sustain the scallop fisheries in Bass Strait, so it is pertinent to ask why. The stock collapse may be due to local overfishing of self-recruiting beds, or it may be unrelated to the stock size of local adults, but due to periodic environmentally driven recruitment fluctuations from the same stocks; or it could result from harvested beds being periodically replenished by low-density unfished beds during favourable oceanic conditions.

Recruitment and distribution of sedentary marine invertebrates such as scallops are strongly influenced by advection and settlement of larvae (Moore and Marshall 1967, Butman 1987). Survival of larvae to settlement ultimately depends on the coincidence of suitable food and oceanic currents. Duration of spawning in *P. fumatus* in the Bass Strait area varies with location (Harrison 1961, Sause et al. 1987). The period between June and November generally coincides with the occurrence of spat settling on collectors (Dix 1981, Hortle and Cropp 1987, Sause et al. 1987). In other scallop species, the abundance of spat in collectors has also been shown to indicate the density of larvae in the water column (Lee & Jo 1980, Yamamoto 1960).

The water structure of Bass Strait changes seasonally from strong stratification between November and April (summer) to a well mixed state from April to September (Baines and Fandry 1983). This may also influence the potential for larval advection.

In this study we examined the advection and settlement of *P. fumatus* in Bass Strait. We first investigated the vertical distribution, area of settlement and the time and place where large numbers of larvae were settling on collectors. We then determined the theoretical advection paths of larvae using a numerical model of the circulation of Bass Strait (Fandry 1982) to estimate whether they came from adjacent or distant scallop beds.

MATERIALS AND METHODS

Collection of Settling Larvae

Collectors consisted of polypropylene 4 mm mesh bags each filled with approximately 1 kg of old monofilament nylon gill netting, previously used for commercial fishing (Dix 1981, Hortle

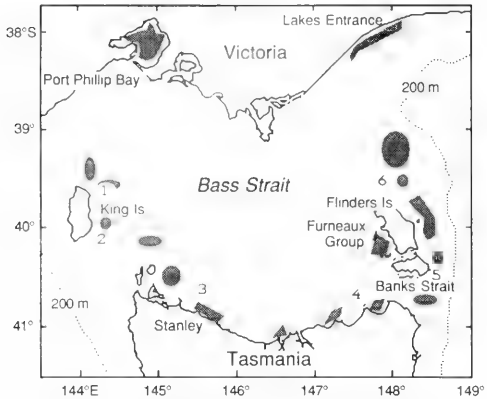

Figure 1. Bass Strait, showing spat collector sites and location of commercially fished beds (shaded areas) of *Pecten fumatus*.

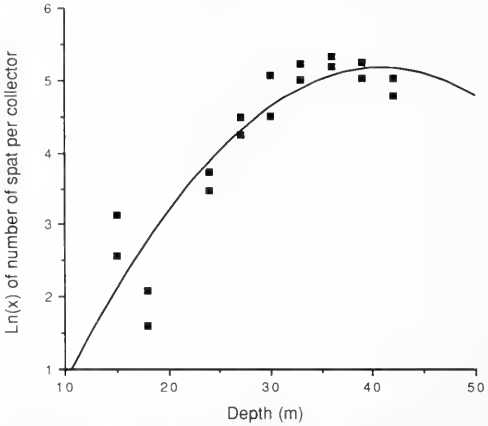

Figure 2. Numbers of *Pecten fumatus* spat collected in paired spat bags at each depth from one line at site 3 in January 1986.

and Cropp 1987). The bags were attached in pairs to an anchored rope held vertically by a subsurface buoy in water depths between 45 and 50 m.

The number and vertical distribution of the pairs of collectors varied between the two sampling years. In 1985/86 they were attached from 5 m above the sea bed to 15 m below the surface at 3 m intervals—a total of 10 pairs. In 1986/87 fewer collector bags were set, the bottom pair was at 5 m above the sea bed, with two more pairs distributed vertically at 6 m intervals. The collectors were deployed at six locations close to existing or previously harvested scallop beds (Fig. 1). Adult scallops from King Island and Banks Strait were collected while the spat lines were set and assessed for gonadal condition. From these data, collectors were deployed from before the stocks were assessed to have spawned until after significant settlement could no longer be detected (September 1985–April 1986; August 1986–April 1987).

Three lines of collecting bags were placed at each site, about 50 m from each other. Each month the oldest line was lifted and replaced with a new one. Apart from the first set in 1985, which was in the water for only two months, each line remained in the water for three months and shared the same water conditions for a month with the line lifted the month before and after. After lifting, the contents of the collector bags were washed into a 0.5 mm mesh plankton net held in a rigid frame. The catch was then frozen for subsequent sorting, identification, and measurement in the laboratory.

Environmental Measurements

Water samples were taken from the surface, midwater (25 m) and bottom with 8 L Niskin bottles after the line was lifted at each site. Initially a Yeo-Kal salinity/temperature meter was used to obtain vertical profiles of salinity and temperature; but it was subsequently replaced by a CTD probe in 1987. Chlorophyll *a* concentration in the water samples was determined by the method of Jones (1979), and the nutrients (silicate, nitrate, nitrite and phosphate) were measured with a Technicon model AA2 autoanalyser.

Data on wind direction and speed used in the circulation model were obtained from the Australian Bureau of Meteorology weather station at Wilsons Promontory, Victoria. These data were obtained at hourly intervals from 1 September 1985 to 14 December 1985, and from 1 August 1986 to 29 December 1986.

Statistical Methods

Spat abundance data were examined by ANOVA after transformation to natural logarithms. Missing samples were replaced,

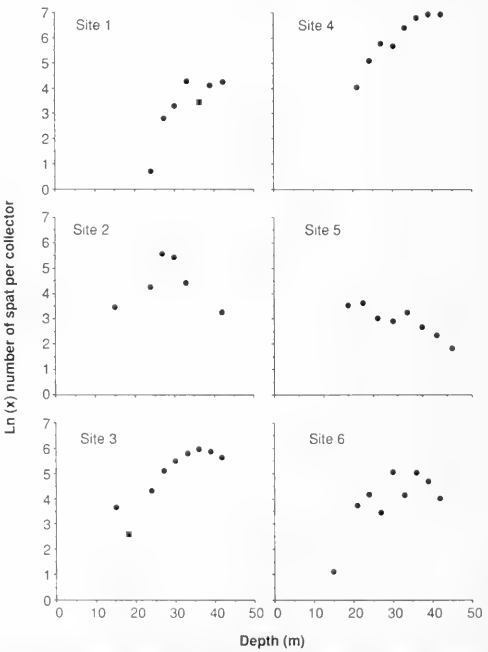

Figure 3. Mean number (from paired spat bags) of *Pecten fumatus* spat collected at each depth from one line at each site in January 1986.

TABLE 1.

Analysis of variance of vertical spat distribution on spat bags from the 1985/86 sampling: Sites 3, 5 and 6—Ln (count per bag) using 7 depths on 6 sampling occasions.

Source	df	Response Variable: Ln(x) of Abundance Per Collector Sum-of-Squares	Mean Squares	F	p
Site	2	8.603	4.302	11.88	<0.001
Time	5	68.929	13.786	38.06	<0.001
Depth	6	7.029	1.172	3.23	0.009
Site × Time	10	136.018	13.602	37.55	<0.001
Site × Depth	12	7.927	0.661	1.82	0.068
Time × Depth	30	22.456	0.749	2.07	0.010
Residual	53	19.197	0.362		
Total	118	258.932			

Missing samples (n = 7) were estimated from least square regression prior to analysis.

where possible, by samples from adjacent collector bags or by interpolated values in the statistical analyses. Where estimation of missing samples was unreliable or inappropriate, only data from the recovered samples were used.

Settling time was determined from an estimate of the age of the collected spat after settlement. Size-at-age of hatchery-grown spat was described by a third order polynomial equation. The proportional difference between this growth rate and that at each site for the size ranges in the samples was then estimated by calculating the differences between the modal size of cohorts in successive samples at a site. This was done for cohorts with more than 50 individuals by the modal analysis of MacDonald & Pitcher (1979), using their program MIX 2.3 (Icthus Data Systems).

Spat of *P. fumatus* settle from 210 to 230 μm shell height (Dix and Sjardin 1975) but the smallest spat detected by our methods was 500 μm. The time taken for growth between 230 μm and the smallest cohort detected in each sample was calculated from the hatchery growth rate data, and standardised by the proportional difference between it and the field rate derived as above for larger animals. The accuracy of the estimated settlement time was confirmed by the presence of the same cohort in samples from all lines immersed during the predicted time of settlement at the site.

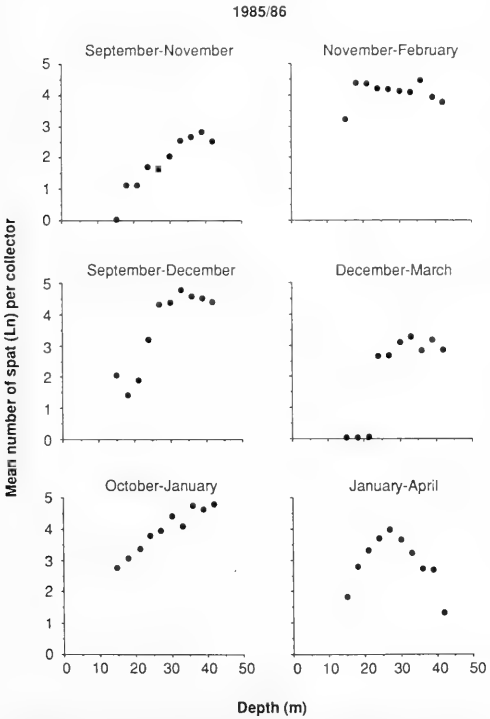

Figure 4. Number of *Pecten fumatus* spat collected at each depth during all sampling periods from sites 3, 5 and 6 in 1985/86.

Determination of Settlement Area

Five spat collector lines were laid off the north-west coast of Tasmania (Site 3, Fig. 1): one as the centre, and four to the north, south, east, and west 0.9 km away. They were deployed from October 1986 to January 1987, and from January 1987 to April 1987.

Advection Modelling

The circulation of Bass Strait has been described and modelled by Fandry (1982, 1983). The model used in the advection study was two-dimensional, based on numerical integration of vertically averaged shallow-water barotropic equations of motion. The model assumes that the larvae are neutrally buoyant passive particles, and that the water column is totally mixed, with no vertical differentiation in current strength or direction. The simulation was run backward in time for 30 days from the estimated time of settling and the track and theoretical position of the larvae noted 14 and 30 days prior to settlement. These times spanned the period from egg to the pediveliger stage (Dix and Sjardin 1975).

RESULTS

Vertical Distribution, Size and Abundance of Settling Spat

The vertical distribution and size of spat were initially examined by counting and measuring all spat in the nine pairs of bags immersed from October 1985 to January 1986 at site 3 (Fig. 1). Abundance was significantly and positively correlated with depth, and while a linear regression gave significant parameter values

TABLE 2.

Analysis of variance of settling area experiment. Two collector bags per line on two sampling occasions (Oct 86–Jan 87, Jan 87–Apr 87) at site 3; five lines were deployed across a 4 km^2 area.

Source	df	Response Variable: Ln(x) of Abundance of Spat Per Collector Sum-of-Squares	Mean Squares	F	p
Time	1	76.728	76.728	1316.593	<0.001
Line	3	0.065	0.022	0.374	0.775
Time × Line	3	0.132	0.044	0.757	0.549
Residual	8	0.466	0.058		
Source	**df**	**Response Variable: Mean Size (Shell Height) of Spat Per Collector Sum-of-Squares**	**Mean Squares**	**F**	**p**
Time	1	120.56	120.56	388.732	<0.001
Line	3	3.512	1.171	3.755	0.059
Time × Line	3	3.164	1.055	3.400	0.074
Residual	8	2.481	0.310		

(adjusted $R^2 = 0.742$, $p < 0.01$), a quadratic function gave a better fit to the data (adjusted $R^2 = 0.811$, $p < 0.01$). The better fit was due to progressively more spat settling to about 35 m depth and then settling in about the same numbers in all deeper bags (Fig. 2). A linear regression of the shell height against the depth of the collector was not significant (adjusted $R^2 = 0.001$, $p > 0.05$).

Differences in vertical distribution of spat were examined further to see whether there were differences between the sampling locations. The number of spat from one collector at each depth from a line recovered at each of the sites in January 1986 were analysed by two-way (sites by depths) ANOVA. The difference attributable to sites was significant ($F = 9.776$, df = 5,32, $p < 0.05$), that due to depth was not ($F = 2.116$, df = 9,32, $p > 0.05$). The pattern of abundance with depth was inconsistent (Fig. 3). Samples from sites 1, 3, 4 and 6 showed similar trends, but site 2 showed increases to 30 m followed by decreases, and site 5 showed a decrease in abundance with depth. Whether the nonsignificant depth factor was due to random variation or to the differences between sites could not be investigated in the absence of replication. So the problem was examined further by three-way ANOVA (sites × times × depths) in samples from sites 3, 5 and 6 collected from all six time periods in 1985/86.

As almost 50% of the collector bags from the top three sampling depths were washed off the lines in storms they were omitted from the analysis. The values for the remaining seven missing samples were estimated from least squares regression prior to the analysis. These data confirmed that although the number of spat differed significantly between the various depths sampled, the differences were consistent between locations. The additional data also showed that the form of these differences changed significantly over time (Table 1). Progressively more spat were present on deeper collectors at the start of the spawning season and through to January 1986 when lines deployed in October 1985 were recovered. However, the collectors lifted in February and March (deployed November and December respectively) had roughly the same numbers of spat at all depths. In contrast, the collectors recovered in April had most spat at 27 m depth, decreasing towards both deeper and shallower depths (Fig. 4).

Settlement Area

The size of an area in which a single cohort of larvae might settle was investigated twice from collectors suspended at 39 m depth from the five lines deployed off the north west coast of Tasmania. Four bags in this series were lost, so these were replaced by two samples collected at 43 m and two at 34 m. One line of the array was lost on the second sampling occasion, and was omitted from the analyses. Analysis of variance of both the mean

TABLE 3.

Analysis of variance of timing of settlement of scallop spat between sites, times and year: Sites 1–6 (Ln abundance per bag) using 2 collector bags at one depth on 6 sampling occasions for both 1985/86 and 1986/87.

Source	df	Response Variable: Ln(x) of Abundance Per Collector Sum-of-Squares	Mean Squares	F	p
Time	5	99.510	19.902	94.28	<0.001
Site	5	3.561	10.712	50.75	<0.001
Year	1	0.224	0.224	1.06	0.306
Site × Time	25	155.296	6.212	29.43	<0.001
Year × Time	5	22.749	4.550	21.55	<0.001
Site × Year	5	51.379	10.276	48.68	<0.001
Time × Site × Year	18	46.772	2.598	12.31	<0.001
Residual	65	13.721	0.211		
Total	129	419.340			

Missing samples (n = 14) were estimated from least square regression prior to analysis.

size and abundance per collector showed that significantly fewer and significantly larger animals were collected on the second (January to April) deployment. However, on each occasion there were no significant differences between lines in either size or number of settled spat (Table 2). This showed that a single cohort of larvae was capable of settling across an area of greater than 4 km^2.

Time of Settlement

The number of spat settling on the two deepest collectors at all sites and sampling periods for both years was examined by a three-way analysis of variance (sites × sampling time × years). Differences were not significant between years but were between sites and sampling periods (Table 3); however, significant interaction terms suggested that these might not be consistent. Progressively more spat were present in both years up to the January lifted collector bags, but numbers then decreased in the February lifted bags in 1985/86 (Fig. 5a). In 1986/87 abundances continued to increase to the February lifted bags, decreasing after that. Differences between sites were also relatively consistent between the two years (Fig. 5b). Sites 3, 4, and 5, traversing from the west to the east of northern Tasmania, all had more spat in 1986/87. In both years their numbers decreased from the west to the east (Fig. 5b). Sites 2 and 6 in contrast, had fewer spat in 1986/87, and site 1 showed little change in both years.

Settlement Dates

The estimated dates of settlement at each of the sites during the two spawning seasons are given in Table 4. Also included are the calculated shell heights of the modes of each settling cohort, their estimated post-settlement age, and the number of spat in the sample that was estimated to belong to each cohort. The calculated times of settlement suggest that the differences in spat abundance described above are due to discrete settlement events (Fig. 5a).

A single settlement occurred everywhere except sites 3 and 6 in 1985/86, which each had two settlements separated by about one and two months respectively. During 1986/87 only sites 4 and 5 had more than one settlement, separated by one and three months respectively. In 1985/86 settlement started in eastern Bass Strait, where it was first recorded from site 6 in September followed by sites 4 and 5 in October. The first settlement at the western sites occurred at sites 1 and 3 in November, followed by site 2 in December. In contrast, although an east–west segregation also occurred during 1986/87, all settlement at the western Bass Strait sites (apart from that in January at site 5) preceded any settlement at the eastern sites. 1986/87 was also characterised by later settlement at almost all sites. Sites with a single settlement in 1985/86 either were settled later in 1986/87 (site 1) or had an additional, later settlement (sites 4 and 5). In sites with double settlements in 1985/86 (sites 3 and 6), settlement occurred later in 1986/87. Only at site 2 did settlement occur during the same month in both years.

Hydrology

During 1985/86 the water column remained well mixed from the surface to bottom at sites 1, 2 and 6. At sites 3, 4 and 5, the water column was stratified from January to April, with the thermocline occurring at 27, 10 and 35 m respectively. During 1986/87 stratification was slight or absent at sites 2, 4 and 5, but occurred at the other sites between December and March. The depth of the surface mixed layer was 16, 42 and 10 m respectively for sites 1, 3 and 6. Settlement occurred during both stratified and unstratified conditions at a single site (e.g., site 5, 1986/87), and at stratified and unstratified sites in a single year (e.g., sites 2 and 3 in both years). The bottom water temperature at the time of settlement varied between and within sites from 12 to 16°C.

Nutrient concentration differed between water depths at most sites. Changes in concentration were correlated across sites during both years, except for phosphates at all sites apart from 3, and silicates and chlorophyll *a* at site 3. No seasonal trend in silicate or chlorophyll *a* was detected, while concentrations of the latter were generally higher in 1986/87 than in 1985/86. Nitrates and phosphates tended to increase in concentration during winter (May–August), particularly at sites 3 and 5, and during 1986/87.

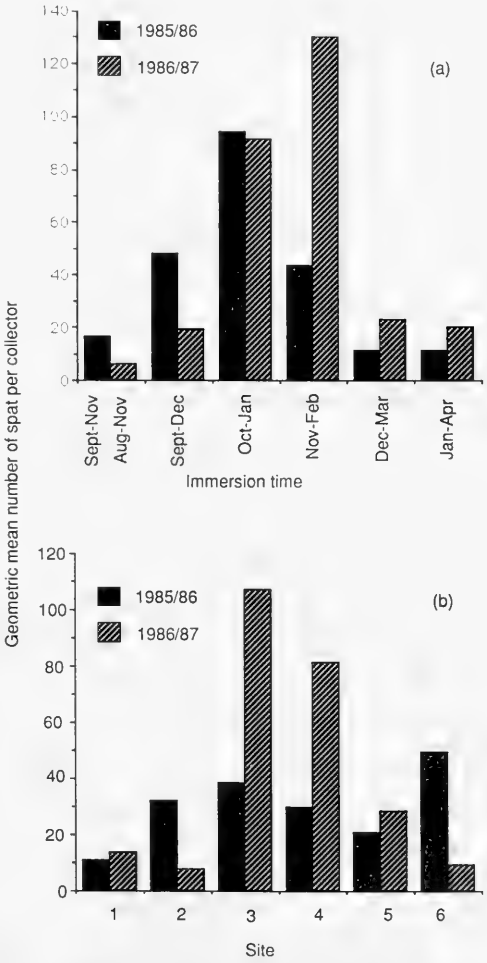

Figure 5. Abundance (geometric mean) of *Pecten fumatus* spat in collectors in 1985/86 and 1986/87. a) during each sampling period; b) at each site.

TABLE 4.

Modal shell heights, number in each mode, and estimated date of settlement for *Pecten fumatus* spat settling in collectors during 1985–86 and 1986–87.

Site	Date Recovered	Mode (mm)	Number in Mode	Estimated Post-settlement Age (days)	Estimated Settlement Date
			1985–86		
1	14-12-85	2.0	3	2	
	13-01-86	5.3	47	52	
	12-02-86	11.5	128	82	22-11-85
2	12-01-86	4.5	151	29	
	13-02-86	8.7	36	61	14-12-85
3	13-12-85	2.7	64	27	
	11-01-86	9.9	241	56	16-11-85
	11-01-86	6.5	56	39	
	17-02-86	11.5	55	79	03-12-85
4	11-11-85	3.1	86	27	
	09-12-85	6.2	408	55	15-10-85
5	12-11-85	3.0	77	27	
	12-12-85	6.8	256	57	16-10-85
6	13-11-85	4.6	77	47	
	10-12-85	6.5	55	74	27-09-85
	14-01-86	5.0	97	47	
	12-02-86	9.4	92	75	28-11-85
			1986–87		
1	20-01-87	1.5	66	17	
	22-02-87	8.2	94	50	03-01-87
	21-03-87	collector not recovered			
2	20-01-87	3.1	37	35	
	22-02-87	7.0	34	70	14-12-86
3	18-01-87	4.4	2086	37	12-12-86
	24-02-87	collector not recovered			
4	11-11-86	2.5	51	20	
	15-12-86	6.0	29	54	22-10-86
	15-12-86	1.0	18	17	
	22-01-87	4.5	302	55	28-11-86
5	12-11-86	2.4	28	29	
	14-12-86	4.3	283	61	14-10-86
	24-02-87	5.0	229	34	
	24-03-87	4.7	87	62	
	25-04-87	6.2	86	94	12-01-87
6	14-12-86	1.1	48	18	
	21-01-87	collector not recovered			
	23-02-87	9.6	7	89	26-11-86

Modal shell heights and number in each mode are averages of two replicate collectors located 5 m above the seabed.

Wind

The wind in both years blew mainly from the west, alternating with short periods of easterlies (Fig. 6). During 1986/87 there were more west winds, and the mean velocities were higher than in 1985/86. East winds in 1986/87 were also generally stronger.

Larval Advection Modelling

During 1985/86 the output of the circulation model suggested that most larvae in the plankton for 30 days came from near (<100 km) to the collection sites (Fig. 7a). This was especially pronounced at sites 1 and 2 and with the second settlement at site 6. Larvae at other sites or times were predicted to originate from various directions and distances between 100 and 200 km from the settlement site. Larvae in the first settlement at site 6 came from the south-west of Flinders Island; those at site 5 came from the north-east; those at site 3 came from the west of the central north coast of Tasmania; and those at site 4 from the east, off the central north coast.

During 1986/87 the estimated paths were very different. The stronger westerly and southwesterly winds during the larval phase produced results that suggested that larvae had much longer paths.

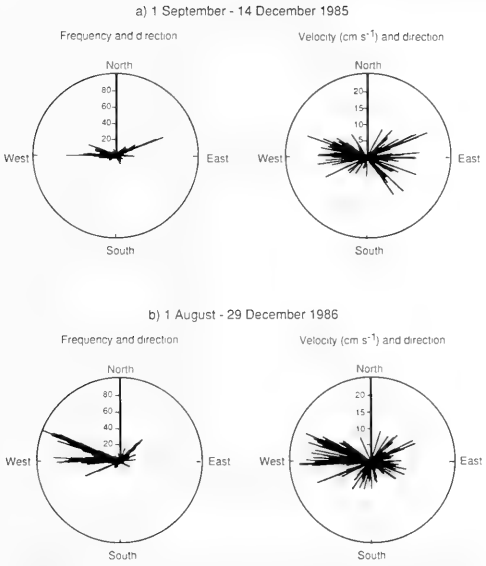

Figure 6. Wind rose records for Bass Strait, as measured at Wilsons Promontory, Victoria. a) 1985; b) 1986.

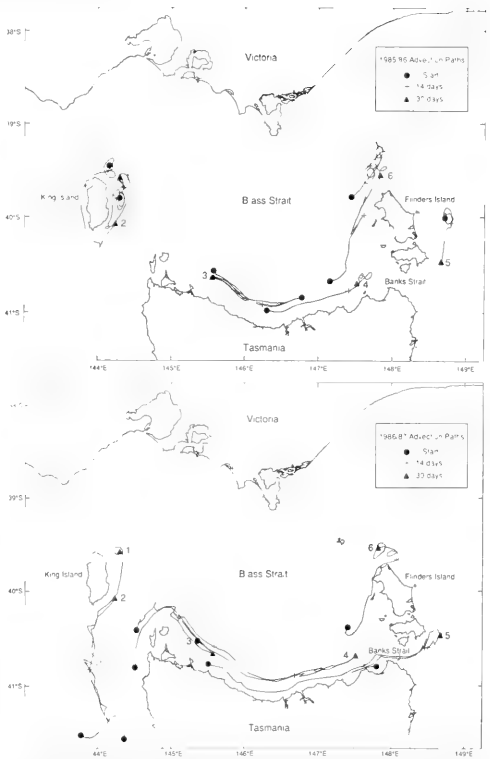

Figure 7. Particle track plot of larval advection from all six sites. Track plots have been run backwards in time and are marked at the 14 d and 30 d position for (a) 1985/86 and (b) 1986/87. Where two significant settlements occurred at a site, the track for both is shown.

These were northward to sites 1 and 2 and easterly elsewhere (Fig. 7b). The paths for larvae settling at sites 1 and 2 indicated a source about 200 km south and to the west of Tasmania. Settlement at site 6 again appeared to have been derived from the west of Flinders Island. The strong westerly wind at the time of advection indicated that the first larval settlement at site 5 came from about 250 km west of the site, while the second settlement, at a time of lighter winds, came from some 80 km to the west. Larvae settling at site 4 were also estimated to come from the west: the first settlement from off the west Tasmanian coast (which also produced larvae for settlement at site 3) and the second from off the central northern Tasmanian coast.

DISCUSSION

More spat were generally found in the deeper bags in both our study and in studies of other scallop species (Brand et al. 1980, Bull 1980, Hortle and Cropp 1987, Tremblay and Sinclair 1990). Our observation that this trend was not consistent during the sampling period also applied to collectors for *Chlamys tehuelchus* (Ruzzante and Zaixso 1985). There it was thought to be due to 'variable conditions of the water mass' containing the larvae—presumably turbulent flow associated with strong currents or topographic gyres that mixed settling stage larvae throughout the water column. In the present study, a predominantly bottom distribution at most sites early in the settling season became variable later in the summer period.

The larvae of *P. maximus* are thought to be initially positively geotactic, swimming continuously. They then alternately swim and sink, and finally explore the substrate, periodically reverting to swimming (Cragg 1980). The increased abundance of *P. fumatus* spat on our deeper collectors may reflect such behaviour, or simply be due to consistently more larvae occurring at depth as suggested for *P. yessoensis* (Yoo and Park 1979, Maru et al. 1973) and *P. maximus* (Kaartveldt et al. 1987). The thermocline did not appear to be significantly associated with settlement in Bass Strait: significant differences in the pattern of vertical distribution of spat were not detected between sites 3, 5 and 6, despite the existence of a thermocline at sites 3 and 5, but not at site 6.

Our observation on the potential size of an area of settling larvae has at least two explanations: the larval patch size may have been greater than the size of our collecting array; or a small patch of settling larvae could have moved past the collectors on successive tidal cycles, with some settlement occurring each time they passed. This settlement mechanism has some support from Gaines et al.'s 1985 study of *Balanus* larvae, where settlement from a water mass passing settling sites significantly reduced the larval concentrations reaching subsequent sites.

The advection paths projected by the model were very much influenced by the strength and direction of the wind. In 1985/86 little net movement of water (or larvae) was suggested; however in 1986/87, when winds were generally much stronger, there was a pronounced displacement to the east, as projected by Baines and Fandry (1983). The flow simulation model used in the present study obviously neglects several critical aspects of larval behav-

iour and circulation processes in the water column. These include the trapping effects of recirculating flows around islands and headlands (Butman 1987), and the interaction of the vertical distribution of larvae and the water flow during stratified conditions. The effect of release of gametes at the sea bed also remains to be quantified. The observed vertical distribution of spat could be a function of the mobility of the larvae in different phases of their planktonic life. If larvae are dispersed near the sea bed, then the critical wind effects in the model suggest that the frictional effects of the sea bed on flow may also restrict advection paths.

Despite these shortcomings, these results do give support to the hypothesis that scallop beds can be self-reseeding, but that during years of strong winds, settling larvae may derive from distant beds. We are aware of only one other similar investigation of scallop larval advection: Darby and Durance's (1989) shelf-flow simulation model of commercially fished regions in the English Channel and Irish Sea. They used summer average conditions in a depth-averaged model to predict the dispersal of up to 5000 particles that could be tracked simultaneously, and ignored thermocline fronts and the vertical distribution of larvae. Their results, like ours, suggested that many commercially fished beds could be considered self-recruiting, while others may depend on parental stocks 'upstream.'

There is now an accumulating body of evidence to confirm that recruitment to scallop beds may follow this general model. Sinclair et al. (1985) examined the history of fisheries for *P. magellanicus, Chlamys opercularis* and *P. maximus*. They suggested that the persistence of widely separated aggregations of characteristic abundance in consistent locations strongly implied that in these species many beds were self-sustaining. Furthermore, the location of the beds was related to physical oceanographic features such as gyres or two-layered circulation. Genetic studies also suggest that stocks of *C. opercularis* occur in discrete populations in United Kingdom and French waters (Beaumont 1982). However, there is strong evidence that the source of recruitment to some stocks may vary from year to year (MacLeod et al. 1985). Fluctuations in recruitment of *Placopecten magellanicus* have been correlated with water temperature during the larval life history (Dickie 1955). They were also related by an eight to nine year periodicity of catches and temperature, and correlated with long term tidal components of the same period (Caddy 1979). Increases in the population size of *Argopecten purpuratus* to over 60 times that in normal years have also been reported. These were related to specific oceanographic conditions, and thought to be due to the influence of warmer *El Nino* waters in Peru (Wolff 1988).

Spawning and fecundity, larval advection, larval survival, settlement success, and post-settlement and juvenile mortality will all combine to affect recruitment strength in succeeding years (Butman 1987, Mann 1988, Palmer 1988). The combination may produce extreme variability in recruitment, although some scallop beds endure many years of fishing and so show at least some measure of regular recruitment. The present study shows that, whilst settlement patterns are variable, some trends do persist between years and sites, and that recruitment in calm years may be a localised event. The implication of these observations for management is that the preservation of minimum spawning stocks must be considered. If it is not, the possibility of recruitment overfishing (Sinclair et al. 1985, Orensanz 1986), ignored in the Bass Strait fishery until now, becomes a very real possibility.

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EFFECTS OF BACTERIAL FILMS ON THE SETTLEMENT OF THE OYSTERS, *CRASSOSTREA GIGAS* (THUNBERG, 1793) AND *OSTREA EDULIS*, LINNAEUS, 1750 AND THE SCALLOP *PECTEN MAXIMUS* (LINNAEUS, 1758)

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ABSTRACT It has been reported that bacterial films are beneficial to the set of the larvae of bivalves. The bacterium, *Shewanella colwelliana* (LST), promoted set of *Crassostrea virginica*. Here we report scale-up experiments on three bivalve species reared in European commercial hatcheries. They were the oysters, *Crassostrea gigas* and *Ostrea edulis*, and the scallop, *Pecten maximus*. Biofilms of LST attracted larvae and promoted set of *C. gigas* and *O. edulis* in one series of experiments. However, these biofilms did not stimulate *P. maximus* set.

KEY WORDS: settlement, *Crassostrea gigas*, *Ostrea edulis*, *Pecten maximus*, bacterial films

INTRODUCTION

Several environmental factors may induce invertebrate larval settlement and/or metamorphosis: particularly, the nature of the substratum, some dissolved compounds, or the biofilm colonizing the substratum. Substrata, immersed in seawater, are rapidly colonized by a biofilm, initially produced by bacteria (Corpe 1970). Multispecies bacterial films have been found to induce settlement of polychaete larvae (Crisp & Ryland 1960, Kirchman et al. 1982), bryozoa (Brancato & Woollacott 1982), and the American oyster *Crassostrea virginica* (Young & Mitchell 1973). Bacteria were also found to induce settlement of the polychaete, *Protodrilus rubropharyngeus* (Gray 1967), and of planula larvae of *Hydractinia echinata* (Müller 1973, Neumann 1979).

In the case of molluscs, Morse et al. (1979) demonstrated that gamma-aminobutyric acid (GABA) induced metamorphosis of the abalone, *Haliotis rufescens*, GABA being an analogue of compounds produced by cyanobacteria living on algal surfaces used as substratum by the gastropod (Morse & Morse 1984). Weiner & Colwell (1982) demonstrated that a particular bacterium, first designed "LST" then classified as *Shewanella colwelliana* (Weiner et al. 1988, Coyne et al. 1989) stimulated set of the oyster *C. virginica*. They showed that an active compound was L-3-4-dihydroxyphenylalanine (L-dopa) which is produced by the bacterium, when fixed on a substratum. Coon et al. (1985) showed that this compound also favored settlement of the Pacific oyster, *Crassostrea gigas*.

The aim of this study was to test the effect of LST on the settlement of different marine bivalve species, which are produced in European commercial hatcheries, namely the oyster, *C. gigas* and *Ostrea edulis*, and the scallop, *Pecten maximus*.

MATERIALS AND METHODS

Origin of Larvae

Larvae of *C. gigas*, provided by the Satmar hatchery (Barfleur, France), were transported to the experimental hatchery of Tinduff (Plougastel-Daoulas, France), out of seawater, on a polyester mesh at 5°C. They were then immersed in filtered seawater, and maintained with aeration at 20°C. Settlement experiments were carried out within 24 hr of arrival. Environmental conditions (temperature, aeration, food, seawater renewal, etc.) were those used by the Satmar hatchery to provide optimum production (Tritar 1987, LeBorgne 1977). Shell chips were used as substrata for spat set. Since it was difficult to count colonizing bacteria on shell, glass slides were included as the substratum (Weiner & Colwell 1982). Set normally occurred within 3 days.

Larvae of *Ostrea edulis* were reared and tested for set in the HEPC hatchery (Carantec, France) (Tritar 1987). Larval collectors were nylon bags (mesh size 3 μm), each containing 600 hundred mussel shells, as usually done in the hatchery. Set normally occurred within 6 days.

Larvae of *Pecten maximus* were reared and tested for set in the Tinduff hatchery (Plougastel-Daoulas, France). For the settlement phase, larvae were transferred to special tanks made of PVC cylinders (500 mm diameter, 450 mm high), closed at the bottom by a polyester net (140 μm mesh size), on which larvae set after 4 to 10 days. Those tanks were immersed in larger tanks with 3000 liters of seawater.

Preparation of Bacterial Films

Bacterial films were prepared from two bacterial strains (LST-D and PS). LST-D strain is a high melanin-producing variant of LST, isolated by Weiner & Colwell (1982) and characterized as *S. colwelliana* (Weiner et al. 1988, Coyne et al. 1989). PS is an unidentified pseudomonad, isolated from a glass slide immersed in seawater, and used in previous experimental surface colonizations (Fera 1985). To form films on the substrata (collectors), the bacteria were inoculated into 700 ml 2216E Marine Broth medium (Oppenheimer & Zobell 1952) and incubated for 48 hrs (beginning of stationary phase; 20°C, PS; 25°C, LST). Culture densities, determined by epifluorescence microscopy (Hobbie et al. 1977), ranged from 9.2×10^7 to 4.8×10^8 cells ml^{-1}. Substrata were cleaned with HCL 0.1N (glass slides) or NaOCl, care-

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fully rinsed in sterile seawater and then immersed in *S. colwelliana* or *Pseudomonas* sp. suspensions (LST-D and PS respectively) for 24 hrs. After colonization, substrate collectors were rinsed in sterile seawater and used for set experiments. Bacterial densities on glass collectors were determined by epifluorescence microscopy (Hobbie et al. 1977). Nylon nets and mussel shells were examined by scanning electron microscopy (SEM), after fixation by glutaraldehyde (2% in 0.2 μm filtered seawater), ethanol dehydration and critical point drying. Autochthonously filmed substrata were prepared by immersion (24 hrs) in hatchery seawater before experiments (SW), while "clean" substrata did not receive prior exposure to seawater bacteria (C).

Bacterial counts of larvae and postlarvae were made before set experiments. Larvae of each species were concentrated and gently homogenized. Twenty 1 ml samples were taken with a pipette (Eppendorf P5000) for direct microscopic bacterial counts.

Design of Experiments

C. gigas: Two parallel experiments were performed, each in two 40 liter PVC tanks, containing 30 liters of seawater. Per tank, 40 glass slides (10 per type of film) in expt. 1 and 60 glass slides (15 per type of film) in expt. 2 were randomly distributed on the bottoms. The salinity of the seawater was 33 ppt, and temperature was 25°C. Larval densities were 100 l^{-1} in expt. 1 and 1000 l^{-1} in expt. 2. Larvae were fed daily with a mixture of unicellular algae. The seawater was aerated and renewed each day. Experiments were terminated after 2d (expt. 1) or 3d (expt. 2).

O. edulis: Two series of experiments were performed. For the first, 12 nylon bags (4 per each experimental surface) were immersed in a 40 liter tank containing 30 liters of seawater (32 ppt, 23°C) with 1000 larvae l^{-1}. For the second, one 370 liter tank with 39 bags (13 per each experimental surface) and one 25 liter tank with 6 bags (2 per each experimental surface) were used. They contained 300 liters of seawater (700 larvae l^{-1}) and 20 liters of seawater (750 larvae l^{-1}) respectively. During both experiments, larvae were fed daily with a mixture of unicellular algae. Seawater was aerated and renewed every two days. Set experiments were terminated after six days of exposure to respective surfaces.

P. maximus: Two experimental series were performed, each using 4 cylindrical tanks. Each tank was partitioned into four 90° sectors (one per experimental surface). The cylinders were immersed in a 3000 liter seawater tank (temperature, 16°C, salinity, 34 ppt). Larval densities for the two series were 170 l^{-1} and 133 l^{-1} respectively. The seawater was aerated and changed each day after gentle cleaning of the substrata. Larvae were not fed. Experiments were terminated after 4 and 2 days respective exposures to the experimental surfaces.

After settlement, fixed larvae were counted under a binocular microscope, metamorphosis being confirmed by new shell growth. In the case of *C. gigas*, all the glass collectors were observed. In the case of *O. edulis*, a sample of 50 to 100 valves was observed, and postlarvae counted. For *P. maximus*, 30 to 40 net areas of 1 cm^{-2} were randomly selected for counting the post larvae. In all cases, results are expressed as total numbers of postlarvae per type of collector (filmed by LST-D, PS or control) and percentages of fixed larvae. In all experiments, after larval set was quantitated, each of the three species of post larvae were reared for two weeks to monitor viability.

TABLE 1.

Densities of bacterial films before and after *C. gigas*, *O. edulis* and *P. maximus* set.

Shellfish	Experimental Series	Experimental Surface	Suspension Density (cells.ml^{-1})	Initial Surface (cells.cm^{-2})	Final Surface Density (cells.cm^{-2})
Crassostrea gigas	1	LST	$1{,}09 \times 10^8$	$4{,}7 \times 10^5$	$1{,}3 \times 10^6$
		PS	$4{,}7 \times 10^8$	$3{,}4 \times 10^8$	$3{,}8 \times 10^6$
		SW	$2{,}8 \times 10^5$	$3{,}1 \times 10^4$	$7{,}5 \times 10^5$
		C		0	$1{,}0 \times 10^5$
	2	LST	$1{,}2 \times 10^8$	$3{,}5 \times 10^5$	ND
		PS	$1{,}2 \times 10^8$	$8{,}6 \times 10^5$	ND
		SW	$2{,}4 \times 10^5$	$3{,}1 \times 10^4$	ND
Ostrea edulis	1	LST	$1{,}6 \times 10^7$	ND	ND
		SW	$9{,}2 \times 10^5$	ND	ND
	2	LST	$1{,}0 \times 10^7$	$1{,}9 \times 10^6$	ND
		SW	$1{,}6 \times 10^5$	$2{,}8 \times 10^5$	ND
Pecten maximus	1	LST	$8{,}5 \times 10^7$	$9{,}5 \times 10^6$	ND
		PS	$1{,}1 \times 10^8$	$9{,}8 \times 10^6$	ND
		SW	6×10^5	0	ND
		C		0	ND
	2	LST	$9{,}1 \times 10^7$	$9{,}7 \times 10^6$	ND
		PS	$9{,}2 \times 10^7$	$6{,}6 \times 10^6$	ND
		SW	$2{,}3 \times 10^5$	0	ND
		C		0	ND

Collectors have been colonized by *Shewanella colwelliana* (LST), *Pseudomonas* sp. (PS), natural seawater bacteria (SW).
C: uncolonized control collectors.

TABLE 2.

C. gigas set on bacterial films

Experimental Series	Tank Number	Experimental Surface	$\bar{X}$	Fixed Larvae Total	%
		LST	1,6	76	29
	1	PS	4,4	44	16,8
		SW	7,4	74	28,2
		C	6,8	68	26
1					
		LST	6,6	66	24,6
	2	PS	9,4	94	35,1
		SW	6	60	22,4
		C	4,8	48	17,9
		LST	40	600	40,1
	1	PS	19,5	293	19,6
		SW	24,3	365	24,4
		C	15,9	263	15,9
2					
		LST	22,5	437	36,8
	2	PS	13,5	202	17
		SW	19,7	295	24,9
		C	15,9	252	21,2

$\bar{X}$ = mean of fixed larvad per type of collector; Total: total of larvae fixed on collectors of one type; %: percentage of fixed larvae per type of collector.

RESULTS

C. gigas *Set*

Larval substrate collectors were colonized as a result of immersion in respective bacterial cultures (Table 1). Surface densities ranged from 3×10^5 to 4×10^6 cells cm^{-2} for LST-D and PS collectors in both the first and second series of experiments. Surfaces exposed to SW were colonized at slightly lower densities (e.g. 3×10^4 cells cm^{-2}). On all collectors, bacterial densities increased slowly during the settlement assay.

The percentages of larval set on each type of filmed surface are presented in Table 2. For the first series, larval set on respective immersed surfaces ranged from 16.8 to 29.0% of the total immersed surfaces in tank 1, and from 17.9 to 35.1% in tank 2. Approximately 6.7% of the total larvae set on these surfaces with the remainder set elsewhere in the tank or unset. Statistical analysis of data (Kruskall & Wallis test) indicated that differences in set from one film to another were not significant. For the second series, percentages of set on test surfaces varied from 15.9 to 40.1% in tank 1, and from 17.0 to 36.8% in tank 2. In both tanks, highest values were found for LST-D collectors, and this difference was statistically significant ($P > 95\%$; Kruskall & Wallis test). The percentages of fixed larvae versus number of pelagic larvae introduced in the settling tanks were 19 and 7% for the series 1 and 2 respectively. 75 to 96% of cemented *C. gigas* metamorphosed and grew normally for 15 days post settlement.

O. edulis *Set*

Where sampled, bacterial surface densities were similar to those of *C. gigas* assays (Table 1). For the first series of set-choice experiments, percentages of fixed larvae on different surfaces varied from 13.5 to 67.6 in tank 1 and from 20 to 50% in tank 2. In both cases, most set occurred on "control" collectors on which the biofilms that formed during the experiment were not examined. For the second series, percentages of fixed larvae varied from 13.3 to 68.3% in tank 1, and from 23 to 46% in tank 2 (Table 3). The highest values were found for LST-D collectors and were significantly different ($P > 99\%$; Kruskall & Wallis test) from the controls. Overall, 7% of the series 1 and 11% of the series 2 larvae set. Most *O. edulis* (71 to 85%) developed and grew normally.

P. maximus *Set*

LST-D and PS colonized the cylindrical collectors at about the same densities as the shell and glass substrata (Table 1). Surprisingly, autochthonous sweater bacteria did not colonize the *P. maximus* collectors after 24 hrs. There was no significant difference in the *P. maximus* choice of one surface over another (Table 4). There was good set on non-filmed surfaces. Consequently, on cylinder substrata, it is likely that neither LST-D or PS positively or negatively influence *P. maximus* set. Furthermore, on cylinder surfaces, films were not a prerequisite for set. For series one, a given surface attracted from 21.0 to 31.7% of the pelagic larvae. When the experiments were repeated (series 2, Table 4), there was more variability (9.1 to 54.5%). Total bacterial densities on a specific surface were not correlated with this variability and, unlike oysters, *P. maximus* larvae were not attracted to biofilms of LST-D. Approximately 5% of the *P. maximus* set on the exptl. surfaces. Those that did, developed and grew normally for at least one week subsequent to their set.

DISCUSSION AND CONCLUSIONS

The percentages of pelagic larvae of a particular species which reach a substratum, settle and metamorphose are not possible to assess in the natural environment. Available data come from laboratory experiments or hatchery data, and are variable because it is difficult to control all of the factors conducive for set (Weiner et al. 1989). Gordon & Jones (1982) reported that percentages of set of *C. virginica* in U.S. commercial hatcheries fluctuated between 10 and 50%. Buestel et al. (1982) reported that set rates of *P. maximus* was about 50% in the hatchery of Tinduff, where the

TABLE 3.

Ostrea edulis set on bacterial films.

Experimental Series	Tank Number	Experimental Surface	$\bar{X}$	Fixed Larvae Total	%
	1	LST	0.5	320	13.5
	(40 litres)	SW	0.7	448	18 5
		C	2.5	1600	67 6
1					
	2	LST	0.9	576	30
	(40 litres)	SW	0.6	384	20
		C	1.5	960	50
	1	LST	4.1	1148	68.3
	(25 litres)	SW	1.1	308	18.3
		C	0.8	224	13 3
2					
	2	LST	5.2	9464	46
	(370 litres)	SW	3.5	6370	31
		C	2.6	4732	23

TABLE 4.

Pecten maximus set on bacterial films.

Experimental Series	Tank Number	Experimental Surface	$\bar{X}$	Fixed Larvae Total	%
		LST	9	4419	23,7
	1	PS	9	4419	23,7
		SW	12	5892	31,6
		C	8	3928	21
		LST	8	3928	28,6
	2	PS	8	3928	28,6
		SW	6	2946	21,4
		C	6	2946	21,4
1					
		LST	9	4419	22
	3	PS	10	4910	24,3
		SW	13	6383	31,7
		C	9	4419	22
		LST	7	3437	22,6
	4	PS	8	3928	25,8
		SW	9	4419	29
		C	7	3437	22,6
		LST	2	982	18,2
	1	PS	2	982	18,2
		SW	6	2946	54,5
		C	1	491	9,1
		LST	1	191	16,7
	2	PS	2	982	33,3
		SW	2	982	33,3
		C	1	491	16,7
2			2		
		LST	1	982	33,3
	3	PS	2	491	16,7
		SW	1	982	33,3
		C		491	16,7
			3		
		LST	3	1473	30
	4	PS	2	1473	30
		SW	2	982	20
		C		982	20

experiments reported here were performed. Le Borgne (1977) estimated that the percentages of settlement for *C. gigas* and *O. edulis* in the Satmar hatchery were 70 and 90% respectively. Obviously, the results obtained in these settlement experiments were lower.

There may be several explanations for these differences. In the case of *C. gigas,* shell debris used in the Satmar hatchery are more inductive to biofilm formation and oyster set than the glass slides used here. For the two other invertebrates, settlement conditions were very close to those used commercially. However, in these studies counts of both pelagic and fixed larvae were more critically evaluated, and only those that set on the collectors were counted. Also, dilutions, transfers, and other manipulations could have affected test larvae, making them less competent for settlement. The percent set did allow conclusions to be made concerning the effects of biofilms.

For the first group of *C. gigas* set experiments, there was no significant difference in the choice of experimental set surfaces. However, for the second group, collectors filmed by LST-D-coated glass surfaces attracted significantly more oysters than those pre-coated by *Pseudomonas,* autochthonous flora or no organisms. Interestingly, the *O. edulis* experiments yielded the same results: no significant difference for the first experiment, and a significant "decision" to set on surfaces coated films of LST-D as opposed to other bacteria in the second series of experiments. Conversely, neither LST nor PS biofilms appeared to be especially beneficial to *P. maximus* set.

Unlike previously reported studies (Weiner et al. 1989) the bivalves in these experiments were presented with a choice of surfaces: two bacterial species, autochthonous organisms and "no film". It was previously reported that LST-D films enhanced set over unfilmed controls. Here we report that these films can preferentially attract motile larvae as well as enhance their set.

It has been frequently noted that, as reported here, set is highly variable in laboratory microcosms and in hatcheries (Weiner and Colwell 1982, Weiner et al. 1989). The failure to search and/or metamorphose on a particular substratum could be attributed to so many variables, ranging from changes in physical conditions to altered larval or bacterial physiology, that the cause often remains elusive. However, observations of the stimulating effect of LST-D films, detected numerous times by several laboratories (Bonar et al. 1990, Tritar S. 1987, Weiner et al. 1989) remain valid and significant even if the effect cannot always be induced to occur. A real challenge, with potential importance for habitats in peril, like Chesapeake Bay, is to learn why not.

The densities of PS and LST-D that colonized the surfaces of the three types of shellfish collectors were each within an order of magnitude of $10^5/cm^2$ at the beginning of the experiments. However counts of colonizing bacteria after 48 hrs exposure to larval and autochthonous bacteria during the first *C. gigas* experiments showed that, as expected, the bacteria multiplied and the biofilm grew. The pure culture films from LST-D and PS were progressively coinhabited by other spécies. Therefore the induction of set by a particular strain could have been "diluted" with the time. Weiner & Colwell (1982), Weiner et al., (1985), reported the induction of *C. virginica* set by LST-D, after 24 hours. In two cases reported here, older LST-D films (48–144 hrs) still attracted larvae and induced *C. gigas* and *O. edulis* set in a more complex hatchery environment. Yet, the dilution of this species by non-inductive organisms could have contributed to the negative results in series one and it is reasonable to suggest that LST-D cued many of larvae during the first hours of exposure, when the biofilms were still largely monospecies. Also it should be noted that, due to its physiology and ability to both produce and resist quinones, LST-D competes well in *in situ* biofilms. Thus, LST-D remains a factor in even long term experiments.

Pecten maximus larvae were neither attracted nor repelled by LST-D biofilms under the experimental conditions employed. The two most likely hypothesis are that first, LST-D may specifically induce oysters (*Crassostrea* and *Ostrea*) and not other shellfish. Among the active compounds produced by LST-D, are L-DOPA analogues. *P. maximus* could respond to neurotransmitters other than L-DOPA as abalone responds to GABA analogues (Morse et al. 1979); or it could set without a biofilm cue as suggested by a comparison of Tables one and four. The second hypothesis is that LST could be inductive but that the experimental biofilms were flawed. For example *C. gigas* and *O. edulis* were reared at 23–25°C, which are also optimum temperatures for the growth of LST-D; however, *P. maximus* larvae were reared at 16°C, a temperature at which LST-D grew and metabolized more slowly.

The designation LST was derived from Lewes spat tank for the oyster hatchery (Lewes, Delaware, USA) from which it was isolated. If a specific induction mechanism exists in natural or hatchery environments, bacteria with some physiology in common with LST must be rather widely distributed. If this assumption is true, collectors immersed for several days in larval tanks may also be colonized by such bacteria, so the interpretation of the results from the control collectors (e.g. Table 3, series 1) must be made cautiously. Specific enrichments for melanin-like pigment producing bacteria in hatchery environments have been done by Tritar (1987). Those bacteria appeared widely distributed, and were found mainly in seawater and algal cultures; however, their biochemical features appeared different from those of LST, except that 50% of them produced a melanin-like pigment and assayed positive for tyrosinase, the enzyme mediating the synthesis of L-Dopa.

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UNEXPLAINED MORTALITIES OF HATCHERY-REARED, JUVENILE OYSTERS, *CRASSOSTREA VIRGINICA* (GMELIN)

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ABSTRACT Survival, growth and pathology of juvenile oysters, *Crassostrea virginica*, in off-bottom culture at Oyster Bay and Fishers Island, New York, were monitored during the summer of 1991 to document and help explain the episodic mass mortalities of cultured seed oysters that have occurred in the northeastern USA over the past several years. At Oyster Bay, where the more detailed study was conducted, 54 to 75% losses affected several 1991 cohorts at mean shell heights ranging from 15 to 24 mm, within 3 to 6½ weeks of transfer from the hatchery to growout trays. Mortalities occurred in July and August, at temperatures between 22 and 25°C, and were reduced significantly at low stocking densities. Deaths were associated with reduced tissue and shell growth, reduced condition index, mantle retraction, the deposition of an abnormal conchiolin layer on the inner shell, and lesions of the mantle surface. No obvious pathogen was identified in soft tissues or shells by light or electron microscopy. The pathology suggested that a toxin-producing agent of bacterial or microalgal origin, or chemical contaminant, caused mantle retraction and secretion of anomalous conchiolin as a defense mechanism. Two potential agents were recognized. Bacteria were found in mantle lesions and within the abnormal conchiolin sheet, but not consistently and with <30% prevalence; it is not clear whether these were primary or secondary invaders. Blooms of a large dinoflagellate, *Gymnodinium sanguineum*, occurred at peak densities of 5×10^5 cells l^{-1} at the time of initial oyster mortalities, although the species is not known to be toxic to bivalves. Follow up studies are planned to identify the etiological agent and culture methods that minimize losses.

KEY WORDS: *Crassostrea virginica*, juvenile oysters, mortalities

INTRODUCTION

Heavy, unexplained mortalities of hatchery-reared, juvenile oysters, *Crassostrea virginica*, have been documented since 1988 by commercial growers in the northeastern U.S. (Rask 1990, 1992; Relyea 1992), and constitute one of the major impediments to the expansion of aquaculture oyster production in this region. Losses typically occur during early field growout of off-bottom cultures. Before 1991, oyster mortalities were observed in the Damariscotta River, ME (>90% losses in 1988, and 40 to 90% losses in 1989); at several sites in MA in 1989 [Orleans (>95% losses), Nantucket (80% loss), and Essex]; in Oyster Bay, Long Island, NY (50% mortalities in 1990), and West Harbor, Fishers Island, NY (1988 through 1990) (authors' 1992 survey). No mortality episodes were documented in Maine or Massachusetts in 1990, but these recurred in the Damariscotta River in 1991.

Juvenile oyster mortalities have thus been widespread and recurrent, but may not be related to a common cause. Some common features were described, however, at most of these sites: 1) mortalities generally occurred during the summer (primarily in July–August) after a period of sustained growth; 2) losses were highly age/size specific, preferentially affecting first-year oysters, at a size of about 6 to 30 mm; 3) mortalities occurred in surface trays or suspended culture, not in bottom plantings; 4) affected oysters

exhibited generalized symptoms of stress, including emaciation of tissues, shell deformity (cupped, left valve outgrowing the right valve), and in some cases, fragile, poorly calcified shells, and 5) other bivalve species cultured in the same system were generally unaffected (e.g., hard clams, *Mercenaria mercenaria* in Oyster Bay, NY, and the European oyster, *Ostrea edulis*, in the Damariscotta River, ME). Mortalities could not be traced to a single hatchery or a common broodstock. Tissue samples were collected at several sites and examined by pathologists at several laboratories. Standard techniques for the detection of known oyster pathogens, including tissue section histology, failed to demonstrate any recognized organism (Rask 1990).

Existing documentation of episodic juvenile oyster mortalities was limited or anecdotal, and insufficient to allow more than speculation of associated causes. Mortalities could be attributed to one or a combination of factors, including primary or secondary infection by a undetected pathogen, or environmental stress due to anthropogenic contaminants, toxic and/or nutritionally unsuitable microalgae, hypoxia, extreme temperatures or overcrowding. Increased susceptibility to disease or other stress factors may also be related to the organisms' genetic makeup (e.g. Ford 1988), as well as their prior history and physiological condition during post-settlement stages in the hatchery, prior to transfer to the field.

The goal of the present study was to implement a rapid-response, comprehensive sampling program that would document juvenile oyster mortalities and help to identify potential cause(s) at two non-contiguous growout sites: Oyster Bay, NY (Frank M. Flower and Sons, Inc.) and Fishers Island, NY (The Clam Farm Inc.). Flower & Sons is the leading producer of oysters in New York State and has successfully grown oysters with current technology for over 30 years. The Clam Farm has been growing oysters on a much smaller scale since 1988. Specific objectives of the study were to describe the relationship between oyster growth, mortality and environmental parameters (temperature, salinity and phytoplankton composition), and to characterize histopathology of the juvenile oyster mortality syndrome as well as to identify potential organisms associated with affected oysters at these two sites.

MATERIALS AND METHODS

Sampling Program at the Oyster Bay Study Site

We monitored growth, mortality, and histopathological condition of two 1991 oyster cohorts produced at the Flower hatchery, which were held in a growout raft system in Mill Neck Creek, Oyster Bay, on the north shore of Long Island, NY. These oysters were the product of two spawnings, conducted on March 18 (large cohort) and April 25 (small cohort) 1991, using local broodstock from Oyster Bay (different individuals for each spawn). Experimental oysters were set on 0.2 to 0.8 mm crushed hard clam shell, and moved from the hatchery to floating trays for field growout on May 25 (large cohort) and June 3 (small cohort). On June 14 mechanical grading of each cohort yielded two experimental groups of relatively uniform size (referred to hereafter as small and large cohorts, SC and LC, respectively), which averaged 6.4 and 16.1 mm in shell height (greatest dimension from the umbo to the posterior margin of the shell) respectively. Oysters were placed in 0.8 m × 1.2 m × 8 cm trays, open at the top and lined on the bottom with 1 mm mesh window screen. Stocking densities included one typically used for commercial growout by the Flower Co. (hereafter referred to as high density) and one lower density (Table 1). The low density treatment was included to determine if growth and mortality in the trays were density-dependent.

Large and small cohorts were suspended in the water column (depth = 3.7 m at low water) in adjacent stacks of six trays each. High-density experimental oysters of a given size were held in the top and bottom trays of each stack (trays 1 and 6, respectively), and low-density groups in trays 2 and 5. The upper tray was suspended about 12 cm below the surface, while the lower tray remained at least 2.5 m off-bottom. Two replicate stacks were maintained for each cohort. During maximum summer production, up to 432 stacks (2592 trays) are typically used to grow oysters and hard clams at this site, with clams occupying only up to 12–23% of available space (J. Zahtila, Flower Co., pers. comm.).

Oysters were sampled (without replacement) approximately every 2 weeks between June 14 and September 20, 1991, although mortalities of small oysters were determined through November 7. A random sample of each replicate was removed after thoroughly mixing the contents of two trays from a given stack (e.g. 1 and 6). Because the growout area is relatively shallow and well mixed, no attempt was made to resolve differences in growth and mortality with depth. Oysters were thinned by random removal of oysters over time (see Table 1). Stocking densities and thinning frequency of the high-density group were decided upon by Flower's personnel. No grading and culling of live oysters, a standard procedure employed by commercial growers, was carried out during the present study. Mechanical grading and culling of dead oysters, however, was conducted on August 9 on a rotary, cylindrical drum sieve. This was necessary because accumulation of shell debris and large numbers of dead oysters, which were by then significantly smaller than live individuals, interfered with effective sampling. Culling removed only dead oysters and did not affect the size distribution of survivors. To allow calculation of cumulative losses, mortality of oysters retained in the system was determined immediately prior to, and following culling. Grading/culling on August 9 effectively removed dead oysters from the small cohort (e.g. 92% of dead oysters from high density trays), but for unknown reasons removed only 34 to 48% of those from the large cohort, and therefore a second culling of this cohort was carried out on September 20 (Fig. 1).

Thus, stocking densities (of the high-density group) and handling protocols were kept similar to standard commercial practices, except that grading and culling was minimal throughout the study, and the identity of experimental oyster groups was maintained over time. Additionally, experimental oysters were held in suspended culture longer than normal at this commercial facility, where oysters are generally removed from growout trays and planted on the bottom at about 20–30 mm in shell height.

On July 26, a third group of oysters, referred to as the "late cohort," was included in the sampling program. These oysters originated from a June 6 spawning of Oyster Bay broodstock (different individuals than those used to produce earlier experimental groups), set on June 27, and were moved to floating trays on July 26, at an initial mean size of 7.7 mm (SE = 0.2, n = 42). This cohort was sampled at weekly intervals, but data are available only through August 29, since the identity of this group was not maintained after this date.

Surface water temperature, determined with a hand-held thermometer, and salinity, determined with a refractometer, were measured at least twice a week at the growout location. Surface water samples were collected weekly and preserved with Lugol's

TABLE 1.

Stocking conditions of oysters cultured off-bottom at the two study sites. Densities are given in numbers of oysters (or volume in liters) per culture unit (tray or pearl net). Number of packed oysters per unit volume was determined in triplicate from subsamples; NT = oysters not thinned at this date.

A) Oyster Bay study site (date of deployment = June 14, water temperature = 21.5°C); $\overline{H}$ = mean shell height; HD and LD = high and low density experimental groups. Date notation = month/day.

	Small 1991 Cohort		Large 1991 Cohort	
Initial $\overline{H}$ (mm)	6.4		16.1	
(SE, n)	(0.12, 50)		(0.30, 50)	
	Stocking densities (#/tray; volume (l)/tray)			
Date	**HD**	**LD**	**HD**	**LD**
6/14	35,700 (2.4)	3,800 (0.25)	7,200 (6.0)	2,000 (1.7)
6/28	19,680 (6.0)	NT	3,680 (8.0)	NT
7/11	3,360 (4.0)	2,232 (3.6)	NT	2,106 (3.6)
7/26	2,340 (6.0)	1,170 (3.0)	1,540 (8.8)	700 (4.0)
8/9	1,424 (8.0)	572 (4.0)	1,167 (9.0)	465 (4.5)
8/23	996 (12.0)	431 (5.2)	484 (11.0)	264 (6.0)
9/6	576 (12.0)	192 (4.0)	400 (10.0)	111 (3.0)
9/20	370 (10.0)	NT	392 (8.0)	NT

B) Fishers Island study site (date of deployment = June 12, water temperature = 18°C).

	Small 1991 Cohort	Large 1990 Cohort
Initial $\overline{H}$ (mm)	8.9	31.7
(SE, n)	(0.23, 49)	(0.92, 30)
Stocking density (#/pearl net)	500	200[a]

[a] Thinned to 100 oysters/pearl net from July 25 onward.

iodine solution, in its concentrated acidic version (Throndsen 1978), for determination of phytoplankton species composition and cell concentrations. Population densities of phytoplankters greater than 5 μm were determined using a Sedgwick-Rafter chamber. A numerically dominant algal species, *Gymnodinium sanguineum* (Hirasaka) (=*nelsoni* = *splendens*), was counted at 200× magnification in unconcentrated samples. For other species, cells in water samples were first concentrated by centrifugation, and enumerated at 400×. The 95% confidence interval was estimated according to Venrick (1978).

Qualitative, visual assessments were made on the degree of siltation and fouling of trays, prevalence of pale digestive glands (a gross indicator of feeding inhibition), and mud blisters (presumably caused by the boring polychaete *Polydora* sp.) determined by dissecting 15 oysters from each cohort.

Determination of Mortality and Growth

Percent mortality was determined *in situ* from a representative sample of at least 100 oysters from each replicate, by prying open the valves with a scalpel, and determining the presence/absence of tissues attached to the shell. Live oysters were returned to the laboratory, where any additional deaths undetected in the field sampling were determined following dissection for dry weight and condition index determination. Counts of disarticulated cupped (left) valves were included in mortality estimates.

At each sampling date, 22 to 50 live oysters from each of two replicate groups were measured with digital calipers (±0.1 mm) to obtain shell height (H). Tissues were dissected and oven-dried to constant weight at 50°C to determine dry weight, using an analytical balance (±0.1 mg) or Cahn electrobalance (±0.01 mg) as appropriate. The sample size was increased to 50 oysters per replicate from July 26 onwards, to accommodate increasing variability in size over time. Whole body weight of tightly-closed oysters, air-dried at room temperature, and dry weight of shells was also determined to estimate the condition index. Soft tissue weight and condition index of small oysters were not determined on the first sampling date (June 14), because oysters could not be reliably shucked at this small size. The following gravimetric condition index (CI) was determined:

$$CI = [\text{Dry meat weight (g)} \times 1000/\text{Internal shell cavity capacity}],$$

where internal shell cavity capacity (g) = (whole live body weight in air) − (dry shell weight in air), following removal of epibionts and debris from the valves (modified from the formula provided by Lawrence and Scott 1982). The gravimetric CI has been recommended as the standard index of choice to measure the nutritive status and meat yield of oysters (Crosby and Gale 1990), and has been ranked as the most sensitive out of 21 indices commonly employed for oysters (Bodoy et al. 1986). The incidence of abnormal conchiolin deposition on the inner shell surface was recorded from July 11 onwards.

Histopathology

Histopathology was performed at the Haskin Shellfish Research Laboratory (HSRL) of Rutgers University, the Battelle Ocean and Marine Sciences Laboratories (Battelle), and the Virginia Institute of Marine Science (VIMS). For light microscopy, a minimum of 25 randomly chosen oysters (including both live and gaping oysters) from each experimental cohort were preserved in

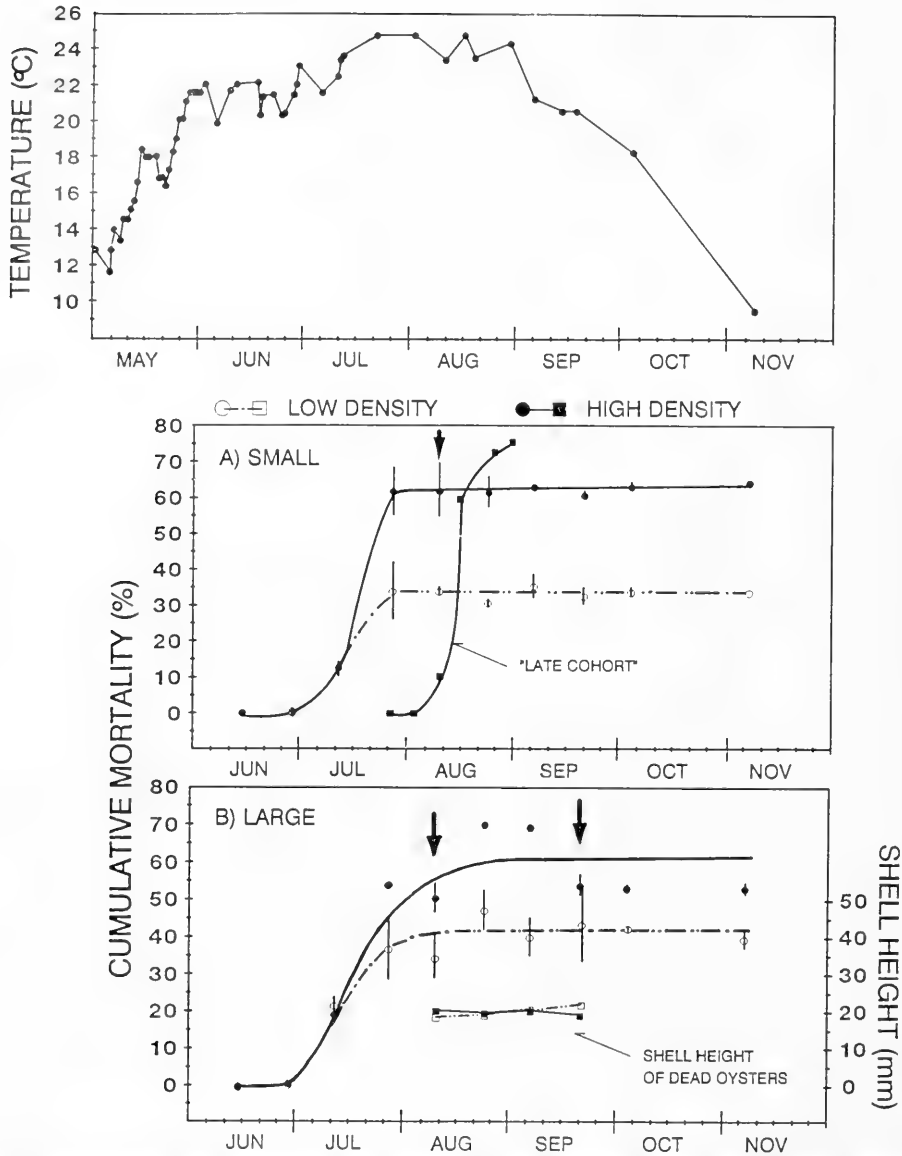

Figure 1. Water temperature at the Oyster Bay, NY oyster growout site (top graph), and mean cumulative, percent mortality of experimental cohorts of juvenile oysters, *Crassostrea virginica* (±standard error, SE; n = 2): A) small cohort and "late cohort," B) large cohort (eye-fitted curves; see text and Table 1 for initial cohort age and size characteristics, and stocking densities). Arrows mark the timing of removal of dead oysters from growout trays by mechanical grading. Bottom graph also shows the mean shell height ($\overline{H}$) of dead oysters (n = 51 to 86, and 23 to 70 for high and low density groups respectively), in order to demonstrate that no additional mortalities occurred after the August 9 sampling date (see Fig. 2 for $\overline{H}$ of live oysters at the corresponding sampling times).

Davidson's fixative (Shaw and Battle 1957) immediately upon collection. After 24–48 h, they were transferred to this same fixative, but without acetic acid, for long-term storage. Samples were shipped to the HSRL for histological processing and analysis. Small oysters which could not be readily dissected *in situ* were fixed whole, following careful prying open of their valves. Large oysters were shucked, and their shells were discarded. The shells of small oysters had decalcified by the time the oysters were processed for histology. Samples examined at Battelle were shipped approximately every 2 weeks, from mid-June through mid-November, by overnight mail from the Flower Co. They were then decalcified and fixed in Dietrich's fixative. A number of individuals were examined prior to fixation for evidence of fungal infection or other organisms that might have penetrated the shell.

Fixed tissues of large oysters were cut laterally from the hinge region, through the adductor muscle, to the posterior margin before being embedded in paraffin. Small individuals with decalcified shells were embedded intact with the ventral side down. This orientation allowed us to view the epithelium under the hinge ligament, the myoepithelial attachment of the adductor muscle to the shell, the abnormal conchiolin layer when present, and the periostracum-secreting mantle edge, as well as various internal organs.

Embedded tissues were sectioned serially at 5–6 μm, and stained with hematoxylin and eosin, or a Masson's Trichrome stain (Humason 1979) modified by the addition of Fast Green and Orange G. An initial sample of 55 individuals, collected during peak mortality and categorized as showing a) no sign of distress, b) early distress (some overgrowth of the left valve, some conchiolin deposition of inner valve, or both), or c) advanced distress (clear overgrowth of left valve, heavy abnormal conchiolin, weak muscle attachment), was examined microscopically for histopathological conditions. Pathological conditions recognized in the initial sample (see Results) were identified and rated (none, light, moderate, or heavy) in subsequent bi-weekly samples.

For transmission electron microscopy, mantle tissue and conchiolin were fixed in 2% glutaraldehyde and 1.5% paraformaldehyde in 0.1 M Millonig's phosphate buffer with 2.7% glucose at pH 7.3 for 2 hr followed by buffer rinses in 0.2 M Millonig's phosphate buffer with 5.4% glucose at pH 7.3, then post-fixed in 1% OsO_4 with 0.1 M Millonig's and 2.7% glucose at pH 7.3 for 1 h. After washing in distilled water, the tissue and conchiolin blocks were stained in 1% aqueous uranyl acetate for 1 h followed by dehydration in a graded series of ethanol solutions and transfer into propylene oxide. Infiltrations and embeddings were made in Spurr's resin and thin sections were stained in Reynold's lead citrate and uranyl acetate. All procedures were accomplished at room temperature except 1) post-fixation which was done in an ice bath, and 2) resin polymerization which was performed at 58°C.

Sampling Program at the Fishers Island Study Site

A similar sampling protocol was implemented at a more oceanic site on the north shore of Fishers Island, NY: 2 cohorts of cultchless juvenile oysters were deployed in pearl nets (34 × 34 cm basal area), in vertical arrays of 4 nets, on June 12, 1991, in eastern West Harbor (depth = ca. 3 m at low water). Both cohorts were produced at the Aquacultural Research Corporation hatchery, Dennis, MA, using broodstock shipped from Island Pond, Fishers Island (G. Matthiessen, Ocean Pond Corp., pers. comm.). The small (1991) cohort (SC) was grown in trays at Ocean Pond Corp.'s nursery site in Island Pond, prior to transfer to West Harbor in mid-June. The large (1990) cohort (LC) overwintered in pearl nets in Island Pond until June 1991. Large cohort oysters were held in pearl nets with a 6 mm mesh, whereas SC oysters were held in 3 mm pearl nets (see Table 1 for initial sizes and stocking conditions). Low stocking-density groups were also maintained at this site (50 and 100 large and small oysters per pearl net respectively), and sampling was without replacement, as described previously. Live oysters were shipped to SUNY Stony Brook by overnight mail in coolers containing freeze-packs. Experimental oysters were thinned but not graded or culled, except at the time of initial deployment.

RESULTS

Sampling at the Oyster Bay Study Site

Mortality Patterns

Mortality estimates obtained *in situ* were generally in excellent agreement with those determined following dissection of oysters in the laboratory. Greatest discrepancies occurred on July 11, when field-determined mortalities were 4–9% and 9–12% for small and large oysters respectively, while laboratory-derived values were 10–15% and 18–21% respectively.

Despite variability between replicate stacks at any given sampling date, especially for small oysters, there was no consistent trend showing greater losses within one of the two replicates. Thus, these differences are attributed to sampling artifact, and data averaged for the two replicates.

Cumulative mortalities remained negligible until July 11, when they reached 12% and 20% for the small and large cohort, respectively, at a time when the water temperature reached 24°C (Fig. 1). Mortalities peaked at 62% and 54% on July 26 for small and large cohorts, respectively, and ceased thereafter. Grading/culling conducted on August 9 was not 100% effective in removing dead oysters from the LC. Therefore, an apparent increase in mortalities of large oysters between August 9 and August 23 is attributed to sampling error, rather than new mortalities. This was confirmed by determining the mean size of dead oysters over time (Fig. 1B), which remained constant, at 20.3–20.8 mm [the size of oysters in early July (Fig. 2)], between August 9 and September 20. Thus the dead oysters present in late August samples are clearly remnants from the July mortality outbreak.

Losses were consistently lower for oysters stocked at the lower density. They attained maxima of only 30–37% for small oysters and 28–38% for large oysters on July 26, but their timing coincided with that of oysters held at high density. Mortalities of the LC levelled off between 40 and 47% after August 23 (Fig. 1B). The outbreak of mortalities occurred during a period of elevated surface temperatures (range = 21.7 to 25.0°C), which were maintained between June and early September (Fig. 1). A comparison of Flower's temperature records from 1987 through 1991 (May 1 to July 26) indicates that late spring water temperatures were higher than usual in 1991. The mean for May was 17.2°C in 1991, compared to 14.2 and 15.3°C in 1990 and 1989, respectively. Cumulative day-degrees calculated for May were 8 to 16% higher in 1991 than in the 4 previous years. However, heavy oyster mortalities were also experienced in 1990, when the lowest May to July temperatures were recorded. Salinities ranged between 24 and 28 ppt, and only moderate to low levels (in late September and early October) of fouling and siltation of trays were observed throughout the study period.

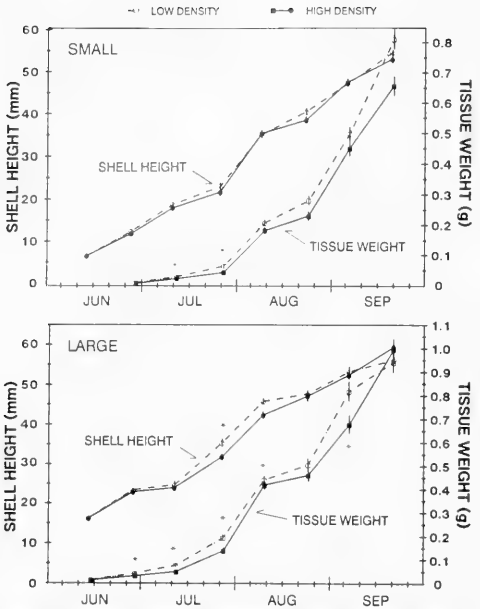

Figure 2. Temporal changes in mean shell height and dry weight of soft tissues of small and large oyster cohorts held at 2 stocking densities in Oyster Bay, NY. Error bars as in Fig. 1 (pooled data from 2 replicate experimental groups, n for samples collected through July 11 = 46–50; n for remaining samples = 94–100). Asterisks indicate dates when significant ($p < 0.05$) differences were observed between density treatments.

The "late cohort," which was the last cohort produced at the Flower Co. in 1991, suffered heavy mortalities 2 to 3 weeks later than the main experimental groups. Cumulative losses increased from 7–13% on August 9 to 75% on August 23, a period during which temperatures remained constant at about 23°C.

On July 4, live, gaping and dead oysters from non-experimental groups, which were experiencing heavy mortalities at the growout site, were examined microscopically for the presence of predatory flatworms, *Stylochus* sp. These were not observed in any of the samples, and thus were eliminated as a potential cause of oyster mortalities. These results were independently corroborated by M. Castagna, who examined live oysters at this site on July 9 (VIMS, pers. comm.). Mud blisters were commonly observed in shells of September 20 samples, and were prevalent in October 3 samples from both cohorts, but were not observed on earlier sampling dates.

Phytoplankton

Analysis of phytoplankton samples revealed that a bloom of the unarmored dinoflagellate *Gymnodinium sanguineum* (cell length 43 to 50 μm, width 31 to 43 μm) occurred in July, at the time when the two main experimental cohorts suffered high mortalities, and again in September. Cell densities of *G. sanguineum* peaked at 5.1×10^5 cells l^{-1} on July 20, declined rapidly by July 26 and remained at relatively high levels, 5.0 to 7.1 $\times$ 10^4 cells l^{-1}, throughout early August (Fig. 3). Cell densities of this species peaked again at 3.5×10^5 cells l^{-1} on September 20, when the small and large cohorts had attained 52.9 and 58.4 mm in shell height respectively. The dominant diatom species was *Skeletonema costatum*, and flagellates were mostly composed of cryptomonads (Fig. 3). During the bloom period, *G. sanguineum* was not necessarily the numerically dominant phytoplankton species. However, if we consider the size difference between a *G. sanguineum* cell and a *S. costatum* cell (5 to 8 μm), the former species was definitively the major contributor of phytoplankton biomass. Starting on July 20, a certain percentage of *G. sanguineum* cells observed were partially degraded or covered by fungus-like hairs. The cell counts reported were determined from surface water samples, and may not be representative of the entire water column, since *Gymnodinium* spp. are motile and positively phototactic, and may not be homogeneously distributed in depth even in shallow estuaries (Fiedler 1982, Chang and Carpenter 1985).

Growth Patterns

Differences in mean height or soft tissue weight between replicates at each sampling date were tested using paired t-tests, ad-

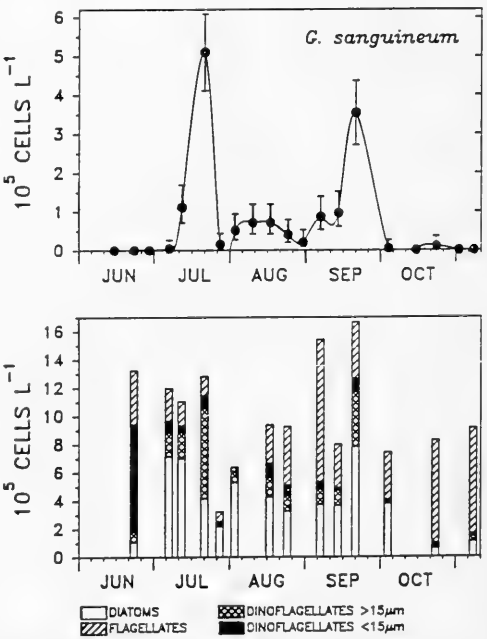

Figure 3. Phytoplankton composition in Oyster Bay surface water samples during the study period. Top graph: population density (mean and 95% confidence interval) of the dinoflagellate *Gymnodinium sanguineum*. Lower graph: abundance of various phytoplankton groups [*G. sanguineum* is included in the large dinoflagellate (>15 μm) group].

justed for the lack of independence of samples over time. Because significant differences ($p < 0.05$) were generally found on only one or two of the sampling dates, data from both replicates were pooled.

Small oysters in both density treatments showed highest absolute (slopes of growth curve between sampling dates, Fig. 2) and relative (instantaneous growth coefficient, k, Table 2) shell and tissue growth rates prior to and following the period of maximum mortalities occurring between July 11 and July 26. Thus, during peak mortalities the soft tissue growth coefficient dropped to about ½ of prior and subsequent levels (Table 2). Interestingly, however, growth did not cease during the mortality episode, as small oysters at both densities continued to deposit shell and doubled in tissue weight during the second part of July. Growth slowed again between August 9 and 23, when the lowest growth coefficients were recorded for this experimental group (Table 2). On this date, histological sections of 18 out of 25 SC oysters and 20 of 26 LC oysters had mature gametes, and in some individuals they were present in the gonoducts. A few males showed empty follicles with remnant sperm, indicative of recent spawning activity. Thus reduced growth in mid-August did not coincide with additional mortalities, and was probably associated with spawning activity.

As expected, relative growth of soft tissues was generally higher for small than large oysters (Table 2). Growth patterns of large oysters were similar to those of small oysters, except that reductions in shell and tissue growth rate during early summer occurred two weeks earlier (June 28 to July 11), i.e. prior to, rather than during, the period of heaviest mortalities. As observed for the SC, there was a second period of slow growth during mid August, marked by minimum values in both shell and tissue growth coefficients for oysters held at high density (Table 2), and coinciding with apparent spawning.

In general, temporal patterns of shell growth within a given cohort were very similar between density treatments. Two-way analyses of variance and *a posteriori* multiple comparisons (Sokal and Rohlf 1971) were used to ascertain the effects of date and density on log-transformed shell height. No significant differences were found between density treatments, except for large oysters on July 26 (Fig. 2). Analysis of covariance (ANCOVA, with log height as covariate) was used to examine the effects of date and culture density on log-transformed tissue weights. Among small oysters, the two density treatments were significantly different ($p < 0.05$) on July 11 and July 26, during the mortality episode, whereas among large oysters significant differences were found at most dates (Fig. 2).

The ranges in mean condition index (CI) values over the study period were 77 to 166, and 92 to 127 for the SC and LC, respectively. Differences in condition with stocking density were less pronounced in the SC, and were significant ($p < 0.05$) only on June 28 and July 26 [ANCOVA with log-height as covariate, and multiple comparisons of arcsine (CI/1000) transformed data (Sokal and Rohlf 1971)] (Fig. 4). Condition dropped markedly in July (by 53 and 39% in high and low density groups respectively), at the time of peak mortalities, and again to a lesser extent (30–31% reduction) in mid-August. The mean CI of large oysters stocked at low density was consistently greater than at high density, but showed a similar seasonal pattern (Fig. 4). Significant differences in condition between density treatments were detected on June 28, July 26 and September 6. The greatest decline in condition (9 to 18%) occurred between late June and early July, coincident with early mortalities in this cohort, and a second decline (11 to 16%) occurred in mid-August when growth rates of soft tissues attained a seasonal minimum (Table 2).

The "late cohort" also exhibited considerable shell growth prior to the mortality outbreak, but ceased growing during peak mortalities (Table 2). Mean shell heights (±SE) were 10.3 (±0.2), 14.9 (±0.3), 16.5 (±0.5) and 16.6 (±0.6) on August 2, 9, 16 and 23, respectively.

Production of live oysters, expressed as total volume or weight per unit time, is the most relevant descriptor of performance in a commercial growout operation, and is a function of both growth and survival. Based on scaling considerations, whole body weight and total volume are expected to show comparable rates of increase over time. In the present study whole animal weight, determined in the laboratory, was subject to less measurement error than packed volume, the unit generally used by commercial growers to estimate production, and allowed an estimate of individual variability. Biweekly changes in biomass (total live weight) of the 2 experimental cohorts held at high density declined markedly during the July mortality episode (Fig. 5). Both cohorts experienced the highest production (2.9 to 4.7 increase in biomass over a 2-week period for small oysters, and 2.5–2.8 increase for large oysters) prior to and immediately following the period of mass mortalities. During August and September, the biomass increment over 2 weeks ranged from 1.1 to 1.8-fold, with lowest increments coinciding with declining temperatures (Fig. 1, top graph).

TABLE 2.

Instantaneous growth coefficients for shell height (k_H) and dry weight of soft tissues (k_{DW}) of large and small experimental oysters held at the high stocking density in Oyster Bay, and k_H of the "late cohort."

	Small		Large		Late Cohort	
Period	k_H	k_{DW}	k_H	k_{DW}	Period	k_H
6/14–6/28	4.27	ND	2.44	5.54	7/26–8/2	4.28
6/28–7/11	3.24	9.07	0.35	3.18	8/2–8/9	5.23
7/11–7/26	1.24	4.32	1.88	6.69	8/9–8/16	1.48
7/26–8/9	3.52	10.17	2.04	7.82	8/16–8/23	0.05
8/9–8/23	0.62	1.68	0.76	0.69		
8/23–9/6	1.50	4.80	0.74	2.74		
9/6–9/20	0.78	2.70	0.90	2.74		

$k = ([\ln x_2 - \ln x_1]/t) \times 100$, where x_2 and x_1 are final and initial heights (mm) or weights (mg) respectively, and t = time interval. ND = not determined; date notation as in Table 1.

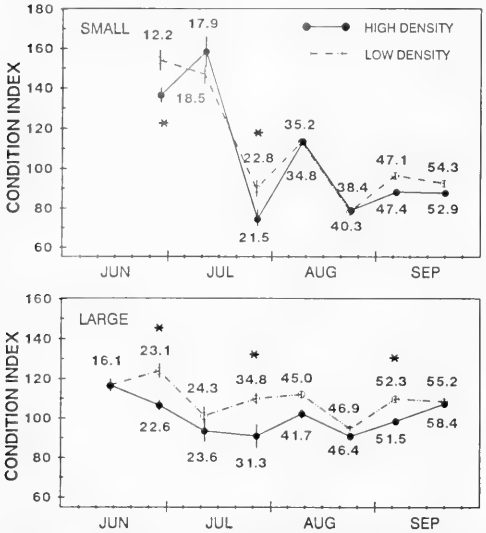

Figure 4. Mean gravimetric condition index (see methods) (±SE) of large and small oyster cohorts cultured at 2 stocking densities in Oyster Bay. Mean shell heights are indicated at each sampling date; asterisks indicate significant differences ($p < 0.05$) in mean condition between density treatments.

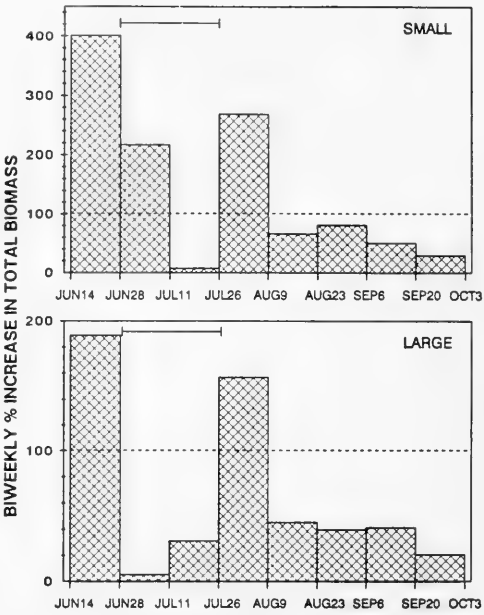

Figure 5. Percent biweekly change in total live biomass of small and large oyster cohorts held at the high stocking density in Oyster Bay, calculated as: $[(n_2W_2 - n_1W_1)/n_1W_1] \times (t_2 - t_1)/15 \times 100$, where n_1 and n_2 = numbers of survivors for each sampling interval ($t_2 - t_1$) = 13 to 15 days, W_1 and W_2 = mean whole body weight of live oysters (pooled data from 2 replicate experimental groups, n as in Fig. 2). Horizontal dashed lines indicate the level corresponding to a doubling of biomass over a 2-week period, and solid horizontal bars mark the period of mortalities. Initial n was arbitrarily selected as that initially deployed in one experimental tray (35,000 and 7,200 oysters for small and large cohorts respectively); 100% survival was assumed after the July 26 sampling date.

Shell Anomalies

Although most juvenile oysters examined had mats of bacteria and other microorganisms on the external shell surface, gross and histological examination of the shell indicated no evidence of penetration by fungus or other shell-boring organism.

Macroscopically, the most consistent correlate with the juvenile oyster mortality syndrome, in both living and dead animals, was a layer of abnormal conchiolin deposited on the inner surface of one or both valves, but primarily on the left valve. It was frequently raised into a ridge several millimeters from the edge of the shell (Fig. 6, right valve). Most often, the ridge formed a completely closed ring (Fig. 6, right valve) on only one valve; however, rings were found on both valves of some oysters. It was not unusual to find the ridge juxtaposed to the adductor muscle along the dorsal to posterior margin of the muscle (Fig. 6, right valve). In some cases, the conchiolin layer was deposited between the adductor muscle and the shell, causing the muscle to detach. Tissues of live oysters were usually found contracted within the bounds of the ridge; however, in some cases the ridge was present inside the free edge of the mantle. Portions of the shell external to the conchiolin ridge were frequently covered by mud and fouling organisms. Some oysters, apparently in early stages of the syndrome, were found with the thin conchiolin sheet covering all or only small portions of the shell surface, but with no ridge. The left valve of affected oysters was often deeply cupped, its edge extending beyond that of the right valve.

Prevalence of abnormal conchiolin increased markedly during July in concert with the increase in mortalities (Fig. 7). Small oysters in both density treatments showed similar patterns, such that 43–48% of living oysters exhibited the syndrome by late July, after which prevalence decreased to nearly zero. In contrast, LC oysters showed a relatively high prevalence (24–34%) as early as July 11. The presence of abnormal conchiolin decreased thereafter in the low density treatment, but remained high (40%) in the high density animals through the end of July, after which it declined (Fig. 7). Prevalence of abnormal conchiolin was also high in the "late cohort," with increasing values of 21%, 40% and 52% on August 9, 16 and 23 respectively.

Longitudinal cross-sections of the lower (cupped) valve of 15 survivors from the SC collected on September 20 were examined for evidence of past alteration in the pattern of shell deposition. No anomalous deposition was apparent on the external surface of the shell or in cross-section, suggesting that survivors were relatively unaffected at the time of mass mortalities.

Histopathology

Light microscopy of samples collected during peak mortality showed that oysters depositing abnormal conchiolin possessed lesions of the mantle characterized by degeneration and sloughing of epithelial cells, infiltration of hemocytes into epithelium and un-

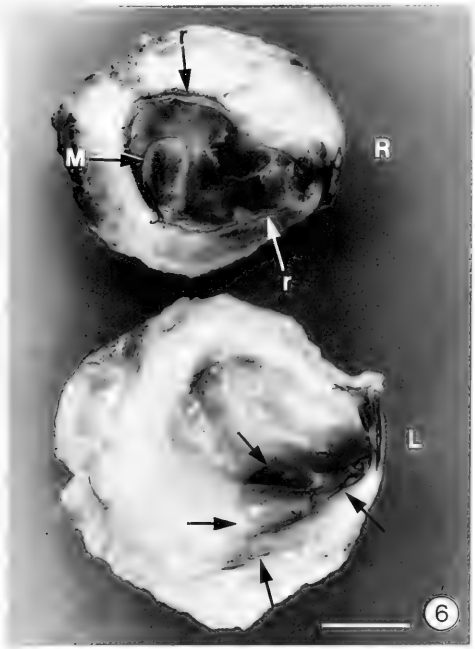

Figure 6. Right (R) and left (L) valves of juvenile oyster where anomalous conchiolin deposits have been formed on the inner shell. The right valve has a conchiolin deposit organized into a peripheral ridge (r) and a thin layer covering the shell within the ridge except where the adductor muscle (M) was attached. Conchiolin on the left valve is layered onto the shell within the area denoted by arrows. The ridge was formed by the mantle beneath and just inside the leading edge of the free mantle and just inside where the visceral mass was in contact with the shell. Bar = 4 mm.

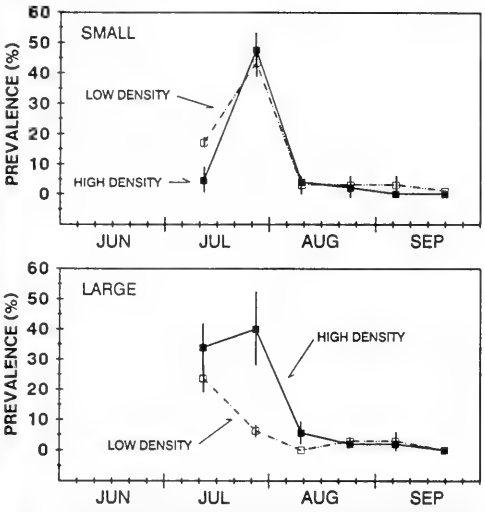

Figure 7. Percent prevalence of abnormal conchiolin deposition on the inner surface of the cupped (left) valve and/or right valve (see Fig. 6) in small and large live, experimental oyster cohorts held at 2 stocking densities in Oyster Bay (prevalence was not determined prior to July 11). Error bars and sample size as in Fig. 2.

derlying connective tissues, and the presence of dense coccoid bodies within phagocytes and epithelial cells (Fig. 8). These bodies often contained one or more punctate, basophilic structures that resembled nuclei of some eucaryotic microbes (Figs. 8 and 11); however, the bodies had a wide range of sizes (<1 to 6 μm) and many contained no basophilic structures (Figs. 8 and 10). Bacteria and ciliates were present in some lesions where the epithelial layer had been eroded and were common in moribund oysters. Degeneration of the myoepithelium was observed in some oysters, accompanied by muscle degeneration and detachment from the shell. Hemocytes, bacteria, and debris were found against both sides of the conchiolin layer and within chambers and spaces between sublayers of the conchiolin (Figs. 9 and 12). The connective tissues in the visceral mass and the digestive epithelia were not affected, and food was present in the guts of most oysters. Hemocyte infiltration and epithelial degeneration were found in the gills of some individuals. No recognizable organism was present consistently in all lesions and all oysters.

Lesions were present, but less severe, in oysters categorized macroscopically as having few or no signs of distress. The smallest, and presumably earliest, lesions consisted of disruptions to the apical portions of epithelial cells under the hinge ligament and in the pallial (peripheral) region of the mantle. Infiltration of hemocytes into the adjacent connective tissue appeared in more advanced cases, followed by epithelial sloughing.

Transmission electron microscopy of mantle tissue from diseased oysters showed that the coccoid bodies seen in light microscopy consisted of portions of oyster cells within phagocytes and epithelial cells, or free in spaces between epithelial cells where they were mixed with cell debris. The substructure of these bodies included mitochondria that resembled those of oyster cells (i.e., having shelf-like cristae), lipoid droplets, concentric arrays of rough endoplasmic reticulum, and electron-dense granular material, delimited by two membranes, which could be chromatin of pycnotic nuclei (Fig. 11). We believe that the latter structures are the basophilic parts of the bodies seen by light microscopy (Fig. 8). When found in whole cells, the coccoid bodies were delimited by a vacuolar membrane. Myelin whorls (Fig. 10) and multivesicular bodies were also characteristically found in the cells containing the coccoid bodies. Most probably these bodies are secondary lysosomes resulting from an extreme example of autophagy. Thin sections of anomalous conchiolin showed bacteria present in the chambers. All of the bacteria had walls characteristic of gram negative species. Some were rods (Fig. 12) and others were filamentous.

Temporal Development of Lesions

No mantle lesions were observed in the initial sample of SC oysters collected on June 16, but they were present in 30% of the sample by June 28 and 83% by July 11 during the height of mortality (Fig. 13). When deaths ceased in late July, nearly 70% of survivors still showed lesions, but they decreased to 16% by

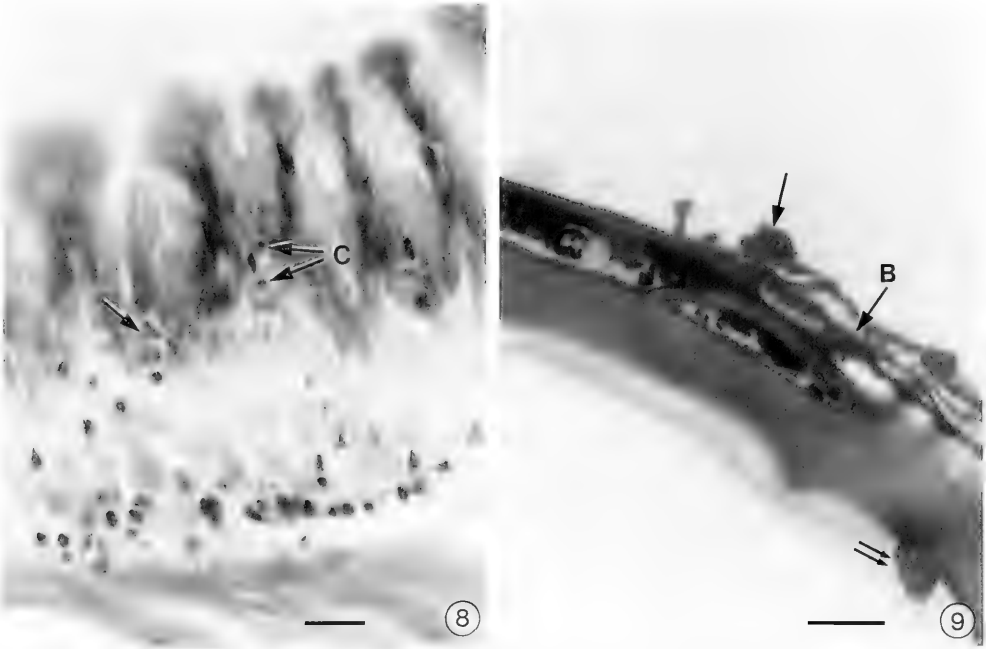

Figure 8. Hematoxylin and eosin-stained histological section of the mantle of a juvenile oyster in early stages of anomalous conchiolin formation. The mantle sample was obtained from the posterior region of the oyster and was found overlying a ring of anomalous conchiolin. Coccoid bodies in mantle epithelium with (C) and without (arrow) basophilic substructure. Bar = 10 μm.

Figure 9. One-half micron, resin-embedded section of anomalous oyster conchiolin. Oyster hemocytes on what is believed to be the outer surface (arrow) and inner surface (double arrow) of the conchiolin deposit; bacteria in chambers (B). Bar = 10 μm.

August 9. In contrast to the SC, lesions were already present in 40% of the LC on June 16. Two weeks later, prevalence was 80%, a figure maintained until July 11. Thereafter, prevalence decreased, reaching a low of 40% of August 9. Interestingly, mantle lesions became more frequent in late September, being found in 60–70% of both groups, before decreasing again in early November (Fig. 13). The prevalence of moderate to heavy lesions was greater than 10% only during the period of heaviest mortality on July 11, and in September 20 samples when lesions were found in digestive, as well as mantle, epithelia.

Oysters examined at Battelle had a somewhat different percentage of lesions, but the pattern was similar. Lesions began to appear in the inner lobe of the mantle and digestive epithelia in September and persisted in the latter through the final samples collected in mid-November.

The prevalence pattern of coccoid bodies mirrored that of the lesions, although they were found in only about half of oysters in which lesions were present (Fig. 13). Bacteria and ciliates were most frequent in late June and July samples, when they were found in up to 30% of the oysters.

Sampling at the Fishers Island Study Site

At Fishers Island only the SC experienced heavy mortalities (up to 96% between July 10 and July 25), whereas cumulative mortality of the LC was only 8 to 11% by the end of the study (Table 3). The number of survivors from the SC was insufficient to continue sampling after July 25.

During early July, when mortalities first started, small oysters exhibited tissue weight loss (27 and 43% reduction over two weeks in high and low density treatments, respectively), and cessation of shell growth. In contrast, large oysters continued to grow at an accelerated rate in both shell and soft tissues during this period (k_H = 1.35 and k_{DW} = 4.92 between June 27 to July 10), but experienced no shell growth and inhibition in soft tissue growth (k_{DW} = 1.43) at the time the SC suffered heaviest losses (July 10 to 25) (Table 3).

Three-quarters of the SC collected on July 10, when mortalities were beginning, had mantle lesions similar to those seen at the Flower Co. Coccoid bodies and bacteria/ciliates were found in 30% and 60%, respectively, of the sample. At the same time, only 20% of the large cohort had lesions and 5–10% showed coccoid bodies, bacteria, or ciliates.

DISCUSSION

At the Oyster Bay site, severe mortalities successively affected several 1991 cohorts of juvenile oysters during July and August, within 3 to 6½ weeks of transfer from the hatchery to the field growout system. Mortalities appeared to be species-specific, since

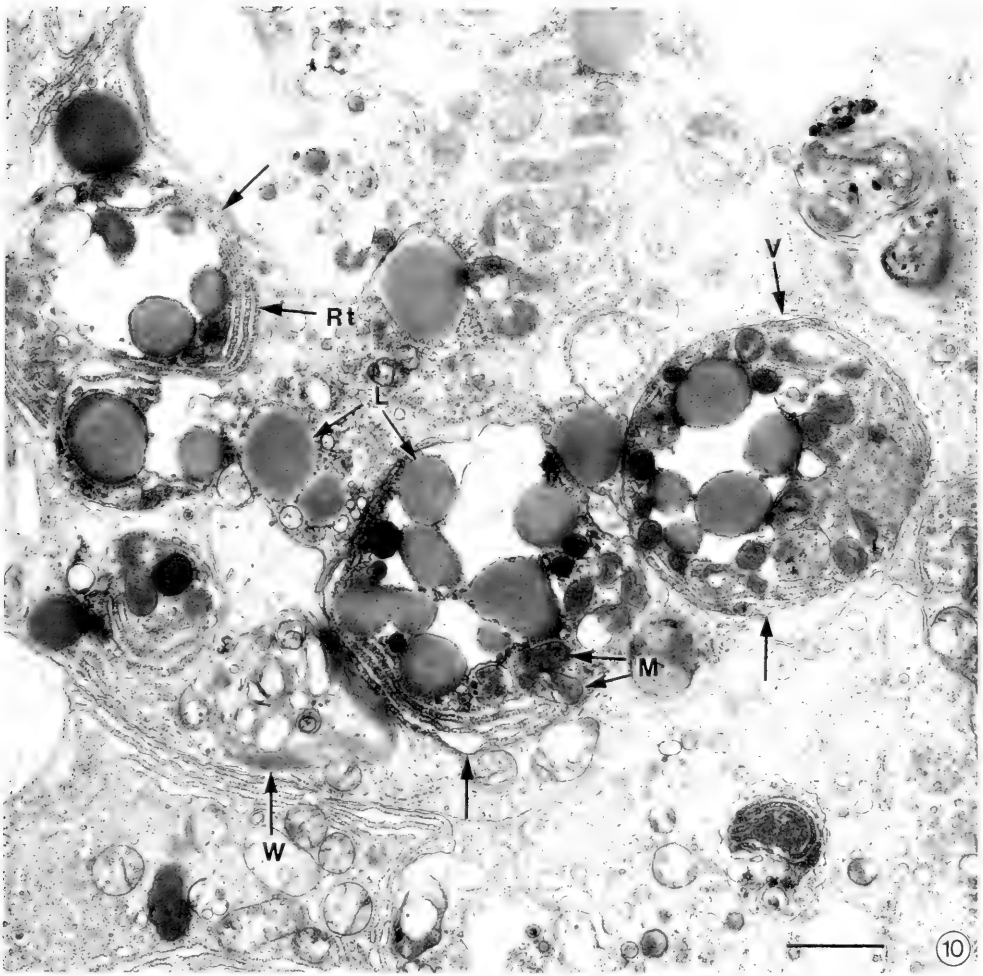

Figure 10. Cytoplasm of mantle epithelial cell from region overlying anomalous conchiolin deposit. Coccoid bodies (arrows) within epithelial cell; lipoid droplets (L); mitochondria (M); rough endoplasmic reticulum (Rt); myelin whorl (W), and membrane of vacuole containing coccoid body (V) Bar = 1 μm.

juvenile hard clams, *Mercenaria mercenaria,* held in adjacent stacks in the same growout system, were not affected. Cumulative mortalities reached 54 to 76% in animals held at commercial densities, and affected animals ranging between 15 mm (late cohort) and 24 mm (LC) in mean shell height at the time of initial losses.

The three experimental cohorts from the Flower's hatchery included in the present study originated from common, Oyster Bay broodstock, although different individuals were spawned to produce each cohort. Juvenile oyster mortalities occurred again at this site in the summer of 1992, with progeny produced from wild broodstock from the Thames River, Connecticut (Borrero and Ford, unpubl. data). Furthermore, seed produced from Oyster Bay broodstock suffered no unusual mortalities when reared at the Bluepoints hatchery, on the south shore of Long Island, in 1992. The causes of mortality thus appear to be site-specific and unrelated to broodstock origin.

Heavy mortalities of juvenile oysters from a different broodstock were also documented at the Fishers Island growout site over a 2-week period in July 1991. Only small cohort oysters were affected, within 4 weeks of deployment, when individuals averaged 12 mm in shell height. Although mortalities coincided at Oyster Bay and Fishers Island, it is not clear at this time that they can be linked to a common cause. Since a more extensive data

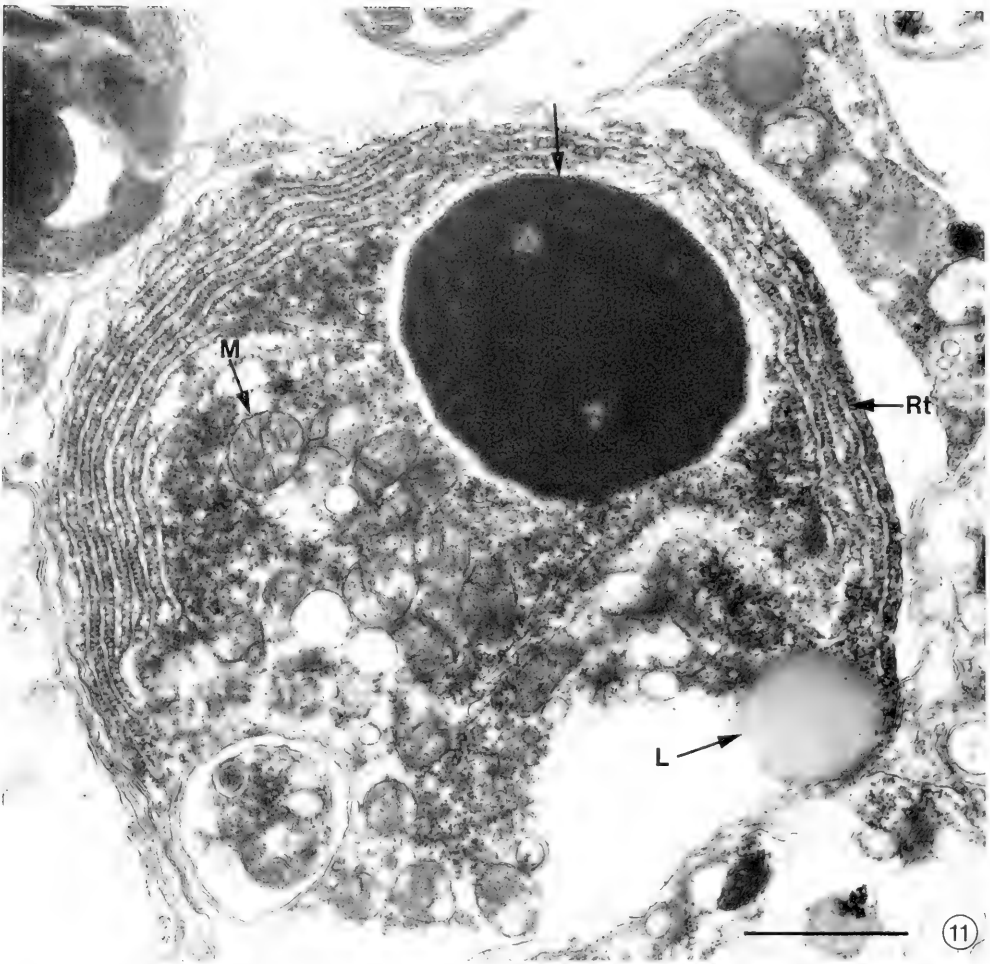

Figure 11. Coccoid body from the same tissue region as in Fig. 10. Electron dense granule (arrow) is believed to be degenerate chromatin material which is the same as the basophilic structures of coccoid bodies when seen in light microscope sections (see Fig. 8). Rough endoplasmic reticulum (Rt); mitochondria (M); lipoid droplet (L). Bar = 1 μm.

base is available for oysters grown in Oyster Bay, our remaining discussion will focus on findings at this site.

Potential Role of Food Limitation and Toxic/Noxious Microalgae

The progression of oyster mortalities in Oyster Bay was remarkably fast, spanning a period of only 2 to 4 weeks. Surprisingly, oysters continued to grow in both shell and tissue weight at this site, albeit at a reduced rate, during the mortality episodes. Positive growth of the population, however, as reflected in mean values, may mask differences in growth and survival among individuals with varying susceptibility to the stressor involved. Visual examination of gut coloration and tissue slides indicated that animals had relatively full alimentary tracts throughout the study. Also, juvenile bivalves are known to survive fairly prolonged starvation [e.g. 10 mm *Mercenaria mercenaria* experienced no mortalities and only 9% dry tissue weight loss when held in filtered seawater in the laboratory for 4 weeks at 24–25°C (Bricelj and Borrero, unpubl. data)], and can minimize weight loss under starvation through a reduction in metabolic rate (Bayne 1973, Malouf and Breese 1978). The above findings therefore suggest that the mortalities documented are unlikely to have resulted from starvation. Dominance in the seston of phytoplankton species of poor nutritional value [e.g. chlorophytes such as *Nannochloris atomus* and *Stichococcus* sp. (Bass et al. 1990)] at high summer temperatures could, however, induce feeding activity, maintain high met-

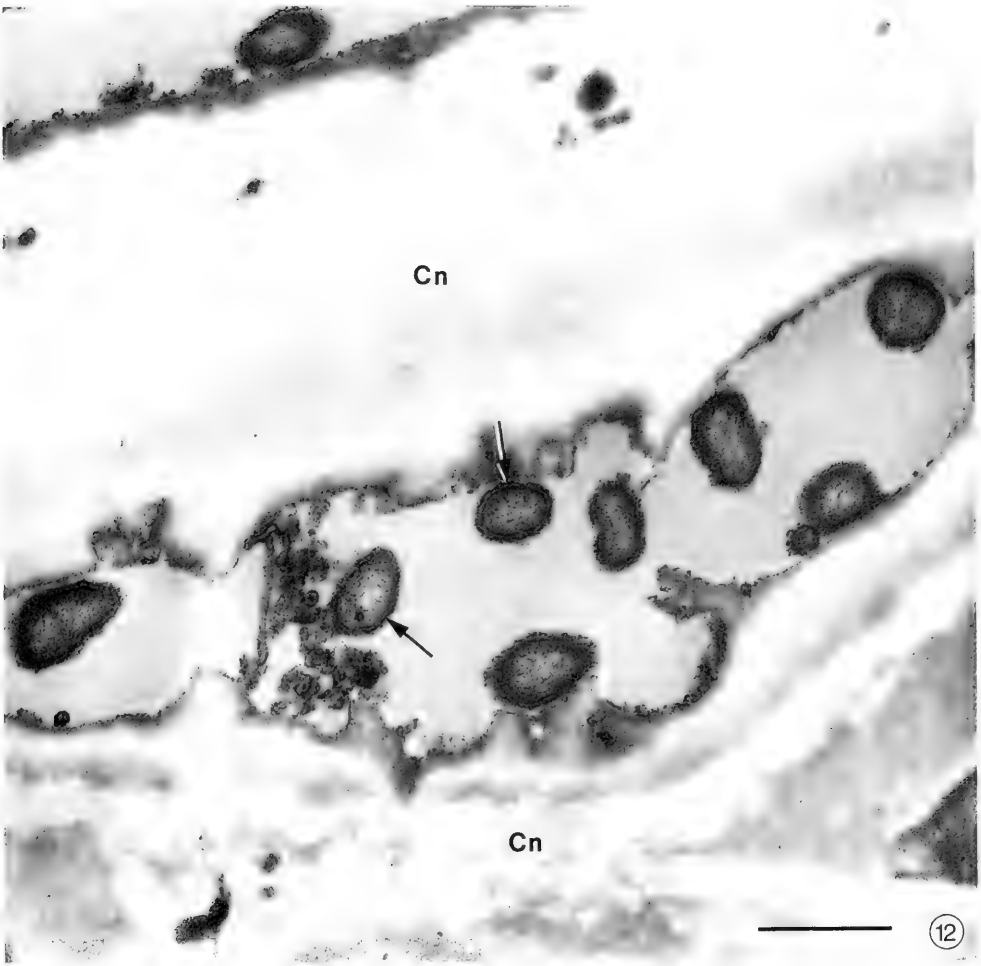

Figure 12. Chamber between layers of anomalous conchiolin (Cn) in which gram negative bacteria (arrows) are present. Bar = 0.5 μm.

abolic rates, and lead to greater weight loss than the absence of food. The time-frame of mortalities and the fact that oysters did not experience weight loss prior to, or during, the period of mortalities, are thus more compatible with stress effects induced by a water or particle-borne contaminant, pathogen and/or the effect of noxious/toxic algae, than food limitation *per se*.

Blooms of the picoplanktonic alga, *Aureococcus anophagefferens*, which is known to cause starvation and mortalities of bivalves (Bricelj et al. 1987, Tracey 1988), have never been recorded in Long Island Sound or adjacent estuaries along the north shore of Long Island. However, a bloom of *Gymnodinium sanguineum* (Dinophyceae) was documented at the Oyster Bay grow-out site between July 11 and July 26, coinciding with peak mortalities of 2 of the experimental cohorts. Cell densities of this dinoflagellate species remained relatively high (at ca. 5×10^4 cells l^{-1}) during early August when the "late cohort" experienced mortalities. No mortalities were associated with a second bloom in September, and only the small cohort in the high density treatment showed a reduction in shell and tissue growth at this time (Table 2). It is noteworthy, however, that the prevalence of mantle lesions and coccoid bodies increased again in both cohorts between August 9 and September 20 (Fig. 13), when dinoflagellate concentrations attained a second maximum. On the other hand, mantle lesions were prevalent in late June, before the increase in *G. sanguineum* cell numbers. Ingestion of toxic dinoflagellates has in some cases been associated with cellular damage (e.g. abnormal vacuolation) of the digestive epithelium in bivalves (Widows et al. 1979, Wikfors and Smolowitz, in press). In the present study

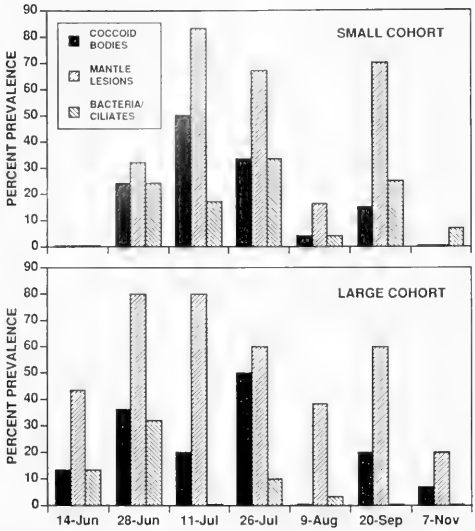

Figure 13. Percent prevalence of 3 pathological conditions observed by light microscopy in tissue sections of juvenile oysters collected at Oyster Bay.

lesions in the digestive gland were observed only in oysters collected in late September and thereafter.

Although *G. sanguineum* has not been previously reported to be toxic to marine fauna, other dinoflagellate species common to east coast estuaries in the summer, are toxic to and cause mortalities of bivalves, including oysters (reviewed by Shumway 1990 and Shumway et al. 1990). The eastern oyster, *Crassostrea virginica,* is particularly susceptible to toxic dinoflagellates. For example, one week's exposure to bloom concentrations of the unarmored dinoflagelate *Gyrodinium aureolum,* (a species closely related to *G. sanguineum,* and associated with fish and shellfish kills [reviewed by Mahoney et al. 1990]), caused 68% mortalities in juvenile *C. virginica* at 10°C, 6 weeks after exposure (Shumway, unpubl. results). Similarly exposed hard clams, *M. mercenaria,* experienced no mortalities. Oysters were able to filter this alga from suspension and were the most affected of 8 bivalve species tested. *Gyrodinium aureolum* was also implicated in mortalities of softshell clams and mussels in Maquoit Bay, Maine, in 1988 (Heinig and Campbell 1992). A bloom of the closely related toxic species *Gymnodinium breve* (*Ptychodischus brevis*) in North Carolina in 1987 resulted in recruitment failure of bay scallops (Summerson and Peterson 1990) and brevetoxin accumulation in oysters and hard clams (Tester and Fowler 1990). The dinoflagellate *Prorocentrum minimum,* which occurs in Long Island Sound, appears to be toxic to some bivalve species, such as the bay scallop, *Argopecten irradians* (Wikfors and Smolowitz, in press).

Early life history stages of bivalves are generally more susceptible to the detrimental effects of toxic algae. Thus, a summer bloom of *Gyrodinium* cf. *aureolum* (= *Gymnodinium nagasakiense*) in the Bay of Brest, France, caused heavy mortalities of postset scallops, *Pecten maximus* (0.25–3 mm), but only cessation of growth and shell abnormalities of juveniles (5–30 mm) held in a shore-based nursery, and growth disturbance rings in wild adults on the bay bottom (Erard-Le Denn et al. 1990). In conclusion, the large biomass contribution of *Gymnodinium sanguineum* at the time of oyster mortalities in Oyster Bay, and the fact that no information is available on this species' toxicity to *C. virginica,* raises the possibility that mortalities could have been caused by noxious/toxic phytoplankton. If so, it is not yet clear why no oyster mortalities were associated with the second bloom of *G. sanguineum.*

Role of Physical Environmental Factors

Environmental factors such as temperature, salinity, and low levels of oxygen can interact synergistically with other stressors. In the present study, however, salinities and temperatures remained within normal levels for this site, although earlier spring warming occurred in 1991 relative to the 4 previous years. The water column is relatively shallow and well mixed, thus precluding oxygen limitation, except in an anoxic microzone that developed within the trays around dead animals. Dense algal blooms could, however, cause transient hypoxia during night hours, when the oxygen demand may exceed supply (see discussion in Heinig and Campbell 1992). Oyster mortalities of three experimental cohorts cultured at this site were restricted to the months of July and August. However, the "late cohort" was the last produced at this study site in 1991; there is no way of determining whether abnormal mortalities would also have affected later cohorts held in the system during the early fall period of decreasing temperatures. Earlier oyster cohorts held in the same growout system, but not tracked by our study, suffered heavy mortalities as early as July 4, when the water temperature was about 22°C. However, the earliest cohort, originating from a spawning in late February, which was deployed in the trays in the first week of April, first reached commercial bottom planting size (20–30 mm) on June 17 without experiencing anomalous mortalities (D. Relyea, Flower Co., pers. comm.).

Dinoflagellate blooms tend to occur during the warmer months of the year. Chang and Carpenter (1985) found that temperature was the principal factor controlling the appearance of summer blooms of *Gyrodinium aureolum* in the Carmans River estuary, Long Island. The rapid decline in *G. sanguineum* cell densities in Oyster Bay in late September, once temperatures dropped below ca. 18°C, may also be temperature related. Thus, while the exact role of elevated summer temperatures in relation to the oyster mortality events cannot be ascertained from our data, we suggest that they may have played an indirect role (e.g. through control of phytoplankton species composition and abundance or microbial activity) in the development and/or progression of mortalities, since these were not documented until temperatures exceeded about 22°C.

Comparative Response of Large and Small Cohorts

The two main cohorts available for comparison at this site, differed by only about 10 mm in mean shell height at the time of deployment. Thus size-dependent mortality can only be assessed over a relatively narrow size range.

In both experimental cohorts deployed on June 14, mortalities coincided with: a) reduction in shell and soft tissue growth (Table 2), b) reduction in gravimetric condition index (Fig. 4), and c) increased prevalence of a distinct ring of conchiolin deposited on the inner shell surface (Fig. 6) and of mantle lesions (Fig. 13).

TABLE 3.

A) Surface water temperature and mean percent cumulative mortalities (SD = standard deviation, n = 2 replicates) of large and small oyster cohorts cultured at Fishers Island at high and low stocking densities (see Table 1). B) Mean shell height ($\overline{H}$, mm) and dry soft tissue weight ($\overline{DW}$, mg) obtained by pooling data from two replicate experimental groups at the high-density treatment [data only available for some of the sampling dates (see text)]. ND = not determined; SE = standard error, n = sample size.

A) % Cumulative Mortality Mean (SD)

		Small Cohort		Large Cohort	
Date	T (°C)	High	Low	High	Low
6/12	18	0.9	0.9	8.8	8.8
6/27	18	0	0	5.4 (5.4)	5.2 (0.6)
7/10	18	15.6 (11.6)	9.0 (5.6)	2.8 (1.9)	1.8 (2.6)
7/25	20	96.4 (1.1)	100	6.8 (6.0)	4.2 (1.2)
8/8	22			6.0 (1.9)	6.2 (1.2)
8/29	ND			6.8 (1.5)	3.3 (2.0)
9/13	21			7.1 (1.0)	3.6 (0.3)
9/26	18.5			10.8 (2.0)	8.2 (3.0)

B) Mean Shell Height ($\overline{H}$) and Tissue Dry Weight ($\overline{DW}$)

	Small Cohort				Large Cohort			
Date	$\overline{H}$	(SE, n)	$\overline{DW}$	(SE, n)	$\overline{H}$	(SE, n)	$\overline{DW}$	(SE, n)
6/12	8.9	(0.2,49)	2.54	(0.13,49)	31.7	(5.0,30)	75.40	(4.32,30)
6/27	12.2	(0.3,60)	7.26	(0.46,59)	33.4	(0.7,60)	120.89	(5.23,60)
7/10	12.0	(0.3,56)	5.28	(0.44,39)	39.8	(0.7,60)	229.24	(12.01,60)
7/25					38.9	(1.0,60)	283.98	(15.98,60)
8/8					44.2	(0.8,98)	280.38	(13.28,98)
9/13					53.9	(1.1,56)	ND	

Several differences in response were observed, however, between the two cohorts. It is noteworthy for example, that the LC showed detrimental effects on growth, and appearance of the abnormal conchiolin syndrome and mantle lesions 2 weeks earlier than the SC. This correlates with the appearance of mortalities 2 weeks earlier than in the SC. The drop in condition index, coincident with mortalities, however, was much more pronounced in the SC (53% compared to only 9% in high-density treatments), yet mortalities peaked at similar levels in both cohorts by July 26. Galtsoff (1964) suggested that young *Crassostrea virginica* have flatter valves and therefore a higher CI than larger oysters (Galtsoff 1964). Rainer and Mann (1992) found no size-dependency of the volumetric CI in *C. virginica,* but tested this only for oysters 36 to 96 mm in height. The difference in initial CI values observed between cohorts in the present study cannot be attributed solely to size-dependency, however, since SC oysters had lower values even when they attained the same size as LC oysters (compare the mean condition index for the SC on July 11 with that of the LC on June 26 in Fig. 4). Difference in mortalities, growth and condition between the 2 cohorts are thus not clearly correlated with size, and may simply reflect inter-batch variability.

A decline in condition represents a sensitive but generalized stress response, of limited value in inferring specific causes of mortality. Reductions in the condition index of oysters have been related to reproductive condition (e.g. Nascimento and Pereira 1980), nutritional stress (Wright and Hetzel 1985), as well as disease (Newell 1985, Paynter and Burreson 1991). Interpretation of changes in the condition index can be confounded if rates of growth in shell and soft tissues are uncoupled (Hilbish 1986). In the present study, however, reductions in growth coincidentally affected both shell and soft tissues.

Mean tissue weight and condition index were generally greater at the lower stocking density, although these differences were not always statistically significant, especially for the SC. Differences in tissue weight between density treatments averaged only 21–24% over the study period in both cohorts, and were more pronounced during the early part of the experiment (through July 26). Mortalities, however, were reduced by as much as 39–45% at the low stocking density. Thus, reduced densities had a greater effect in reducing mortalities (especially in the small cohort), than in increasing growth rates, suggesting that the former largely resulted from reduced incidental anoxia, associated with fewer total numbers of dead animals within growing trays. These results suggest that thinning of cultured oysters may provide a management alternative to partially mitigate losses during the critical period of summer mortalities.

Histopathology

Both gross pathology and histopathological evidence suggests an irritant or toxin affecting the epithelial cells of the mantle, causing retraction of that organ and an attempt to "wall off" the oyster's soft tissues from the irritant. We found no evidence to support the contention that juvenile oyster mortality is associated with a newly described protozoan of uncertain affinity (Farley et al. 1992). The coccoid bodies present in many lesions appear to be remnants of degenerated oyster cells, not a protist, and the ciliates seen in some lesions are undoubtedly opportunistic invaders. We conclude that if a microbe is the causative agent of the "disease," it is either not found consistently or in significant numbers in tissues of affected oysters and induces lesions from a distance by production of an exotoxin, or it is easily washed free of the tissues

during specimen preparation for microscopy. It is highly unlikely, however, that a whole population of microbes would be washed from a histological preparation. Furthermore, enough material has been examined at three laboratories (Battelle, HSRL, and VIMS) to make it most unlikely that even a small population of highly virulent microbes would be overlooked.

Juvenile oyster mortality syndrome, including the formation of conchiolin deposits around the contracted oyster tissues, resembles "brown ring disease" of cultured Manila clams, *Tapes philippinarum*, in western Europe (Paillard et al. 1989, Paillard and Maes 1990). The disease was first reported in 1987 when it caused heavy mortalities in cultured clams. Nearly all of the moribund clams exhibited abnormal deposits of organic material on the inner shell. Neither protozoan nor metazoan parasites were detected in histological sections, but a bacterium of the genus *Vibrio* was isolated from diseased clams and caused the brown ring syndrome when injected into healthy clams (Paillard and Maes 1990). Similarly, anomalous conchiolin deposits, generally around the posterior edge of the shell margin, are associated with mortalities of the golden lip pearl oyster, *Pinctada maxima*, in Western Australia. Pass et al. (1987) suggested that *Vibrio harveyi*, isolated from affected pearl oysters, was involved in causing the disease. In both *P. maxima* and *T. philippinarum*, the conchiolin deposits differed somewhat from those of *C. virginica* in that the former were not consolidated into a distinct thin ridge, but rather were spread into a wider band with more irregular borders.

The bacterial etiology of "brown ring disease" in Europe and the fact that histopathological lesions in affected oysters are similar to those found by Dungan and Elston (1988) in association with bacterial destruction of the hinge ligament in juvenile Pacific oysters, *C. gigas*, indicates that a bacterium cannot be ruled out as the cause of juvenile oyster mortality. Whether the bacteria which we found in tissues and in chambers within the anomalous conchiolin deposits are one or several species of opportunistic bacteria or are the causative agent(s) of the mortality remains to be determined. It should be noted that deposition of conchiolin can be affected by other stressors, including handling (e.g. in bay scallops, Palmer 1980) and exposure to anthropogenic contaminants (Hillman unpubl. observations).

In conclusion, the results obtained suggest that oysters may have been affected by a toxin-producing agent (most likely of bacterial or microalgal origin), or by a chemical contaminant which caused mantle retraction and secretion of an abnormal conchiolin layer as a defense mechanism. Death presumably occurred when the muscle became detached from the valve due to conchiolin deposition between the muscle and the shell, and/or degeneration of myoepithelial cells accompanied by bleeding. Mortalities were probably aggravated by entry of secondary invaders into lesions, and by the development of anoxic conditions (in turn aggravated by high summer temperatures) within the trays as oysters began dying. Future work should further investigate site-specificity of mortalities, the influence of rearing temperature, and age/size of affected oysters, the potential role of bacteria, and especially the association of mortalities with the occurrence of blooms of dinoflagellates or other potentially toxic phytoplankton species.

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IN VITRO CULTURE OF PRESUMPTIVE NERVOUS TISSUE OF *CRASSOSTREA VIRGINICA* (GMELIN, 1791)

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ABSTRACT The absence of an *in vitro* culture system of marine pelecypod cells and tissue has impeded efforts to investigate physiological and developmental processes, both normal and pathological, of these organisms. Utilizing dissociated tissue, explants and techniques described herein, we report the successful primary culture of presumptive nervous tissue of *C. virginica*.

KEY WORDS: *C. virginica*, nervous tissue, tissue culture, visceral ganglion, pelecypod

INTRODUCTION

Compared with other invertebrates, there exists a paucity of literature concerning the *in vitro* culture of marine pelecypod cells and tissues, indicating the difficulties these endeavors present. Certainly, organ cultures of heart, gonad and mantle have been readily achieved. These cultures typically remain viable for considerable periods, although all will eventually deteriorate (Machii 1974, Ieyama et al. 1979, Hetrick et al. 1981). Development of anchorage-dependent cell lines such as cultures of epithelial and muscle cells, has had limited success. Historically, viability, growth and maintenance of cells obtained from dissociated tissue, organ cultures that have lost their histiotypic integrity or cells that have migrated from organ cultures, have been short term and limited (Perkins and Menzel 1964, Cecil 1969, Hetrick et al. 1981). Because of these limitations, many physiological, developmental and pathological studies requiring *in vitro* viability, measurement and response to natural or induced interactions and perturbations have not been possible. We report herein the *in vitro* primary culture of presumptive nervous cells and tissue from the visceral ganglion of *Crassostrea virginica* (Gmelin), partially fulfilling the above requisites.

MATERIALS AND METHODS

Mature specimens of *Crassostrea virginica* were obtained from local packing plants and maintained at the Haskin Research Laboratory, Port Norris, New Jersey. Tissue culture flasks and 0.22 μm nylon filters were obtained from Corning Glass Works and the antimicrobics and powdered medium from Sigma Chemical Co.

Preparation of Ganglionic Explant

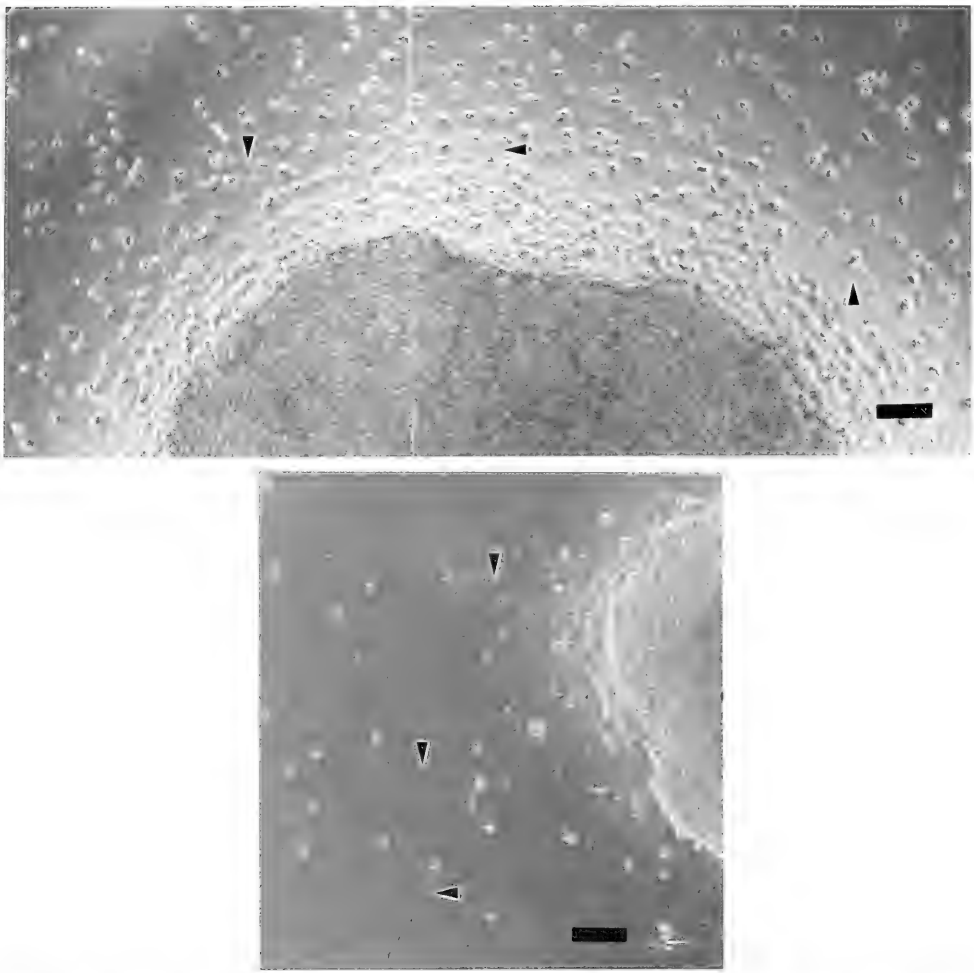
In order to eliminate as much contamination as possible, oysters were initially brush-scrubbed vigorously, immersed in a 1% Clorox (5.25% sodium hypochlorite) solution for 1 minute and rinsed in sterile sea water. Sterile, cell free hemolymph was obtained by aspiration from the adductor muscle sinuses and subsequent filtration through a 0.22 μm nylon filter. The oysters were then shucked by separating the hinge and cutting the adductor muscle. The visceral ganglion was dissected as aseptically as possible using a stereo-dissecting microscope. Each ganglion so obtained was never completely free of extraneous surrounding tissue due to the cortical location of the nerve cell bodies which generally surround the medullary neuropil of the ganglion (Galtsoff 1964). The isolated ganglia were rinsed several times in sterile sea water and subjected to two 20 minute rinses in sea water containing antimicrobics (penicillin, 20,000 U/ml, streptomycin, 20 mg/ml and amphotericin B, 25 μg/ml). Each ganglion was transferred to a deep well Maximow slide, flooded with sterile sea water containing the antimicrobics and minced into 1 mm^3 pieces. Following mincing, the pieces were rinsed twice with a sterile sea water/antimicrobic mixture to remove debris and unwanted tissue. An autologous sterile hemolymph/sea water mixture (50/50) with antimicrobics was added to the slide containing the minced tissue.

Preparation of Dissociated Ganglion

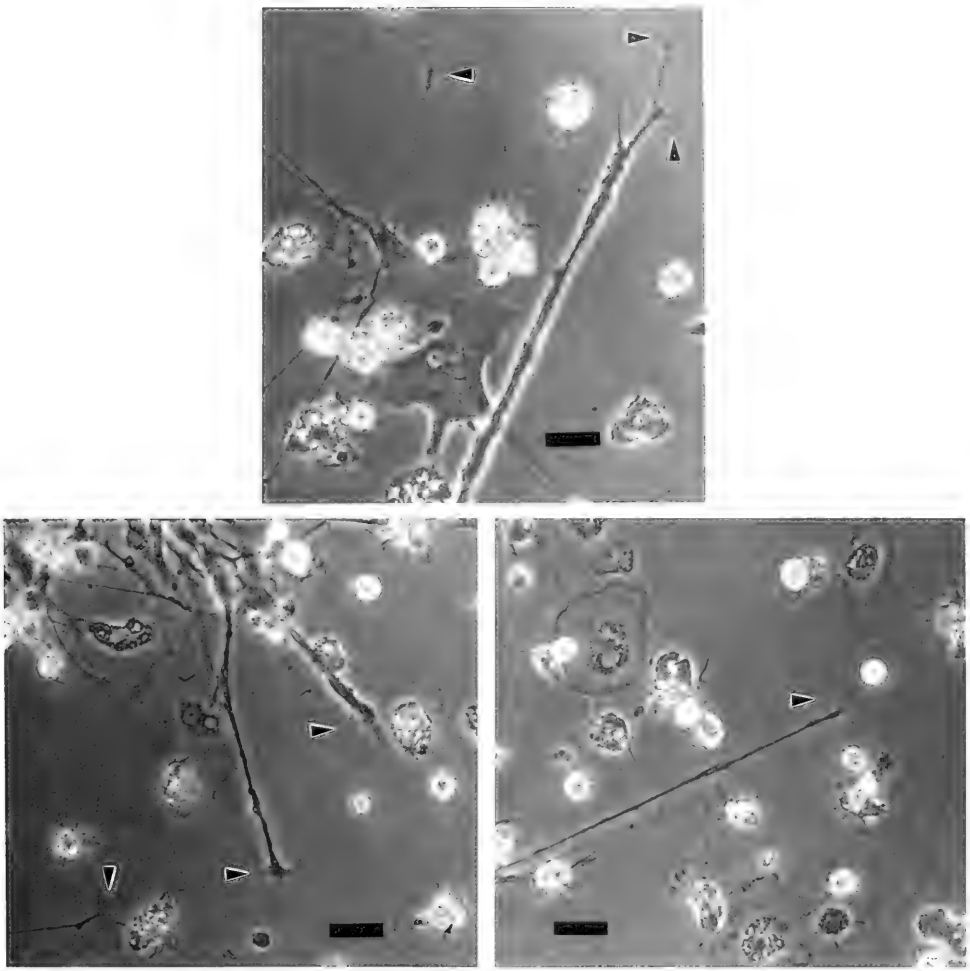
Tissue was obtained as described above. Minced pieces were placed into a sterile 15 ml centrifuge tube containing 10 ml of Ca^{2+}- and Mg^{2+}-free sea water (CMF) for 10 minutes. The CMF sea water was aspirated and replaced with a solution of 0.25% trypsin and 0.02% EDTA dissolved in CMF sea water and incubated at room temperature for 25 minutes. Following incubation, the dissociation medium was aspirated and replaced with stoichiometric quantities of soybean trypsin inhibitor (TI) in sterile sea water. Following a gentle agitation of the tissue, the TI solution was removed by several sterile sea water rinses and replaced with a sterile autologous hemolymph/sea water solution (50/50) containing antimicrobics. The minced pieces of tissue were dissociated into single cells and small clumps of cells by trituration with a series of sterile Pasteur pipets each with a decreasing diameter obtained by fire-polishing and sizing. The diameter used for the final pipeting was approximately 0.3 mm. Considerable care is needed to insure intact cells with minimum damage. In these experiments, the use of DNAase to prevent artifactual clumping following dissociation was not necessary.

Culture of Ganglionic Explant

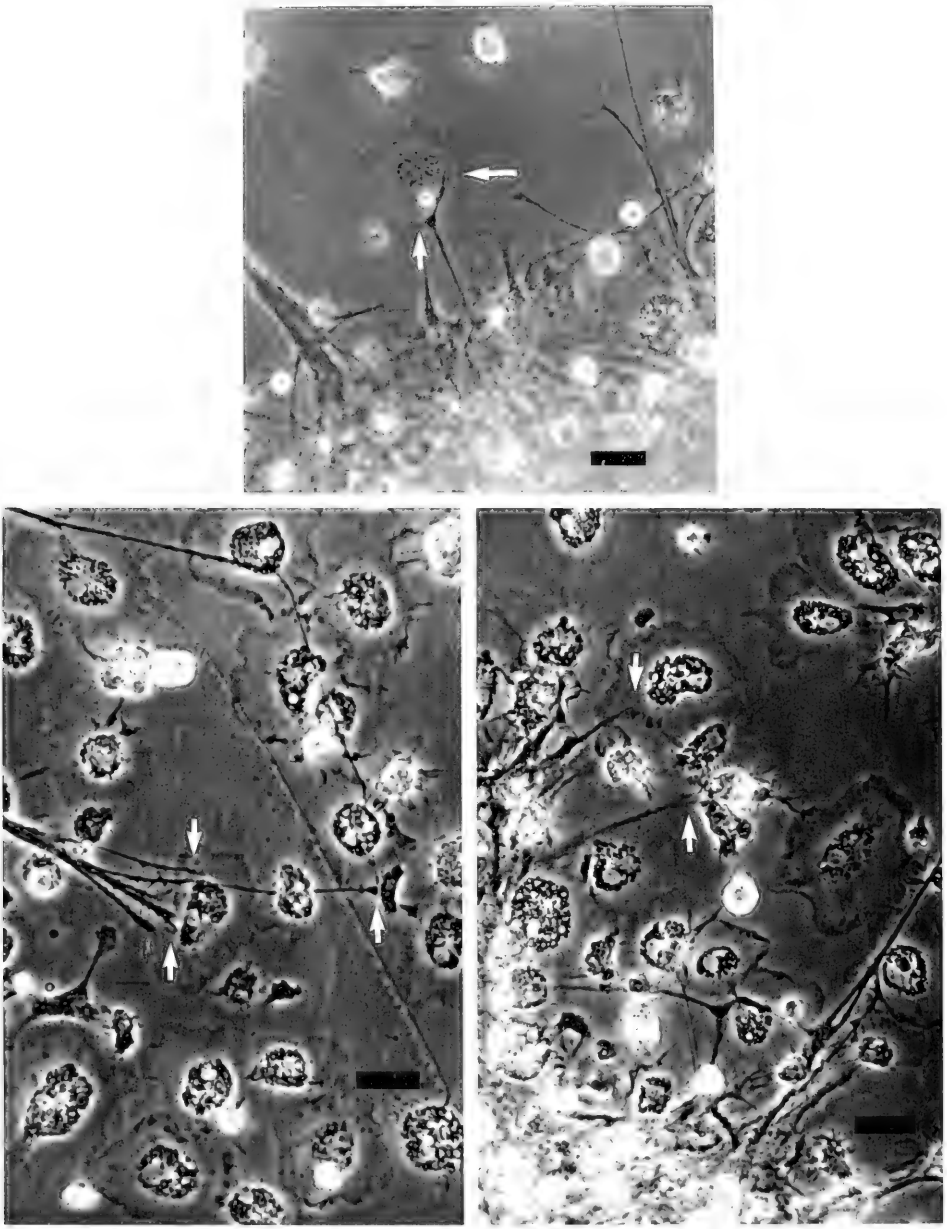
Ordinary T25 culture flasks, tissue culture grade, were used in all experiments. The culture medium consisted of a (50/50) mixture of autologous hemolymph and 3% Leibovitz's L-15 medium (w/v) dissolved in sterile sea water containing antimicrobics (Leibovitz 1963). The final volume of medium in each flask was 2.5 ml. Flasks were inverted several times to humidify the internal atmosphere and prevent dehydration of the explant. Flasks were placed on a slight incline so that the mouth was slightly elevated.



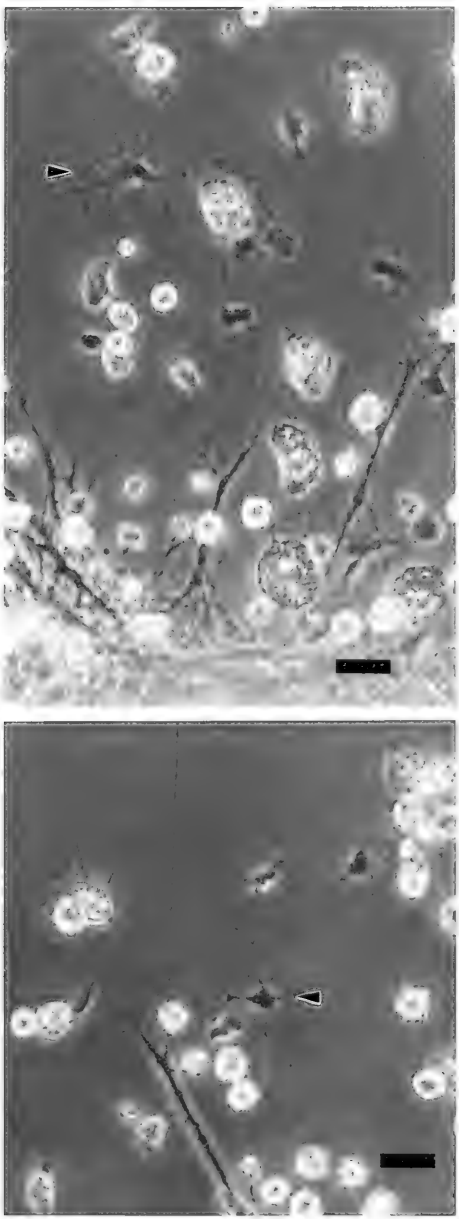
Figures 1 and 2. Explant culture of visceral ganglion of *Crassostrea virginica*. Neurite sprouting of the explant indicated by arrows. Scale bar = 0.20 mm.



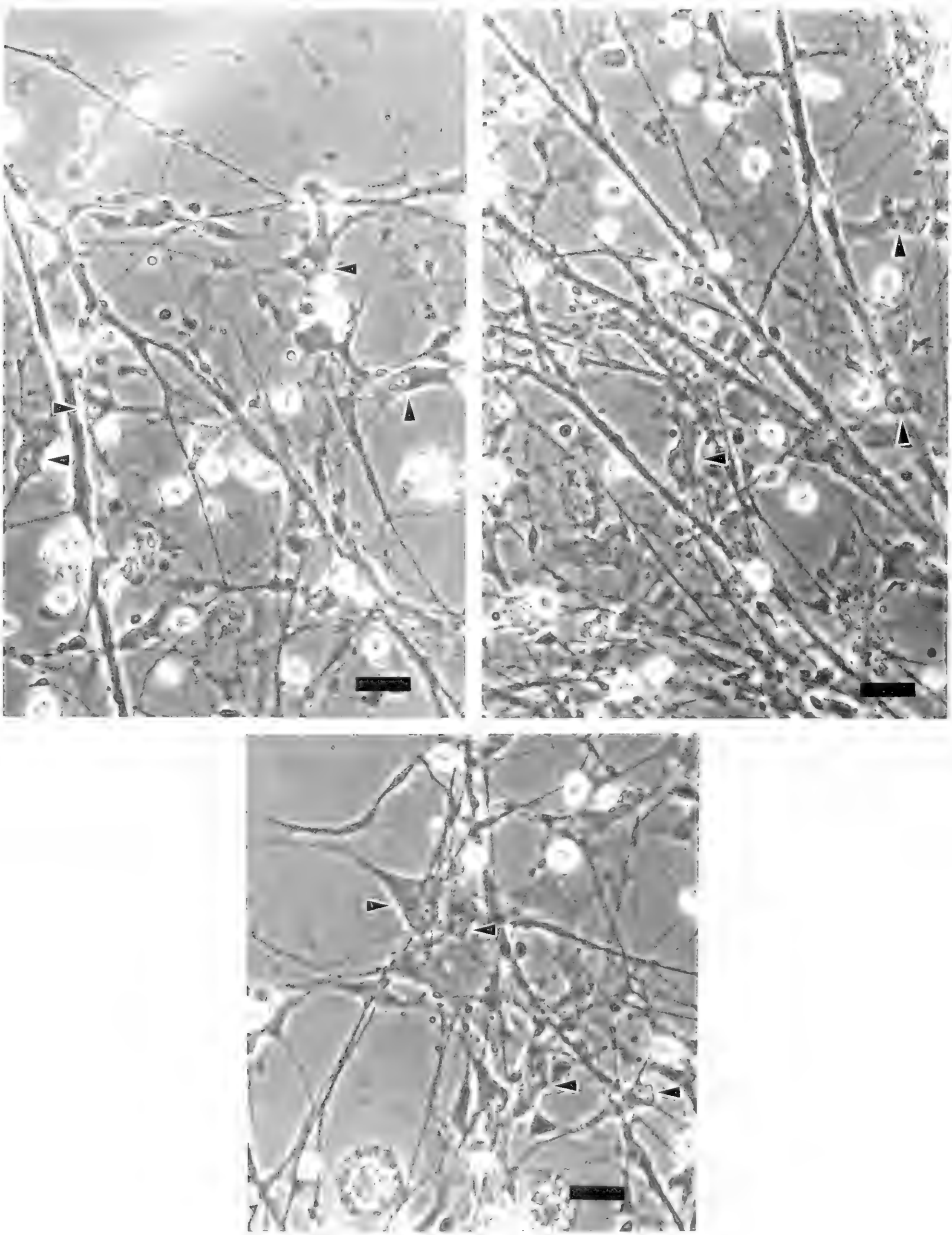
Figures 3–5. Neurite sprouting and neurofilaments in cultured visceral ganglion of *C. virginica*. Growth cones typical of cultured neurons indicated by arrows. Scale bar = 0.05 mm.



Figures 6–8. Neurite and neurofilaments in cultured visceral ganglion of *C. virginica*. Neurofilaments and growth cones exhibiting axiosomatic tropism indicated by arrows. Scale bar = 0.05 mm.



Figures 9–10. Neurites with growth cones and support cells (presumptive glial cells) which have migrated out of the cultured explant of the visceral ganglion of *C. virginica*. Support cells indicated by arrows. Scale bar = 0.05 mm.



Figures 11–13. Photomicrographs of a portion of the developing neuronal network of explant cultures of *C. virginica*. Arrows point to presumptive nerve cell bodies which have migrated out of the explant. Scale bar = 0.05 mm.

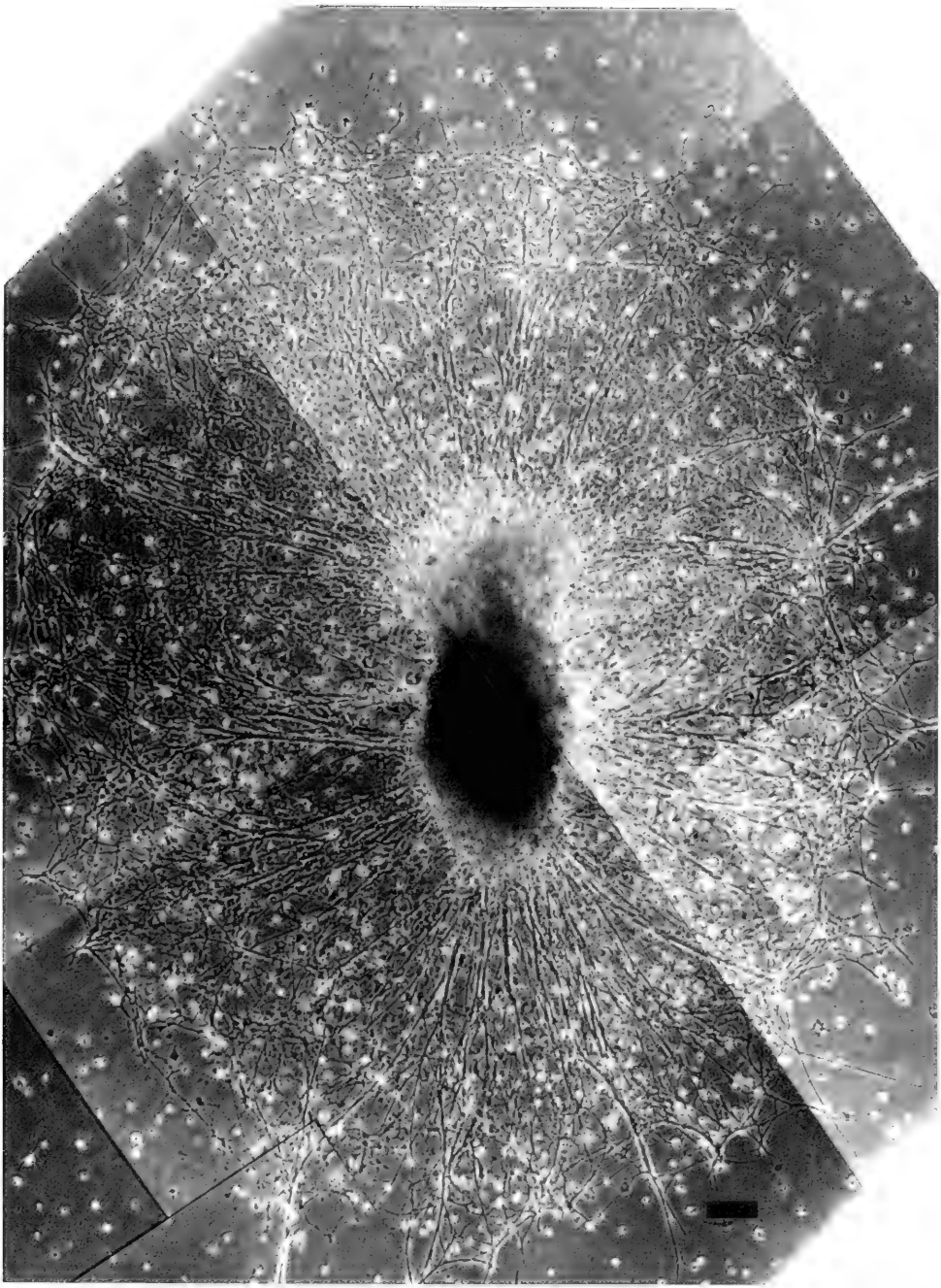
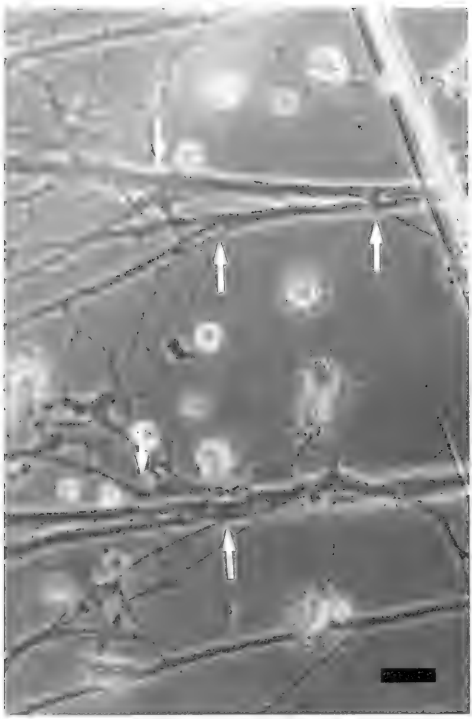
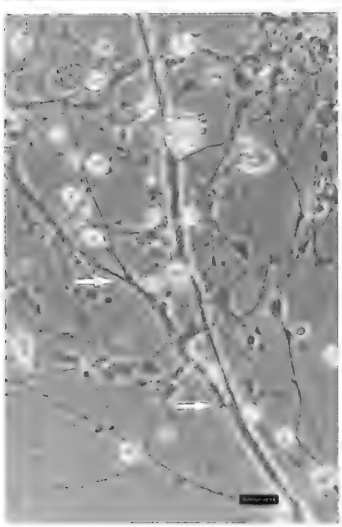
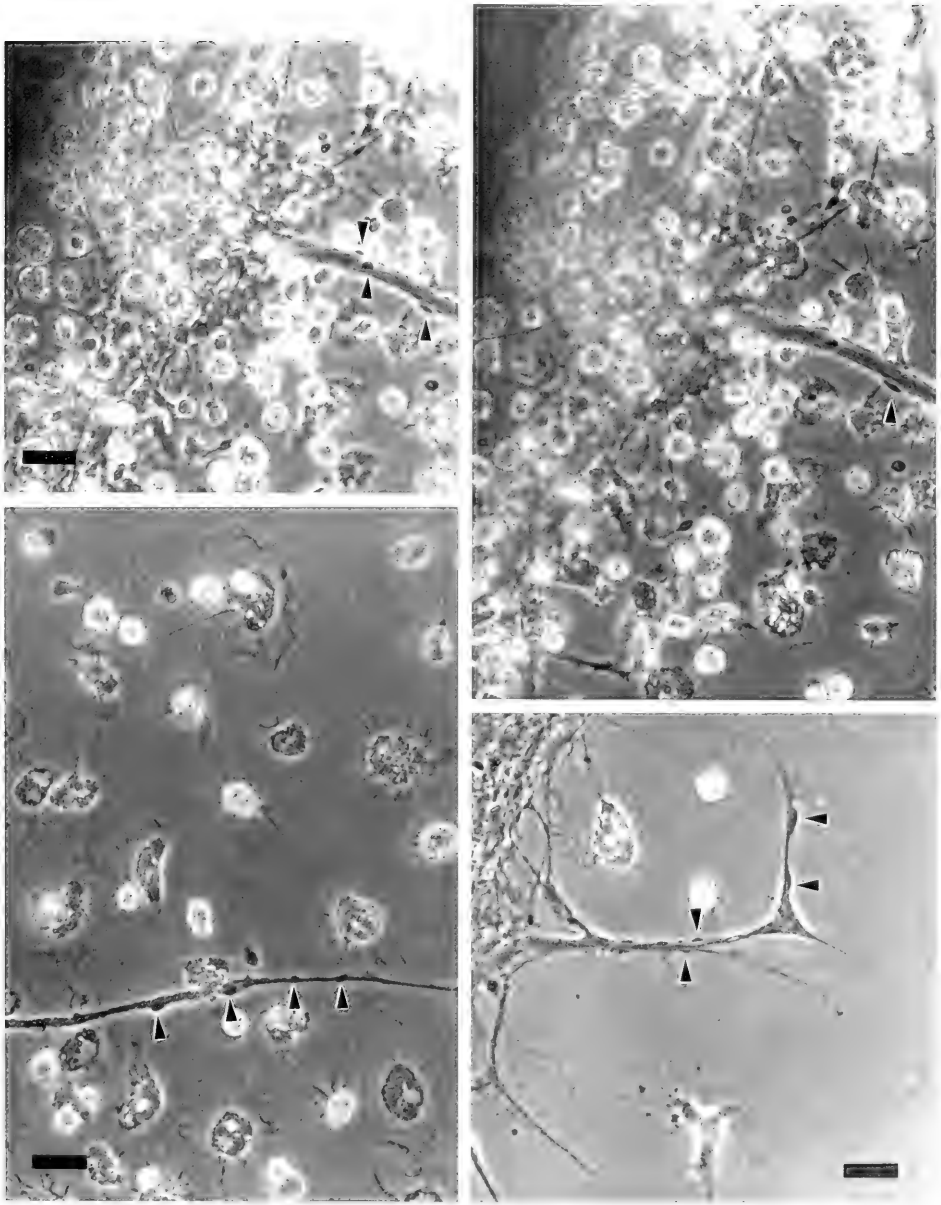


Figure 14. Explant culture of a 14 day old explant culture of the visceral ganglion of *C. virginica*. Notice the complex network of cells, anastomoses and interrelations throughout the culture. Scale bar = 0.20 mm.





Figures 15–16. Presumptive nerve fibers formed by the consolidation of smaller neurites and fibers. Notice the involvement of support cells and finer neurofilaments in the complex. Arrows indicate points of consolidation. Scale bar = 0.05 mm.



Figures 17–20. Large nerve fibers exhibiting presumptive nuclei identified by arrows. Scale bar = 0.05 mm.

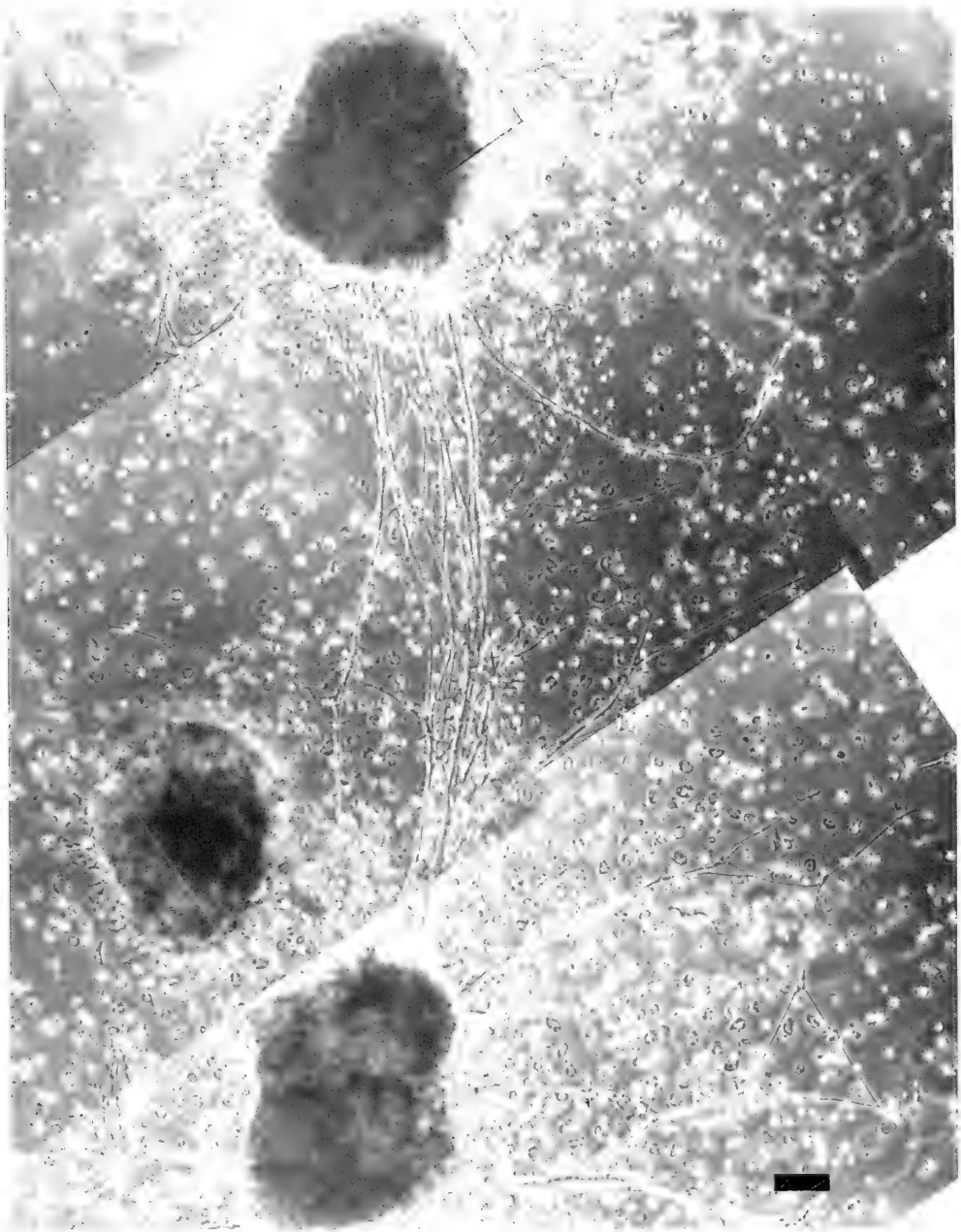


Figure 21. Anastomoses between presumptive neurons and nerve fibers from two different explant cultures of the visceral ganglion of *C. virginica*. Scale bar = 0.20 mm.

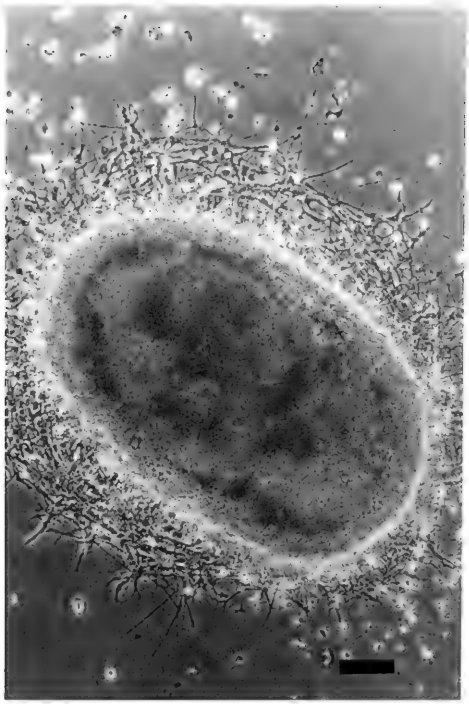


Figure 22. Micrograph of the same cultured explant as Figure 14 one week later (3 week culture). Notice withdraw of nervous cells and processes into the explant as presumptive histotypic orientation occurs. Scale bar = 0.20 mm.

The minced tissue was then placed on the wet surface, with care taken not to touch the pooled medium at the bottom of the flask. The cultures were left inclined at least 18 hrs during which time migrating cells and presumptive nervous tissue could attach to the bottom of the flask. Following attachment, inclination of the flasks was lowered slightly so that the leading edge of the medium was as close to the explant as possible allowing diffusion to occur. In this fashion, both explant, migrating cells and neurites could be provided with necessary nutrients without detachment of floating. Culture temperatures ranged from 17 to 24°C and a 50% medium exchange was provided every 4 days.

Culture of Dissociated Ganglia

Culture vessels were prepared as above. Following dissociation, the resultant suspension of single cells and small clumps of cells from a single ganglion was added to the flask. A minimum amount of medium was added to encourage cell settling and attachment as well as to discourage floating. The cultures were left undisturbed for 3 days, after which time additional medium was gently added. Culture temperatures and feeding rates were as above.

RESULTS AND DISCUSSION

Explant Primary Cultures

Within 24 hours after initiation, the main migrating cell types present in the explant culture were cells typically found in oyster hemolymph (hemocytes). However, in many instances, neurite (axonal) sprouting of the explant could also be demonstrated (Figs. 1 and 2). Initially, the number of neurites was relatively small as was the diameter of the presumptive neurofilaments. Growth cones, characteristic of cultured neurons, were quite evident (Figs. 3–5). Over a period of two weeks, the explant profile became more complex as the presumptive neurites increased in length and diameter. Growth appeared to be stimulated by axiosomatic contact with other cells (Figs. 6–8) as support cells (presumptive glial cells) become evident (Figs. 9–10). In many instances, the support cells were found at the leading edge of the complex, along with small diameter neurites and growth cones. As the complex grew to involve many cells, migration of presumptive neuronal cell bodies out of the explant was seen (Figs. 11–13). These cells exhibited typical neuronal morphology including large central nuclei, large prominent nucleoli, Nissl substance, numerous dendrites and neurofilaments. Branching of nerve cell axons with multiple associations and anastomoses was also common. Proximally, these cells along with the support cells formed a remarkably complex network of interconnections and presumptive synapses as they became more numerous (Fig. 14). The relationship between the presumptive nerve and support cells also appeared to increase in complexity.

Presumptive nerve fibers were formed as the consolidation of neurites occurred (Figs. 15–16). Larger fibers appeared to involve large numbers of nerve cells as presumptive nuclei could be identified along the fiber (Figs. 17–20). These fibers reached a length of approximately 2000 μm with a diameter of 12.5 μm. Individual neurites immediately proximal to the growth cone could be identified with diameters of approximately 0.5 μm.

Additionally, as seen in Figure 21, anastomoses between presumptive neurons and nerve fibers also readily occurred. These anastomoses were the result of the consolidation of earlier relationships between multiple neurites from adjacent explants. Interestingly, if the peripheral axons did not establish an appropriate relationship (as yet unknown) with other tissue, the neurite out-

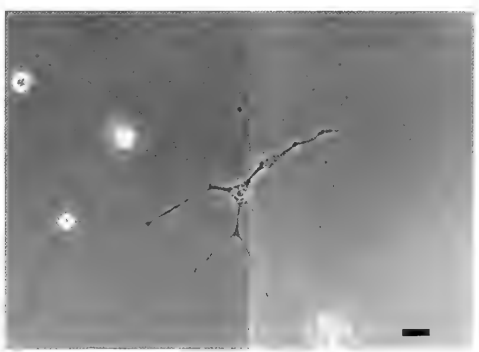


Figure 23. Individual presumptive nerve cell from dissociated visceral ganglion. Scale bar = 0.05 mm.

reach retreated and the network began to condense. The regression would continue over a period of several days until consolidation was complete with the resultant appearance of the explant reminiscent of histotypic orientation (Fig. 22).

Cultured cells from dissociated ganglia exhibited phenomena generally similar to that seen in explant cultures. Immediately following ganglion dissociation and culture initiation, all cells were rounded and not attached to the substrate. After 4–7 days in culture, individual presumptive nerve cells became evident (Fig. 23). Long neurites (neurofilaments) with growth cones appeared and anastomosed with other cells (Fig. 24). Presumptive support cells were also evident and exhibited diffuse outreach patterns (Fig. 25–26).

CONCLUSION

A substantial amount of the neurological knowledge available to the scientific community has been generated from investigations on molluscs, primarily, cephalopods and gastropods. Studies of cultured *Aplysia, Helix, Helisoma,* and *Melampus* neurons and ganglia have reached the molecular level and will continue to elucidate neurological fundamentals common to many species. Bullough (1985), in reviewing these investigations, provides ample evidence of the value of these *in vitro* systems. Extended studies and observations utilizing the *in vitro* system described

Figure 24. Nerve cells and small clumps of cells from dissociated visceral ganglion. Notice the long neurites and anastomoses with other cells. Scale bar = 0.20 mm.

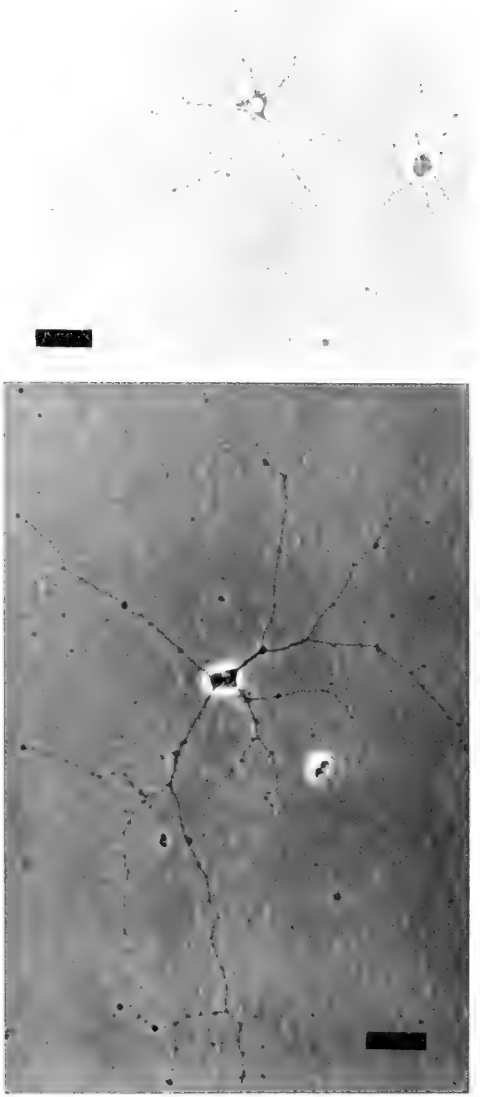


Figure 25–26. Presumptive support cells from dissociated visceral ganglion. Scale bar = 0.05 mm.

herein, could contribute not only to our knowledge of basic neuroscience, but should permit investigations of the development, morphology, physiology and pathology of the pelecypod nervous system. Current studies are ongoing to confirm the cells described in this study as nerve cells.

ACKNOWLEDGMENTS

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INFECTION INTENSITY OF *PERKINSUS MARINUS* DISEASE IN *CRASSOSTREA VIRGINICA* (GMELIN, 1791) FROM THE GULF OF MEXICO MAINTAINED UNDER DIFFERENT LABORATORY CONDITIONS[1]

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ABSTRACT A protozoan parasite, *Perkinsus marinus*, has been responsible for infection and mortality of eastern oysters, *Crassostrea virginica*, since before 1950. Studies on the course of infection intensity in individual animals have been restricted by the need to sacrifice animals for diagnosis, so quantitative association of disease intensity with environmental conditions and individual survival has not been accomplished. A recently developed hemolymph assay provided the means to quantitate infection intensity from live oysters. Application of this technique demonstrated progression of *P. marinus* intensity in Gulf of Mexico oysters maintained in laboratory aquaria in fed and unfed conditions at different test temperatures (18°–27°C) and salinities (6–36 ppt). In one experiment, the infection intensities over eight weekly samplings increased $10^{0.09}$ hypnospores mL^{-1} hemolymph $week^{-1}$ for low temperature/low salinity conditions and $10^{0.36}$ hypnospores mL^{-1} hemolymph $week^{-1}$ for high temperature/high salinity conditions. Temperature was more influential than salinity in *P. marinus* intensity and oyster mortalities. Oysters containing 10^3–10^4 hypnospores mL^{-1} hemolymph survived in low temperatures, but not in high. Feeding did not affect the intensity of *P. marinus*, but may have been a factor in survival of infected oysters.

KEY WORDS: *Crassostrea virginica, Perkinsus marinus*, oyster, invertebrate pathology, invertebrate immunology, agglutinins

INTRODUCTION

The oyster parasite *Perkinsus marinus*, formerly identified as *Dermocystidium marinum*, has infected eastern oysters, *Crassostrea virginica*, in the Gulf of Mexico since before 1950 (Mackin et al. 1950) and has been held responsible for severe oyster mortalities. Numerous investigators have described the characteristics of *P. marinus* disease, its subsequent discovery in Chesapeake Bay (Andrews 1988 for review) and most recently, its re-emergence in Delaware Bay (S. E. Ford, Rutgers University, personal communication). Disease prevalence and severity is known to vary seasonally and has been associated repeatedly with high water temperatures and high salinity (Hewatt & Andrews 1955, Mackin & Boswell 1956, Andrews & Ray 1988).

A culture and staining method was developed by Ray (1952) that not only diagnosed *P. marinus* but also provided a semi-quantitative evaluation of disease intensity. The method has been modified slightly (Ray 1966) but has remained the standard diagnostic procedure for nearly 40 years. Diagnosis requires shucking the oyster to obtain mantle or rectal tissue, so it has been impossible to study the disease in individual animals over time. However, recent application of the Ray diagnostic technique to oyster hemolymph (Gauthier & Fisher 1990) has provided a fully quantitative method to evaluate infection intensity in live oysters. The new method permits the study of diseased progression or regression on individual oysters in different environmental conditions and can be used to better establish the relationship of disease intensity with mortality.

Although "bleeding" of hemolymph from the adductor muscle creates an artificial condition, Ford (1986) determined that oyster hemolymph samples may be drawn once a month without excessive physiological harm to the animal. She found that twice monthly withdrawals caused a decrease in hemolymph serum protein levels. Bivalve hemolymph samples have been used for a variety of immunological and physiological assays (Anderson 1981, Bayne et al. 1979, Cheng 1977, Feng & Canzonier 1970, Feng et al. 1977, Fisher & Newell 1986, Ford 1986, Hardy et al. 1977, McDade & Tripp 1967, Nakamura et al. 1985, Tripp 1966, Wittke & Renwrantz 1984). Hemolymph sampling can thus monitor both the disease condition and a variety of host responses and physiological conditions.

A host condition that has been linked to defense is the ability of cell-free hemolymph (serum) to agglutinate homologous foreign particles. Several studies have indicated that serum agglutinins, or lectins, may play a role in nonself recognition in invertebrates (Olafsen 1988) by opsonizing invasive foreign material. In a recent study, serum agglutinins were found to be significantly higher in laboratory selected, MSX-disease resistant oysters during the late summer infection period than in unselected, susceptible oysters (Chintala & Fisher 1991).

Experiments were undertaken at two laboratories to evaluate the influence of temperature, salinity and feeding on the course of infection of *P. marinus* disease in Gulf of Mexico oysters. Hemolymph analysis allowed repetitive sampling of individual oysters and quantitation of infection intensity. Serum levels of agglutinin and protein were also monitored to establish their relationship with disease and host physiology.

[1]Contribution number 769 Gulf Breeze Environmental Research Laboratory.

MATERIALS AND METHODS

Experimental Protocol and Assay Procedures

Three oyster studies were performed at two laboratories, the University of Texas Medical Branch at Galveston (UTMB) and the U.S. Environmental Protection Agency Gulf Breeze Environmental Research Laboratory (GB/ERL). All experiments were conducted in 10 gallon (~40 L) aquaria and gaping or dead oysters were removed daily.

Hemolymph Diagnosis of *P. marinus*

Hemolymph drawn (0.5 mL) from the adductor muscles of individual oysters were centrifuged for 4–5 min at low speed (800 × g) on a microcentrifuge. The supernate (cell-free hemolymph, or serum) was collected, preserved with 0.01% sodium azide and held at 4°C for lectin and protein analysis as described below. The pelleted hemocytes were covered with 0.5 mL fluid thioglycollate medium containing 5 μL mL^{-1} mycostatin stock solution and 5 μL mL^{-1} chloromycetin stock solution (Ray 1966), incubated in the dark for 5–7 days, then centrifuged, treated with 2M sodium hydroxide, re-centrifuged and finally resuspended in 0.5 mL distilled water. Hypnospores were enumerated according to the methods of Gauthier & Fisher (1990) or as modified in the third experiment described here.

Hemolymph Serum Agglutinin and Protein Assays

Protein concentrations were measured on preserved oyster serum within two months of withdrawal using the Pierce* Protein BioAssay Kit with bovine serum albumin standards. Agglutination titers were measured within one week of withdrawal on two-fold serial dilutions of preserved oyster serum according to the method of Fisher & DiNuzzo (1991) using horse erythrocytes (Cocalico Biologicals, Inc. Reamstown PA) and human O^+ erythrocytes from the UTMB Blood Bank.

Data analysis was performed using Statistical Analysis Systems (SAS) correlation programs (Schlotzhauer & Littel 1987) and Duncans Multiple Range Tests. Regressions were determined by the Sigma Plot Scientific Graph System (Jandel Scientific, Sausalito CA 94965).

Experiment 1: Salinity Variables (UTMB)

Seventy-two oysters were collected on October 17, 1990 from Eckerd's Bayou (25 ppt salinity) in Galveston, Texas and randomly placed, without salinity acclimation, into six 40-L aquaria (12 oysters each) containing 20 L sea water at 6, 12, 18, 24, 30 or 36 ppt salinity maintained at 25 ± 2°C. One-half the water in each aquarium was replaced three times a week and oysters were fed approximately 2% of their body weight of laboratory-grown algae (*Chlorella* sp. and *Isochrysis galbani*) three times each week after each water change. Prior to immersion in the aquaria, oyster shells were notched and 0.5 mL hemolymph was withdrawn from all oysters. Six of the twelve oysters in each aquarium were sampled 2, 4, 6 and 8 weeks after immersion and the remaining six were sampled 3, 5 and 7 weeks after immersion. This alternating sample sequence provided weekly data points but only bi-weekly samplings for each oyster.

Experiment 2: Salinity × Temperature Variables (UTMB)

Seventy-two oysters were collected from East Lagoon, Galveston on January 26, 1991 (15 ppt salinity) and randomly placed in six 40-L aquaria (12 oysters each) containing 20 L of sea water at 15 ppt salinity and 20°C. Salinities in the aquaria were altered 3 ppt every 2 days until two aquaria were at 12, 24 and 36 ppt salinity. Water temperature was then adjusted in replicate aquaria to low (L = 18 ± 2°C) or high (H = 27 ± 1°C) temperatures and initial hemolymph samples were drawn on February 13. Unfiltered sea water from Galveston Bay was adjusted for salinity and temperature and used to replace one-half the volume of each aquarium three times per week. Oysters were fed three times a week (after each water change) approximately 2% of their body weight in *Isochrysis galbani* cultured in the laboratory.

Hemolymph from oysters in all aquaria was sampled on a monthly basis with the exception of those oysters held at high temperature, 36 ppt salinity (H/36) which were rapidly dying; these were sampled bi-weekly. Because of heavy mortalities, sampling of all high temperature aquaria was suspended after 2 months; monthly sampling of low temperature aquaria continued an additional 2 months (until June 12).

Experiment 3: Salinity × Temperature Variables (GB/ERL)

Oysters for the studies performed at GB/ERL were collected from Apalachicola Bay, Florida, on March 20, 1991 and held for one week in flow-through seawater tanks at GB/ERL where the average salinity was 17 ppt and temperature 19°C. On March 28, oysters were randomly placed into 18 40-L aquaria (12 oysters each) with 20 L Instant Ocean (Aquarium Systems, Inc., Mentor OH 44060) artificial sea water at the temperature and salinity conditions described below.

Conditions for the Experiment 3 oysters varied in a multifactorial scheme that involved two feeding levels, three temperature and three salinity factors. Sea water in different aquaria was maintained at low (LT = 18°C), high (HT = 25°C) or variable (VT = 15–27°) temperature and low (LS = 10 ppt), high (HS = 25 ppt) or variable (VS = 10–25 ppt) salinity. Variable temperature (VT) conditions were established by changing the thermostat to 18°C or 25°C temperature on alternate weeks; low initial temperatures were thus elevated gradually during the first week. Variable salinity (VS) conditions were initiated at low salinity, changed to high after one week and alternated thereafter with each water change.

For each condition described above, one aquarium received 350 mL of laboratory-cultured *Isochrysis galbani* five times per week (fed) and a replicate aquarium received no cultured algae (unfed). All sea water in each aquarium was replaced each week with artificial sea water adjusted to the appropriate temperature and salinity.

Hemolymph (0.5 mL) was drawn four days after immersion (April 1) from three of 12 oysters in each aquarium. On this first sampling date only, hemolymph was also drawn from 25 additional reference oysters still maintained in the flow-through sea water tank. Each week hemolymph was drawn from three different oysters until, after four weeks, all 12 oysters were sampled and the cycle was repeated. This staggered schedule was maintained so

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that individual oysters were sampled only twice (once every four weeks) but data points were obtained weekly for eight weeks.

The high temperature portion of the experiment was terminated on May 21, 1991 due to heavy mortalities. To provide tissue samples from both low and high temperature conditions, the VT oysters were elevated and maintained at 25°C for an additional month (until June 24). After this time, tissue burdens of *P. marinus* were quantified from hemolymph, mantle, digestive gland and gill tissues of 5–6 oysters from each LT and each VT (25°C for final month) condition. Hemolymph analysis was performed as described for Experiments 1 and 2 except for the following modification: Digested and stained hemolymph samples (0.1 mL) were vacuum-filtered onto filter paper (0.45 μm pore size) and the total number of stained hypnospores counted. This vacuum-filter modification was also incorporated into the methods of Choi et al. (1989) to enumerate the hypnospores per gram wet weight of mantle, digestive gland and gills.

RESULTS

Experiment 1: Salinity Variables (UTMB)

Perkinsus marinus was detected in all 72 oysters in Experiment 1 prior to immersion in different salinities. The infection intensity averaged $10^{3.16\pm0.21}$ (±standard error) hypnospores mL^{-1} hemolymph. The number of hypnospores per mL hemolymph increased at all salinities over the eight-week period (Fig. 1A) at average rates of $10^{0.09}$ (6 ppt; r = 0.51), $10^{0.11}$ (12 ppt; r = 0.80), $10^{0.18}$ (18 ppt; r = 0.65), $10^{0.12}$ (24 ppt; r = 0.77), $10^{0.19}$ (30 ppt; r = 0.77) and $10^{0.27}$ (36 ppt; r = 0.94) hypnospores $week^{-1}$.

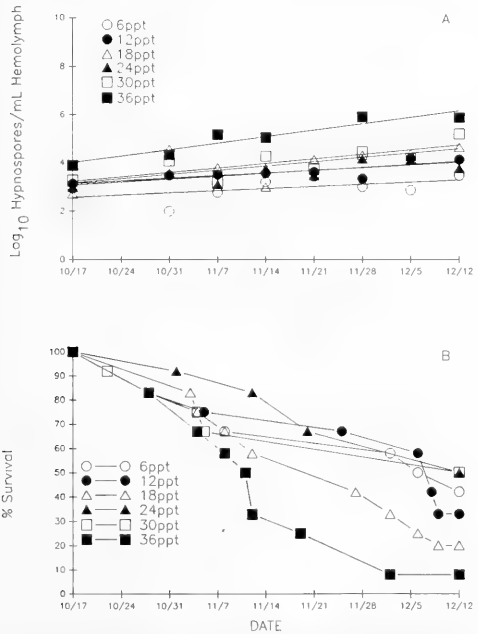

Figure 1. Experiment 1 oysters held in six different salinity conditions from 6–36 ppt. (A) Progression of *P. marinus* (log_{10} hypnospores mL^{-1} hemolymph) for oysters held eight weeks. (B) Percent survival of the same oysters.

Mortalities were high at all salinities in Experiment 1 (Fig. 1B) which featured bi-weekly hemolymph withdrawal from the same oysters and an abrupt salinity change. The highest rate of mortality was at 36 ppt salinity.

Initial serum protein concentration before immersion averaged 3.71 ± 0.20 mg mL^{-1} (n = 70) and this decreased to <0.5 mg mL^{-1} at all salinities within eight weeks. Serum protein in oysters held at 24 and 30 ppt salinity declined gradually whereas those at 6, 12 and 36 ppt were <1.0 mg mL^{-1} within three weeks.

Agglutination titers for horse and human erythrocytes (RBC) decreased in serum of oysters held at 6 ppt from 2^8–2^9 down to 2^2 within eight weeks. Titers decreased gradually for oysters at 12 ppt salinity (2^9 to 2^6) but did not decrease for oysters at 24, 30 and 36 ppt. Titers in oysters held at 18 ppt salinity remained relatively level until they collapsed to $<2^2$ in the seventh week.

Over all conditions and for all eight samplings, highly significant positive correlations ($p < 0.01$) were calculated for *P. marinus* intensity with time (r = 0.338, n = 225), and salinity (r = 0.492, n = 153), for serum protein with horse RBC agglutination (r = 0.474, n = 220) and human RBC agglutination (r = 0.359, n = 220), and for salinity with horse RBC agglutination (r = 0.216, n = 151). A significant ($p < 0.05$) positive correlation was calculated for salinity with human RBC agglutination (r = 0.178, n = 151). There was a highly significant inverse correlation for time with serum protein concentration (r = −0.632, n = 220), horse RBC agglutination (r = −0.384, n = 221) and human RBC agglutination (r = −0.383, n = 221). A significant inverse correlation was determined for *P. marinus* intensity with human RBC agglutination titers (r = −0.156, n = 221).

Experiment 2: Salinity × Temperature Variables UTMB

Perkinsus marinus was detected in all Experiment 2 oysters and intensity increased over time in all conditions (Fig. 2A). The greatest increase in intensity (per mL hemolymph) was found in oysters from H/36 ($10^{0.37}$ hypnospores $week^{-1}$, r = 0.99) and H/24 ($10^{0.37}$ hypnospores $week^{-1}$, r = 0.97). Oysters in L/36 had intermediate increases in intensity ($10^{0.12}$ hypnospores $week^{-1}$, r = 0.99) and those in L/12, L/24 and H/12 had the lowest increases (ranging from $10^{0.02}$ – $10^{0.06}$ hypnospores $week^{-1}$, r = 0.53 – 0.77).

Mortalities were greater in high temperatures than low temperatures regardless of salinity (Fig. 2B). Of the oysters in high temperature conditions, those at H/36 experienced the greatest number of mortalities and those at H/12 experienced the fewest. Mortalities in the low temperature aquaria were low at all salinities.

Infection intensity of *P. marinus* from all aquaria and sample dates showed a highly significant ($p < 0.0001$) positive correlation with time (r = 0.298, n = 180), temperature (r = 0.432, n = 180) and salinity (r = 0.316, n = 180). When aquaria were analyzed separately, infection intensity showed a highly significant positive correlation with time for oysters held in L/36, H/24 and H/36.

Serum protein concentrations over the first two months decreased only slightly from approximately 3 mg mL^{-1} to approximately 2 mg mL^{-1}. Agglutination of both horse and human RBC remained relatively steady. Protein concentrations were positively

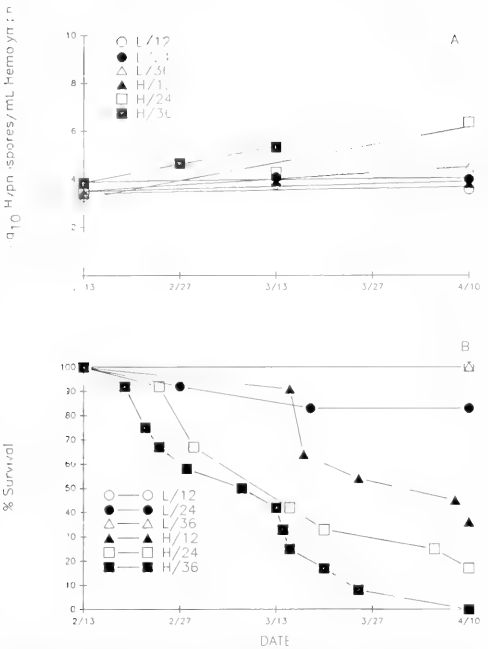

Figure 2. Experiment 2 oysters held in low (L = 18°C) or high (H = 27°C) temperature and in 6, 24 or 36 ppt salinity sea water. (A) Progression of *P. marinus* ($\log_{10}$ hypnospores mL^{-1} hemolymph) for oysters held eight weeks. (B) Percent survival of the same oysters.

correlated with both agglutinins ($p < 0.01$), and horse RBC titers were inversely correlated ($p < 0.05$) with time. Both horse and human RBC titers correlated positively ($p < 0.01$) with salinity.

During the last two months, serum protein concentrations decreased to <1 mg mL^{-1} in all low temperature conditions. Horse and human RBC agglutination titers remained relatively constant (12 ppt = 2^5–2^8; 24 ppt = 2^7 – 2^{10}; 36 ppt = 2^8 – 2^{10}) until termination of the experiment.

Experiment 3: Salinity × Temperature Variables (GB/ERL)

Perkinsus marinus was positively diagnosed from all 25 oysters held in the flow-through sea water tank (April 1 sample) with an average intensity of $10^{3.32 \pm 0.14}$ hypnospores mL^{-1} hemolymph. Hypnospores were also detected (Fig. 3) in the hemolymph of all 54 Experiment 3 oysters sampled on April 1 after four days in the aquaria. In low salinity/low temperature conditions, intensity increased $10^{0.09}$ hypnospores mL^{-1} $week^{-1}$ ($r = 0.58$) and at high temperature/high salinity conditions increased $10^{0.36}$ hypnospores mL^{-1} $week^{-1}$ ($r = 0.92$) (Table 1). The greatest extremes in intensity were found in VS conditions, where low temperature, fed conditions resulted in $10^{0.01}$ hypnospores mL^{-1} $week^{-1}$ ($r = 0.09$) and high temperature, unfed conditions resulted in $10^{0.44}$ hypnospores mL^{-1} $week^{-1}$ ($r = 0.88$). There were significant differences in *P. marinus* intensity between all high temperature and low temperature conditions averaged over time (Table 1).

Oyster mortalities in Experiment 3 were greatest at high temperature and lowest at low temperature for all salinity conditions tested (Fig. 4). High salinity was not associated with high mortalities at low temperature conditions (Fig. 4B). Variable temperature (VT) resulted in intermediate mortalities in (a) high or variable salinity and (b) low salinity, unfed conditions; there were no mortalities for fed oysters in VT and low salinity conditions. There were reduced mortalities in fed oysters held at VT in both 25 ppt and VS conditions.

Oysters from the low temperature and the VT aquaria (VT aquarium maintained at 25°C for the last month) were sacrificed on June 24 and *P. marinus* hypnospores enumerated from five different tissues. Analysis of variance (SAS: General Linear Models Procedure) calculated significant differences among environmental conditions for hemolymph ($F = 6.64$, $p < 0.001$), digestive gland ($F = 3.18$, $p = 0.011$), gill ($F = 2.86$, $p = 0.018$), and

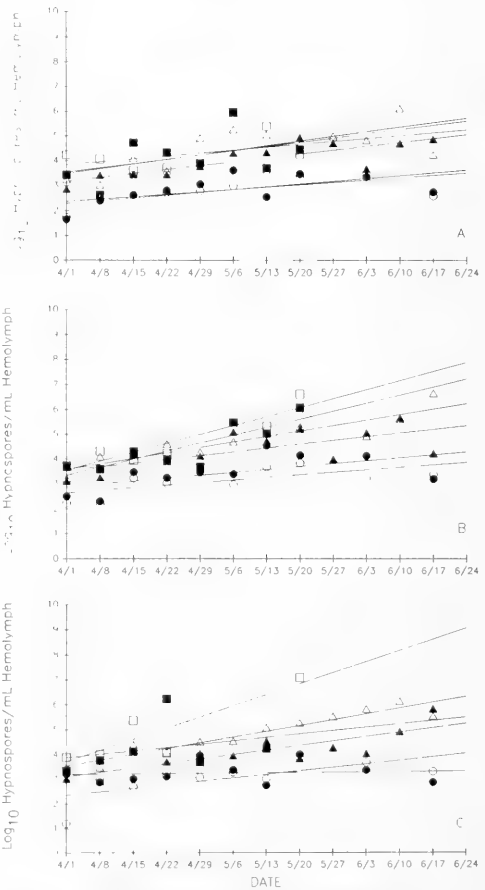

Figure 3. Progression of *P. marinus* ($\log_{10}$ hypnospores mL^{-1} hemolymph) for Experiment 3 oysters held at 18°C (circles), 25°C (squares), or variable temperature (triangles), and at 10 ppt (graph A), 25 ppt (graph B) or variable salinity (graph C) and either fed (filled symbols) or unfed (open symbols).

TABLE 1.

The intensity of *P. marinus* (log_{10} hypnospores mL^{-1} hemolymph) averaged over eight weekly samples from Experiment 3 oysters are listed in decreasing order according to the 18 different environmental conditions tested.

Environmental Condition: Temp	Sal	F/U	Weekly Progression: Log_{10} Slope	Average Intensity (Log_{10})
25	VS	U	0.44 (.88)	4.55^{A}
25	25	U	0.36 (.92)	4.43^{A}
VT	25	U	0.22 (.91)	4.34^{AB}
VT	VS	U	0.23 (.95)	4.33^{AB}
25	25	F	0.32 (.84)	4.26^{AB}
VT	10	U	0.17 (.70)	4.22^{AB}
VT	25	F	0.15 (.70)	4.19^{AB}
25	10	U	0.12 (.52)	4.16^{AB}
25	VS	F	0.14 (.29)	4.15^{AB}
25	10	F	0.19 (.47)	4.07^{AB}
VT	10	F	0.16 (.83)	3.82^{BC}
VT	VS	F	0.18 (.83)	3.77^{BC}
18	25	F	0.12 (.59)	3.39^{CD}
18	VS	F	0.01 (.09)	3.32^{CDE}
18	25	U	0.10 (.64)	3.10^{DE}
18	VS	U	0.14 (.69)	2.92^{DE}
18	10	F	0.09 (.58)	2.78^{E}
18	10	U	0.11 (.54)	2.77^{E}

Environmental conditions included low (18°C), high (25°C) or variable (VT) temperatures, low (10 ppt), high (25 ppt) or variable (VS) salinities, and fed (F) or unfed (U).

Average intensities with the same superscript notations were not significantly different (alpha = 0.05, Duncan's Multiple Range Test).

The average weekly progression, log_{10} slope, and regression (r) values are presented for comparison.

mantle ($F = 3.30$, $p = 0.009$). Calculation with Duncan's Multiple Range Test demonstrated significant differences among environmental conditions for all tissues (Table 2). There were higher levels of *P. marinus* hypnospores at the higher temperature (VT) and the higher salinity (25 ppt). Generally, animals that were not fed had higher tissue burdens than fed animals under the same conditions. A gram of wet tissue had approximately ten times as many hypnospores as one mL of hemolymph.

Agglutination titers for horse RBC and human O^{+} RBC were not significantly affected by different salinity, temperature or feeding, nor were they correlated with *P. marinus* intensity.

DISCUSSION

Infection intensity of *P. marinus* in aquarium-held eastern oysters collected from the Gulf of Mexico was found to increase over time for fed and unfed oysters at all test temperatures (18°–27°C) and salinities (10–36 ppt). Although environmental factors were undoubtedly interactive, oyster mortalities appeared most closely associated with high temperatures. This was most clearly shown in Experiment 2 where there was a high rate of oyster mortality at 27°C/12 ppt salinity in spite of relatively low parasite intensities (Fig. 2). Oysters held at low (18°) temperatures survived 10^{3}–10^{4} hypnospores mL^{-1} hemolymph at all salinities, whereas those at high temperatures did not (Experiments 2 and 3). According to the results of Gauthier & Fisher (1990), this level of hemolymph infection corresponds to moderate/heavy tissue infections, commonly found in living oysters. Nutrition may have also been a factor in survival of infected oysters, since Experiment 3 oysters at low salinity and variable temperature (VT) were found to survive 10^{5} hypnospores mL^{-1} hemolymph if fed, but not if unfed (Figs. 3 and 4). Also, unfed VT oysters generally had higher tissue burdens of *P. marinus* than fed oysters under the same conditions (Table 2).

The high mortalities in Experiment 1 may have been due to a combination of stresses that included laboratory culture conditions, the absence of a gradual salinity acclimation period, and bi-weekly hemolymph withdrawals. For all experiments with heavy mortalities, it is likely that disease intensity was underestimated due to the loss of heavily infected oysters during the course of the experiment. Nonetheless, results indicated that intensity of *P. marinus* in oyster hemolymph increased at 6 ppt salinity, with increasing rates at higher salinities. These findings agree with those of Perkins (1966) and Chu & Greene (1989) who found that

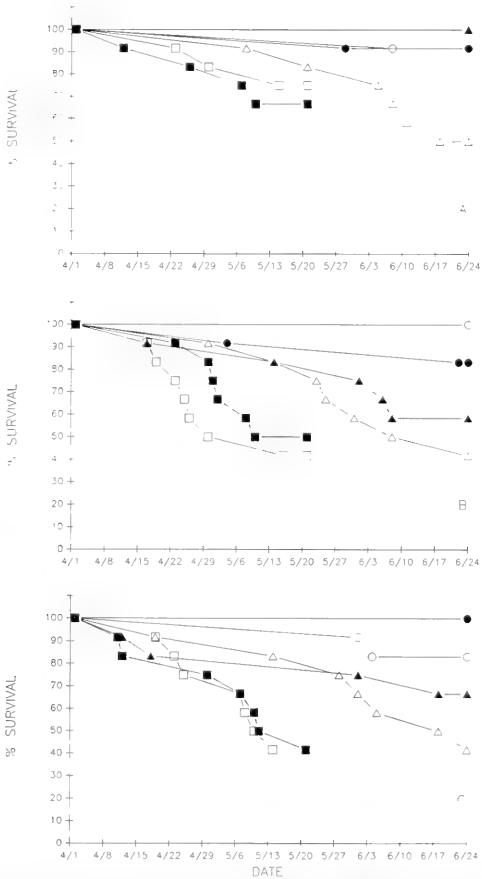

Figure 4. Percent survival of Experiment 3 oysters held at 18°C (circles), 25°C (squares), or variable temperature (triangles), and at 10 ppt (graph A), 25 ppt (graph B) or variable salinity (graph C) and either fed (filled symbols) or unfed (open symbols).

TABLE 2.

The average number of *P. marinus* hypnospores (log_{10}) per mL hemolymph or per gram wet weight of digestive gland, gill and mantle cavity tissue (±standard error) from Experiment 3 oysters held for 12 weeks in eight different environmental conditions.

Environmental Condition	Number of Hypnospores (Log_{10}) Hemolymph	Digestive Gland	Gills	Mantle Cavity
VT	5.23^{BC}	5.94^{BC}	5.63^{ABC}	5.63^{BC}
25 ppt	±.39	±.46	±.51	±.52
Fed				
VT	6.64^{A}	7.30^{A}	6.85^{A}	7.15^{A}
25 ppt	±.29	±.82	±.72	±.57
Unfed	(2)	(2)	(2)	(2)
VT	4.29^{CD}	5.65^{BC}	5.24^{BC}	5.55^{BC}
10 ppt	±.10	±.12	±.10	±.12
Fed				
VT	5.62^{AB}	6.83^{AB}	6.40^{AB}	6.44^{AB}
10 ppt	±.24	±.30	±.24	±.23
Unfed	(5)	(5)	(5)	(5)
LT	4.38^{CD}	5.86^{BC}	5.42^{BC}	5.30^{BC}
25 ppt	±.36	±.26	±.30	±.34
Fed				
LT	4.03^{D}	5.59^{C}	4.76^{C}	4.59^{C}
25 ppt	±.21	±.22	±.34	±.36
Unfed				
LT	3.73^{D}	5.00^{C}	4.87^{C}	5.24^{BC}
10 ppt	±.47	±.36	±.45	±.52
Fed				
LT	3.66^{D}	5.38^{C}	4.71^{C}	4.55^{C}
10 ppt	±.31	±.53	±.45	±.39
Unfed				

LT = low temperature (18°C), VT = variable temperature (18–27°C, but held at 25°C for the last 4 weeks).
N = 6 except where noted in parentheses.
Means for different environmental conditions that were not significantly different (alpha = 0.05, Duncan's Multiple Range Test) are noted with the same superscript letter.

low salinity (<6 ppt) restricted sporulation of *P. marinus* but did not affect survival of the zoospores. These data also sustain field data (Andrews & Ray 1988) that relate higher infection intensity with higher salinity.

Parasite intensity in Experiment 2 was positively correlated with both salinity and temperature. There was increased intensity of *P. marinus* over time in all conditions, but very slow progression at (a) all low temperatures and (b) at high temperature, low (12 ppt) salinity conditions. These data underscored the interactive effects of temperature and salinity in the progress of infection, although temperature appeared to be the dominant factor. This agrees again with the work of Perkins (1966) and Chu & Greene (1989) who reported that *P. marinus* sporangia required at least 18°C to sporulate and that salinities of less than 5–10 ppt could decrease sporulation activity.

Temperature was clearly the dominant factor in survival of Experiment 2 oysters (Fig. 2). This observation is particularly important because mortalities in nature are often believed to be a consequence of *P. marinus* infection if the parasite is found in neighboring or moribund oysters. Many mortalities in nature might actually be associated with high water temperatures: Temperatures in Galveston Bay in 1990 were consistently above 27°C for over five months (June–Oct), whereas the laboratory aquaria, where heavy mortalities occurred, were maintained at 27°C for only 2 months.

High mortalities occurred at high temperatures in Experiment but were greater when combined with high and variable salinities than with low salinity (Fig. 4). Low temperature oysters consistently had low parasite levels at all salinities (Fig. 3) and exhibited low mortality.

The influence of salinity, temperature and food availability was also demonstrated by tissue burdens assessed at the end of Experiment 3 (Table 2). There was relatively even distribution of *P. marinus* across body tissues; this finding contrasts with histological sections of oysters collected from nature, where *P. marinus* are more abundant in the digestive gland epithelia. This discrepancy may be due to the heavy infections found in this study that diminish differences among tissues. Lower *P. marinus* levels found in hemolymph than other tissues must be viewed with the understanding that only hemocytes, a small fraction of the hemolymph, were assayed.

The decline in protein content of oysters from Experiment 1 may have been due to stress from the frequent (bi-weekly) withdrawal of hemolymph from the adductor muscle. In a study conducted by Ford (1986), oysters with hemolymph withdrawal at greater than monthly frequencies showed decreased protein levels. Although agglutination titers for horse and human RBC were highly correlated to serum protein content and declined with repeated withdrawals, serum protein is probably a better indicator of poor animal health since it decreased more dramatically and consistently.

Experiment 2 showed that decreasing the frequency of hemolymph sampling to once per month alleviated the serum protein decline witnessed in Experiment 1. Nonetheless, oysters at the

higher temperature died at about the same rate (Figs. 1 and 2). It is noted also that protein levels in low temperature oysters monitored beyond two months showed a decrease in protein, whereas agglutination activity was not reduced. It is possible that repeated hemolymph withdrawal, even at monthly intervals, will eventually adversely impact oyster physiology.

Both horse and human RBC agglutination titers in Experiments 1 and 2 correlated positively ($p < 0.01$) with salinity. This result was previously reported by Tamplin and Fisher (1989) and probably reflects an increase in agglutinin activity rather than content. In this study, no association was found between agglutinin levels and parasite intensity.

ACKNOWLEDGMENTS

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OCCURRENCE AND SIGNIFICANCE OF INGESTED HAPLOSPORIDAN SPORES IN THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN, 1791)

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ABSTRACT The spore stage of *Haplosporidium nelsoni*, cause of MSX disease in the eastern oyster, *Crassostrea virginica*, is presumed to play a role in transmission, but has never been found in the water. We have found ingested haplosporidan spores, resembling in size and shape those of *H. nelsoni*, within the digestive tract lumina of eastern oysters in Delaware Bay. Ingested spores were found in oysters of all ages; were present throughout Delaware Bay and in other locations where *H. nelsoni* is present; and were also found in oysters during the original MSX epizootic in 1958. They predominated from May through October when they were present in 20% to 40% of the oysters examined, with a mean frequency of 0.5 spores per tissue section. We calculated that this would extrapolate to several thousand spores in the entire digestive tract and several hundred per liter in the water filtered by the oysters. Although the spores were present during the infection period for *H. nelsoni*, their frequency showed a weak, negative correlation ($r = -0.55$; $p < 0.02$; $N = 17$ years) with *H. nelsoni* prevalence the following year, suggesting that if they are a stage in the life cycle of that parasite, they are not directly infective to oysters, but may infect an alternate host. We are attempting to identify the ingested spores and to determine their place, if any, in the life cycle of *H. nelsoni*.

KEY WORDS: spore, MSX, oyster, *Haplosporidium nelsoni*, life cycle, transmission

INTRODUCTION

The life cycle and means of transmission of *Haplosporidium nelsoni* (Haskin et al. 1966), cause of MSX disease in the eastern oyster, *Crassostrea virginica*, have remained an enigma since the parasite's discovery in 1957 (Haskin & Andrews 1988). Experimental transmission has never been achieved and new infections in nature do not depend on proximity to infected oysters. The spore stage, a typical part of the haplosporidan life cycle (Perkins 1990), is produced only rarely in adult oysters, in which infections typically consist of plasmodial stages (Couch et al. 1966, Farley 1967, Andrews 1979, Haskin & Andrews 1988). Barber et al. (1991) recently reported that *H. nelsoni* spores are formed regularly in infected oysters less than a year old (spat), although it is not yet clear whether they are infective to other oysters. Spore structure, however, indicates that they are important in transmission. Their thick walls (Rosenfield et al. 1969) imply that they are capable of resisting environmental extremes and may survive for extended periods outside the host. Filamentous projections (Perkins 1979, McGovern & Burreson 1990) may be flotation devices (Lom 1990) facilitating long-distance water transport between hosts.

In 1989, while examining stained sections of eastern oysters collected in Delaware Bay, we noted an operculated spore, resembling in size and shape that of *H. nelsoni*, within the digestive tract lumen of an oyster. The spore was among an abundance of diatoms and was in an oyster that was not patently infected with *H. nelsoni* plasmodia, suggesting that it had been ingested rather than produced from a sporulating stage of the parasite within that oyster. A preliminary scan of sections from other oysters showed that this was not an isolated incident. Because the finding of haplosporidan spores in the digestive tract lumina of oysters had never been reported before and because of the possibility that the spores might be a stage in the life cycle of *H. nelsoni*, we have made a systematic search for ingested spores in tissue slides of oysters collected over the past 34 years in Delaware Bay. We wished to document seasonal, annual, and spacial variability in the abundance of ingested spores; to compare their size with that reported for known haplosporidan species, including *H. nelsoni;* to correlate ingested spore numbers with prevalence of *H. nelsoni* infections; and to estimate the abundance of the spores in the water column.

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METHODS

Examination of Tissue Slides

Archived tissues, prepared from samples collected beginning in 1958, had been fixed in Davidson's fixative; sectioned at 5–6 μm; and stained with hematoxylin, aniline blue, and acid fuchsin. More recent sections were stained with a modification of a Masson Trichrome stain (Humason 1979) by the addition of Fast Green and Orange G, which stains the spores bright red. Most samples contained 20 individuals and had been collected throughout the year at various locations in Delaware Bay (Ford & Haskin 1982, Haskin & Ford 1982). Tissue sections, which included gill, stomach, intestine, and digestive diverticula, were scanned at 312× with closer examination at 1250× as needed. The number of ingested spores, as well as the presence of *H. nelsoni* infection, was recorded for each oyster examined. Data from each sample included the number of oysters in which ingested spores were present, the mean number of ingested spores per section, the prevalence of oysters patently infected with *H. nelsoni*, and the frequency of ingested spores in infected and uninfected oysters. Length and width of mature *H. nelsoni* spores in sporocysts of infected oysters, as well as ingested spores in oysters collected at several locations in Delaware Bay, were measured at 500× with an ocular micrometer.

Examination of Fresh Gut Contents

During October and November, 1991, a number of oysters were placed in a tray suspended 1.7 meters off the bottom in the Maurice River, a tributary of the Delaware Bay. Periodically, oysters were collected, shucked, and their stomach contents removed by pipet. Slide preparations of the gut contents were examined microscopically at 312×. Measurements were made with an ocular micrometer at 500×.

Estimates of Spore Abundance

The total number of spores in an oyster digestive tract was extrapolated from the number in a tissue section by calculating the fraction of gut volume represented in a standard section. To do this, we made latex stomach casts of oysters with dry weights ranging from 1 to 2.5 grams and determined their volumes by displacement. We then estimated the volume of the intestine/rectum by measuring the length of this tube in dissected oysters and multiplying the result by its cross-sectional area, as determined by image analysis measurements of tissue sections. We then obtained a conversion factor for extrapolating the number of spores per section into total number of spores by dividing the gut volume present in 6-μm sections (from image analysis measurements) into the estimated total volume of the digestive tract.

Statistics

Differences among samples in the frequency of sections containing spores, in the mean number of spores per section, and in the size of spores were examined by analysis of variance using a general linear model. Proportional data were arcsine transformed for analysis and retransformed for presentation. Differences were considered significant at $\alpha = 0.05$.

RESULTS

Examination of Tissue Slides

Capped haplosporidan spores were found in the digestive tract lumina (stomach, mid-gut, and intestine) of 818 of 3292 (25%) oyster tissue sections examined (Fig. 1). The great majority appeared to be intact, although a few looked as though the lid had opened. They were found from April through December, but predominated from May through October, when water temperature was above 10°C (Fig. 2A and B). The prevalence of ingested spores between May and October ranged from 20% to 40%, with a peak in September. They were absent from January through March, at which time intestinal lumina were usually empty. The mean number of spores per section increased through the summer to a high of approximately 0.8 in September, then decreased rapidly through December (Fig. 2C).

Spores were found in oysters of all ages. One quarter of the oysters examined were patently infected with *H. nelsoni*, although only 12% of all ingested spores were found in these animals. When present, the number of ingested spores per tissue section ranged from 1 to 12, with a mean of 0.5. They occurred in oysters from all locations sampled in Delaware Bay, including sites as far up the estuary as oysters grow (Fig. 3). The prevalence of oysters with ingested spores was higher on the seed beds than in the lower bay (31% vs 17%), as was the mean number of spores per section (0.68 vs 0.38) ($p < 0.001$ in both comparisons).

The mean (SE) length and width of all ingested spores (N = 193) was 5.5 (0.1) × 7.5 (0.1) μm. In comparison, the mean size of *H. nelsoni* spores produced in infected Delaware Bay oysters (N = 76) was 5.3 (0.1) × 7.5 (0.1) μm. There were no significant differences in the size of ingested spores in oysters collected from upper seed beds (N = 68), lower seed beds (N = 75), and planted grounds (N = 50). In addition to Delaware Bay, we found ingested spores of the same appearance and dimensions in oysters from Wellfleet and Cotuit Harbors, Massachusetts and at Deal Island, Maryland, all areas where *H. nelsoni* is present.

The earliest finding of ingested spores was in oysters collected on 30 July and 14 November, 1958, during the height of the first *H. nelsoni* epizootic in Delaware Bay. Thereafter, the frequency varied from year to year. There was no correlation between the abundance of ingested spores and *H. nelsoni* infection prevalence in the same year; however, there was a weak, but significant, negative correlation ($r = -0.55$; $p < 0.02$; N = 17 years) between their frequency in oysters on the planted grounds and *H. nelsoni* prevalence the following year.

Examination of Fresh Gut Contents

Fresh spores were found in the gut contents of oysters suspended in the Maurice River during October and November, 1991. The mean size of these spores, 9.7 (0.1) × 11.6 (0.1) μm (N = 105), was similar in dimension to those of *Haplosporidium louisiana* (8.4 × 12.1 μm in fixed material), a parasite of xanthid

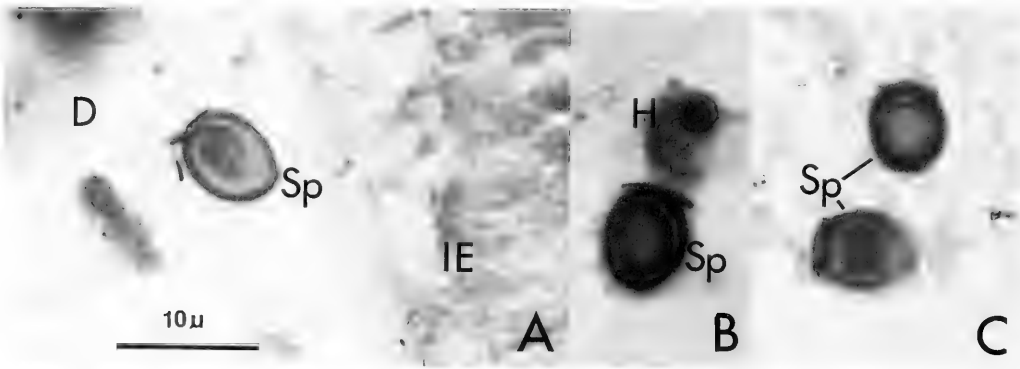

Figure 1. Photomicrographs of haplosporidan spores in oyster digestive tracts. D–diatom; Sp–spore; IE–intestinal epithelium; H–hemocyte.

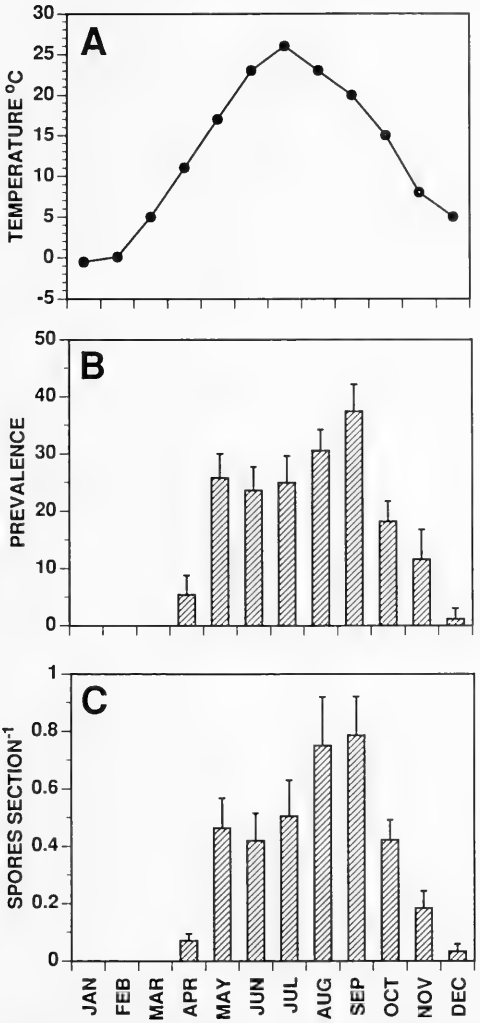

Figure 2. Mean water temperature in Delaware Bay obtained from collecting vessel cruise sheets (A); mean monthly prevalence of oyster tissue sections containing ingested spores in Delaware Bay (B); mean number of spores per tissue section by month in Delaware Bay oysters (C).

Figure 3. Chart of Delaware Bay showing locations where oysters with ingested haplosporidan spores have been found.

crabs (Sprague 1963, Perkins 1979), which are known to be present in the River. A total of 3108 spores was counted in a 70-μL sample of stomach contents from a single small oyster (shell dimensions 25 × 21.5 mm). Subsequent examination of tissue sections from 21 oysters in the same group revealed spore counts ranging from 0 to 9 per section, with a mean of 4.1. Apparently intact spores were found in feces of oysters brought from the River into the laboratory and placed in aquaria.

Estimates of Spore Abundance

We estimated that the fraction of the digestive tract volume represented in a tissue section ranged from 1/10,000 in an oyster of 1 gdwt to 1/12,500 in an oyster 2 gdwt. By extrapolation, the grand mean of 0.5 spores per section would represent about 5,000 to 6,250 spores in the entire digestive tract lumen, assuming equal distribution of spores along its entire length. Preliminary measurements indicated that at temperatures of 21–23°C, gut passage rates varied from 1.7 to 2.2 hr in oysters the same size (K. Tammi, Haskin Shellfish Research Laboratory, personal communication). During this time, the oysters would be capable of clearing from 6 to 14 liters of water (Newell 1985). Assuming 95–100% retention efficiency for particles in the spore size range (Palmer & Williams 1980, Riisgard 1988), we calculate that the concentration of spores in the water processed by the oysters must be on the order of 500 to 800 L^{-1}. Our estimates could be high because tissues are probably stretched during the making of casts so that our calculation of digestive tract volume may be artificially high. Also, spores may not be equally distributed along the digestive tract or may be retained in the tract longer than algae. Even if our estimates are two or three times too high, spore concentration in the water would still be several hundred per liter.

DISCUSSION

At the light microscope level, the ingested haplosporidan spores found in tissue sections of oysters throughout Delaware Bay were indistinguishable in size and morphology from those of the oyster pathogen, *Haplosporidium nelsoni*. Of the approximately

TABLE 1.

Haplosporidan species potentially present in Delaware Bay with spore sizes.

Species	Size (μm)	Reference	Host
H. louisiana	12.1 × 8.4 (Fixed)	(Sprague 1963)	Xanthid crabs
Minchinia sp.	UNKNOWN	(Newman et al. 1976)	*Callinectes sapidus*
Digestive tract (Maurice River)	11.6 × 9.7 (Fixed) 11.6 × 9.6 (Fresh)	Barber (personal observation)	Unknown
Digestive tract (Delaware Bay)	7.5 × 5.5 (Fixed)	Barber (personal observation)	Unknown
H. nelsoni	7.5 × 5.4 (Fixed) 7.4 × 5.3 (Fresh)	(Couch et al. 1966) Barber (personal observation)	*Crassostrea virginica*
M. teredinis	7.5–5.5 L (Fixed) 6.0–4.5 W 10.0–11.0 L (Fresh) 8.0–9.0 W	(Hillman et al. 1990)	*Teredo spp.*
Minchinia sp. (probably *M. teredinis*)	5.7–3.8 L (Fixed) 5.2–3.2 W	(McGovern & Burreson 1990)	*Teredo spp.*
H. costale	3.1 × 2.6 (+25% when fixed and undehydrated)	(Wood & Andrews 1962)	*Crassostrea virginica*

40 described haplosporidan species with spores generally similar to *H. nelsoni* (genera *Haplosporidium, Minchinia,* and *Urosporidium*), at least five are potentially present in Delaware Bay because their hosts inhabit the estuary (Table 1). The ingested-spore dimensions were the same in samples from throughout the Bay, suggesting that they belong to a single species. *Minchinia teredinis,* a parasite of shipworms, has spores that cannot be clearly distinguished from those of *H. nelsoni* by light microscopy (Hillman et al. 1990). Based on our experience, *Teredo* spp. are not particularly abundant in Delaware Bay, but we have found *M. teredinis* in the lower bay and cannot rule out the possibility that the ingested spores, or at least some of them, belong to this, or some as yet undescribed species. Because *H. nelsoni* is typically very prevalent in the estuary, however, we must consider the prospect that the ingested spores are a part of the *H. nelsoni* life cycle.

The finding of ingested spores in 1958 samples confirms the fact that they were present during the original MSX epizootic of 1957–59 in Delaware Bay, although we were not able to determine whether spores were present earlier than this because oyster tissues were not collected before the first mortalities occurred in 1957. In addition, the spores are abundant during the infective period for *H. nelsoni,* which occurs from early June through October in Delaware Bay (Ford & Haskin 1982).

The wide distribution of ingested spores within Delaware Bay indicates that they are disseminated by water currents or vector species if they are formed primarily in one section of the Bay (e.g., in the lower bay if in oysters), or that they are produced in non-sedentary (e.g., non-oyster) hosts, which are themselves widely dispersed. They may well be present during the entire year, but not appear in oysters during winter because the latter are not feeding. If the spores are stages in the life cycle of *H. nelsoni,* it is puzzling that their abundance in oyster digestive tracts is not positively correlated with subsequent infection prevalence, but is, in fact, negatively correlated with the prevalence of infected oysters in the following year. This suggests that the spores are not directly infective to oysters, but may infect an intermediate host. It is also puzzling that ingested spores are found more frequently on the seed beds than in the lower bay, which is the opposite of the typical distribution pattern of *H. nelsoni* infection in oysters (Haskin & Ford 1982). The higher ingested-spore prevalence may not necessarily mean that spores were more numerous in the upper bay, however, because oysters on the seed beds had fewer infections, and consequently probably had higher clearance rates (Newell 1985) than those in the lower bay. Alternatively, high salinity might enhance rates of excystation, resulting in a lower abundance of intact spores in the lower bay.

We do not know whether spores pass through oyster guts more than once, although this seems possible based on our finding apparently intact haplosporidan spores in oyster feces. We also do not know whether spores are distributed uniformly in the water column. Delaware Bay, however, is a shallow, well-mixed estuary, which suggests that spores would not be confined to the bottom layers, as does the finding of large haplosporidan spores in digestive tracts of oysters suspended 1.7 m from the bottom in the Maurice River. In any case, our estimates suggest that the spores are very abundant, as well as widely distributed, in Delaware Bay.

We are attempting to identify the ingested spores and to determine their place, if any, in the life cycle of *H. nelsoni.* If they are *H. nelsoni,* the large numbers that may be present in oyster digestive tracts suggests a mechanism by which parasites could be transferred into non-enzootic waters. Allowing oysters to clear their digestive tracts in "clean" water before shipment would minimize this possibility.

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OCCURRENCE OF HAPLOSPORIDIAN AND *PERKINSUS*-LIKE INFECTIONS IN CARPET-SHELL CLAMS, *RUDITAPES DECUSSATUS* (LINNAEUS, 1758), OF THE RIA DE VIGO (GALICIA, NW SPAIN)

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ABSTRACT In November 1987, an abnormally high mortality was observed in a depuration plant at Meira, Spain, in clams *Ruditapes decussatus* (Linnaeus, 1758), imported from Portugal. Trying to clarify the causes of this mortality, samples were taken from the depuration plant and from several natural beds in different months of the year. A *Perkinsus*-like organism and haplosporidian plasmodia were detected. The *Perkinsus*-like organism had the aspect of round "ring" cells with a diameter varying between 3 and 15 μm. The parasite was present on all the different organs of the clam. The haplosporidian plasmodia were found in the epithelia of the stomach, intestine and, primary and secondary digestive ducts of different clams. The observed plasmodia were mostly spherical to elongated in shape (some of them ameboid), with the longest axis varying between 5.5 and 16 μm and containing 3 to 16 nuclei.

KEY WORDS: *Ruditapes decussatus*, clams, protozoan parasites, haplosporidian, *Perkinsus*-like, parasitism

INTRODUCTION

Perkinsus species have historically been considered the cause of extensive mortalities in commercially important molluscs (Ray & Chandler 1955). *Perkinsus marinus* has caused mass mortalities of the oyster *Crassostrea virginica* Gmelin along the east coast of America (Andrews & Hewatt 1957) and in the Gulf of Mexico (Ray 1966a). A similar species of *Perkinsus* has been thought to be the cause of mortalities of *C. virginica* in Hawaii (Kern et al. 1973) and more recently has been associated with mortalities of the clam *Ruditapes decussatus* and other bivalves in the Mediterranean (Da Ros & Canzonier 1985) and in the Atlantic European coast (Ruano & Cachola 1986). Unidentified *Perkinsus* species are common in bivalves. Perkins (1988) reported that 34 species of bivalve molluscs from the Pacific and Atlantic Ocean and the Mediterranean Sea harbour *P. marinus* or related organisms.

Several investigators have described different haplosporidian species as pathogens of many groups of marine animals (Perkins 1968, 1971, 1979, Newman et al. 1976, van Banning 1977, Marchand & Sprague 1979, Ball 1980, Ormieres 1980, Azevedo 1984). Recently several descriptions of haplosporidian plasmodia have been reported in the carpet-shell clam *Ruditapes decussatus* (Joly & Comps 1979, Chagot et al. 1987, Villalba & Navas 1988).

In November 1987, an abnormally high mortality was observed in a depuration plant at Meira, Spain, in clams of this species imported from Portugal. By Spanish law these animals have to be held in a depuration plant for at least 48 hours before they can be sold fresh in the market for human consumption. Trying to clarify the causes of this mortality, samples were taken from the depuration plant and from different nearby natural beds in Spain on different months of the year. A *Perkinsus*-like organism and a haplosporidian were detected.

This is the first description of a *Perkinsus*-like organism and a haplosporidian from clams (*Ruditapes decussatus*) collected in natural beds in Galicia (NW of Spain), the area of highest production of clams (320,170 Kg) in Spain.

MATERIALS AND METHODS

Clams were collected from three natural beds (Cesantes, Meira and Rodeira) (Fig. 1). The salinity was: Cesantes 23‰, Meira (natural bed and depuration plant) 30‰ and Rodeira 33‰. It remained constant throughout all the year in the different sampling locations. The temperature varied from 9°C (November–December) to 16°C (June) in all the sampling places. During the sample period, natural beds were monitored monthly for dead clams.

One hundred clams (40–50 mm of length of major axis) from each place were fixed whole in Davidson's. A transversal section approximately 5 mm thick including foot, gills and visceral mass was cut in each clam, embedded in paraffin, sectioned at 5 μm and stained with iron hematoxylin, acid fuchsin and aniline blue. The thioglycollate procedure of Ray (1966b) was used to detect the presence of *Perkinsus*-like organisms. Weighted prevalence was calculated following Ray (1954).

RESULTS

As a result of the histopathological and microbiological studies, a *Perkinsus*-like and a haplosporidian were found in clams taken from the depuration plant and the natural beds. The results are presented in Table 1. The highest prevalence for the *Perkinsus*-like organism and the haplosporidian was found in the depuration plant. Interestingly, the prevalence of the *Perkinsus*-like organism in clams from the depuration plant was extremely high in comparison with the prevalence found in the samples taken from natural beds. The same happened with the weighted prevalence that attained a 2.68 value whilst the highest value found in the natural beds was 0.85. The haplosporidian was found in all the places sampled, depuration plant and natural beds except in Rodeira, but the difference of frequencies of appearance among the samples was less important. This haplosporidian had the highest prevalence in winter, decreasing in the warmer months.

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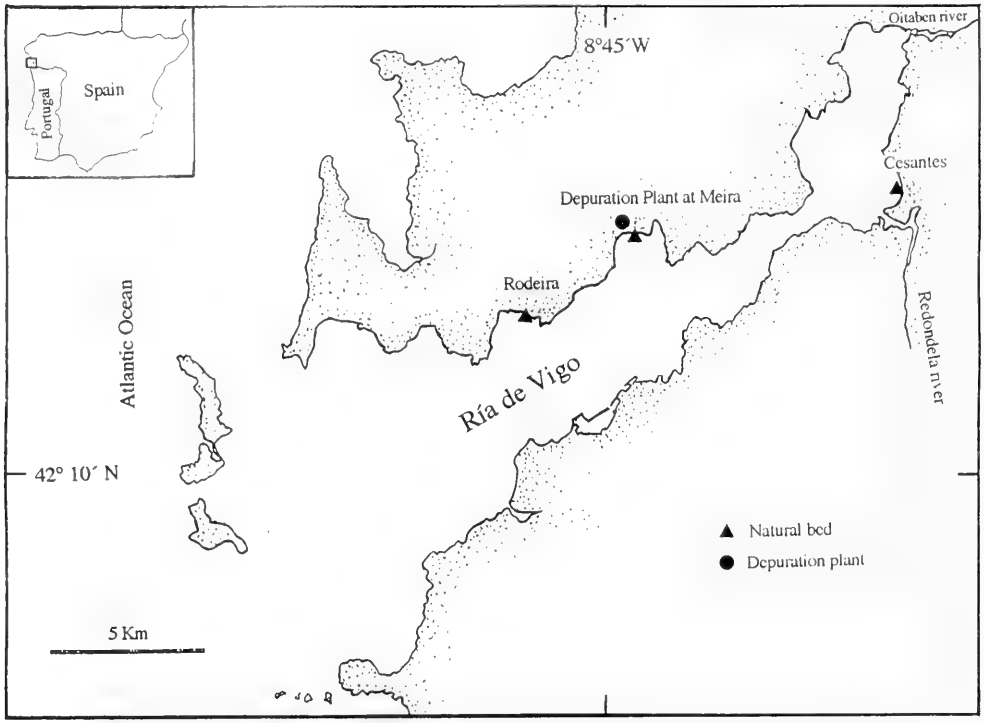


Figure 1. Places sampled in the Ría de Vigo (NW Spain) and situation of the depuration plant.

Differences in the frequency of appearance of the *Perkinsus*-like organism were not found using the thioglycollate method and histology.

The *Perkinsus*-like organism had the aspect of round cells with a diameter varying between 3 and 15 μm (Fig. 2). The host usually develops a strong hemocytic response (Fig. 3A–B). The parasite was present on all the different organs of the clam.

The haplosporidian plasmodia were found in the epithelia of the stomach, intestine and, primary and secondary digestive ducts of different clams (Fig. 4). The observed plasmodia were mostly spherical to elongated in shape (some of them ameboid), with the longest axis varying between 5.5 and 16 μm and containing 3 to 16 nuclei. Two different kinds of nuclei could be observed: (1) the largest one (2 to 2.5 μm), is the most abundant form, enclosing an

TABLE 1.

Weighted prevalence and frequency of appearance of *Perkinsus*-like and a haplosporidian in carpet shell clams *Ruditapes decussatus* from the Ría de Vigo (NW Spain).

Location	Month/Year	Parasites (% Appearance)		
		Weighted Prevalence *Perkinsus*-like	*Perkinsus*-like	Haplosporidian
Depuration plant at Meira	Nov./1987	2.68	80	40
Meira*	Dec./1987	0.60	32	25
Rodeira*	Dec./1987	0	0	0
Cesantes*	Dec./1987	0.85	15	30
Cesantes*	April/1988	0	0	25
Cesantes*	June/1988	0	0	5

Natural beds)

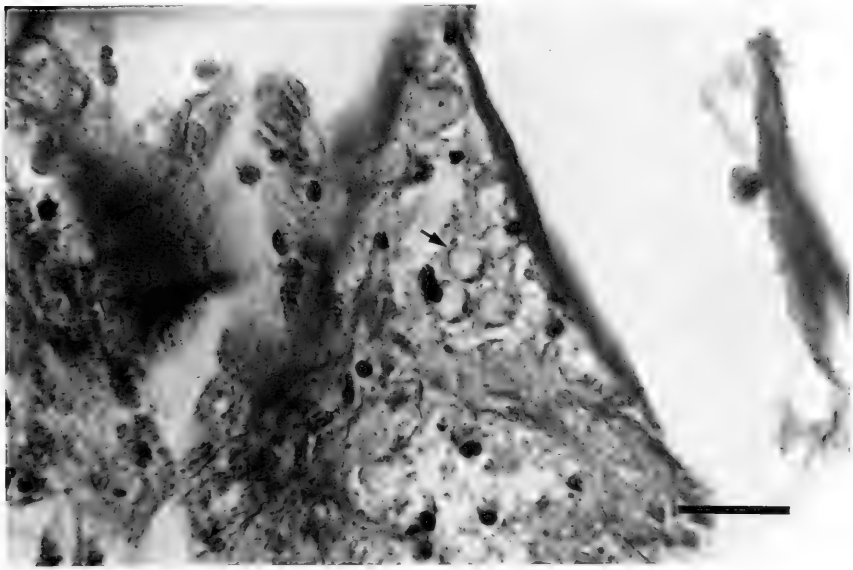


Figure 2. Aspect of the *Perkinsus*-like organism (arrows) (scale bar 50 μm).

apparent endosome, and (2) the smallest one (1 to 1.5 μm) without endosome.

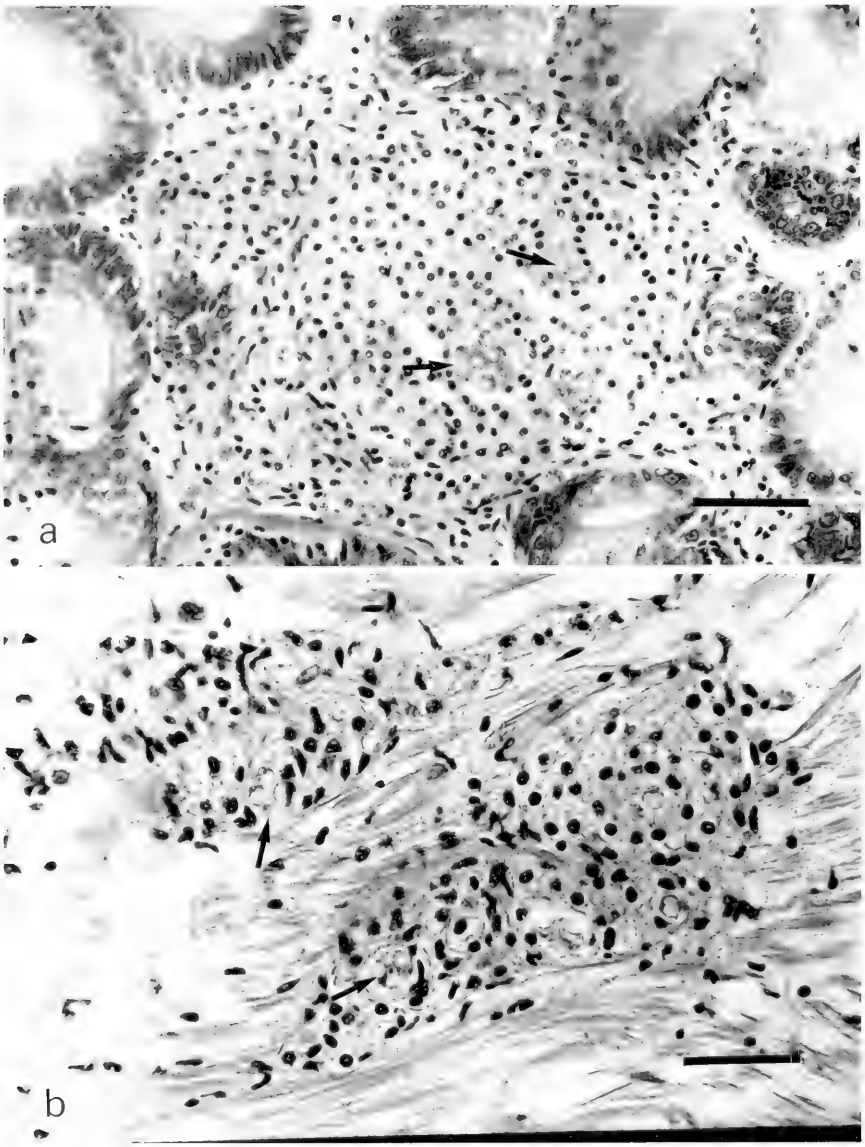
No mortalities were detected in the natural beds.

DISCUSSION

The *Perkinsus*-like organism has far greater similarities with the *Perkinsus*-like organism described by Da Ros & Canzonier (1985) in Italy and Comps & Chagot (1987) in Portugal from clams (*R. decussatus*), than it does to *P. marinus*. In tissue sections, *P. marinus*, typically shows multicellular meronts in various stages of development, as well as the unicellular trophont stage, which is the only one found in the histological sections examined in the present paper. Since no observations of the flagellated stage ("zoospores") essential for the diagnoses of the Perkinsea class (Levin 1978) was made, it is not possible to ascribe this parasite, found in the Ría de Vigo, to one particular species. It is clear that the parasite causes localized tissue lesions. This phenomenon is characterized by the infiltration with granulocytes encapsulating the parasites. This coincides with the description by Comps & Chagot (1987) of the infection of *R. decussatus* with *Perkinsus* in Portugal.

The only haplosporidian species that has been described as a parasite of *R. decussatus* is *Haplosporidium tapetis* (Vilela 1950). Chagot et al. (1987) transferred this species to the genus *Minchinia* after studying the ultrastructure of the spore. Sporulation was not detected in any of these samples, although plasmodia penetrated the digestive epithelia and this is normally previous to sporulation. Without the spores, the identification of the species cannot be done. Infections were accompanied with hemocytic infiltration of the tissues, but it cannot be assumed that the haplosporidian was the cause of the hemocytic reaction since there was a concurrent infection with *Perkinsus*-like organisms. The smallest nuclei (1 to 1.5 μm) without endosome, found in the plasmodia was probably an artifact: tangential sections that missed the nucleolus giving the appearance of small size. Electron microscopy is being conducted to clarify this point.

Andrews (1988) reviewed the epizootiology of *Perkinsus marinus* in *Crassostrea virginica* and reported that the infection and subsequent development of the disease are favoured by temperatures in excess of 25°C and high salinities. These conditions are different and opposite to the ones that favour the infection in the Ría de Vigo, maybe the reason is that they are different species of parasites. The mortality detected in the depuration plant could not be explained by a difference in the water temperature, since this was the same in both the depuration plant and the natural beds. The clams stored in the depuration plant died after one week and the intensity of the infections was much higher in the animals from this place, as shown by the weighted prevalence value. The weighted prevalence attained a value of 2,68 in the depuration plant. Andrews (1988) stated that a value of 1,5 or higher for live hosts indicates that most eastern oysters (*Crassostrea virginica*) are infected and those with severe cases die. The only differences between the depuration plant and the natural beds were the overcrowding of the clams and the high prevalence and intensity of the *Perkinsus*-like organism in the plant. Since the haplosporidian was found in the clams from the depuration plant and from the natural beds and the prevalence of the *Perkinsus*-like organism was very high in the depuration plant, the mortality could be attributed, mainly, to the *Perkinsus*-like organism. The situation of overcrowding in the tanks of the depuration plant with a possible



Figures 3. *Perkinsus*-like organism (arrow) and host hemocytic response in the digestive gland (A) (scale bar 100 μm) and in the muscle (B) (scale bar 75 μm).

transportation abuse (delay in transit, loss of refrigeration or rough handling) of the clams after being harvested may have contributed to increase the loss of clams weakened by the parasites.

The fact that no mortalities were detected in the natural beds could be explained by the lower prevalence of both parasites and the lack of stress due to the process of harvesting and holding in the plant.

Other occasional parasites found were chlamydias, trematodes

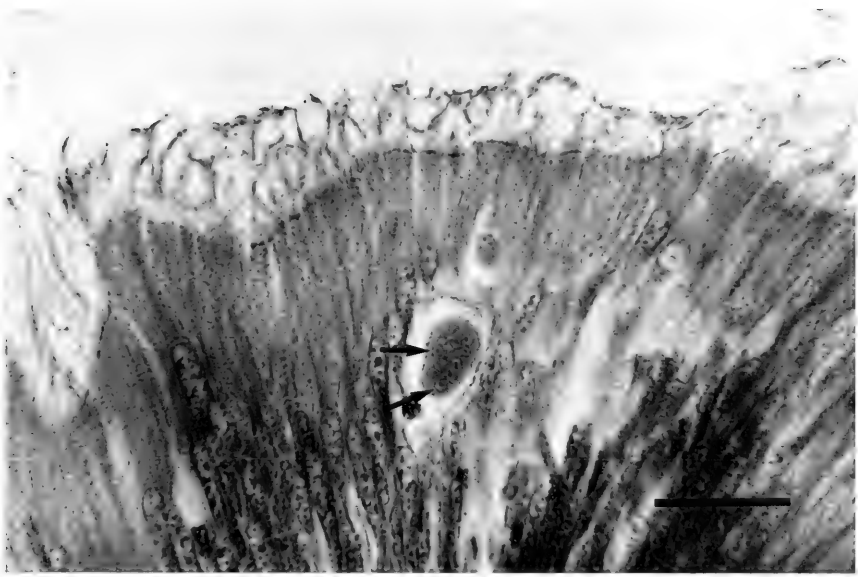


Figure 4. Haplosporidian plasmodia in the epithelia of the stomach (arrows) (scale bar 30 μm).

and turbellarians with no clear pathogenic effect on the host because of the very low prevalence and intensity.

The presence of the *Perkinsus*-like organism and the haplosporidian could endanger the production of clams in the area of Spain with highest harvest of this species in natural beds (320.170 kg). Since all the clams should go through the depuration plant process for human consumption the *Perkinsus*-like organism should be consistently monitored to minimize losses.

Further studies should be conducted to characterize the *Perkinsus*-like organism and the haplosporidian, and its possible role as a potential pathogen agent for the clam culture.

ACKNOWLEDGMENTS

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EFFECTS OF HYPOXIC AND HYPEROXIC CONDITIONS ON HEMOCYTE ACTIVITY AND ABIOTIC PARTICLE RETENTION BY THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN, 1791)

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ABSTRACT Fluorescent polystyrene microspheres resistant to intra-hemocytic digestion were introduced by gastric intubation into the stomachs of eastern oysters, *Crassostrea viginica* (Gmelin) which were then placed in seawater at various partial pressures of dissolved oxygen ranging from 9 to 480 mm Hg. The oysters were assayed at one and three days post-intubation for the number of residual microspheres in the tissues and compared to the number of microspheres in the tissues at one hour post-intubation. To test the effect of oxygen partial pressure on hemocytes, oysters in the different oxygen environments were bled after three days and hemocyte concentration, size, viability and phagocytic uptake of microspheres *in vitro* was determined.

The number of residual microspheres in the tissues after one day in the various oxygen environments was one order of magnitude lower than the number present at one hour after intubation. No further decrease was seen at three days post-intubation. The number of tissue microspheres did not change over the range of O_2 partial pressures tested. Analysis of hemocyte viability, size and phagocytic activity *in vitro* also showed no major differences among oysters exposed to the various oxygen levels. Hemocyte concentration in the hemolymph of oysters in the near anaerobic environment was one order of magnitude lower than those in the environments with higher oxygen tensions. These data suggest that while hemocyte numbers in the circulation may be lowered by anaerobic conditions, hemocyte viability, phagocytic activity and diapedesis of particles from the tissues are independent of the partial pressure of oxygen in the water surrounding the oyster.

KEY WORDS: oyster, *Crassostrea*, hemocyte, hypoxia, hyperoxia

INTRODUCTION

A major defense against infection in bivalve molluscs consists of motile phagocytic cells which circulate in the hemolymph, permeate the connective tissues and lie on the luminar surfaces of digestive epithelia (Schmid 1975, Howland and Cheng 1982, Fisher 1986, Adema et al. 1991). These hemocytes effect protection by encapsulation and/or phagocytosis of the invading organisms. If the invading cells are digestible, they are destroyed within phagosomes. Those species or stages in the life history of the invading organism which are resistant to intracellular digestion may be stored in the hemocytes and later removed by diapedesis through epithelial membranes (Cheng 1981, Sminia et al. 1987). Certain microorganisms, however, may succeed in permeating tissues of the oyster through infective stages which overcome the cellular defense system of the host by resisting intra-phagosomal digestion followed by proliferation inside the host cell. Thus, the rate of diapedesis may be too slow to eliminate the intracellular resident before completion of the proliferative phase. In such cases, if conditions are right, hemocytes may serve to spread the parasitic cells resulting in systemic infection which frequently leads to the death of the host.

Since oysters are filter feeders, it is reasonable to suspect that an important route of invasion may be through the gastric epithelium. We have shown in a recent study that indigestible fluorescent polystyrene microspheres introduced into the stomach of American oysters are phagocytosed by hemocytes in the lumen of the stomach and are carried through the gastric epithelium into the underlying tissues where many have been observed for up to ten days after introduction by gastric intubation (Alvarez et al. 1992).

Studies of energy requirements for hemocyte phagocytosis in the oyster have shown that the process is independent of oxygen, most likely deriving ATP through glycolysis (Cheng 1976, Alvarez et al. 1990). However, the effect of oxygen partial pressures above and below one atmosphere has not been examined nor has the effect of oxygen availability on the depuration of indigestible particles from the tissues by diapedesis been studied. Thus, the present study was conducted to examine the role of oxygen in these processes and to determine if variations in oxygen availability might have an effect on particle removal and hemocyte activity. Such information might be a consideration in the design and management of depuration processes.

MATERIALS AND METHODS

Oysters were collected in lower Old Tampa Bay at a site 27°52′2″ north latitude, 82°38′13″ west longitude and held in a biologically filtered recirculating artificial seawater system at 20°C and 27 ppt salinity. Bacteria in the biological filter oxidize ammonia to nitrite and then to nitrate, the latter being less toxic than ammonia. Nitrate levels were monitored weekly and typically kept below 40 mg/L.

Particle Intubation Procedure

Gastric intubation was used as the method for introducing the experimental particles in order to ensure a uniform volume and concentration. The detailed procedure has been described (Friedl et al. 1992). A window was cut on the anterior ventrolateral aspect of the left valve just caudal to the beak using a Dremel #409 cutting disc in an osteological drill. Four cuts were made forming

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a rectangle about 1 cm across its narrow dimension. The depth of the cuts was adjusted to penetrate the prismatic layer of the shell but not the deeper nacrous (calcitostracum) layer. The prismatic layer was gently pried away and the nacrous layer perforated to expose the mantle of the anterior ventral region of the oyster. Retraction of the mantle margins exposes the labial palps allowing ready access to the mouth.

An 18 gauge stainless steel feeding tube with a bulbous end was connected by polyethylene tubing to a Manostat 2 ml microburette filled with an ultrasonically dispersed suspension of 2.16 μm mean diameter fluorescent polystyrene latex microspheres (Fluoresbrite, Polysciences, Inc.) in artificial seawater. The feeding tube was inserted between the labial palps up and into the mouth. The tube was advanced up to a stop serving to position its bulbous tip within the stomach. A 0.05 ml volume of the microsphere suspension was slowly delivered to the stomach. The volume of the gut was previously determined by water displacement by latex casts prepared by gastric intubation. The volume used in the present experiments was sufficient to fill the gut without undue distention or visible loss through the mouth or anus. Examination of sections of the gut of several intubated oysters showed no lesions of the epithelium.

Following gastric intubation, the window was closed using the plate of prismatic shell removed when the opening was made. Strips of Parafilm "M" Laboratory Film rolled to a cylindrical diameter of 1–2 mm were used to line the opening and the plate pressed into place and coated with Trim Dental Plastic (Harry J. Bosworth Co., Skokie, IL).

Microspheres were introduced as described above into oysters from which the right valve was removed. These oysters were used for determination of the number of microspheres present in the tissues 1 h after introduction into the stomach. These values served as a reference for subsequent determinations. Ten oysters were used in each experiment for microsphere recovery for each oxygen environment. The oysters were sampled for residual microspheres in the tissues at one and three days post-intubation. In a series of concurrent experiments, non-intubated oysters were maintained in the oxygen environments for three days after which time hemocytes were removed from an adductor muscle sinus and tested for viability and phagocytic activity *in vitro*. Hemocyte numbers and sizes were determined from pooled samples of three oysters from each of the oxygen environments. This experiment was repeated five times.

Oxygen Environments

Immediately following intubation the oysters were placed in 40 l of water at 27 ppt salinity in closed tanks. A slow mixing current was maintained with a stirrer. Temperature was regulated at 20°C throughout the experiment. The partial pressure of oxygen in the water was maintained by a dissolved oxygen controller (New Brunswick Scientific) sensing through membrane electrodes. These robust, autoclavable electrodes (polarographic or galvanic) are designed for fermentation control. The electrodes were calibrated at 160 mm Hg in air-saturated sea water at 20°C and at zero dissolved oxygen in a saturated sodium sulfite solution. Calibration was done prior to each experiment and typically checked and readjusted in 160 mm Hg control artificial sea water during each experiment. A continuous record of the controller operation was made throughout each experiment. The controllers operated valves which dispensed purging or augmenting gases by bubbling into the tank water. Partial pressures below 160 mm of Hg were maintained by purging with nitrogen and above 160 mm Hg by injection of oxygen. The partial pressure of dissolved O_2 was monitored throughout the experiments. The following partial pressures were used: 9, 80, 160, 240, 320 and 480 mm of Hg, 160 representing normal air saturation.

Residual Tissue Microsphere Determination

Soft parts of oysters were removed from the valves, rinsed in glass-distilled water (GDW), placed one each in double plastic bags and weighed. Thirty ml of 5.25% sodium hypochlorite was added and the bags were placed in a Stomacher Laboratory Blender (Tekmar Co., Cincinnati, Ohio) operated at half speed for 1 min. The homogenates were decanted into centrifuge tubes (A), diluted to a volume of 45 ml with DW, and centrifuged at 3000 × g for 30 min. The supernates were decanted into centrifuge tubes (B) and the pellets resuspended in 20 ml DW. The supernates and the resuspended pellets were again centrifuged at 3000 × g for 30 min. The supernates in B were discarded and the supernates in A were decanted into B which were again centrifuged. The pellets in B were resuspended in DW and added to A where the pellets were resuspended and the volume adjusted to 10 ml in DW. The final suspension was gently sonicated and vortexed. Five 10 μl aliquots of the homogenate were placed on microscope slides and air dried. The number of microspheres present in each were counted with a fluorescence microscope. The average counts for five oysters were combined and the results extrapolated to an average number of microspheres per gram wet weight of tissue.

Hemocyte Analysis

Hemocyte concentration and diameter was determined from pooled hemolymph of three oysters maintained for three days in each of the oxygen environments. The oysters were bled from an adductor muscle sinus with a 1 ml syringe fitted with a 16 gauge needle. Hemocyte counts and size measurements were done on vapor-fixed hemocytes (37% formaldehyde) suspended in Isoton® using an Elzone® 280 PC Particle Analyzer.

Percent hemocyte viability was determined by adding 0.1 ml of a 0.1 mg/ml solution of fluorescein diacetate in filtered artificial sea water (27 ppt) to freshly drawn hemolymph and incubating for 15 min. Aliquots of the suspensions were placed on slides and the number of fluorescent and non-fluorescent cells were counted using simultaneous phase contrast and fluorescence epi-illumination microscopy (Mishell et al. 1980, Alvarez et al. 1991).

The phagocytic activity of hemocytes *in vitro* was determined for three oysters from each oxygen environment. Plastic culture flasks (25 ml) were coated with cell-free hemolymph. Two ml of a suspension of microspheres in filtered 0.54 M NaCl giving a concentration of 1.08×10^7 microspheres/ml was gently centrifuged to form a uniformly distributed monolayer on the bottom of the flask. The supernate was decanted and two ml of hemolymph drawn from adductor muscle sinuses of oysters in each of the oxygen environments were added to the flasks and gently centrifuged so that all of the hemocytes contacted the microsphere monolayer at approximately the same time. The cells were allowed to remain in contact with the microspheres for 30 min at room temperature after which the overlying fluid was pipetted off and the cells detached by trypsinization (0.4% trypsin in 0.54 M NaCl). The suspended hemocytes were vapor fixed for 30 min by inserting a filter paper wick soaked in 37% formaldehyde into the

opening of the flask. The hemocytes were scored for phagocytosis from wet mounts examined with a fluorescence microscope (Alvarez et al. 1989).

Statistical Analysis

Two statistical analyses were applied to each set of data: a one-way analysis of variance (ANOVA) and Scheffe's Multiple Contrast test.

RESULTS

Tissue Microsphere Retention in Relation to Environmental Oxygen

Microscopic examination of mucus on the gill surfaces and in the shell liquor of oysters exposed to various oxygen levels in the interval between 1 and 24 h after the introduction of microspheres showed large accumulations of hemocytes, most of which contained intracellular microspheres.

The observed retention patterns of tissue microspheres are remarkably similar considering the over 50-fold change in oxygen tensions (Table 1). No significant differences were found between means derived from ten oysters tested at each level ($P < 0.05$). Although a log drop in number is apparent between 1 and 24 hours, there is little further clearance of particles over the subsequent 48 hours.

Effects of Environmental Oxygen on Hemocyte Parameters

Figure 1 shows the phagocytic activity of hemocytes from oysters held under differing oxygen tensions. These data are expressed as mean percent of hemocytes which contained at least one microsphere. It can be seen that the *in vitro* ability to ingest microspheres is nearly constant over the entire range of oxygen tensions tested (9–480 mm Hg). No significant differences in activity were noted between the oxygen environments ($P < 0.05$).

The proportion of viable hemocytes from oysters exposed to different levels of dissolved oxygen are also shown in Fig. 1. Each mean is derived from five repeats with hemocytes pooled from three oysters in each environment. Hemocyte viability was high under the conditions of the experiments, ranging from 82 to 93%. No significant differences in viability were found between environments.

The median diameters of hemocytes from experimental oysters did not vary much upon exposure of oysters to low and high oxygen environments (Fig. 2). However, the concentration of circulating hemocytes was found to be significantly lower at 9 and 80 mm Hg than at the higher oxygen levels ($P < 0.05$).

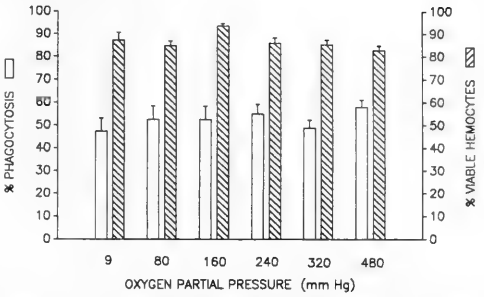

Figure 1. ***In vitro*** **hemocyte phagocytic activity and hemocyte viability are shown as functions of oxygen partial pressures (mm Hg). Phagocytic activity is expressed as percent hemocytes containing one or more beads. Viability is expressed as percent hemocytes showing fluorescence after staining with the vital stain, fluorescein diacetate. No significant differences in these parameters were found between oxygen levels ($P < 0.05$). Standard errors indicated.**

DISCUSSION

Oysters inhabit environments rich in suspended particles. Though a certain amount of size selectivity results from filtration through the gill filaments, microbial size particles enter the gut together with larger particulates. Some of the small particles may serve as food, while others are passed out in the feces (Galtsoff 1964).

We have recently shown by scanning electron microscopy that the inner surfaces of the stomach contain large numbers of motile phagocytes. Thus, we suggest that these cells may carry phagocytosed microbial size particles into the intercellular spaces of the tissues underlying the gastric epithelium (Alvarez et al. 1992). Some particles are eliminated from the tissues by intraphagosomal

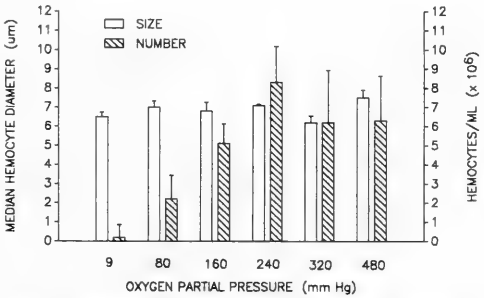

Figure 2. The effect of environmental oxygen partial pressure on hemocyte size and concentration. Hemocyte size, expressed as the mean of the median diameters of hemocytes from five oysters in each oxygen environment, was measured with a particle analyzer. Hemocyte concentration, also measured with a particle analyzer, is expressed as cells/ml. No significant changes in hemocyte size were noted. Hemocyte concentration, however, was significantly lower at 9 and 80 mm Hg than at the higher oxygen partial pressures ($P < 0.05$). Standard errors indicated.

TABLE 1.

Number of microspheres retained per gram of soft parts wet weight at 1, 24, and 72 hours after introduction by gastric intubation as a function of oxygen partial pressure.

Po_2 (mm Hg)	Microspheres/ gm 1 hour	Microspheres/ gm 24 hours	Microspheres/ gm 72 hours
9	2.30×10^4	5.60×10^3	2.88×10^3
80	2.30×10^4	2.28×10^3	2.64×10^3
160	1.99×10^4	3.26×10^3	3.07×10^3
240	1.60×10^4	6.15×10^3	1.05×10^3
320	8.64×10^3	4.57×10^3	2.51×10^3
480	1.50×10^4	3.13×10^3	2.55×10^3

digestion. Particles that resist intracellular digestion, however, can be cleared from the spaces by diapedesis of motile phagocytes through epithelial membranes exposed to the exterior of the body (Robohm 1984, Adema et al. 1991). Thus, the process of clearing indigestible particles may involve both phagocytosis and active movement of particle-containing hemocytes through the tissue spaces. If, however, non-self recognition or digestion is defeated, it is possible that some foreign material may remain.

It has been previously shown that treatment of hemocytes of *Mercenaria mercenaria* with potassium cyanide fails to alter particle uptake (Cheng 1976). More recently, oyster hemocytes exposed to abiotic particles *in vitro* in anaerobic environments showed no inhibition of phagocytosis (Alvarez et al. 1989). These observations imply that the phagocytic phase of the clearing process is independent of oxygen and is energetically associated with anaerobic metabolism. The purpose of the present study was to determine the effect of oxygen availability on phagocytic uptake of particles and diapedesis *in vivo*.

We observe an initial ten-fold decrease in the number of indigestible particles in the tissues 24 h after their introduction into the gut in oysters maintained in air-saturated atmospheres at one atmosphere of pressure (160 mm Hg). Large numbers of particle-containing hemocytes are found embedded in mucus on the outer surfaces of the gills suggesting that a major path of exit is through the simple columnar ciliated epithelium of the gills. Counts of microspheres in tissues 48 h later show no significant further decline in number. Our previous histological study of abiotic particle clearing demonstrated the presence of internalized microspheres in the connective tissue spaces as long as 10 days post intubation, although in far fewer numbers than were seen at 2 and 5 days (Alvarez et al. 1992). It is remarkable that this pattern persists over a more than 50 fold range of oxygen tensions (9–480 mm Hg). Introduced particle uptake and retention thus seem to be relatively independent of oxygen availability, not being inhibited at either hypoxic or hyperoxic levels.

The present data also show that hemocyte viability, size and phagocytic activity are unaffected by the same range of tensions of environmental oxygen in *C. virginica*. The concentration of hemocytes in the hemolymph, however, was found to be ten-fold lower in hypoxic conditions. These data suggest that while low oxygen tensions are not lethal to hemocytes, circulation or replacement of these cells following diapedesis may be extensively impaired. This suggests that long term hypoxia could result in a reduction in the rate of clearing of tissue particulates by this mechanism. Whether the reduced number of hemocytes in the hemolymph is due to reduced levels of hemopoesis or to a lower level of mobilization of circulating hemocytes is not known and is the subject of further investigation.

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MODELING OYSTER POPULATIONS I. A COMMENTARY ON FILTRATION RATE. IS FASTER ALWAYS BETTER?

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ABSTRACT The measurements reported in the literature that relate bivalve size to filtration rate tend to fall on one of two curves. The upper curve predicts filtration rates at a given size which are about three times those of the lower. A time-dependent numerical model for population dynamics and energy flow in post-settlement oyster populations was used to compare the effect of these two filtration versus size relationships on simulations of population growth and reproductive effort. The growth rates, fecundity, size and reproductive season of the simulated populations agree with measurements obtained from field populations only if the lower curve is used; unless the present consensus for the oyster's assimilation efficiency, the effect of high food supply on the oyster's feeding efficiency, or the measurement of oyster food supply are substantially in error. The results of these simulations question the tendency to accept higher filtration rates as more accurate for modeling field populations and suggest that an evaluation of measurements of components of the energy budget can only be made within the context of the species' complete energy budget.

KEY WORDS: bivalve, oyster, filtration rate, energy budget

INTRODUCTION

One goal of physiological research is to extract general relationships from diverse measurements of physiological rates that can be used to predict growth rates and fecundity of a particular species under selected environmental conditions. This is the basis for the use of scope for growth, for example, in environmental monitoring. Once general relationships are available, they can be combined into an energetics model and used to simulate the response of a population to a range of environmental conditions. Using an integrated modeling approach, the consistency of general relationships can be tested within the context of the entire energy budget of a species.

Bivalves can be important components of estuarine ecosystems because, if population abundances are sufficient, they have the capability to filter a large fraction of the water in a relatively short time and can, therefore, impact phytoplankton standing crop and production. Ecosystem models have been used to determine the significance of bivalve filtration in estuaries. However, most measures of bivalve energetics used in ecosystem models come from laboratory measurements. These relationships, normally obtained from disparate sources, when combined in a model, must provide growth rates and fecundities comparable to field observations. Otherwise the effect of the species on the environment may be poorly estimated.

One important component of the energy budget of a bivalve is filtration rate. Filtration rate can be related to weight by an allometric equation [$FR = aW^b$ where b normally varies from 0.4 to 0.6 (Officer et al. 1982, Winter 1978)]. Values in this range suggest that filtration rate may scale with length or surface area rather than biomass, and, indeed, Doering and Oviatt (1986) offer an alternative linear relationship with size which was used successfully by Klinck et al. (1992) to model oyster (*Crassostrea virginica*) populations.

This contribution stems from a consideration of how to parameterize bivalve filtration rates in models. That attempt led to a broader investigation of the adequacy of physiological measurements for bivalves for predicting growth and fecundity in the field. Our approach is to compare field observations of growth and reproduction for the American oyster, *Crassostrea virginica*, with the same rates obtained from an energetics model. In particular, we consider the effect of the choice made for parameterization of oyster filtration rate. Oysters are a good choice for such a study because most aspects of oyster physiology fall within the range typical of bivalves (Powell and Stanton, 1985). Therefore, the results of this study can be generalized to provide guidance for modeling bivalve physiology and population dynamics as a whole.

A GENERAL FILTRATION RATE RELATIONSHIP

The measurements shown in Figure 1 are from studies of various bivalve species in which filtration rate was related to size. In many cases, studies were directed towards understanding the effects of temperature (e.g. Newell et al. 1977, Buxton et al. 1981), salinity (e.g. Loosanoff 1953, Navarro 1988), variations in food quality (e.g. Ballantine and Morton 1956, Riisgård 1988) or food quantity (e.g. Griffiths and King 1979, Higgins 1980b), or other environmental parameters (e.g. Loosanoff and Tommers 1948, Walne 1972, Shumway et al. 1983) on bivalve filtration rate. Where possible, the data shown in Figure 1 are typical of summer

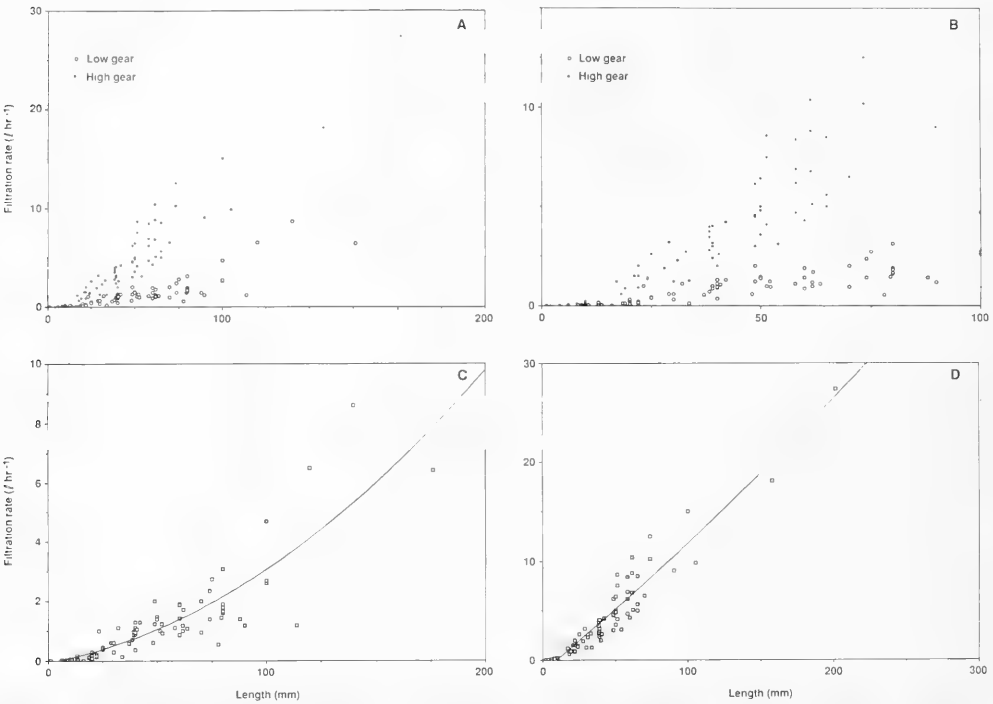

Figure 1. The relationship between filtration rate (1 hr^{-1}) and length (mm) in suspension-feeding bivalves; a, all filtration rates used in the study; b, filtration rate measurements for animals less than 100 mm long; c, the lower (low gear) curve [filtration rate (1 hr^{-1}) = $-7.4437 \times 10^{-2} + 1.3317 \times 10^{-2} *$ length(mm) + $1.7957 \times 10^{-4} *$ length $(mm)^2$]; d, the upper (high gear) curve [filtration rate (1 hr^{-1}) = −1.1994 + .12137 * length(mm) + $8.1652 \times 10^{-5} *$ length$(mm)^2$]. The data were obtained from: *Mercenaria mercenaria*, Hibbert (1977), Coughlan and Ansell (1964), Rice and Smith (1958); *Mytilus californianus*, Rao (1953), Fox et al. (1937), Bayne et al. (1976); *Geukensia demissa*, Jordan and Valiela (1982); *Dreissena polymorpha*, Dorgelo and Smeenk (1988); *Cardium edule*, Vahl (1972a); *Choromytilus meridionalis*, Griffiths (1980), Griffiths and Buffenstein (1981); *Argopecten irradians*, *Crassostrea virginica*, Palmer (1980); *Mytilus edulis*, Riisgård and Møhlenberg (1979), Riisgård and Randløv (1981), Riisgård et al. (1980); *Mytilus chilensis*, Navarro and Winter (1982); *Crassostrea rhizophorae*, Castro et al. (1985); *Ostrea edulis*, *Crassostrea angulata*, Mathers (1974); *Mya arenaria*, *Venus striatula*, *Ostrea edulis*, Allen (1962); *Ostrea edulis*, Buxton et al. (1981), Newell et al. (1977); *Ostrea chilensis*, Winter et al. (1984); *Cardium edule*, *Cardium lamarcki*, Brock and Kofoed (1987); *Chlamys islandica*, Vahl (1980); *Crassostrea gigas*, Gerdes (1983); *Ostrea edulis*, Wilson (1983); *Crassostrea virginica*, Loosanoff (1958), Loosanoff and Nomejko (1946), Langefoss and Maurer (1975); *Argopecten irradians*, Chipman and Hopkins (1954); *Chlamys opercularis*, Vahl (1972b); *Cardium lamarcki*, *Didacna longipes*, *Didacna trigonoides*, *Hypanis angusticostata*, *Mytilaster lineatus*, Sanina (1976); *Mytilus edulis*, *Cardium echinatum*, *Cardium edule*, *Modiolus modiolus*, *Arctica islandica*, Møhlenberg and Riisgård (1979); *Mytilus edulis*, *Cardium edule*, *Venerupis pullustra*, Foster-Smith (1975); *Ostrea edulis*, *Crassostrea gigas*, *Mercenaria mercenaria*, *Venerupis decussata*, Walne (1972); *Lasaea rubra*, Ballantine and Morton (1956); *Brachidontes exustus*, *Spisula solidissima*, *Argopecten irradians*, *Crassostrea virginica*, *Geukensia demissa*, *Mercenaria mercenaria*, Riisgård (1988); *Mulinia lateralis*, Shumway et al. (1983); *Choromytilus chorus*, Navarro (1988); *Aulacomya ater*, Griffiths and King (1979).

temperatures, without turbidity, and represent the highest rates obtained when food quantity or quality was varied. These, then, are usually the highest rates attained by the species in the study. We present data only for filter feeders; data on deposit feeders (e.g. Meyhöfer 1985) were excluded. When a continuous relationship was given, we chose 3 to 7 points encompassing the range of sizes used to generate the relationship so that no one data set was weighted much more heavily than the others.

To include as many data as possible, filtration rate was plotted as a function of length rather than the preferred measure of biomass. Powell and Stanton (1985) show this to be satisfactory for addressing the general questions of interest here. As data for the same species cover the range of values to be discussed, any errors introduced by using length rather than biomass are insignificant to this discussion. A comparable range of values would be present after a conversion to biomass. The oyster population model to be presented subsequently is in terms of biomass and the conclusions drawn from the model concerning the trends in filtration rate are presented in terms of biomass.

Three aspects of Figure 1 are of interest. (1) A general relationship between filtration rate and size exists that would permit estimation of filtration rates in unstudied species. (2) Although the relationship is curvilinear, a linear relation of the type used by Doering and Oviatt (1986) would suffice over a wide size range.

(3) Two distinct filtration rate versus size trends exist in the data; one predicting a substantially higher filtration rate at size than the other. It is this third observation, the bimodality present in Figures 1a and 1b, that will be considered further here. Which relationship, the upper or lower one, should be used to estimate filtration rate? Why do two relationships exist?

A simple explanation for the bimodal form of Figures 1a and 1b is not apparent. In many cases, filtration rates measured for the same species are found on both curves. With the possible exception of scallops, the bimodality is not a taxonomic effect. All rates for scallops fall on the upper curve. The bimodality does not appear to be an effect of technique, the measurement of clearance rates versus pumping rates for instance. Most of the data are derived from measurements of clearance rates and these fall on both curves. Simple variations in food quality and quantity also seem to be excluded. Both curves contain a preponderance of data where pseudofeces were not produced, for instance, and clearance rates were measured using organic and inorganic materials. Both recent and classical data are present in both curves. Bimodality would not appear to be related to technological advancement or other temporally-associated phenomena.

The higher filtration rates of the upper curve have been noted in most of the studies reporting them. Typical explanations for the higher rates include more careful experimentation, improved technology, etc. We see no evidence to accept the higher curve on the assumption that higher rates are inherently more accurate. Were these trends due to some improvement in methodology, temporal effects should be observed, the bimodality in the filtration rate versus size relation would not be as apparent, and some data would fall between the two curves. We cannot explain the bimodal nature of the filtration rate versus size dependency, but suggest that a physiological switch is a potential explanation.

Regardless, we infer from the bimodal nature of the filtration rate versus size relationship that one of the two curves may not be representative of normal filtration rates in the field and, hence, not adequate for modeling bivalve energetics. Certainly, Figure 1 suggests that the choice of a filtration rate versus size relationship is an important one because the ingestion rates predicted may vary by a factor of 3 or more as a consequence. Accordingly, we have compared growth rates and fecundities predicted from physiological measurements by a mathematical model with field observations on growth and fecundity to assess how the bimodal form of the filtration rate versus size relationship affects the species' entire energy budget.

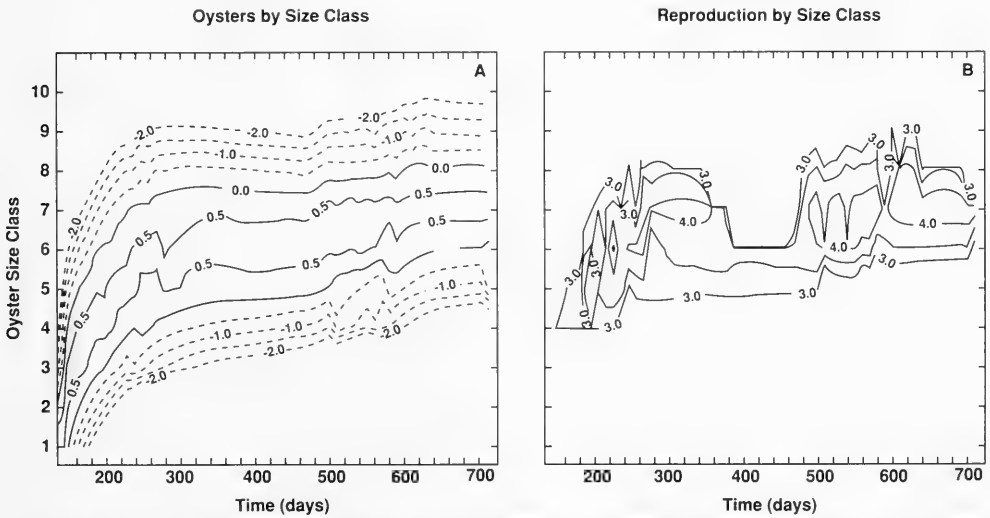

Figure 2. The growth rate, gonadal development and size obtained by simulated oyster populations using the lower filtration rate curve of Figure 1c. Assimilation efficiency = 75%. Temperature, salinity and food time series are for Galveston Bay as discussed in the text. A, a plot of oyster numbers in each size class versus time. B, a plot of the amount of gonadal material present in each size class versus time. Simulation was initiated with the recruitment of a cohort of 10 individuals on Julian day 140 (January 1 = day 1) into size class 1. The size class dimensions are: 1, 0.3–25.0 mm; 2, 25.0–35.0 mm; 3, 35.0–50.0 mm; ≥4, 50.0–63.5 mm; 5, 63.5–76.0 mm; 6, 76.0–88.9 mm; 7, 88.9–100.0 mm; 8, 100.0–110.0 mm; 9, 110.0–125.0 mm; 10, 125.0–150.0 mm. Size classes 1–3 are juveniles; ≥4, adults. The size class boundaries between 4 and 5, 5 and 6, and 6 and 7 represent size limits for market-size oysters: 2.5 inches, 3.0 inches and 3.5 inches, respectively. Contours in A are the logarithms of the number of oysters ($\log_{10}$ N) with a contour interval of 0.5. The mean size of the population can be traced by following the area between the two contours labeled 0.5. Contours in B are the logarithm of calories ($\log_{10}$ cal) with a contour interval of 0.5. Caloric values can be converted into joules by multiplying by 4.16 J cal^{-1}; into biomass by using 6100 cal g dry wt^{-1}; and into the equivalent number of fully developed eggs by 13 ng $egg^{-1} \times 6.133 \times 10^{-6}$ cal ng^{-1}. Numbers or calories are plotted opposite the size class designation, not halfway between; hence on day 140 all individuals are in size class 1 opposite the grid mark labeled 1. In this simulation, by day 430, most oysters are in size class 6. On the graph of reproduction (B), downward jags or triangles just before, for instance, day 285 or after day 575 indicate spawnings. Gentle changes, for instance between days 285 and 430 indicate gonadal development or resorption.

THE MODEL

Perspective and Basic Characteristics

The model presented subsequently considers the dynamics of the post-settlement phase of oyster population dynamics from newly-settled juvenile through adulthood. The population model consists of a system of ten coupled ordinary differential equations, with each equation representing a size class of oyster. Some of the details, assumptions, and justifications of the relationships used are given in Klinck et al. (1992), White et al. (1988), and Powell and Stanton (1985). For simplicity, we will outline the size class model briefly and discuss in detail just those relationships important to the present study.

In our simulations, we have divided the oyster's size spectrum into 10 size classes (denoted by the subscript j) defined in terms of biomass (g dry wt) from 1.3×10^{-7} g [~0.3 mm using White et al.'s (1988) biomass-size conversion] to 25.9 g (~150 mm), as detailed in Figure 2. The lower size limit represents the size at settlement (Dupuy et al. 1977); the upper size limit represents an oyster larger than those normally found in the Gulf of Mexico. The size class boundaries between size classes 4 and 5, 5 and 6, and 6 and 7 represent size limits that have been used or considered for market-size oysters [converted to biomass using White et al.'s (1988) biomass-size conversion]: 2.5 in, 3.0 in, and 3.5 in, respectively. The size classes are, therefore, not equally apportioned across the oyster's biomass spectrum. We define adults, individuals capable of spawning, as individuals weighing more than 0.65 g ash-free dry weight, about 50 mm in length (Hayes and Menzel, 1981), although gonadal development has been observed at somewhat smaller sizes (Coe 1936, Burkenroad 1931); hence size classes 1 to 3 are juveniles.

The following four conversions and scaling factors were used throughout the model; for simplicity, we will not include them in the equations given subsequently. (1) All calculations were done in terms of energy in cal m^{-2}. When necessary, we convert energy to biomass using a caloric conversion of 6100 cal g dry wt^{-1} for oysters (Cummins and Wuycheck 1971) [Garton (1986) report a somewhat lower value] and 5168 cal g dry wt^{-1} for the food available to the oysters. The biomass-length conversion given in White et al. (1988) was used to convert between biomass and length. This conversion is only an approximation given the range in growth forms found in oysters throughout their latitudinal range. (2) Gains, losses or transfers of energy (or biomass) between oyster size classes were expressed as specific rates (day^{-1}) which were then applied to the caloric content in a size class. For example, ingestion (cal day^{-1}) divided by a caloric value in cal gives a specific rate (cal day^{-1}/cal = day^{-1}) which is then used to calculate incremental changes in a size class. (3) Transfers between size classes were scaled by the ratio of the average weight of the current size class (in g dry wt or cal) to that of the size class from which energy was being gained or to which energy was being lost. This ensured that the total number of individuals [converted from biomass using White et al.'s (1988) biomass-size conversion] in the model was conserved, in the absence of recruitment and mortality. (4) Because the size classes in the model are not equivalent in dimension, each specific rate for each transfer between size classes was scaled by the ratio between the two size classes

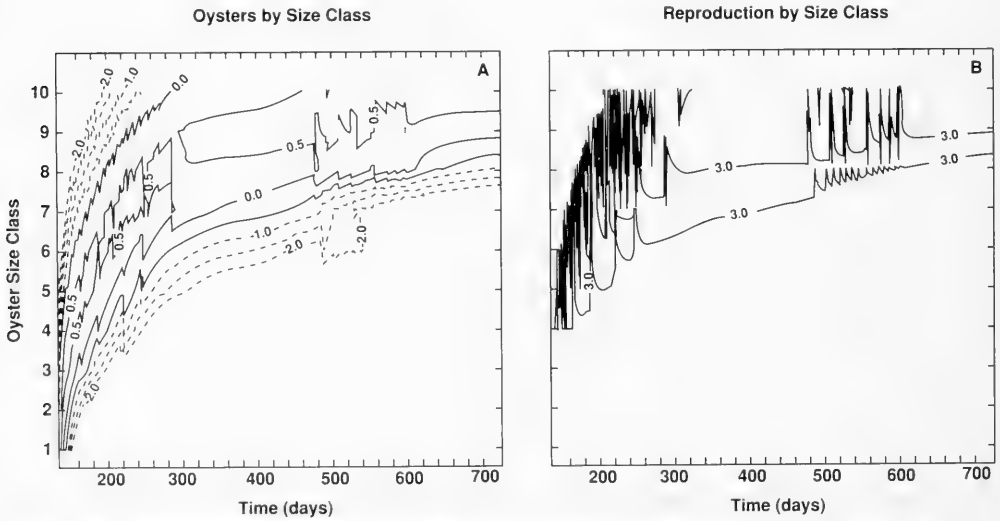

Figure 3. The growth rate, gonadal development and size obtained by simulated oyster populations using the high gear filtration rate curve of Figure 1d. Simulation was initiated with the recruitment of a cohort of 10 individuals on Julian day 140 (January 1 = day 1) into size class 1. A: The number (log_{10} N) of oysters per size class versus time. The mean size of the population can be traced by following the area between the two contours labeled 0.5. B: The amount of gonadal material present (log_{10} cal) versus time with a contour interval of 1.0. Caloric values can be converted into joules by multiplying by 4.16 J cal^{-1}; into biomass by using 6100 cal g dry wt^{-1}; and into the equivalent number of fully developed eggs by 13 ng $egg^{-1} \times 6.133 \times 10^{-6}$ cal ng^{-1}. Otherwise same as in Figure 2.

for transfers up: $W_j/(W_{j+1} - W_j)$
for transfers down: $W_j/(W_j - W_{j-1})$

where W is the median value for biomass (in g dry wt) in the size class.

Governing Equation

Following White et al. (1988), net production (NPj) is assumed to be the difference between assimilation (A_j) and respiration (R_j)

$$NP_j = P_{gj} + P_{rj} = A_j - R_j. \quad (1)$$

where Pg is growth and Pr is reproduction.

Excretion is not included, it being a minor component of the energy budget (Boucher and Boucher-Rodoni, 1988). The change in oyster standing stock with time in each size class (O_j) is the result of changes in net production and the addition of individuals from the previous size class or loss to the next largest size class by growth. Stated mathematically,

$$\frac{dO}{dt}j = P_{gj} + P_{rj} + (\text{gain from } j - 1) - (\text{loss to } j + 1) \quad (2)$$

for j = 1,10 recognizing $P_{rj} = 0$ for J = 1,3.

Resorption of either gonadal or somatic tissue results in loss of biomass. When $NP_j < 0$, oysters lose biomass and transfer into the next lower size class. This is an important difference between our size class model and a size class model based on linear dimensions: shell size does not change, however biomass does during periods of negative scope for growth. This is the basis for the use of condition index as a measure of health in oysters (e.g. Newell 1985, Wright and Hetzel 1985). To allow for a negative scope for growth, equation (2) must be modified as

$$\frac{dO_j}{dt} = P_{gj} + P_{rj} + (\text{gain from } j - 1) - (\text{loss to } j + 1) + (\text{gain from } j + 1) - (\text{loss to } j - 1) \quad (3)$$

for j = 1,10. The last two terms on the right side of equation (3) represent the individuals losing biomass and, thus, translating down to the next lower size class.

Feeding and Assimilation

Ingestion rate depends upon the filtration rate and the ambient food concentration. We adapted Doering and Oviatt's (1986) equation for filtration rate to oysters by using Hibbert's (1977) biomass-length relationship to obtain filtration rate as a function of biomass and temperature:

$$FR_j = \frac{L_j^{0.96}T^{0.95}}{2.95} \quad (4)$$

and

$$L_j = W_j^{0.317}10^{0.669} \quad (5)$$

where filtration rate is in ml filtered ind^{-1} min^{-1} and W_j is the ash-free dry weight in g for each size class. This equation approximates the low gear curve of Figure 1c at 20°C between 0 and 100 mm. We modified Doering and Oviatt's (1986) equation to approximate the upper curve between 0 and 100 mm (Figure 1d); thus retaining the relationship of filtration rate with temperature described in more detail by Loosanoff (1958). Loosanoff's (1958) measurements suggest that the rate of increase of filtration rate moderates at temperatures above 25°C, in accordance with a gen-

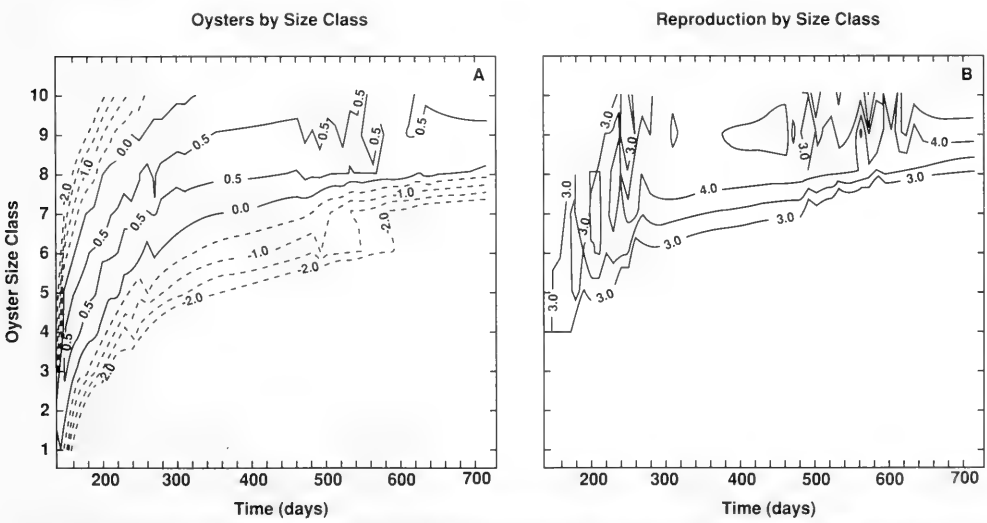

Figure 4. The growth rate, gonadal development and size obtained by simulated oyster populations using the high gear filtration rate curve of Figure 1d and an assimilation efficiency of 0.54. Simulation was initiated with the recruitment of a cohort of 10 individuals on Julian day 140 (January 1 = day 1) into size class 1. A: The number (log_{10} N) of oysters per size class versus time. The mean size of the population can be traced by following the area between the two contours labeled 0.5. B: The amount of gonadal material present (log_{10} cal) versus time. Caloric values can be converted into joules by multiplying by 4.16 J cal^{-1}; into biomass by using 6100 cal g dry wt^{-1}; and into the equivalent number of fully developed eggs by 13 ng egg^{-1} × 6.133 × 10^{-6} cal ng^{-1}. Otherwise same as in Figure 2.

eral trend for bivalves described by Winter (1978), and declines above 32°C. However, equation (4) and its modification for the high gear curve approximate measured values throughout the normal temperature range when compared to appropriate data referenced in Figure 1, so we use them without further modification.

Filtration rate was further modified by salinity as described by Loosanoff (1953). Filtration rate decreases as salinity drops below 7.5‰ and ceases at 3.5‰. In mathematical terms:

$$\begin{aligned} &\text{at } S \geq 7.5‰ && FR_{sj} = FR_j; \\ &\text{at } 3.5 < S < 7.5‰ && FR_{sj} = FR_j(3.5 - S)/4.0; \\ &\text{at } S \leq 3.5‰ && FR_{sj} = 0; \end{aligned} \tag{6}$$

where S is environmental salinity and FR_j is the rate obtained from equation (4).

The reduction in feeding efficiency at high particulate loads, characterized by pseudofeces production, was included as a depression in filtration rate rather than as a separate function as used by Soniat (1982). From data presented in Loosanoff and Tommers (1948), total particulate content can be related to a reduction in filtration rate as

$$\tau = (4.17 \times 10^{-4})(10^{(0.418x)}) \tag{7}$$

where τ is the total particulate content (inorganic + organic) in g l^{-1} and x is the percent reduction in filtration rate. Solving equation (7) for the percent reduction in filtration rate gives a modified expression for filtration rate of the form:

$$FR_{sj} = FR_j\left[1 - .01\left(\frac{\log_{10}\tau + 3.38}{0.418}\right)\right]. \tag{8}$$

Equation (8), if applied to total particulate content (inorganic + organic), approximates the results of Haven and Morales-Alamo (1966) and limits ingestion rate to approximately the maximum value found by Epifanio and Ewart (1977). Therefore, an additional term to lower ingestion efficiency at high food concentrations was not used. We assume all particles are removed by filtration, a slight overestimate (Palmer and Williams 1980), that oysters feed more or less continuously (Higgins 1980a), and that filtration rate does not vary with food availability (Higgins 1980b; Valenti and Epifanio, 1981).

Filtration rate times the ambient food concentration gives oyster ingestion. To the extent that oysters can select nitrogen-rich particles from the filtered material for ingestion, equation (8) yields an underestimate of ingestion (Newell and Jordan, 1983). Assimilation is obtained from ingestion using an assimilation efficiency of 0.75, an average value obtained from Tenore and Dunstan (1973), Valenti and Epifanio (1981) and Langefoss and Maurer (1975).

Respiration

Oyster respiration as a function of temperature and oyster weight was obtained from Dame (1972) as

$$R_j = (69.7 + 12.6T)W_j^{b-1} \tag{9}$$

where R_j is in µl O_2 consumed hr^{-1} g dry wt^{-1} and b = 0.75. Equation (9) conforms to the more general relationship for all bivalves obtained by Powell and Stanton (1985).

Salinity effects on oyster respiration were parameterized using data given in Shumway and Koehn (1982) as follows:

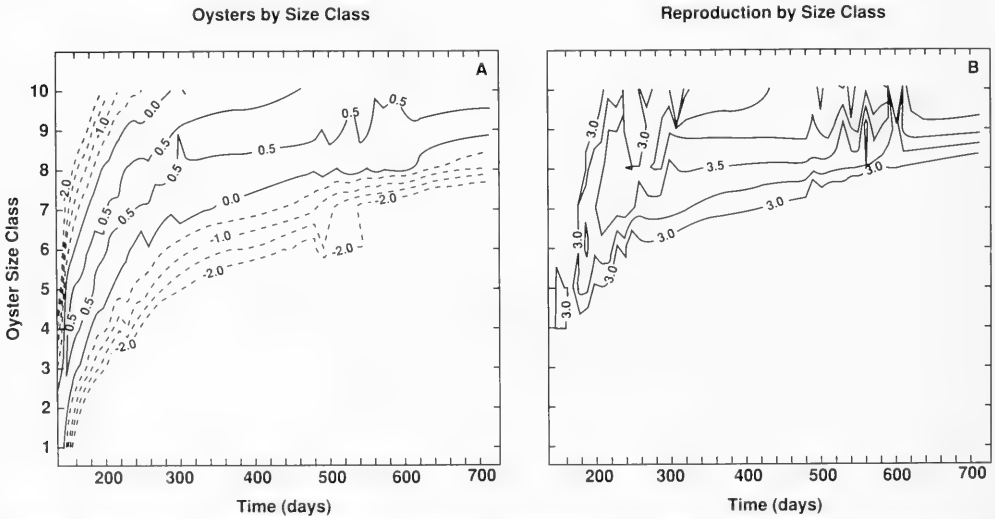

Figure 5. The growth rate, gonadal development and size obtained by simulated oyster populations using the high gear filtration rate curve of Figure 1d, an assimilation efficiency of 0.75 and with the effect of food content on filtration efficiency added. Simulation was initiated with the recruitment of a cohort of 10 individuals on Julian day 140 (January 1 = day 1) into size class 1. A: The number (log_{10} N) of oysters per size class versus time. The mean size of the population can be traced by following the area between the two contours labeled 0.5. B: The amount of gonadal material present (log_{10} cal) versus time. Caloric values can be converted into joules by multiplying by 4.16 J cal^{-1}; into biomass by using 6100 cal g dry wt^{-1}; and into the equivalent number of fully developed eggs by 13 ng egg^{-1} × 6.133 × 10^{-6} cal ng^{-1}. Otherwise same as in Figure 2.

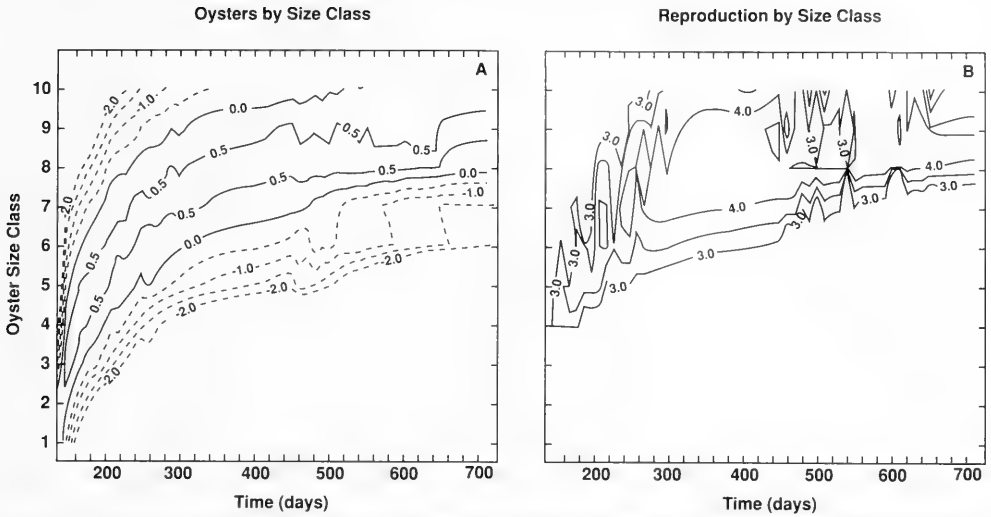

Figure 6. The growth rate, gonadal development and size obtained by simulated oyster populations using the high gear filtration rate curve of Figure 1d, an assimilation efficiency of 0.75 and the effect of food content on ingestion efficiency double that of Figure 5. Simulation was initiated with the recruitment of a cohort of 10 individuals on Julian day 140 (January 1 = day 1) into size class 1. A: The number ($\log_{10}$ N) of oysters per size class versus time. The mean size of the population can be traced by following the area between the two contours labeled 0.5. B: The amount of gonadal material present ($\log_{10}$ cal) versus time. Caloric values can be converted into joules by multiplying by 4.16 J cal^{-1}; into biomass by using 6100 cal g dry wt^{-1}; and into the equivalent number of fully developed eggs by 13 ng egg^{-1} × 6.133 × 10^{-6} cal ng^{-1}. Otherwise same as in Figure 2.

$$\begin{aligned} &\text{At } T < 20°C \qquad R_r = 0.007T + 2.099 \\ &\text{At } T \geq 20°C \qquad R_r = 0.0915T + 1.324 \end{aligned} \tag{10}$$

where R_r is the ratio of respiration at 10‰ to respiration at 20‰:

$$R_r = \frac{R_{10‰}}{R_{20‰}}.$$

$$\begin{aligned} &\text{At } S \geq 15‰ \qquad R_{js} = R_j. \\ &\text{At } 10‰ < S < 15‰ \qquad R_{js} = R_j(1. + [(R_r - 1)(15. - S)/5.]) \\ &\text{At } S \leq 10‰ \qquad R_{js} = R_j R_r. \end{aligned}$$

Shumway and Koehn (1982) identified effects of salinity on respiration at 20‰; however, we used a 15‰ cutoff to conform to Chanley's (1958) observations on growth.

Reproduction

For adult oysters (j = 4,10), net production was apportioned into growth and reproduction by using a temperature-dependent reproduction efficiency of the form

$$R_{effj} = 0.054T - 0.729 \tag{12}$$

for January to June and

$$R_{effj} = 0.047 - 0.809 \tag{13}$$

for July to December. Equations (12) and (13) were derived empirically from the field observations of Soniat and Ray (1985). Disagreement exists in the literature concerning the extent to which oyster reproduction is temperature acclimatized (Loosanoff and Davis 1953, Stauber 1950, Loosanoff 1969). However, from the studies of Butler (1955), Kaufman (1979) and Quick and Mackin (1971) acclimatization appears unimportant over the latitudinal range of Chesapeake Bay to the southern Gulf of Mexico. Equations (12) and (13) may not hold north of Delaware Bay.

The portion of net productivity going into reproduction is given by

$$P_{rj} = R_{effj} NP_j, \quad \text{for } j = 4, 10. \tag{14}$$

Somatic growth is the remaining fraction. In cases where $NP_j < 0$, we assume preferential resorption of gonadal tissue to cover the debt, although some data suggest the contrary (Pipe 1985). Gonadal resorption is commonly observed in stressed oysters (e.g. Gennette and Morey 1971) and in the fall and winter when food is reduced (Kennedy and Battle 1964). For juveniles and adults with no gonadal tissue, resorption of somatic tissue occurs. We assume reduced reproduction at low salinity (Engle 1947; Butler 1949) results from decreased filtration rate and increased respiratory rate and so include no specific relationship for this effect.

Although a considerable literature exists on factors controlling the initiation of spawning (e.g. Loosanoff 1965, Stauber, 1950, Dupuy et al. 1977), including empirical temperature-dependent relationships (Kaufman 1979, Loosanoff and Davis 1953), little is understood about factors controlling the frequency of spawning over the entire spawning season (e.g. Davis and Chanley 1956). In our model, spawning occurs when the cumulative reproductive

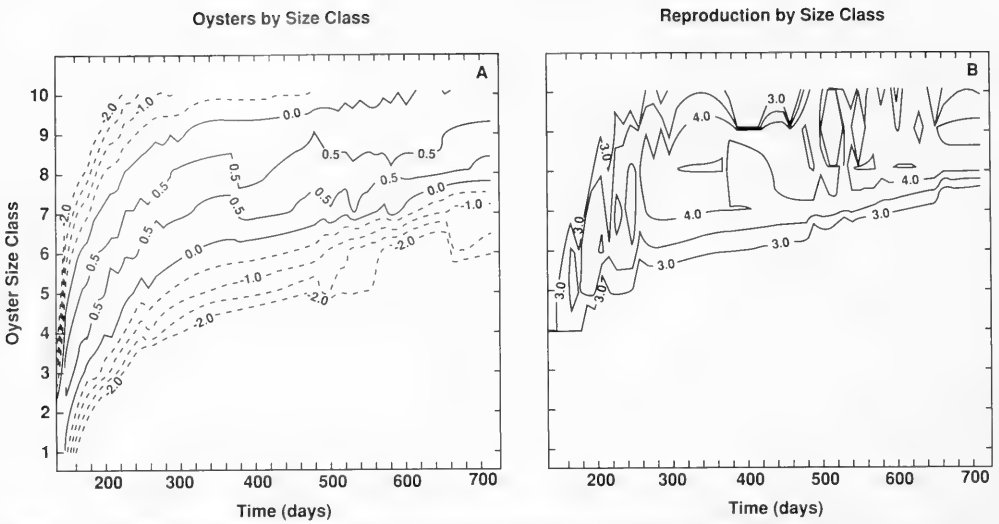

Figure 7. The growth rate, gonadal development and size obtained by simulated oyster populations using the high gear filtration rate curve of Figure 1d, an assimilation efficiency of 0.54 and with the effect of food content on ingestion efficiency added as in Figure 5. Simulation was initiated with the recruitment of a cohort of 10 individuals on Julian day 140 (January 1 = day 1) into size class 1. A: The number (log N) of oysters per size class versus time. The mean size of the population can be traced by following the area between the two contours labeled 0.5. B: The amount of gonadal material present (log_{10} cal) versus time. Caloric values can be converted into joules by multiplying by 4.16 J cal^{-1}; into biomass by using 6100 cal g dry wt^{-1}; and into the equivalent number of fully developed eggs by 13 ng egg^{-1} × 6.133 × 10^{-6} cal ng^{-1}. Otherwise the same as in Figure 2.

biomass of a size class exceeds 20% of the standing stock, an estimate based on data presented in Gallager and Mann (1986) and Choi et al. (in press).

Characteristics of Simulations—Standard Approach

The model described by equation (3) was solved numerically using an implicit (Crank-Nicolson) tridiagonal solution technique. The time step for model integration was one day. Simulations were run for 6 yr which is sufficient time for transient adjustments of the model to disappear.

Environmental parameters used in the model were from Galveston Bay (April Fool's Reef): the food time series was obtained from Soniat et al. (1984); the temperature and salinity time series came from Soniat and Ray (1985). The salinity time series contains only one monthly value in 2 yr low enough to affect the physiological processes in the model. Accordingly, variability in food content and temperature dictated the effect of the environment on the simulated oyster population distributions presented here. Klinck et al. (1992) show that results of the model compare adequately to field data for Gulf of Mexico oyster populations in bays as diverse as Galveston Bay, Apalachicola Bay and Tampa Bay.

MODEL SIMULATIONS

Expectations

We will compare the results of the model to four expectations obtained from field data and observations.

1. Juvenile growth rates are taken from Hayes and Menzel's (1981) data for Apalachicola Bay. In accordance with those data, we consider the case of larval recruitment on May 20 (Julian day 140). Hayes and Menzel (1981) observed that the 25 largest oysters grew to a mean length of 66 mm in 44 days (Julian day 184) and 84 mm in 62 days (Julian day 202). These growth rates are high for Gulf coast oysters (e.g. Ingle and Dawson, 1952). Higher rates would generally not be expected.
2. Like many bivalves (e.g. Stickney 1964), adult oysters usually lose biomass during the late winter months (Dame 1972, 1976, Shaw and Merrill 1966). Gonadal resorption may be observed in late fall and winter. Negative net production derives from a decrease in food supply and a more rapid temperature-dependent decrease in filtration rate than in respiration rate so that maintenance costs decline less than does the rate of food acquisition. We expect large oysters to lose weight during the winter.
3. Among the factors controlling the maximum size (biomass) of the average adult oyster in a habitat is food availability. At some point, growth of somatic tissue in the warmer months will balance loss during the winter. This process is abetted by the relationship of respiration and filtration rate to size. Respiration rate increases faster ($b = 0.75$ in $R = aW^b$ versus $b = 0.4$ to 0.6 in $FR = aW^b$) so that an upper limit is placed on size by the balance between the requirement of tissue maintenance and the capacity to acquire food. In Galveston Bay, this size is about 8 to 10 cm (Wilson et al. in press). [For convenience we convert from

biomass as used in the model to length using the length to biomass conversion of White et al. (1988). Obviously, since shell length does not shrink in the winter, while somatic content does, this conversion serves only as an approximation to the true shell length attained].

4. Spawning in the northern Gulf of Mexico is normally restricted to the summer months [in the southern Gulf of Mexico spawning may occur all year around—Wilson et al. (1990)]. Reproductively-advanced oysters typically make up the majority of the population only from April through October. Large oysters ready to spawn would not be expected in the winter.

Simulations

In Figures 2 and 3, we compare the simulated time-dependent behavior of oyster growth and reproductive development obtained for the two filtration rate curves. The lower filtration rate curve produces each of the expected effects:

1. Oysters reach size class 5 (64–76 mm) in about 45 days and size class 6 (76–89 mm) in about 72 days post-settlement.
2. Little winter growth occurs in adults.
3. Average adult size (size class 6) is 76–89 mm.
4. Gonadal tissue is present in the larger oysters from April to October. Spawning occurs in the spring and fall, as indicated by an abrupt decrease in gonadal tissue (downward jags in Figure 2b). Gonadal resorption occurs in the winter.

In contrast, the results of simulations using the upper (high gear) curve do not conform to the above criteria. Oysters spawn all year. Post-settlement growth rates are too fast (88–100 mm in 40 days). Adult size is too large.

The simulations shown in Figures 2 and 3 provide a quandary: either the high gear filtration rate curve produces unrealistic rates of growth and reproduction in the field or some other aspect of oyster energetics is incorrectly modeled. The components of oyster energetics that might be incorrectly modeled are those that could reduce net productivity below the calculated levels; respiration rate, assimilation efficiency, ingestion efficiency, and food supply.

The function for respiration rate used in the oyster model is typical of all bivalves (compare Powell and Stanton 1985); accordingly the calculated respiration rate should be appropriate for oysters. Although some range does exist in the various measurements of respiration rate for oysters (Shumway 1982), the variation present produces only minor changes in the model and will not be considered further.

The assimilation efficiency, 0.75, though representative of values reported for oysters, might be too high. Powell and Stanton (1985) found an average assimilation efficiency of 0.54 for bivalves. To test the effect of varying assimilation efficiency, we reduced assimilation efficiency to 0.54 but retained the high gear filtration rate curve (Fig. 4). Growth rates are still too high, size too large and reproductive season too long. The assimilation efficiency must be reduced to 0.25 to obtain results similar to Figure 2. This factor-of-three reduction about balances the factor-of-three increase in food acquisition produced by the high gear filtration rate function. An assimilation efficiency of 0.25, however, is nearly outside the range of assimilation efficiencies measured for bivalves (Powell and Stanton 1985) and well below efficiencies typical for oysters.

The rate of food ingestion was not limited in the simulations depicted in Figures 2 through 4. At high rates of food acquisition, pseudofeces are produced so that not all filtered food is ingested. Adding ingestion efficiency to the model (approximated as an effect of turbidity or total particulate content) has little effect on the results using the low gear filtration rate curve because rates of food acquisition are not too high. However, adding ingestion efficiency to the model has a substantial effect on simulations using the high gear filtration rate curve (Fig. 5). The simulation depicted in Figure 5 includes the function for ingestion efficiency and an assimilation efficiency of 0.75. Growth rates are still too high, size too large and reproductive season too long. A doubling of the effect would result in pseudofeces production at food concentrations below levels where pseudofeces production is normally observed. Even so, doubling the effect of particulate content on filtration rate, hence reducing ingestion efficiency still further (Fig. 6), although improving the result, nevertheless yields an expected reproductive season that is still too long, growth rates that are still too high and adult size that is still too large. The same pattern occurs if an assimilation efficiency of 0.54 is combined with reduced ingestion efficiency (Fig. 7).

Reducing food supply would also provide realistic rates of growth, adult size, and reproduction. However, we have used food supplies well within the range normally measured by a variety of estimators such as chlorophyll, particulate organic carbon, or carbohydrate, lipid and protein. However, oysters may filter water faster than the ambient current velocity, which may result in a local depletion in food supply. A local depletion of food supply has been shown in mussels (Wildish and Kristmanson 1985, Frechette and Bourget 1985) and field evidence for the same over oyster reefs is relatively strong (Powell et al. 1987, Dame et al. 1984). However, the significance of local food depletion over open-bay reefs is still open to question (Powell et al. 1987). Nevertheless, we cannot exclude the possibility that standard water column measurements of food supply yield values much above the food supply actually available to oysters under normal environmental conditions.

CONCLUSIONS

If the high gear filtration rate curve is used, the simulated oyster populations behave according to the four criteria established from observation only by lowering assimilation efficiency to a value much below the average for all bivalves, in the range of 0.25, by producing pseudofeces at unrealistically low food contents, or by assuming that the real food supply is well below that indicated by water column measurements. Assuming that the assimilation efficiencies and ingestion efficiencies reported for bivalves are even approximately correct and that food supply is adequately measured, only the low gear filtration rate curve yields results comparable to field measurements. This result does not preclude the accuracy of the high gear curve. However, without a reevaluation of other aspects of the energy budget, parameterization of filtration rate using the high gear curve cannot produce results typical of field conditions. As oysters are fairly typical bivalves and as measurements of filtration rate for oysters appear on both curves, we assume that the same would be true for most filter-feeding bivalves; the possible exception being scallops, the data for which fall predominantly on the high gear curve.

The explanation for the bimodal form of Figures 1a and 1b does

not seem to reside in the methodology used. Rather, the bimodal form more likely indicates a real physiological feature of bivalves. The filtration process has two characteristic rates, a high and a low gear. Because so few rates fall in between the two curves, one possible explanation is that the two curves result from two anatomically different configurations for the gill. Regardless, low gear would appear to be the standard setting for feeding in the field. Perhaps high gear is typically used for gamete release, for instance, or for clearing debris from the mantle cavity (see Jørgensen, 1954). Loo and Rosenberg (1989) observed that bivalves fed at rates considerably below their maximum potential rates in the field, a finding supported by the results of this modeling study.

These calculations point to the necessity that components of the energy budget not be measured in isolation; only when all aspects are considered can the adequacy of the measurement of individual components be assessed (e.g. Buxton et al. 1981). The calculations also suggest that higher filtration rates are not inherently better or more accurate, as has sometimes been claimed. The tendency to take the higher rate as more accurate can result in the overestimation of the filtration capacity of bivalve populations. Rather, models must use parameterizations for filtration rates that are consistent with measured growth rates, adult size and reproduction in field populations, if the role of bivalves in determining water clarity, phytoplankton standing stocks and production is to be quantified (e.g. Cloern 1982, Officer et al. 1982, Hily 1991, Loo and Rosenberg 1989). Our model suggests that the low gear, not the high gear, curve is a better representation of filtration rate and is the form that should be used in models of the energetics of suspension-feeding bivalves.

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MODELING OYSTER POPULATIONS III. CRITICAL FEEDING PERIODS, GROWTH AND REPRODUCTION

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ABSTRACT A time-dependent population dynamics model for oyster (*Crassostrea virginica*) populations is used to test the hypothesis that variations in the seasonal sequence in temperature and food supply are responsible for observed variations in oyster reproductive effort. Simulations show that a few degrees change in temperature or a small (2 to 4 week) shift in timing of the spring or fall bloom can considerably alter the duration of spawning and the seasonal spawning patterns. Furthermore, the timing of the spring and fall plankton blooms (the oyster's food supply) relative to the spring increase and fall decrease in temperature is crucial in determining reproductive effort over a spawning season. Delay of the spring bloom with respect to the spring temperature rise increases reproductive effort and affects the number and timing of spawning pulses. Simulations using environmental conditions appropriate for Laguna Madre, Galveston Bay and Chesapeake Bay show that reproductive effort decreases with increasing latitude and that the timing of increases in food supply relative to rising temperature becomes more important. Other environmental factors such as low salinity events and turbidity have a lesser effect on population growth and reproduction than do changes in temperature and food supply. The model results suggest that oyster reproductive patterns at higher latitudes (e.g. Chesapeake Bay) are characterized by discrete spawning pulses; continuous spawning becomes more frequent at lower latitudes. The results of this modeling study suggest that the characteristically-wide range of oyster reproductive efforts recorded in the literature may result from seemingly minor changes in the environment.

KEY WORDS: population models, oyster reproduction, spawning

INTRODUCTION

One interesting result from the many studies of oyster reproduction is the frequency with which generalities fail to apply to specific times and locations. For example, although spawning may occur nearly all year, at least at southern latitudes (Wilson et al. 1990, Quick and Mackin 1971), spring and fall spawning pulses are often observed (e.g. Gauthier and Soniat 1989, Butler 1949, Loosanoff and Nomejko 1951, Hayes and Menzel 1981). In some years, however, only one pulse occurs (e.g. Butler 1949, Hopkins et al. 1953). Other times, spawning is most intense in the summer; no spring or fall pulse is observed (e.g. Heffernan et al. 1989). Spawning throughout the summer season with no defined spawning pulse has also been recorded (Ingle 1951, Littlewood and Gordon 1988). In Galveston Bay, as a specific example, the spawning season has variously been observed to be from March to September, May through August, or all year around. Spawning peaks have been recorded in mid-April to mid-June, June to July, or later in the year (Hopkins 1935, Hofstetter 1977, Soniat 1982).

Not surprisingly, given the diversity of observations, the timing and reasons for spawning pulses and the critical temperatures necessary to initiate spawning have been the topic of considerable discussion (e.g. Stauber 1950, Loosanoff and Davis 1953, Butler 1955, and many others). The variability in the observations suggests that important but subtle changes in environmental conditions from one year to the next may produce substantial variations in reproductive effort. For example, oyster filtration rate, which determines the rate of food acquisition, increases with increasing temperature. The amount of ingested food depends on this rate as well as on the ambient food concentration. Both temperature and food supply vary on seasonal and yearly time scales. Furthermore, temperature determines the allocation of oyster net production between somatic and reproductive tissue, with more energy going to reproduction as temperature increases. Thus temporal variations in food supply and temperature combined with the basic physiology of the oyster can potentially produce unexpected and variable patterns in oyster reproductive effort. The importance of interactions between temperature and food supply has been documented for other shellfish (e.g. Ansell 1986), a number of other invertebrates (e.g. Sarma and Rao 1991, Himmelman 1980) and finfish (e.g. Cushing 1990, Lasker 1978). Also, Rand (1973) showed that the interplay of a varying environment and animal physiology can produce the suite of reproductive patterns observed in many animal species.

Under favorable conditions, oysters can become ready to spawn in a very short time, 10 days or less (Loosanoff and Davis 1953). This suggests that many spawnings may be possible per female each year. This possibility is not considered the normal situation, but is in accordance with observations on reproductive effort reported by Davis and Chanley (1956). Rapid development of gonadal material, with a subsequent spawning pulse, suggests that oysters may respond rapidly to strongly varying environmental conditions, particularly temperature and food supply.

Given the above observations, it is reasonable to expect that the juxtaposition of changes in temperature and food supply, such as the timing of the spring and fall phytoplankton blooms relative to the major warming and cooling periods during the spring and fall, respectively, might be crucial determinants of spawning success

during the year. Furthermore, the success of the yearly reproductive effort and the pattern of the spawning sequence (one pulse, two pulses, continuous) may result from the relationship of temperature and food supply during these critical spring and fall feeding periods.

In this modeling study, we examine the likelihood that variations in the seasonal sequence of temperature and food supply are responsible for the observed variations in oyster reproductive effort. In particular, we consider the processes underlying the success or failure of spring, summer and autumn spawning pulses in populations of the American oyster (*Crassostrea virginica*) in a particular habitat, Galveston Bay, Texas. This analysis is then extended to oyster populations in habitats that range from Laguna Madre, Texas to Chesapeake Bay to consider the effect of latitudinal gradients in environmental conditions on reproductive effort and spawning pattern.

THE MODEL

Basic Characteristics

The oyster population model (Fig. 1) is designed to simulate the dynamics of the post-settlement phase of the oyster's life from newly-settled juvenile through adult. Therefore, the oyster's size spectrum was partitioned into 10 size classes (Table 1), which are not equally apportioned across biomass. The lower size limit represents the size at settlement (Dupuy et al. 1977); the upper size limit represents an oyster larger than those normally found in the Gulf of Mexico. The boundaries between size classes 4 and 5, 5 and 6, and 6 and 7 represent size limits that have been used or considered for market-size oysters: 2.5 in, 3.0 in, and 3.5 in, respectively. Adult oysters, those individuals capable of spawning, are defined as individuals weighing more than 0.65 g ash-free dry weight, about 50 mm in length (Hayes and Menzel 1981), although gonadal development has been observed at somewhat smaller sizes (Coe 1936, Burkenroad 1931). Hence, size classes 1 to 3 are juveniles.

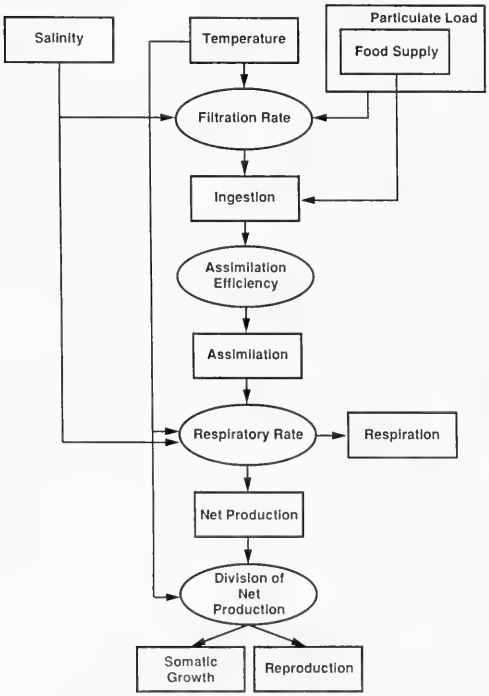


Figure 1. Schematic of the oyster population model.

TABLE 1.

Biomass and length dimensions of the oyster size classes used in the model.

Model Size Class	Biomass g Ash Free Dry Wt	Length mm
1	1.3×10^{-7}–0.028	0.3–25
2	0.028–0.10	25–35
3	0.10–0.39	35–50
4	0.39–0.98	50–63
5	0.98–1.94	63–76
6	1.94–3.53	76–88
7	3.53–5.52	88–100
8	5.52–7.95	100–110
9	7.95–12.93	110–125
10	12.93–25.91	125–150

Biomass is converted to size using the relationship given in White et al. (1988).

The following three conversions and scaling factors were used in the oyster model. For simplicity, these are not explicitly shown in the governing equations that are described in the following section. First, all calculations were done in terms of energy (cal m^{-2}). Oyster caloric content was obtained by applying a caloric conversion of 6100 cal g dry wt^{-1} (Cummins and Wuycheck 1971), to biomass calculated from the biomass-length conversion given in White et al. (1988). This conversion is only an approximation, however, given the range in growth forms found in oysters throughout their latitudinal range. The food available to the oysters was converted to caloric equivalents by using 5168 cal g dry wt^{-1}. Second, gains, losses or transfers of energy (or biomass) between oyster size classes were expressed as specific rates (day^{-1}) which were then applied to the caloric content in a size class. For example, ingestion (cal day^{-1}) divided by a caloric value in cal gives a specific rate (cal day^{-1}/cal = day^{-1}), which is then used to calculate incremental changes in a size class. Finally, because the size classes in the model are not of equal size, transfers between size classes were scaled by the ratio of the average weight of the current size class (in g dry wt or cal) to that of the size class from which energy was being gained or to which energy was being lost:

$$\frac{W_j}{W_{j-1}} \text{ or } \frac{W_j}{W_{j+1}}$$

where W is the median value for biomass (in g dry wt) in size class j. This ensured that the total number of individuals in the simulated population was conserved in the absence of recruitment and mortality. In addition, each specific rate for each transfer between size classes was scaled to the relative size of the respective classes:

$$\text{for transfers up:} \quad W_j/(W_{j+1} - W_j)$$
$$\text{for transfers down:} \quad W_j/(W_j - W_{j-1}).$$

Governing Equation

The change in oyster standing stock with time in each class (O_j) is the result of changes in net production and the addition of individuals from the previous size class or loss to the next largest size class by growth. Excretion was not included since it is a minor component of the oyster's energy budget (Boucher and Boucher-Rodoni 1988). Following White et al. (1988), net production in any size class, NP_j, is the sum of somatic (P_{gj}) and reproductive tissue (P_{rj}) production which is assumed to be the difference between assimilation (A_j) and respiration (R_j):

$$NP_j = P_{gj} + P_{rj} = A_j - R_j. \tag{1}$$

Therefore, a governing equation for each oyster size class can be written as

$$\frac{dO_j}{dt} = P_{gj} + P_{rj} + (\text{gain from } j-1) - (\text{loss to } j+1) \tag{2}$$

for $j = 1, 10$, with $P_{rj} = 0$ for $j = 1, 3$.

Resorption of either gonadal or somatic tissue results in loss of biomass. When $NP_j < 0$, oysters lose biomass and transfer into the next lower size class. This is an important difference between this size class model and a size class model based on linear dimensions: shell size does not change, however biomass does during periods of negative scope for growth. This is the basis for the use of condition index as a measure of health in oysters (e.g. Newell 1985, Wright and Hetzel 1985). To allow for a negative scope for growth, equation (1) must be modified as

$$\frac{dO_j}{dt} = P_{gj} + P_{rj} + (\text{gain from } j-1) - (\text{loss to } j+1) + (\text{gain from } j+1) - (\text{loss to } j-1). \tag{3}$$

The last two terms on the right size of equation (3) represent the individuals losing biomass, and thus, translating down to the next lower size class. Implementation of the model given by equation (3) requires that the processes that result in production and/or loss of somatic and reproductive tissue be described in mathematical terms. The functional relationships used in the model and the rationale for particular choices are given in the following sections.

Filtration Rate, Ingestion and Assimilation

For this model, the filtration rate relationship given by Doering and Oviatt (1986) was adapted to oysters using Hibbert's (1977) biomass-length relationship to obtain filtration rate for each size class as a function of temperature (T) and biomass:

$$FR_j = \frac{L_j^{0.96} T^{0.95}}{2.95} \tag{4}$$

and

$$L_j = W_j^{0.317} 10^{0.669} \tag{5}$$

where filtration rate, FR_j, is given as ml filtered ind^{-1} min^{-1} and length (L_j) is obtained from W_j, the ash-free dry weight in g for each size class. Powell et al. (this issue) show that equations (4) and (5) yield results comparable to a more general equation derived for all bivalves, including oysters, over the size range appropriate for this model. In addition, equation (4) has the advantage of containing the temperature-dependency described in more detail by Loosanoff (1958), an attribute not present in most other filtration rate equations (Doering and Oviatt 1986). Measurements (Loosanoff 1958) suggest that the rate of increase of filtration rate moderates at temperatures above 25°C, in accordance with a general trend for bivalves described by Winter (1970), and declines above 32°C. However, equation (4) yields realistic values throughout the normal temperature range, so it is used in the model without modification for lower filtration rates at even higher temperatures.

Equation (4) was modified to allow for salinity effects on filtration rate as described by Loosanoff (1953). Filtration rate decreases as salinity drops below 7.5‰ and ceases at 3.5‰. In mathematical terms:

$$\begin{aligned} S &\geq 7.5‰ & FR_{aj} &= FR_j \\ 3.5 < S &< 7.5‰ & FR_{aj} &= FR_j(3.5 - S)/4.0 \\ S &\leq 3.5‰ & FR_{aj} &= 0 \end{aligned} \tag{6}$$

where S is the ambient salinity and FR_j is the rate obtained from equation (4).

The reduction in feeding efficiency at high particulate loads, characterized by pseudofeces production, was included as a depression in filtration rate rather than as a separate function as used by Soniat (1982). From data presented in Loosanoff and Tommers (1948), total particulate content can be related to a reduction in filtration rate as

$$\tau = (4.17 \times 10^{-4}) 10^{0.418x} \tag{7}$$

where τ is the total particulate content (inorganic + organic) in g l^{-1} and x is the percent reduction in filtration rate. Solving equation (7) for the percent reduction in filtration rate gives an expression for filtration rate modified by total particulate content, $FR_{\tau j}$, of the form:

$$FR_{\tau j} = FR_{aj}\left[1 - \left(.01\left(\frac{\log_{10}\tau + 3.38}{0.418}\right)\right)\right]. \tag{8}$$

Equation (8), if applied to total particulate content (inorganic + organic), approximates the results of Haven and Morales-Alamo (1966) and limits ingestion rate to approximately the maximum value found by Epifanio and Ewart (1977). Therefore, an additional term to lower ingestion efficiency at high food concentrations was not used. It is assumed that all particles are removed by filtration, a slight overestimate (Palmer and Williams 1980), that oysters feed more or less continuously (Higgins 1980a), and that filtration rate does not vary with food availability (Higgins 1980b, Valenti and Epifanio 1981).

Filtration rate times the ambient food concentration gives oyster ingestion. To the extent that oysters can select nitrogen-rich particles from the filtered material for ingestion, equation (8) yields an underestimate of ingestion (Newell and Jordan 1983). Assimilation is obtained from ingestion using an assimilation efficiency of 0.75, an average value obtained from Tenore and Dunstan (1973), Valenti and Epifanio (1981) and Langefoss and Maurer (1975).

Respiration

Oyster respiration, R_j, as a function of temperature and oyster weight in each size class was obtained from Dame (1972) as

$$R_j = (69.7 + 12.6T) W_j^{b-1} \tag{9}$$

which conforms to the more general relationship for all bivalves obtained by Powell and Stanton (1985).

Salinity effects on oyster respiration over a range of temperatures, were parameterized using data given in Shumway and Koehn (1982) as follows:

$$T < 20°C \quad R_r = 0.007T + 2.099$$

and

$$T \geq 20°C \quad R_r = 0.0915T + 1.324, \tag{10}$$

where R_r is the ratio of respiration at 10‰ to respiration at 20‰: $R_r = R_{10‰}/R_{20‰}$. Equations (9) and (10) were combined to obtain respiration over a range of salinities as:

$$\begin{aligned} S &\geq 15‰ & R_j &= R_j, \\ 10‰ < S &< 15‰ & R_j &= R_j(1 + [((R_r - 1)/5)\ (15 - S)]), \\ S &\leq 10‰ & R_j &= R_jR_r. \end{aligned} \tag{11}$$

Shumway and Koehn (1982) identified effects of salinity on respiration at 20‰; however, we used a 15‰ cutoff to conform to Chanley's (1958) observations on growth.

Reproduction

For adult oysters ($j = 4, 10$), net production was apportioned into growth and reproduction by using a temperature-dependent reproduction efficiency of the form

$$R_{effj} = 0.054T - 0.729 \tag{12}$$

for January to June and

$$R_{effj} = 0.047T - 0.809 \tag{13}$$

for July to December. Equations (12) and (13) were derived empirically from the field observations of Soniat and Ray (1985). Disagreement exists in the literature concerning the extent to which oyster reproduction is temperature acclimatized (Loosanoff and Davis 1953, Stauber 1950, Loosanoff 1969). However, from the studies of Butler (1955), Kaufman (1979) and Quick and Mackin (1971), acclimatization appears unimportant over the latitudinal range of Chesapeake Bay to the southern Gulf of Mexico. Equations (12) and (13) may not hold north of Delaware Bay.

The portion of new production that goes to reproduction is given by

$$P_{rj} = R_{effj}NP_j, \quad \text{for } j = 4, 10. \tag{14}$$

Somatic growth is the remaining fraction. In cases where $NP_j < 0$, it is assumed that preferential resorption of gonadal tissue occurs to cover the debt, although some data suggest the contrary (Pipe 1985). Gonadal resorption is commonly observed in stressed oysters (e.g. Gennette and Morey 1971) and in the fall and winter when food is reduced (Kennedy and Battle 1964). For juveniles and adults with no gonadal tissue, resorption of somatic tissue occurs. It is further assumed that reduced reproduction at low salinity (Engle 1947, Butler 1949) results from decreased filtration rate and increased respiratory rate and so no specific relationship is included for this effect.

Although a considerable literature exists on factors controlling the initiation of spawning (e.g. Loosanoff 1965, Stauber 1950, Dupuy et al. 1977), including empirical temperature-dependent relationships (Kaufman 1979, Loosanoff and Davis 1953), little is understood about factors controlling the frequency of spawning over the entire spawning season (e.g. Davis and Chanley 1956). In the model, spawning occurs when the cumulative reproductive biomass of a size class exceeds 20% of the standing stock; an estimate based on data presented in Gallager and Mann (1986) and Choi et al. (in press).

Model Implementation and Environmental Forcing

The model described by equation (3) was solved numerically using an implicit (Crank-Nicolson) tridiagonal solution technique with a one day time step. All simulations were initialized with settlement of a cohort of 10 individuals on Julian day 140 (about mid-May). No recruitment or mortality was allowed. The external forcing for the model is from time series that specify ambient temperature, salinity, food concentration and turbidity conditions. Each simulation was run for 6 yr which is sufficient time for transient adjustments to disappear and for the oyster population to reach an equilibrium in response to a given set of environmental conditions.

Numerous simulations (not shown) were performed initially using real and idealized time series for the environmental variables. These simulations, some of which are reported by Powell et al. (this issue) and Hofmann et al. (submitted), were used to calibrate and verify the transfers between size classes and the overall population characteristics and to provide guidance as to model sensitivity to various parameters. These simulations demonstrated that temperature and food concentration had more of an effect on the structure and character of the simulated oyster populations than variations (i.e. ±10%) in individual model parameters. It should be noted that all of the parameters in the model are specified from either field or laboratory measurements; no free parameters need to be empirically determined. Therefore, the focus of this modeling study is on variations in environmental forcing. Other applications of this model have focused on growth and characteristic adult oyster size in Galveston Bay (Powell et al. this issue) and growth, characteristic adult size and reproductive effort in several bays and estuaries (Klinck et al. 1992, Powell et al. this issue, Hofmann et al. submitted).

The simulations described in the following sections used observed monthly-averaged time series of temperature of two years length from Galveston Bay (Soniat and Ray 1985), the Laguna Madre (Powell et al. in press) and Chesapeake Bay (Galtsoff et al. 1947). The temperature values were linearly interpolated to obtain values at one day intervals to be consistent with the time step used in the model. For a six-year simulation, the two-year temperature time series was repeated three times.

For most of the simulations described in the following section, salinity values were held constant at 24‰, which is an optimal salinity for oyster physiology, to simplify the discrimination between salinity and temperature effects. For some Galveston Bay simulations, a low salinity (7‰) event was imposed and one Chesapeake Bay simulation used the salinity time series given in Galtsoff et al. (1947).

In general, food supply is higher in summer than in winter and higher under bloom conditions than normal summer levels. This general trend was used to develop an idealized time-varying food supply. Except where noted, each simulation used 5 winter months with a food ration of 0.5 mg l^{-1} and 3 or 5 summer months of 0.75 mg l^{-1}. Spring and fall phytoplankton blooms, each of 2 months duration at 1.25 mg l^{-1} were then imposed on this background food supply. Some simulations have only a spring or fall bloom and 5 normal summer months; others contain both blooms and only 3 normal summer months. All simulations with a single bloom had the same total food available during the year; only the timing of the blooms changed. All simulations having two blooms

received a higher food supply (0.5 mg l^{-1} higher for 2 months). With this exception, variations in food supply were determined by the timing of the blooms. Some of the Chesapeake Bay simulations used the food time series presented in Berg and Newell (1988) as well as their values for turbidity. A summary of the environmental conditions used for each simulation is given in Table 2.

RESULTS

Approach

The oyster population simulations are presented in three parts. The first set of simulations considers the effects of environmental variations in temperature and food supply on the pattern and frequency of spawning in oyster populations in Galveston Bay, Texas, a typical temperate latitude bay. The second set of simulations considers the effect of latitudinal variations in temperature, with different food supply conditions, on the patterns and frequency of spawning. Finally, the effects of other environmental factors, such as salinity and turbidity, on oyster reproduction and spawning are considered.

Galveston Bay

Spring Bloom Response

Seasonal temperature variations in Galveston Bay (Fig. 2ii) are characterized by a temperature increase from March to May and a decrease that begins in October and extends through January. Maximum summer temperatures are 28° to 30°C; minimum winter temperatures are about 10°C. Overall the second year of the 2-yr temperature sequence shown in Fig. 2ii averaged somewhat warmer (19.8°C vs. 21.8°C) over most of the year. The spring temperature increase was also earlier (March vs. April) in the second year.

A spring bloom that occurs in March–April coincides with the spring increase in temperature (Fig. 2ii). The simulated oyster population that results when the spring temperature and food increases are in phase (Fig. 2) will be taken to be the reference simulation to which other simulated populations that result from different environmental forcings are compared. When describing the results of subsequent simulations, only differences or exceptions to the trends in the reference simulation will be noted. The reference simulation is described by the following characteristics.

The time development of the oyster population over the six year simulation is shown in Figure 2i. Initially all individuals are in the smallest size class and during the first two years of the simulation these individuals grow to the larger size classes. At the end of the simulation, the population has stabilized with the majority of the individuals in size classes 6 and 7.

Reproductive effort, defined as the total number of calories spawned (eggs and sperm released), is larger in the first two years of the simulation when the oyster population is of smaller average size (Fig. 2i, iii). As the population stabilizes with larger size individuals in the last three years of the simulation, reproductive effort declines (Fig. 2iii). In the latter part of the simulation, years 5 and 6, the largest 10% of the population (size classes 8–10) accounts for most of the spawning effort (Fig. 2, Table 3); however, this is largely an artifact of the use of biomass as a measure of individual size, rather than some linear dimension. Individuals ready to spawn will have a higher biomass. Individuals already spawned or not yet in spawning condition will weigh less and will likely be found in a lower size category.

Reproductive effort in the second, fourth and sixth years (Fig. 2ii, iii) is higher because the spring increase in temperature occurs earlier and temperatures are warmer throughout the summer. Therefore, the rate of food acquisition is higher at the same level of food supply because filtration rates are faster at higher temperatures.

TABLE 2.

Summary of environmental conditions used for the oyster population simulations.

Area	Temperature	Salinity	Spring Bloom	Fall Bloom	Turbidity	Figure
Galveston Bay	V	24	March–April	N	N	2
Galveston Bay	V	24	May–June	N	N	3
Galveston Bay	V	24	N	August–September	N	4
Galveston Bay	V	24	March–April	August–September	N	5
Galveston Bay	V	24	April–May	September–October	N	6
Laguna Madre	V	24	March–April	N	N	7
Laguna Madre	V	24	N	August–September	N	8
Laguna Madre	V	24	March–April	August–September	N	9
Chesapeake Bay	V	24	March–April	N	N	10
Chesapeake Bay	V	24	May–June	N	N	11
Chesapeake Bay	V	24	April–May	September–October	N	12
Galveston Bay	V	March–April 7	March–April	August–September	N	13
Galveston Bay	V	August–September 7	March–April	August–September	N	14
Chesapeake Bay	V	V	B&N	B&N	V	15

The figure displaying the results for each set of environmental conditions is indicated. For all simulations, a time-varying temperature regime (V) was imposed. For simulations that used a constant salinity (‰), the value is given. All simulations, except those shown in Figure 15, use time-varying food distributions that include a spring or a fall phytoplankton bloom or both. The months in which blooms were imposed are indicated for each simulation. For the Galveston Bay simulations shown in Figures 13 and 14, a period of low salinity was imposed; the time of the event and salinity (‰) are indicated. The Chesapeake Bay simulations shown in Figure 15 used time-varying food concentrations and turbidity levels given in Berg and Newell (1986) designated as B&N. Exclusion of an environmental variable is denoted by N.

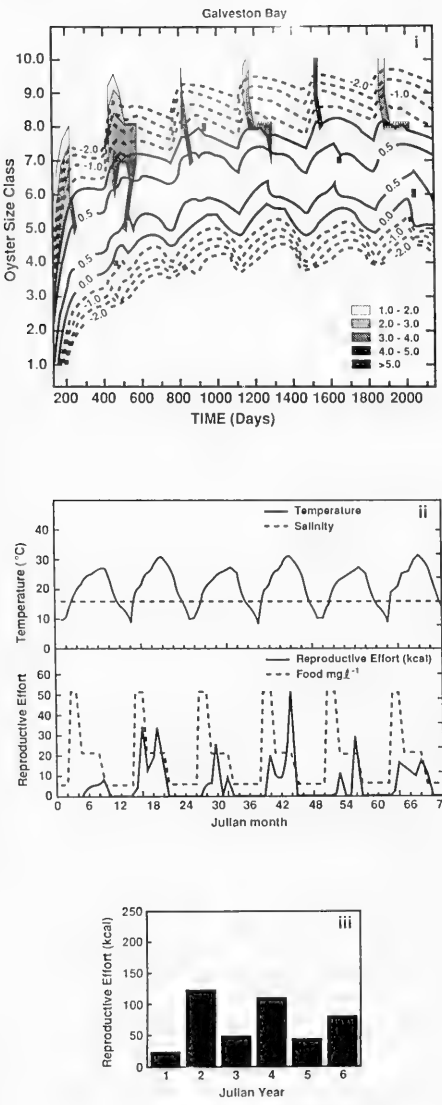


Figure 2. The effect of a March–April spring bloom on population growth rate, size frequency, spawning pattern and reproductive effort in Galveston Bay. The simulation was initiated with the recruitment of a cohort of 10 individuals on day 140 into size class 1. i) Simulated population distribution of oyster size class versus time in Julian days beginning January 1 of year 1 (day 1). Isolines for the number of individuals are the logarithms of the number of oysters ($\log_{10} N$). Hence, the zero contour corresponds to one individual. Population concentrations less than this are indicated by dashed lines; solid lines are population values greater than one individual. Shading for the amount of spawn production is the logarithm of cal ($\log_{10}$ cal) with the darkest shades corresponding to the highest values. Contour interval is 0.5 for the number of individuals m^{-2} and 1.0 for spawn production. Numbers of individuals or calories are plotted opposite the size class designations, not halfway between; hence on day 140 all individuals are in size class 1 opposite the grid mark labeled 1. ii) Temperature, salinity and food time series used for the simulation and the simulated total reproductive effort (kcal spawned) for each month. iii) Integrated population reproductive effort for each model year.

The spawning season is longer in the initial portion of the simulation (years 1 and 2) when the population consists primarily of smaller sized oysters (Fig. 2ii). In the latter part of the simulation, the spawning season extended from March through September (Table 3), with maximum spawning occurring in the late summer. Throughout the spawning season, spawning tends to be mostly continuous, on a monthly averaged basis, but distinct spawning pulses, defined by months of relatively higher reproductive effort, also occur (Fig. 2i, ii, Table 3). The magnitude of the spawning pulses becomes less pronounced as the population ages and increases in size. Higher ingestion rates in the warmer years (2, 4, 6) permit a larger fall spawning pulse, following nearly continuous spawning during the summer. Lower ingestion rates in the cooler years (3 and 5—most of the population is not adult in year 1) result in two, more discrete spawning pulses per year.

The fall spawning pulse tends to be larger even with a single phytoplankton bloom in the spring (Fig. 2ii, Table 3). Reduced temperatures in the spring result in a relatively high portion of the spring bloom going to somatic growth rather than reproductive tissue. Consequently, spring and early summer spawning pulses are reduced. Fall spawning pulses are more intense even without a fall bloom because average summer food supplies and temperature-dependent filtration rates are higher.

The timing of spawning pulses is variable, with spawning pulses typically occurring in April–May or May–June and August. The spring pulse occurs about one month or more after the start of the spring bloom. Years with warmer spring temperatures are characterized by an earlier spring spawning pulse in April to May and, in those years, younger oysters tend to spawn earlier.

Delaying the spring bloom to April and May places it just after the spring increase in temperature. As in the previous simulation, smaller oysters have a higher reproductive effort, the spawning pulses become less pronounced with increased size, and warmer years permit a higher reproductive effort. However, unlike the previous simulation, the spawning season is longer (Table 3) so that the total reproductive effort for the 6-yr simulation is about 28% higher. Spawning tends to be continuous throughout the spawning season (Table 3), but several spawning pulses do occur in the summer and late fall. The timing of the spring pulse varies, but usually occurs in May or June.

Delaying the spring bloom one month further (May–June) places it considerably after the temperature increase in the spring (Fig. 3ii). This simulation corresponds to measurements made by Soniat et al. (1984) who observed the highest food concentrations in Galveston Bay during this time. Total reproductive effort (Fig. 3iii) is higher than the two previous cases (40.2% and 16.9%, respectively). The spawning season remains about the same length (Table 3) but occurs one month later (May–October). Spawning continues throughout the spawning season, but intense spawning pulses tend to occur in June, sometimes July, and in August. The timing of the pulses varies considerably from year to year, but the

TABLE 3.

Summary of spawning characteristics and patterns from the fifth and sixth years of the Galveston Bay, Laguna Madre and Chesapeake Bay simulations.

Location	Bloom Time	Maximum Spawn		Spawning Pattern		Spawning Season		Spawn Size Class–Sp or Su		Spawn Size Class–Fall	
		Yr 5	Yr 6	Yr 5	Yr 6	Yr 5	Yr 6	Yr 5	Yr 6	Yr 5	Yr 6
Galveston Bay	March–April	Aug	Aug	Sp, F	CP	May–Aug	March–Sept	8–10	8–10	7	6, 8
Galveston Bay	April–May	Aug	April	Sp, Su, F	CP	April–Nov	April–Nov	8–10	6, 8–10	6, 8	6, 8
Galveston Bay	May–June	July	June	Su	Su, F	May–Sept	May–Sept	8–10	7–10	5, 7, 8	5, 7, 8
Galveston Bay	Aug–Sept	Sept	Aug	F	F	July–Oct	June–Sept	N	N	5, 8–10	6–10
Galveston Bay	March–April Aug–Sept	Sept	Aug	C	C	Aug–Oct	April–Oct	N	6, 7, 9	6–10	6, 8–10
Galveston Bay	April–May Sept–Oct	Oct	Sept	Sp, F	CP	May–Dec	April–Nov	8, 9	6, 8, 9	6, 8–10	6, 8–10
Laguna Madre	March–April	April	April	CP	CP	March–Sept	March–Sept	5, 7–10	5, 7–10	5, 7–10	7, 8
Laguna Madre	April–May	May	May	CP	CP	April–Sept	April–Sept	5, 8–10	8–10	5, 8	5, 8
Laguna Madre	Aug–Sept	Sept	Aug	CP	C	May–Oct	May–Oct	7, 8	7	5, 8–10	5, 7–10
Laguna Madre	Sept–Oct	Sept	Sept	CP	CP	June–Nov	June–Dec	N	N	5, 6 8–10	5, 6 8–10
Laguna Madre	March–April Aug–Sept	Oct	Aug	CP	CP	March–Oct	March–Oct	6, 8–10	6, 8–10	6, 8–10	6, 8–10
Chesapeake Bay	March–April	June	Aug	Su	F	June–Oct	Aug–Sept	N	N	5, 7	7
Chesapeake Bay	April–May	July	Aug	Sp, F	Sp, F	May–Oct	May–Sept	8–10	8–10	6–10	5, 7, 8
Chesapeake Bay	May–June	June	June	Su, F	CP	May–Oct	May–Aug	7–10	8–10	6	6–9
Chesapeake Bay	March–April Aug–Sept	Sept	Sept	Sp, Su, F	Su, F	May–Oct	June–Dec	7, 8, 9	8	5	5, 6, 8

Continuous spawning is denoted by C; continuous spawning with discrete pulses is denoted by CP. Spring, summer and fall spawning pulses are indicated by Sp, Su, and F, respectfully. Times when no size class spawns are indicated by N.

trend is for the spring–early summer pulse to be distinctly larger than the fall pulse. The late spring bloom results in spawning pulses that are more pronounced each year than those that result from blooms earlier in the year.

Overall delaying the spring bloom into the year results in a larger reproductive effort, a switch from a stronger fall spawning pulse to a stronger spring–early summer spawning pulse, the more frequent occurrence of two or more spawning pulses, more pronounced spawning pulses throughout the continuous spawning season characteristic of the latitude, and a longer reproductive season.

Fall Bloom Response

A fall bloom (Fig. 4, Table 2) results in an overall reproductive effort that is nearly identical (120 kcal $6yr^{-1}$ vs. 128 kcal $6yr^{-1}$) to that obtained from a late spring bloom. One strong fall spawning pulse occurs, however, amongst continuous low level spawning throughout most of the summer season (Fig. 4i,ii, Table 3). Relative to the spring bloom simulations, the reproductive season is delayed and shortened; July–October in the colder year, June–September in the warmer year. Significant reproductive development begins 2 months prior to the first spawning event, but low ingestion rate (caused by a low food supply) slows gonadal development.

Spring and Fall Bloom Response

The inclusion of both a spring (March–April) and fall (August–September) bloom (Fig. 5ii) introduces an increase in food supply as well as a change in the distribution of food throughout the year. Two blooms result in increased reproductive effort (143 kcal $6yr^{-1}$; Fig. 5iii) relative to the single bloom simulations. The general characteristics of the oyster population are similar to those obtained for the single bloom conditions (Fig. 5i). The spawning season extends from April to October and is characterized by a meager spring pulse in warm years, and a strong fall spawning pulse in each year (Fig. 5ii, Table 3). Fall spawning ceases with the end of the fall bloom.

If the spring and fall blooms are delayed by one month, total reproductive effort (Fig. 6iii) is similar to the previous case, but the distribution of the effort throughout the spawning season changes markedly. Reproductive effort in the spring increases, whereas that in the fall decreases relative to the earlier double bloom (Fig. 5i, ii vs. Fig. 6i, ii). The September–October bloom extends into the fall period when temperatures markedly decline. Spawning effort drops prior to the termination of the fall bloom in response to the colder temperatures. In contrast, spawning effort associated with the earlier August–September bloom in the previous simulation decreases as the bloom decays. Decreasing food supply stops spawning in this case.

Latitudinal Temperature Effects

Laguna Madre

The Laguna Madre is located south of Galveston Bay (26°N vs. 29°N) on the Texas coast. Therefore, the seasonal temperatures in the Laguna Madre, particularly during the winter, average warmer than in Galveston Bay (Fig. 7ii). Spring warming occurs about one month earlier (March vs. April); fall cooling about one-half month later (late November). A March–April phytoplankton bloom which coincides with the spring warming in the Laguna Madre results in the stimulated oyster distributions shown in Figure 7i. In

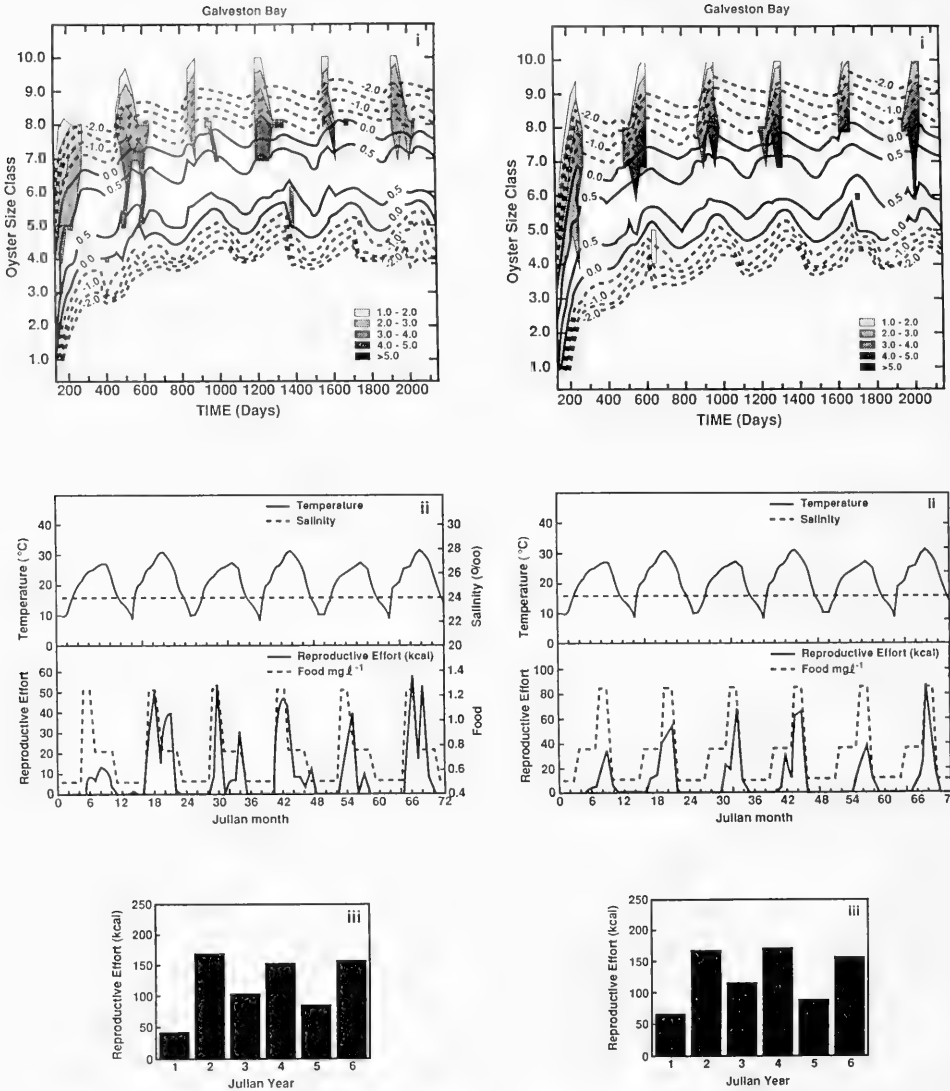


Figure 3. The effect of a May–June spring bloom on population growth rate, size frequency, spawning pattern and reproductive effort in Galveston Bay. Otherwise same as Figure 2.

Figure 4. The effect of an August–September fall bloom on population growth rate, size frequency, spawning pattern and reproductive effort in Galveston Bay. Otherwise same as Figure 2.

comparison to the equivalent for Galveston Bay (April–May bloom), total reproductive effort is somewhat higher (110 kcal $6yr^{-1}$ vs. 100 kcal $6yr^{-1}$) and the decline in reproductive effort with increasing size (Fig. 7i) is less pronounced. Unlike Galveston Bay, the spawning season in Laguna Madre is longer (Table 3) and smaller-sized animals contribute more to the total reproductive effort. However, the pattern of spawning is similar; spawning is essentially continuous with stronger spawning pulses occurring during a few summer months.

Delaying the spring bloom in the Laguna Madre by one month (April–May) produces a pattern similar to the equivalent case for Galveston Bay, where the spring bloom occurs one month after the spring rise in temperatures (Fig. 4, May–June). Relative to the earlier spring bloom in the Laguna Madre, reproductive effort is

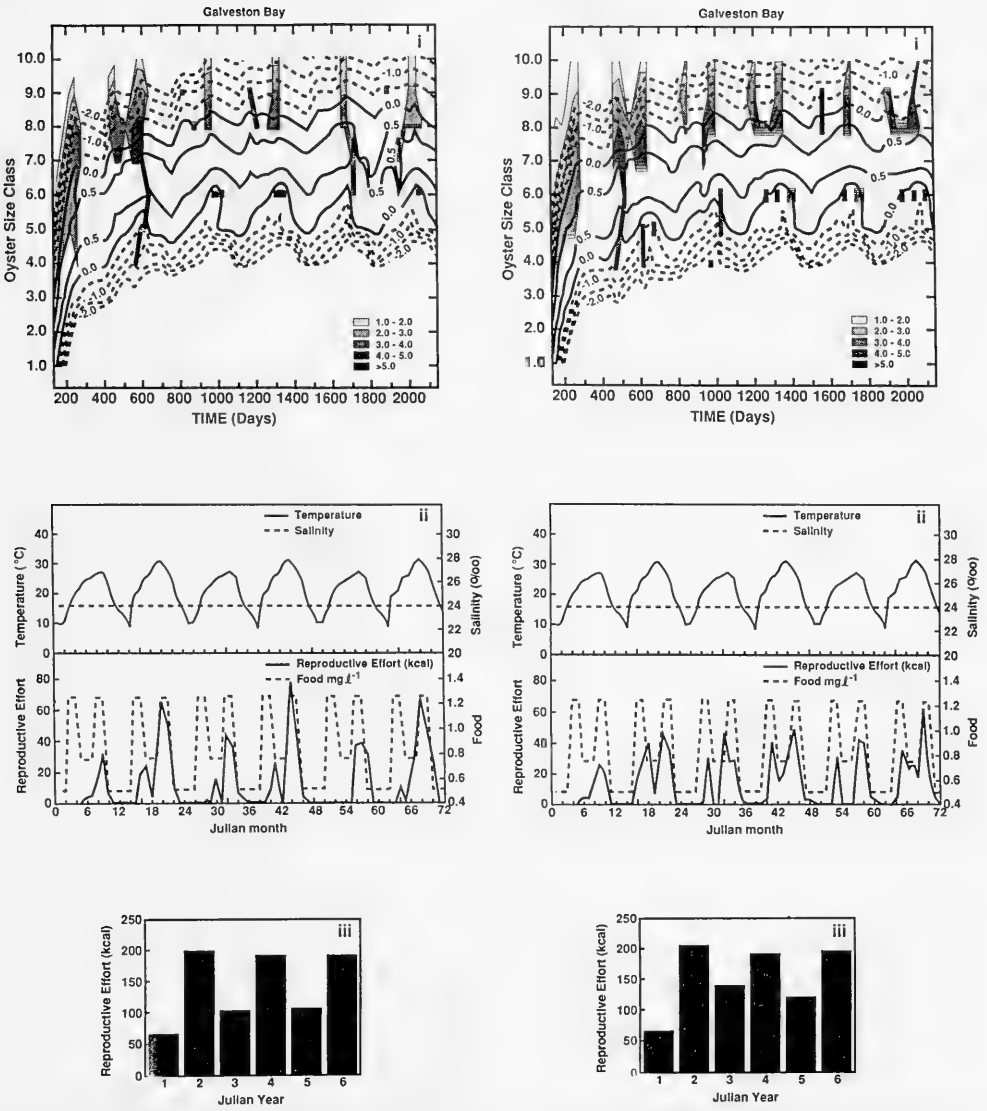


Figure 5. The effect of a spring and fall bloom on population growth rate, size frequency, spawning pattern and reproductive effort in Galveston Bay. Otherwise same as Figure 2.

Figure 6. The effect of delaying the spring and fall blooms by one month on population growth rate, size frequency, spawning pattern and reproductive effort in Galveston Bay. Otherwise same as Figure 2.

higher (13%), but the spawning season is essentially unchanged (Table 3). The spawning pattern is still characterized by continuous spawning throughout the season with discrete peaks in the spring and fall. Unlike Galveston Bay, the drop in reproductive effort at large oyster size is less pronounced. Overall, when compared to Galveston Bay, the same trends occur, albeit one month earlier, reproductive effort is larger, and spawning is more continuous throughout the spawning season.

A fall phytoplankton bloom in the Laguna Madre (Fig. 8) results in patterns similar to those for the spring bloom except that higher temperatures permit a spring spawning pulse with just the normal rise in food supply to higher summer levels and spawning

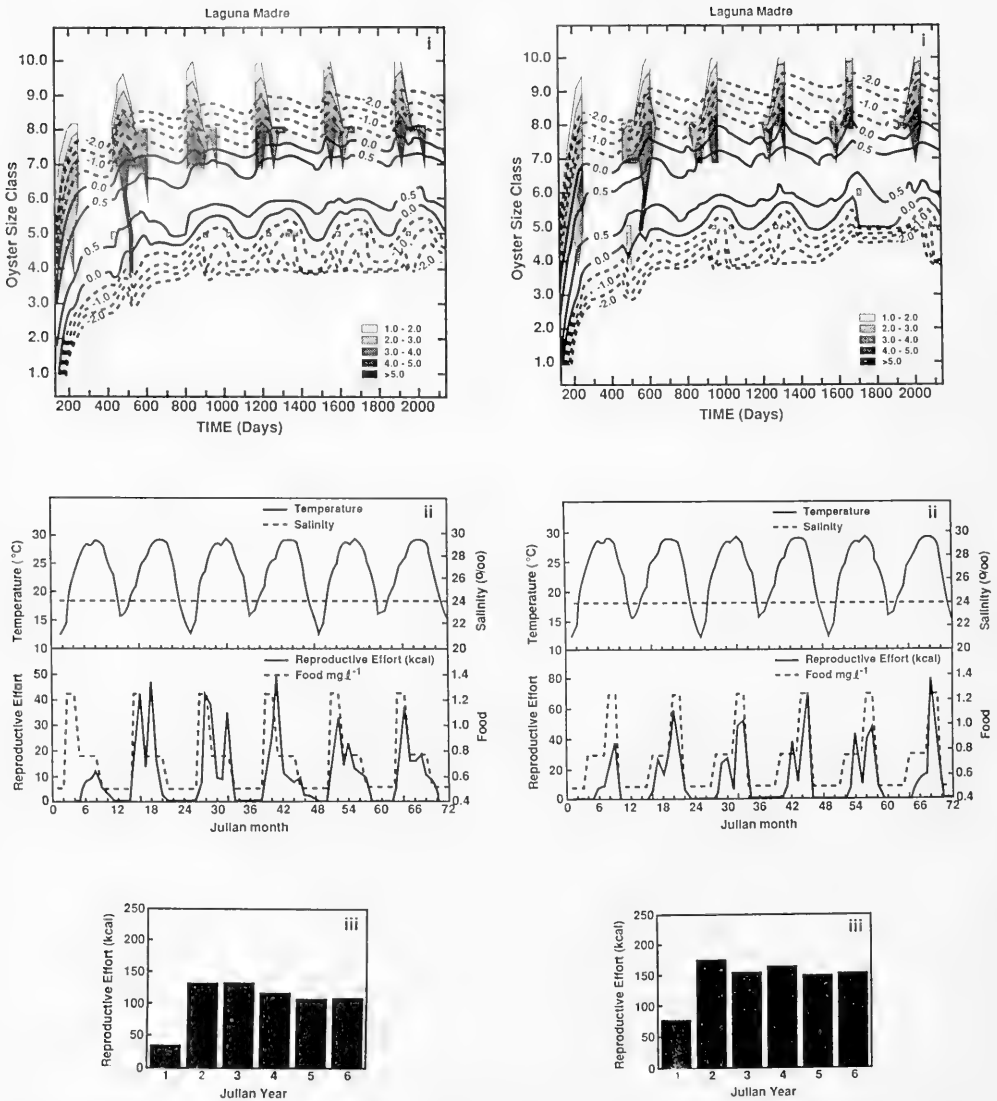


Figure 7. The effect of a spring bloom (March–April) on population growth rate, size frequency, spawning pattern and reproductive effort in Laguna Madre. Otherwise same as Figure 2.

Figure 8. The effect of a fall bloom (August–September) on population growth rate, size frequency, spawning pattern and reproductive effort in Laguna Madre. Otherwise same as Figure 2.

continues into the late fall. Delaying the bloom by one month (September–October) results in lower reproductive effort, spawning into November (Table 3), and a distinct decrease in reproductive effort with increased oyster size. As in Galveston Bay, termination of the spawning season is produced by decreased food upply in the case of the August–September bloom and lower mperature in the case of the September–October bloom. For the later fall bloom, spawning declines before food supplies are exhausted. A spring and a fall bloom in the Laguna Madre (Fig. 9) produces a spawning pattern similar to that observed in Galveston Bay oyster populations that experience the double bloom one month later (Fig. 6). The one month offset is roughly equivalent to the offset in the spring rise in temperature between the two bays. However, in the Laguna Madre, overall reproductive effort is

higher (22%) and spawning is more continuous than in Galveston Bay (Fig. 9 vs. Fig. 6, Table 3).

Chesapeake Bay

The seasonal temperature cycle in Chesapeake Bay, a northern (30°N) temperate bay, is characterized by warming in April and May and cooling in October (Fig. 10ii). Overall, the trend in the occurrence and variation in timing of spawning pulses with changing bloom conditions in Chesapeake Bay (Fig. 10i) resembles that of Galveston Bay and Laguna Madre. However, when the spring bloom in Chesapeake Bay precedes the spring temperature increase, spawning is essentially limited to a fall spawning pulse (Fig. 10i, ii). As the spring bloom is delayed into the year and becomes more aligned with warmer temperatures (Fig. 11), the spring pulse becomes stronger and reproductive effort increases. However, even with the development of a spring spawning pulse, oyster populations in Chesapeake Bay have significantly lower reproductive effort, more discrete spawning pulses, and a shorter reproductive season than oyster populations in Galveston Bay (Table 3).

An early spring and fall bloom in Chesapeake Bay (Fig. 12ii) results in lower reproductive effort than a single late spring bloom (Fig. 11iii vs. 12iii). Food supply increases in the spring before temperature permits reproductive development. Increased food supply in the fall extends into the time of decreasing temperature, which reduces oyster filtration rate. As a result, a portion of the spring and fall bloom cannot be used for reproduction. Though reproductive effort is lower in Chesapeake Bay than in Galveston Bay, oyster size is larger because the lower temperatures result in more energy going into somatic growth. Also the spawning pattern of the Chesapeake Bay oyster population is characterized by 2 or 3 distinct spikes throughout the spawning season, rather than the nearly continuous spawning that characterizes Galveston Bay oyster populations (Table 3).

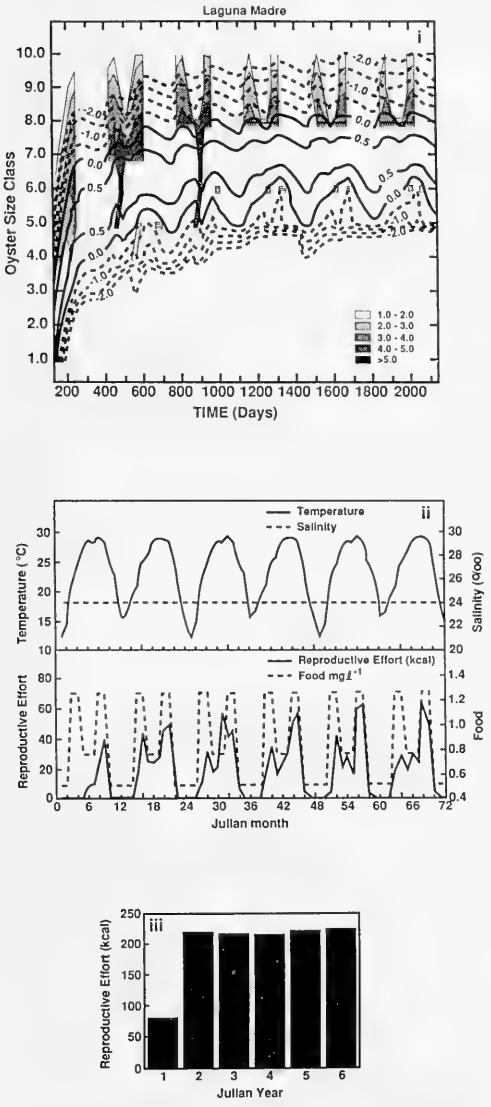


Figure 9. The effect of a spring and fall bloom on population growth rate, size frequency, spawning pattern and reproductive effort in Laguna Madre. Otherwise same as Figure 2.

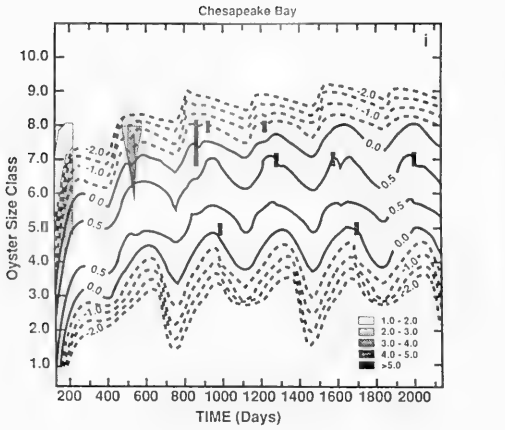
Other Environmental Factors

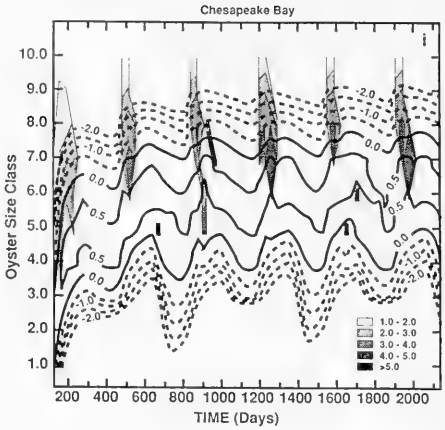
Salinity

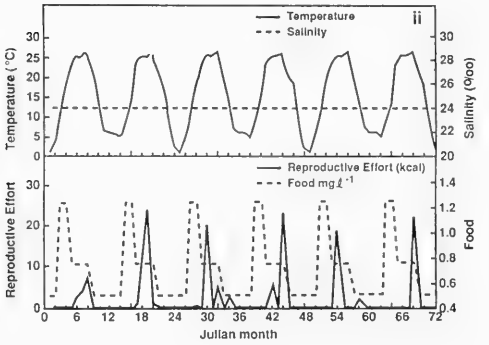
Oyster filtration [equation (6)] and respiration [equation (11)] rates are reduced at low salinities. Hence, low salinity events can potentially alter the size class structure and reproductive capacity of oyster populations. To test the effect of low salinity events, Galveston Bay oyster populations were exposed to salinities of 7‰ for 2-month periods (Table 2) that coincided with the spring bloom (Fig. 13) or fall bloom (Fig. 14). In each simulation, the low salinity event had a minimal effect on the oyster population structure and reproductive effort was reduced only slightly relative to the corresponding constant high salinity simulations (cf. Fig. 5). As a comparison, Butler (1949) found significant effects only at even lower (e.g. 3‰) salinities. Overall, a large change in salinity has considerably less effect on the oyster populations than does a small change in temperature or food supply.

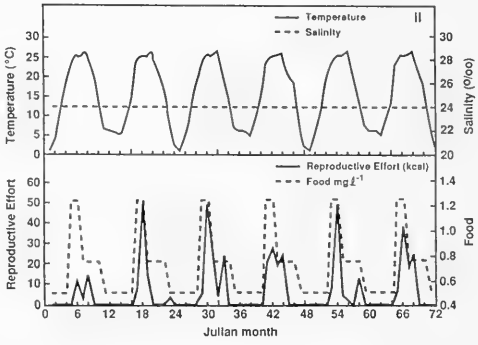
Turbidity

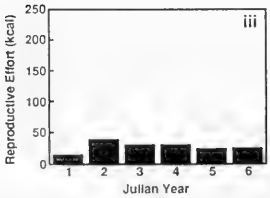
Increased turbidity decreases oyster filtration rate [equations (7) and (8)]; consequently, turbidity is another environmental variable that can potentially affect reproductive effort. To examine the effect of turbidity, we used a time series from Chesapeake Bay (Berg and Newell 1986) that contained turbidity maxima coincident with the spring and fall maxima in food supply (Fig. 15ii). The primary effect of turbidity is to reduce food supply. Lower food supply reduces the adult size of the oyster population from size class 8 and 9 for the no-turbidity case (not shown) to size class 7. Turbidity, through its effect on filtration rate, also reduces population reproductive effort by about one-half. Spawning pulses become less discrete when turbidity is included, but they continue to coincide with the spring and fall phytoplankton blooms as they do in the simulation lacking turbidity. Hence, the spawning patterns do not change, only the magnitude.











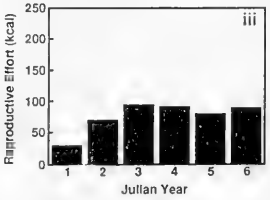


Figure 10. The effect of a spring bloom (March–April) on population growth rate, size frequency, spawning pattern and reproductive effort in Chesapeake Bay. Otherwise same as Figure 2.

Figure 11. The effect of a delayed spring bloom (May–June) on population growth rate, size frequency, spawning pattern and reproductive effort in Chesapeake Bay. Otherwise same as Figure 2.

DISCUSSION

Perspective

Comparison and/or verification of the simulations shown in Figures 2–15 with observations reported in the literature can only be made on the coarsest level. No studies exist that simultaneously document physical parameters, food supply and reproductive effort in oysters. Therefore, we present a discussion of the characteristics and trends in the temporal pattern of reproductive effort and the latitudinal variability in reproductive effort in oysters as suggested by the simulated populations. The discussion, then, focuses on expectations rather than on observations. Rigorous tests

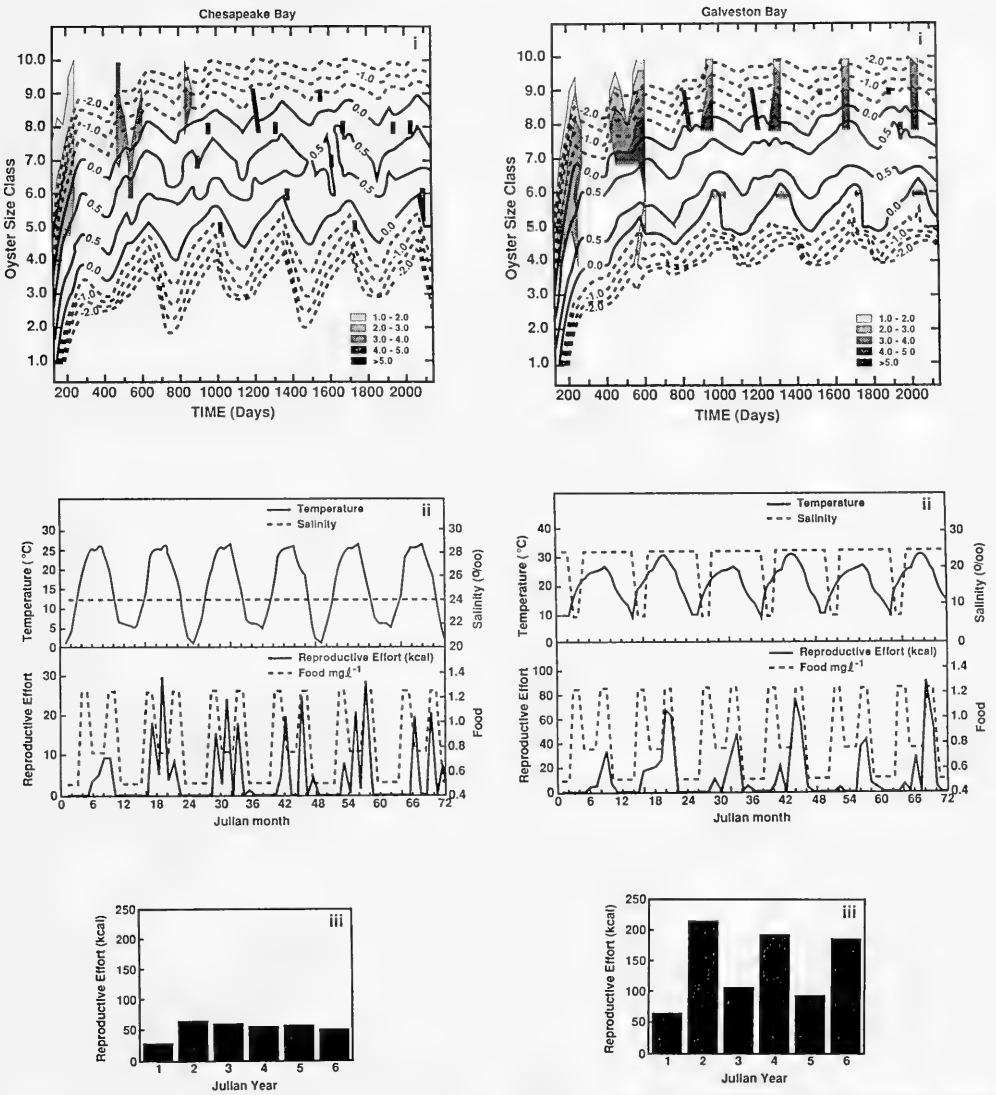


Figure 12. The effect of a spring and fall bloom on population growth rate, size frequency, spawning pattern and reproductive effort in Chesapeake Bay. Otherwise same as Figure 2.

Figure 13. The effect of lowered salinity coincident with the spring bloom on population growth rate, size frequency, spawning pattern and reproductive effort in Galveston Bay. Otherwise same as Figure 2.

of the accuracy of the simulated oyster populations must await availability of adequate data sets.

Timing of Spawning Pulses

Little information is available on the factors determining the timing of spawning pulses for oyster populations. Early studies by Nelson (1955), Prytherch (1929) and others emphasized the importance of food supply as well as temperature in oyster reproduction. The simulated oyster populations suggest three factors that are of crucial importance in oyster reproduction: food supply, the timing of food supply, and the timing of temperature change. In particular, the reproductive effort depends critically on the timing of increases in food supply relative to increases or decreases in temperature.

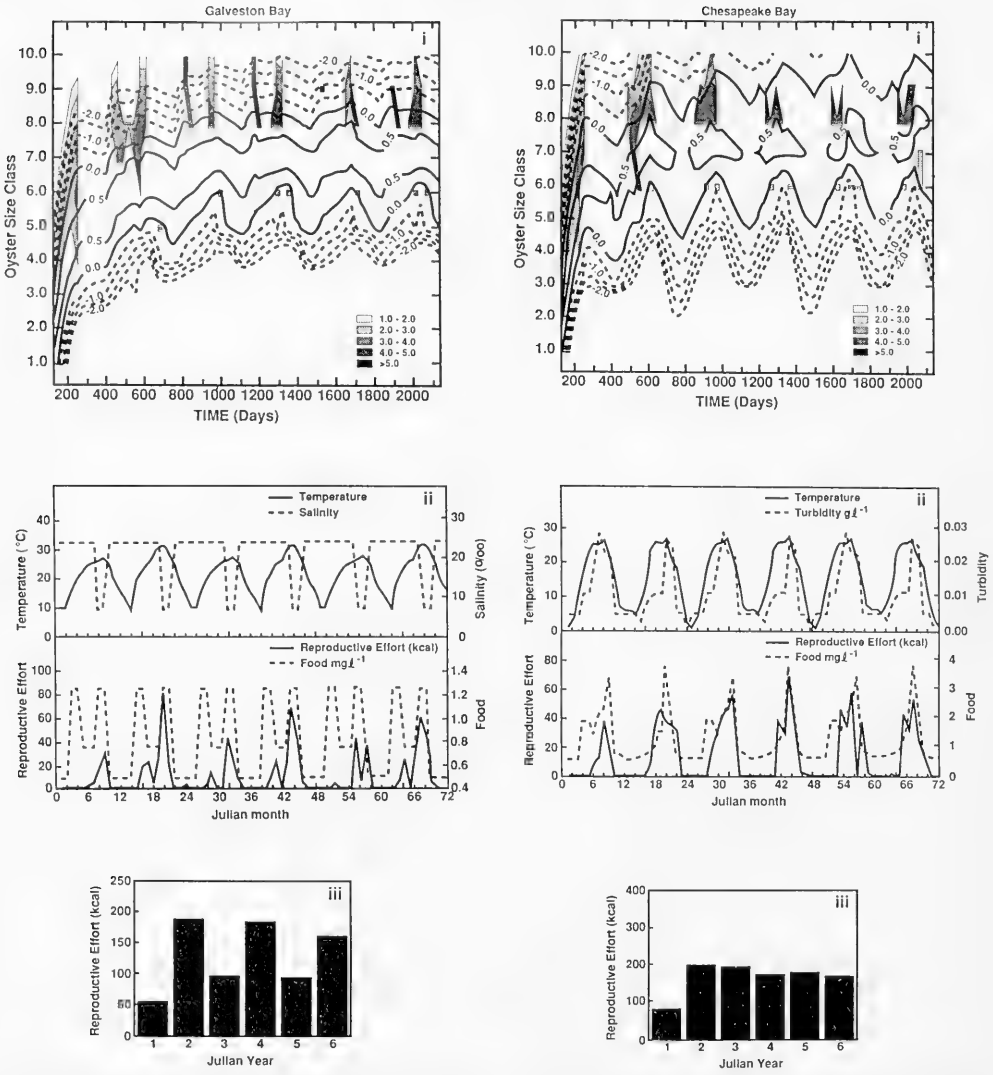


Figure 14. The effect of lowered salinity coincident with the fall bloom on population growth rate, size frequency, spawning pattern and reproductive effort in Galveston Bay. Otherwise same as Figure 2.

Figure 15. The effect of turbidity on population growth rate, size frequency, spawning pattern and reproductive effort in Chesapeake Bay. Otherwise same as Figure 2.

As the phytoplankton bloom translates through the year from early spring to late fall, the pattern of spawning changes. When the spring bloom precedes the spring temperature increase, a strong fall spawning pulse occurs because reproductive effort depends solely on the generally higher levels of food characteristic of summer months. As the spring bloom occurs later and aligns with the spring increase in temperature, the spring spawning pulse becomes larger and reproductive effort increases because the warmer temperatures result in more of the food supply going to the growth of reproductive tissue. The classic spring and fall spawning pulses begin to occur. Moving the bloom still later into the year increases the strength of the fall spawning pulse while retaining the strong spring pulse. Two strong spawning pulses, one in the spring-summer and one in the fall, normally require two food signals during the year. No data are available to corroborate these expectations.

Many factors facilitate the occurrence of a fall spawning pulse, whereas the careful coincidence of several factors is required to realize a spring spawning pulse. To have a strong spring pulse, the spring bloom must occur just after the temperature increase and the population must contain mostly small individuals. Larger animals may require too long to prepare for spawning even when food supplies are high. Accordingly, spring pulses should occur much more rarely than pulses in the summer and fall in oyster populations. Pollard (1973) provides observations from Louisiana oyster populations that support this expectation.

In cases where two strong spawning pulses occur, the fall spawn generally, but not always, is larger. A larger fall reproductive effort accrues from a longer time at high temperatures permitting a greater acquisition of food to support the spawning effort. Heavier sets in the fall noted in Galveston Bay by Quast et al. (1988) probably result from this phenomenon. Kennedy and Krantz (1982) suggested that variability in the timing of spawning pulses was due to year-to-year variations in food supply. The simulated oyster populations show that the specific timing of spawning pulses can be quite variable, especially if the reproductive potential of the oyster population is weakened by slight offsets between the timing of the spring temperature increase and the occurrence of the spring bloom. Delays in spawning of a month or more are easily achieved by small misalignments in the timing of environmental conditions. Knowing food supply and the timing of food supply is crucial to understanding the temporal variability in reproductive effort.

Latitudinal Effect on Reproductive Effort

The simulations for the Laguna Madre, Galveston Bay and Chesapeake Bay oyster populations show that spawning season (Kennedy and Krantz 1982) and reproductive effort decline at higher latitudes (Table 3). The timing of the spring and fall blooms relative to the temperature regime is a critical feature in determining the reproductive effort of oysters in all three bays. As latitude increases, the period of time when temperature and food availability coincide to produce a good reproductive effort declines. As variation in both temperature and food supply and the timing of both are characteristic of all bays and estuaries, an increase in the critical dependency of the simultaneity of events should reduce the likelihood of success at higher latitudes. Successful recruitment should occur more rarely at higher latitudes. Loosanoff (1966) observed significant year-to-year variability in reproductive success in Long Island Sound. Whether reproductive success is more dependable in the Gulf of Mexico (e.g. MacKenzie 1977) remains unknown.

Comparison of the simulated reproductive characteristics across latitudes shows that reproductive effort varied considerably but that the pattern of spawning pulses and the influence of the coincidence of blooms and changes in temperature on that pattern varied much less. Accordingly, the total reproductive effort and the timing of that effort are to some extent independently controlled.

Continuous Spawning

The reproductive season may be characterized by discrete spawning pulses, continuous spawning, or pulses embedded within a more continuous spawning condition. The latter occurs commonly in the Gulf of Mexico (e.g. MacKenzie 1977 vs. Loosanoff and Nomejko 1951). The simulated distributions suggest that continuous spawning should occur more frequently at lower latitudes and should be associated with populations dominated by larger oysters (Table 3). Spawning pulses should be more discrete at higher latitudes. Reproductive rate is higher at lower latitudes because the temperature regime is milder, so that filtration rates average higher. Higher filtration rates result in ingestion rates sufficient to support spawning over a larger fraction of the year. In addition, as the warmer months encompass a larger segment of the year, more of the yearly net production is allocated to reproduction and less to somatic growth. At higher latitudes, food supply is more crucial because the warm period of the year is shorter and lower temperatures reduce filtration rate and allocate more of net production to somatic growth. For these systems, spawning pulses directly following increases in food supply are more likely to occur and spawning between blooms is less likely to occur. Discrete spawning pulses rather than continuous spawning is the result.

The simulated oyster populations show that larger individuals require more food and a longer time to become ready to spawn. This time period increases significantly with small increases in size (biomass). Accordingly, this implies that oyster populations consisting primarily of larger individuals which are variable in size (biomass) will become ready to spawn later and spawn longer. The initial delay in spawning period reduces the impact of the signal produced by food and temperature.

Spawning Season

In the simulations, spawning season is longest when the spring and fall blooms just follow or precede the changes in temperature in the spring and fall. The duration of the spawning season decreases for all other environmental conditions and also decreases as the population ages. The beginning and ending of the spawning season for the simulated oyster populations is determined primarily by temperature and secondarily by food supply. In the spring, early spawning only occurs when maxima in temperature and food supply coincide. Any offset of one relative to the other results in a delay in the initiation of the spawning season. In the fall, temperature and food supply are less strongly coupled. The large decline in fall spawning intensity occurs in response to either a decrease in temperature or food supply. High food supply or warm temperatures in the fall may extend the spawning season (as defined by the last observable spawn) into times of lower temperature or food supply, respectively, albeit at a low level, because individuals may already be nearly ready to spawn. Accordingly, little additional food or only slightly elevated temperatures are required to generate the last small spawning effort of the season. This final spawn, however, adds little to the yearly reproductive effort which effectively ceases with the fall drop in temperature or food supply, whichever occurs earlier. Winter spawning should be extremely rare and require high levels of food; note that gonadal tissue can be present without spawning.

Animal Size

The simulated populations for all bays examined show that changes in the spawning pattern are coincident with changes in population size and size-dependent reproductive effort. Larger animals often spawn less (per g or even per individual) because rates of food acquisition decline relative to biomass at larger size more than does the cost of respiration and because more time is required to accumulate the gonadal material necessary to trigger a spawning

event. The effect is more significant in Galveston Bay and Chesapeake Bay. In the Laguna Madre, scope for growth is larger because temperatures average higher and filtration rate rises faster with increasing temperature than does respiratory rate. Therefore, increased food acquisition minimizes the effect of larger size because increased maintenance costs become a smaller component of the energy budget and more of net production is devoted to the reproductive effort. Conversely, smaller oysters spawn more, earlier, have a larger spawning season and more discrete spawning pulses. Population size frequency should be an important determinant of spawning pattern and spawning effort. Unfortunately, however, no data exist on the relationship of size and reproductive effort [per year, not per spawn (see Choi et al. in press)].

The simulations show that the simple hypothesis that more food results in a proportionately larger reproductive effort is not necessarily correct. If increases in food supply occur very early or late in the year when temperatures are low, a higher fraction of the food is allocated to somatic growth and adult size increases. Additional food should increase reproductive effort more predictably at lower latitudes because warmer temperatures occur for a larger fraction of the year and, thus, the increase in food supply is more likely to occur during the reproductive season.

Variability in Reproductive Effort

Prytherch (1929), among others, noted that reproductive effort was not correlated with population abundance. The results shown in Figures 2–15 support this observation. Small changes in temperature, population size frequency and the timing of spring and fall blooms cause sufficient variability in reproductive effort within a single oyster population to overshadow effects resulting from changes in population abundance. Turbidity, as it affects ingestion rate, is also important. In contrast, marked changes in salinity should affect reproductive effort much less strongly. Reproductive effort is most sensitive to changes in temperature, then food, followed by age and lastly salinity.

The characteristically-wide range of reproductive efforts recorded in the literature may result from seemingly minor changes in the environment (see Thompson 1990 for an interesting example). Reproductive effort for the season may depend on the timing of the spring bloom relative to the spring temperature increase. Summer and fall reproductive efforts should be more insulated from the vagaries of a changing climate and thus be more dependable components of the reproductive season. Accordingly, the wide range in recruitment success noted in the literature may not necessarily be due to the vagaries of life in the plankton but to the reproductive effort of the adults, which may be more than doubled by a small increase in temperature or the fortuitous coincidence of temperature rise and the spring bloom. Prytherch (1929) describes one good example in oysters.

CONCLUSIONS

The great variability in spawning pattern and spawning intensity observed in oysters from site-to-site, from year-to-year, and across latitudes may originate in simple and seemingly minor changes in the available food supply, the yearly temperature regime, and the juxtaposition of temperature change and the timing of the seasonal plankton blooms. Observations as divergent as the failure of broodstock abundance to correlate with yearly spawning effort, the presence of continuous spawning or discrete pulses during the spawning season, and the degree of predictability of spawning success across latitudes and over the years may be explained, at least partially, on this basis. The results of the simulations, however, can only be compared in the crudest way to data in the literature. Understanding the temporal and spatial variations in oyster reproduction requires simultaneous measurements of food supply and environmental variables to determine the degree to which our expectations meet reality. These studies remain to be done.

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THE USE OF CEMENT-STABILIZED GYPSUM AS CULTCH FOR THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN, 1791)

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ABSTRACT Cement-stabilized gypsum, clamshell (*Rangia cuneata*), and Mexican limestone were tested for their attractiveness to larvae of the eastern oyster, *Crassostrea virginica*. Gypsum was previously found to attract larvae but its rapid dissolution in seawater precludes its use as a cultch material. Crushed gypsum was mixed in a 1:1 w:w ratio with Portland cement to produce a stable material (gypment); solubility tests showed no significant difference between initial and final weights after exposure to seawater for two months. The three cultch materials were tested under field and laboratory conditions. Field results show that clamshell attracted significantly ($P < 0.05$, Non-parametric ANOVA) fewer spat per dry liter of cultch than did gypment or Mexican limestone, which were not significantly different from one another. Laboratory results show no significant difference among any of the cultches in their larval attracting capabilities.

KEY WORDS: *Crassostrea virginica, Rangia cuneata,* oysters, clamshell, cultch, gypsum, limestone

INTRODUCTION

Public oyster grounds in Louisiana are managed by the Louisiana Department of Wildlife and Fisheries (LDWF), mainly for the production of seed oysters. Since 1926, the LDWF has used clamshell (*Rangia cuneata*) as cultch for the production of seed oysters. Clamshell was used because it was abundant and inexpensive and produces well-formed single oysters (Dugas et al. 1981). The major source of this shell was from vast deposits in Lake Pontchartrain; dredging however was recently banned, severely reducing supplies. A search for an alternative to clamshell was thus initiated to supply the seed grounds, maintain current reefs, and build new ones.

Soniat et al. (1991) reviewed the literature on alternatives to clamshell and tested gravel, limestone, and crushed roadbed (concrete with some asphalt). They also found that gypsum attracted larvae, but its rapid dissolution in flowing seawater (25% per week in 6 *l*/min flow) eliminated it as a feasible alternative. (Gypsum is a by-product of fertilizer production, is currently present in vast quantities, and inexpensive.) The purpose of this work was to stabilize gypsum, decrease its rate of dissolution, and compare its effectiveness to limestone and clamshell.

MATERIALS AND METHODS

Physical Properties of Cultch

Physical properties of cultch materials were determined to transform spat per wet liter data to spat per cm^2 and spat per dry liter of cultch. Measured properties included density, surface area, weight, and wet volume per dry liter. One dry liter of cultch was poured into a large graduated cylinder to obtain a wet volume by water displacement. The surface area (cm^2) of a dry liter of cultch was obtained by wrapping aluminum foil around individual rocks.

A gypsum/cement cultch (gypment) was produced by mixing gypsum and cement in a 1:1 weight to weight ratio. Initially, several different mixes of gypsum to cement were made (i.e. 9:1, 8:2, 7:3, 6:4, 5:5) to determine the most suitable mix. Mixtures of <40% cement appeared brittle and a 1:1 mix was chosen for subsequent experiments. The gypsum was obtained from Louisiana Stone Aggregates, Inc. (Gonzales, LA) in sand-sized particles; the Portland cement was purchased locally. The 1:1 mix (w:w) was prepared on site (Grand Terre, LA) on 2 May 1991 using a gas powered cement mixer. Several molds were constructed with plywood as a base and molding as borders. The gypment slurry was poured into the molds to a height of about 1.5 cm and allowed to dry for one week. The hardened gypment was then broken into pieces ranging in size from 3 × 4 to 10 × 10 cm.

Solubility Experiments

Solubility experiments were conducted from 7 May 1991 to 24 July 1991 at the Louisiana Universities Marine Consortium (LUMCON) Lab in Cocodrie, LA. Thirty rocks each were placed in a static tank and in a flow tank. The tanks were 3 m long by 0.6 m wide by 0.3 m deep into which ambient seawater was pumped. The static tank was filled to a depth of 9 cm and the water was changed once every two weeks, whereas water was delivered to the flowing tank at a rate of 7.5 *l*/min. Initial and final weights were compared using a nonparametric analysis of variance (NPAR1WAY, SAS Institute 1990) to determine if a significant loss of gypment occurred.

Field Experiment

A field experiment was conducted from 1 May 1991 to 9 August 1991 at the Lyle St. Amant Marine laboratory on Grand Terre Island, Louisiana. It employed three cultches (clamshell = *Rangia cuneata,* Mexican limestone = fossil coral, and 1:1 gypment) in 40 replicates each for a total of 120 plots. Plots were assigned a cultch using a random number generator and arranged in a 12 tray by 10 tray grid on the bottom of a 0.25 hectare (60 × 40 m) pond. The trays (50 × 50 × 6 cm) were constructed of plastic coated heavy wire mesh (1.27 × 1.54 cm), and contained cultch material to a height of 3 cm. The pond was filled to a depth of 0.6 m with ambient seawater delivered at a rate of 285 *l*/min for the

duration of the experiment. Separate monitoring trays of the three cultches were checked once a week for spat set. Weekly water parameter tests included salinity (refractometer; Behrens 1965), dissolved oxygen (Azide Winkler modification; American Public Health Association 1985), temperature (mercury thermometer), and flow rate (volume/time). Plankton samples were taken weekly by slowly pulling a plankton net along the length of the pond (60 m) and later observed for oyster larvae. The cultches were in the water from 1 May 1991 to 9 August 1991, at which time the pond was drained and the number of spat per tray counted. The cultch from each tray was poured into a 40 *l* graduated bucket to determine the wet volume of cultch. Oyster settlement as a function of substrate was examined using NPAR1WAY.

Laboratory Experiment

The three cultches were tested in a smaller-scale experiment at the LUMCON wet laboratory using hatchery reared larvae (Gulf Shellfish Farms of Louisiana). Cultches were placed in four 40 × 36 cm plastic trays that were divided into 15 sections each for a total of 60 subplots (20 for each cultch). The bottoms of the trays were lined with plastic screening and the plots were separated by 1 cm × 2 cm wooden molding. The trays were placed in a 2 m diameter circular tank 9 cm off the bottom. Ambient (2 ppt, 26.8°C) and high salinity (32 ppt, 26.8°C) seawater were each pumped in at a rate of 6.0 *l*/min to a height of 15 cm and circulated through the tank; the salinity was maintained at about 15 ppt. Approximately 422,000 larvae were placed in the tank in a ready-to-set eyed stage. The experiment was conducted from 17 July 1991 to 24 July 1991, at which time the spat were counted. The wet volume of each subplot was obtained by the method described above for the field experiment and converted to dry volume to yield spat per dry liter of cultch. Oyster settlement as a function of cultch was analyzed using NPAR1WAY.

Flume Experiment

An experiment was performed to determine if the gypsum or cement component of the gypment attracted the oyster larvae. Three cultches were tested: the original gypment composed of a 1:1 (w:w) cement:gypsum mix, a pure cement cultch, and a 1:1 (w:w) cement:sand mix (sandment). The cultches were mixed in plastic containers and poured into each subplot.

Cultches were randomly assigned to 21 subplots (7 each) using a random number generator. Each subplot was 2.85 cm^2 and constructed with a plexiglass sheet with numerous holes drilled in it. The substrates were placed in a flume at the University of New Orleans which contained 95 *l* of 16 ppt synthetic seawater (Instant Ocean) circulating at a rate of 1.14 m/min. Salinity and temperature were maintained at 16 ppt and 22°C. Approximately 5,000 hatchery reared larvae (Gulf Shellfish Farms of Louisiana) were introduced into the system and allowed to set for 24 hrs. The plexiglass sheet was removed and spat were counted using a stereomicroscope. Temperature, salinity, and flow rate were measured daily. The experiment was conducted from 24 June 1992 to 27 June 1992.

RESULTS

Physical Properties of Cultch

Results from physical tests on cultch indicate that clamshell (0.69 kg/dry liter) was the lightest cultch, followed by gypment (1.89 kg/dry liter) and limestone (2.18 kg/dry liter). Clamshell 2671 cm^2/dry liter cultch) and limestone (2645 cm^2/dry liter cultch) had virtually the same value for surface area per dry liter of cultch, whereas gypment (1813 cm^2/dry liter cultch) had the lowest value.

Solubility Experiments

Seawater salinity ranged from 2 ppt to 8 ppt with a mean and standard deviation of 3.6 ppt ± 2.15; temperature ranged form 23.8°C to 27.4°C with a mean of 26.3°C ± 1.36. No significant difference was found between the initial and final weights of gypment in either the static or flow systems.

Field Experiment

Water temperature in the pond ranged from 22.8 to 32.8°C (mean = 29.2°C ± 3.0), whereas mean salinity was 11.2 ppt ± 4.2 with a range of 4 to 17 ppt. Dissolved oxygen values varied from 5.5 to 8.3 ppm with a mean of 6.3 ppm ± 1.0 and were, on average, 87% of saturation. The rate of water flow into the pond was 285 *l*/min and water depth was maintained at 0.6 meters. All measurements were taken at approximately 10:30 a.m.

Based on spat per liter of cultch, clamshell (mean = 1.04, range = 0.19–2.31, S.D. = ±0.54) attracted significantly ($P < 0.05$) fewer spat than did limestone (mean = 1.66, range = 0.50–4.49, S.D. = ±0.81) or gypment (mean = 1.69, range = 0.61–3.30, S.D. = ±0.67), which were not significantly different from each other. When data are expressed as spat per cm^2 of cultch, clamshell (mean = 0.39, range = 0.07–0.86, S.D. = ±0.20) attracted significantly ($P < 0.05$) fewer spat than did gypment or limestone, and limestone (mean = 0.63, range = 0.19–1.70, S.D. = ±0.31) attracted significantly ($P < 0.05$) fewer spat per cm^2 than did gypment (mean = 0.93, range = 0.34–1.82, S.D. = ±0.37) (Table 1). Spat were not removed in the determination of wet volume of cultch. (The error due to including spat volume with cultch volume was 0.80% at most.)

Laboratory Experiment

Spat per liter data show no significant differences between clamshell (mean = 466.3, range = 104.1–1230.8, S.D. = ±339.6), limestone (mean = 482.13, range = 176.87–1017.45, S.D. = ±272.86), or gypment (mean = 498.75, range 239.75–863.54, S.D. = ±155.52). When transformed to spat per cm^2,

TABLE 1.

Oyster spat set per liter and per cm^2 of cultch from field experiments.

	Gypment	Limestone	Clamshell
Mean number of spat per cm^2 of cultch	0.93	0.63	0.39
Range	0.34–1.82	0.19–1.70	0.07–0.86
Standard Deviation	0.37	0.31	0.20
Mean number of spat per dry liter of cultch	1.69	1.66	1.04
Range	0.61–3.30	0.50–4.49	0.19–2.31
Standard Deviation	0.67	0.81	0.54

Nonparametric ANOVA showed that clamshell attracted significantly ($P < 0.05$) fewer spat per liter of cultch than did limestone or gypment (gypsum and cement), which were not significantly different from each other. In units of spat per cm^2 all three cultches were significantly ($P < 0.05$) different from each other.

gypment (mean = 0.28, range = 0.13–0.48, S.D. = ±0.09) attracted significantly ($P < 0.05$) more spat than limestone (mean = 0.18, range = 0.07–0.38, S.D. = ±0.10) or clamshell (mean = 0.18, range = 0.04–0.46, S.D. = ±0.13). Limestone and clamshell were not significantly different from each other (Table 2).

Flume Experiment

Spat per subplot data were analyzed using non-parametric ANOVA. Gypment (mean = 21.19, range = 4–48, S.D. = ±10.30) attracted significantly ($P < 0.05$) more spat per subplot than did sandment (mean = 9.90, range = 2–23, S.D. = ±4.98) or cement (mean = 7.52, range = 1–21, S.D. = ±5.32) which were not significantly different from each other (Table 3).

DISCUSSION

Spat set data were analyzed in units of spat per dry liter and spat per square centimeter of cultch. Volume was used since it is easily measured and of practical significance, whereas an area measurement was needed to insure that differences between cultches were not simply due to differences in surface area. (In the flume experiment, the manufactured cultches were identical in size and shape and thus data are reported as spat per subplot.)

Both field and laboratory results suggest that gypment performed as well as or better than clamshell and limestone in attracting spat. Mexican limestone also performed well by attracting at least as many spat per liter or per cm^2 as did clamshell. The greatest difference among cultches was found in the field experiment. The spat used in the laboratory experiment were reared in a hatchery and purchased at a ready-to-set stage. When placed in the tank at the laboratory they possibly had less time to be selective in their cultch choice. Therefore, this could have resulted in less variation in spat set numbers. In contrast, larvae used in the field experiment were pumped in from lower Barataria Bay and thus were at natural concentrations. Variation in spat set numbers here was much more evident, perhaps because they had a greater amount of time to be selective.

Despite the low spat set numbers from the field experiment, significant differences were observed. The poor set is attributable to the excessively low salinity which likely caused a reduction in spawning activity (Butler, 1949). Cumulative rainfall was two times higher than the annual norm at the termination of the experiment. The low set is not attributable to low dissolved oxygen. Oxygen concentrations were about 87% of saturation and taken in the morning hours when values are characteristically lower due to the absence of photosynthesis during the night.

Louisiana has a surplus of gypsum to the extent that it is a solid waste problem. The rapid dissolution of raw gypsum makes it impractical for use as cultch. However, we have demonstrated that it can be stabilized with cement and it is as good as or better than clamshell at producing spat. A 1:1 mix of gypsum and cement was used although lesser amounts of cement should be more economical and equally effective. A 6:4 mix of gypsum to cement should prove successful yet a 7:3 mix appeared to be excessively brittle. The flume experiment suggests that it is the gypsum component, and not the cement which is most attractive to the larvae.

TABLE 2.

Spat set per liter and per cm^2 in laboratory experiments.

	Gypment	Limestone	Clamshell
Mean number of spat per cm^2 of cultch	0.28	0.18	0.18
Range	0.13–0.48	0.07–0.38	0.04–0.46
Standard Deviation	0.09	0.10	0.13
Mean number of spat per dry liter of cultch	498.75	482.13	466.32
Range	239.75 – 863.54	176.87 – 1017.45	104.05 – 1230.77
Standard Deviation	155.52	272.86	339.56

Nonparametric ANOVA shows no significant ($P < 0.05$) difference among any of the cultches based on spat attracted per dry liter.

Gypment (gypsum and cement) attracted significantly ($P < 0.05$) more spat per cm^2 than did clamshell or limestone, which were not significantly different from each other.

TABLE 3.

Oyster spat set per subplot in the flume experiments.

	Gypment	Sandment	Cement
Mean number of spat per subplot	21.19	9.90	7.52
Range	4–48	2–23	1—21
Standard Deviation	±10.30	±4.98	±5.32

Nonparametric ANOVA showed that gypment (gypsum and cement) attracted significantly ($P < 0.05$) more spat per subplot than did cement or sandment (sand and cement) which were not significantly different from each other.

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POPULATION STRUCTURE OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN, 1791), ON TWO OYSTER BARS IN CENTRAL CHESAPEAKE BAY: FURTHER CHANGES ASSOCIATED WITH SHELL PLANTING, RECRUITMENT AND DISEASE

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ABSTRACT Flag Pond oyster bar on the western shore of central Chesapeake Bay was sampled by divers using random quadrats in spring and fall 1979 and 1983–91. Governors Run oyster bar, located 10 km northwest of Flag Pond, was similarly sampled in 1984 and 1986–91. During this time a total of 3085 m^2 (9255 samples) was examined. Low oyster density on Flag Pond in 1979 increased in the early and mid 1980s following the planting of 369×10^3 bushels of shells and above-average spatfall during five of the years from 1980 to 1986. Low density on Governors Run also increased after above-average spatfall occurred there in 1985 and 1986. Above-average salinity, which ranged from 15 to 20‰ for 4 years beginning in 1985, was partly responsible for improved larval setting, but it also allowed the invasion of MSX from southern Bay waters. As oyster populations were peaking, they were devastated by disease; legal oyster density on Flag Pond of 5.5 m^{-2} in 1985 fell to 1.0 m^{-2} by 1988, and sublegal density of 18.2 m^{-2} in 1987 fell to 9.0 m^{-2} by 1988. Boxes, which are two intact empty shells connected by the hinge ligament, gave an indication of the mortality caused by MSX and increased from 1.8 m^{-2} in 1985 to 12.8 m^{-2} in 1987. Governors Run showed similar effects of disease as sublegal oysters decreased from 23.8 m^{-2} in 1987 to 7.4 m^{-2} in 1988. Increased spatfall in 1991 on Flag Pond and throughout much of the Chesapeake Bay in Maryland gives renewed hope for the oyster fishery, but it does so at a time when Dermo is increasing its range and intensity and mortality is rising again.

KEY WORDS: oyster, *Crassostrea virginica*, Chesapeake Bay, shell planting, larval recruitment, MSX, Dermo

INTRODUCTION

The eastern oyster, *Crassostrea virginica* (Gmelin 1791), inhabits Gulf and Atlantic coast estuaries as far north as the Gulf of St. Lawrence, and is frequently found where salinities range from 5‰ to 30‰, provided other requirements are met including, but not limited to, a solid substrate, good water movement, temperatures between 0° and 32°C, and an adequate food supply (Galtsoff 1964). Since the 1800s the oyster has been the basis for the most valuable commercial fishery in Chesapeake Bay. Maryland annual landings totaled 15×10^6 bushels (bu) in 1885 (Kennedy and Breisch 1981), and the exvessel value (the amount received by the fishermen) has approached $18 million (for the 1984 season; NMFS 1985, 1986). Although the value per bushel continues to rise, total landings decreased so dramatically during the 1980s (Fig. 1) that the overall value of the fishery has become second to that of the blue crab fishery which had an exvessel value of $26.9 million in 1989 (NMFS 1991).

During most of the 1970s, recruitment of young oysters into Chesapeake Bay populations was limited by low average salinities (Davis et al. 1981, Krantz et al. 1982, Krantz and Davis 1983). Ulanowicz et al. (1980) determined that larval oyster recruitment was closely related to sustained high salinity (>16‰) and the size of the oyster harvest the season before. Despite relatively poor setting, commercial landings remained between 2 and 3×10^6 bu annually (Fig. 1). Several years of above average salinity during 1980–82 and 1985–86 led to improved larval recruitment and spat settlement and survival (Krantz et al. 1982, Krantz and Davis 1983, Abbe 1988), and indicated potential increases in commercial oyster landings. Yet despite improved setting in the 1980s, commercial landings declined almost steadily in sharp contrast to the 1970s when setting was poorer but harvests were much better. The above average salinities, which led to the increased setting in the early and mid 1980s, also allowed the parasitic protozoan *Haplosporidium nelsoni* (Haskin, Stauber and Mackin) (MSX) to return to Maryland waters from the southern Chesapeake Bay where it has existed since at least 1959 or before (Andrews 1964, Andrews and Wood 1967). Mortalities occurred in 1982 following the dry years of 1980–82, but the epizootic of 1986–88 was the most devastating. These were the first documented incursions of MSX into Maryland since 1965 (Rosenfield and Sindermann 1966). MSX along with increased activity of *Perkinsus marinus* (Mackin, Owen and Collier) (Dermo) caused extensive mortalities in much of the Bay that may never have experienced MSX before. As a result, annual landings in Maryland were reduced to only about 400×10^3 bu during the 1987–90 seasons. But reduced oyster harvests can also result from factors other than disease, including loss of habitat caused by excessive siltation and summertime low oxygen concentrations, reproductive failure of adults or low larval recruitment and spat survival, and predation by numerous organisms from flatworms to crabs, fish and waterfowl (Kennedy and Breisch 1981, Abbe 1986).

Although oysters are found throughout much of the Chesapeake Bay, low population densities often limit the extent to which some oyster bars can be worked commercially. The Flag Pond bar on the western shore of Chesapeake Bay exemplified this situation prior to the 1980s; only about 29% of its 275 hectares (ha) had bottom stable enough for oyster production. Studies conducted in 1968 (ANSP 1968) and 1979 (Abbe 1988) estimated a population size of $5–7 \times 10^3$ bu of oysters on 81 ha of bottom (60–85 bu ha^{-1}). This small population size reflected not only the poor recruitment of the 1960s and 1970s, but also the shortage of habitat where oyster larvae could set and grow successfully. In 1980, in an effort to increase population size, 102×10^3 bu of shells were planted by the Maryland Department of Natural Resources (DNR) in the discharge area of the Calvert Cliffs Nuclear Power Plant (CCNPP), and in 1982 another 197×10^3 bu were planted at two sites just south of the CCNPP near Camp Conoy (W. Outten,

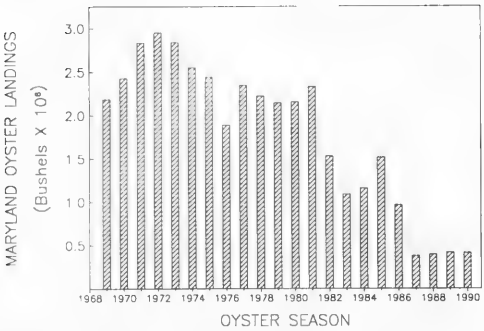

Figure 1. Maryland oyster landings in millions of bushels by season from 1969 to 1990.

Maryland DNR, Annapolis, Maryland, pers. comm.). In 1984, an additional 70 × 10^3 bu of shells were distributed near Camp Conoy between the two sites planted in 1982. All shell plantings were within the boundaries of Flag Pond bar, and no shells have been planted in this area since 1984.

The studies of this area from 1979 to 1986 (Abbe 1988) gave an encouraging outlook for the oyster community and for the oyster fishery within the area. From low levels in 1979 to peak levels reached between 1983 and 1986, legal size oysters increased threefold, sublegals increased by 40 times and spat increased by 180 times. These increases in relatively small areas of Flag Pond bar (4 to 20 ha) allowed hope for improvements in the fishery on a larger scale, but much has happened on Flag Pond and baywide since then. This paper presents a continuation of the work begun in 1979. Abbe (1988), however, presented mostly positive changes associated with shell planting and increased recruitment, whereas the present study adds the element of disease and shows many negative changes that have occurred since 1986. The diseases themselves were not monitored as part of this study (they were by Maryland DNR), but their effects on the populations were easy to detect. This effort is one of the more detailed, long-term studies of its type conducted in Maryland, and although the effects of disease on other bars will differ because of differences in initial population size and prevalence and intensity of disease, it may provide some idea of how the population structure was changing on other oyster bars throughout the state during a period of drought and disease.

MATERIALS AND METHODS

Description of Study Areas

The center of the Flag Pond bar is 12 km north of the mouth of the Patuxent River on the western shore of Chesapeake Bay (38°25′50″N 76°26′20″W). The upper and lower extremes are 8.1 km and 3.7 km, respectively, from the tip of Cove Point (Fig. 2). At its widest point the bar extends approximately 1.1 km from shore, and is about 0.2 km offshore at its narrowest.

In 1979 the estimate of suitable oyster bottom on Flag Pond bar was 81 ha (200 acres) or 29% of the total area (Abbe 1980). The remainder was either too sandy (inshore) or too muddy and soft (offshore) to support an oyster population. Much of the inshore bottom was rocky, and although oysters were found there, harvesting by conventional methods of tonging or dredging was probably difficult.

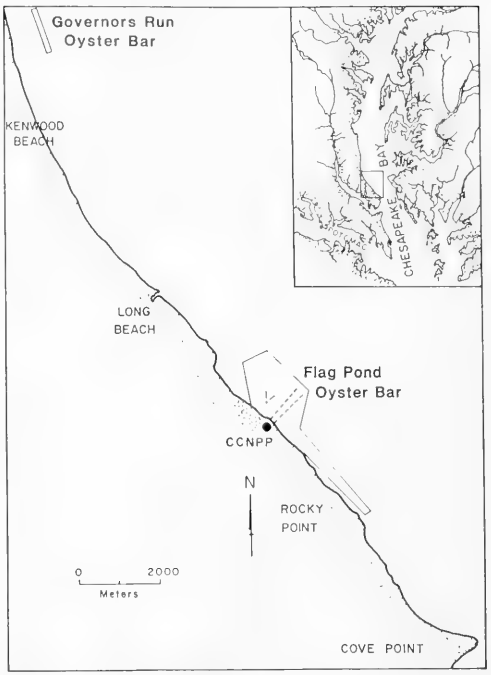

Figure 2. Location of Flag Pond oyster bar and a portion of Governors Run oyster bar in central Chesapeake Bay.

Water depth ranged from less than 1 m to 10 m, but most oysters were found in water less than 6 m deep. Water temperature and salinity over the bar as determined by a Beckman RS5-3 portable salinometer ranged from near 0°C in winter to 28°C in summer; salinity from May to November, when oyster activity is greatest, averaged about 14‰ with annual means during these months ranging from about 9 to 18‰ (Fig. 3). Dissolved oxygen (DO) concentrations in summer were measured with a YSI Model 57 Dissolved Oxygen Meter and normally ranged from 3 to 9 mg O_2 l^{-1} in water less than 8 m, but were often 1 mg O_2 l^{-1} or less at depths greater than 9 m. DO concentrations less than 3 mg O_2 l^{-1} occasionally occurred in shallow water for up to a day as a result of upwelling caused by west or southwest winds (Breitburg 1990, Osman et al. 1991), but low DO conditions were not a serious problem.

A second bar (Governors Run at 38°31′0″N 76°30′10″W) lies 10 km northwest of the CCNPP, and at one time covered 500 ha (1236 acres) of Bay bottom (Yates 1913). Presently, however, much of the area consists of a soft black mud bottom (offshore) and sand bottom (inshore), both unsuitable for oysters. In 1984 we determined that oysters were confined to a narrow strip parallel to shore about 0.7 km from shore (Fig. 2). The bottom within this area consisted of sand, hard mud, silt, shell or some combination of these elements, but had none of the large rocks characteristic of certain areas of Flag Pond bar. Highest densities were found in a 2-ha (5-acre) area at the up-bay end of this strip, and most of the efforts since 1984 were confined to this small area.

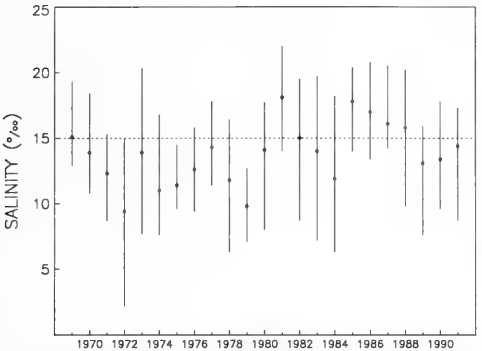

Figure 3. Salinity means and ranges during May to November from 1969 through 1991 on Flag Pond oyster bar. Recruitment was better when mean salinity was above 15‰ (dotted line), but disease mortalities were also higher.

Water depth was 6 to 7 m, temperatures and salinities were similar to those on Flag Pond, and DO concentrations were also similar to those on Flag Pond, but low DO events were more frequent and longer lasting than on Flag Pond because of differences in bottom topography.

Sampling

The study was conducted from the 12.8-m (42-ft) research vessel *JOSEPH LEIDY* during May–June and September–November 1979 and 1983–1991 in the areas of the Flag Pond bar outlined in Figure 4. The Governors Run bar was examined during the same months of 1984 and 1986–1991. Areas 2, 4, and 5 of Flag Pond bar were planted with shells during 1980, 1982 or 1984,

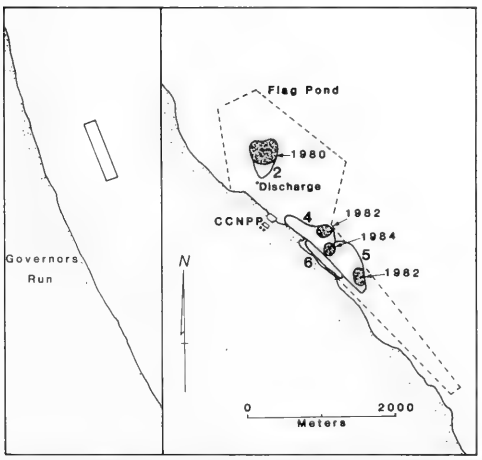

Figure 4. Outlines of areas sampled on Flag Pond bar during 1979 and 1983–91 and at Governors Run during 1984 and 1986–91. Shaded areas on Flag Pond show dates and approximate areas of shell planting.

and all, with the addition of the unplanted inshore Area 6, were surveyed semiannually from 1983 to 1991 (Abbe 1992).

Methods used in this study were similar to those of May (1971) in his survey of the Alabama oyster resources. May determined that a sample density of 2.1 m^2 ha^{-1} (1 yd^2 $acre^{-1}$) was sufficient to adequately sample the oyster populations that he investigated. The present study used higher sample densities on both bars—6 m^2 ha^{-1} on Flag Pond and 24 m^2 ha^{-1} on Governors Run.

Sampling methods were detailed by Abbe (1988) so they will only be summarized here. A series of transects over each area was made, with several locations sampled along each. Three steel squares (each 0.33 m^2 and painted white to enhance underwater visibility) were deployed from both sides of the boat, and all oyster components were removed from each square by divers who brought the material to the surface for examination. Shells and boxes (two empty shells connected by the hinge ligament) were counted, and live oysters were counted by size class: legal (≥76 mm), sublegal (<76 mm that had set at least the prior year), and spat (newly-set oysters that had attached during the present setting season). After the components from each square were examined and recorded they were returned to the water. The number of sampling locations in each area varied according to area size, bottom type, and season, but generally ranged from 12 to 24 (24 to 48 m^2) per area per season (spring or fall).

Statistical Analysis

Data were analyzed using a nested analysis of variance (General Linear Model Procedure; SAS Institute, Inc., 1982) in which all factors were assumed to be random. The model takes the form,

$$log(y_{ijkl} + 1) = M + A_i + B_j(A_i) + C_k(B_j) + D_l(C_k) + E$$

where y_{ijkl} = parameter measurement (number of shells, boxes, or oysters) for $area_i$, $location_j$, $position_k$, and $replicate_l$.

M = overall mean
A_i = area effect
B_j = location-within-area effect
C_k = position (port or starboard)-within-location effect
D_l = replicate-within-position effect
E = type III MS for location-within-area as an error term

When significant differences among areas were detected ($p < 0.01$), a Student-Newman-Keuls (SNK) test was used to rank the areas according to their mean densities and determine where the differences occurred.

RESULTS

A mean of 266 m^2 were sampled on Flag Pond bar annually, and 60 m^2 were sampled on Governors Run bar. This equalled 3085 m^2 for the entire study or 9255 individual squares. Since much of the 152 m^2 sampled on Governors Run in 1984 was on barren sand bottom, the mean was reduced to only 45 m^2 during subsequent years.

Shell density on Flag Pond increased from 82 m^{-2} to 228 m^{-2} in 1984 as a result of shell planting by Maryland DNR, but it decreased steadily after 1985 as shells fractured into smaller pieces due to biological and physical processes and reached 84 m^{-2} by late 1991. Shell density also increased on Governors Run in 1984 from 26 m^{-2} to 195 m^{-2}, but this resulted from a decrease in the amount of barren bottom examined in fall compared to spring of that year. Shell densities also declined on Governors Run over

time, but at a slower rate than on Flag Pond, probably because of less commercial activity than on Flag Pond.

Annual legal oyster density on Flag Pond ranged from 4.3 to 5.5 m^{-2} during 1983–86 (Fig. 5). In spring 1986 the legal density was 5.8 m^{-2}, but by fall it decreased to 4.0 m^{-2}, the first indication from Flag Pond of disease (MSX) that was devastating other oyster bars throughout the state. The decline continued to an annual density of 2.0 m^{-2} in 1987 and to 1.0 m^{-2} in 1988 because of disease, although a slight increase in 1989 to 1.6 m^{-2} occurred as some of the oysters from the 1986 and 1987 year classes reached legal size. With continued growth of these recruits, legal density climbed above 3 m^{-2} in 1990 and 1991 (Fig. 5).

On Governors Run the legal density was only 0.4 m^{-2} in 1984, an artificially low level that resulted in part from the large number of samples collected from unproductive bottom. From 1986 to 1990, legal oysters ranged from 1.4 to 2.4 m^{-2}, with the lowest density in 1987 after the outbreak of disease (Fig. 6). In 1991, however, the legal density declined to 0.7 m^{-2}, a level below that seen during the worst of the MSX years. Although spat setting was good on Governors Run in 1986, and appeared to have been good in 1985 based on the increase in number of sublegals observed in 1986, only a slight increase in legal density resulted from it by spring 1988, with no improvement thereafter.

Sublegal density on Flag Pond was only 0.7 m^{-2} in 1979, but by 1983 it reached 27.7 m^{-2} because of successful spat setting during the previous two years (Fig. 5). As some of these oysters grew to legal size in 1984 and 1985, sublegal density decreased because the small 1983 and 1984 year classes were unable to contribute to population growth. The strong year classes of 1985 and 1986, which could have increased sublegal density tremendously, and ultimately could have increased the density of harvestable oysters, failed to do so. The 23.4 spat m^{-2} in 1985 resulted in an increase of only 5.8 sublegals m^{-2} by 1986, and the even larger 1986 year class (36.8 spat m^{-2}) resulted in no increase

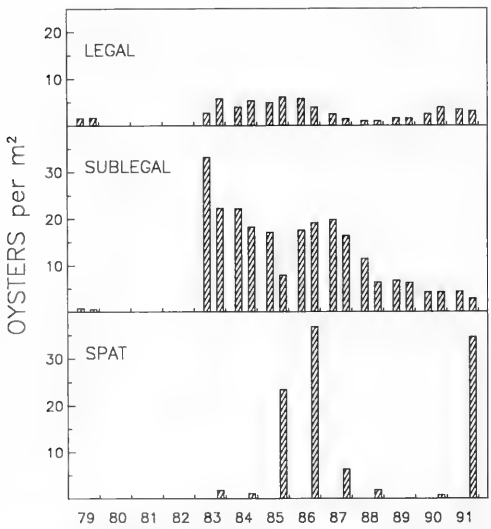

Figure 5. Densities of legal oysters, sublegals, and spat on Flag Pond (all areas combined) during spring and fall 1979 and 1983–91.

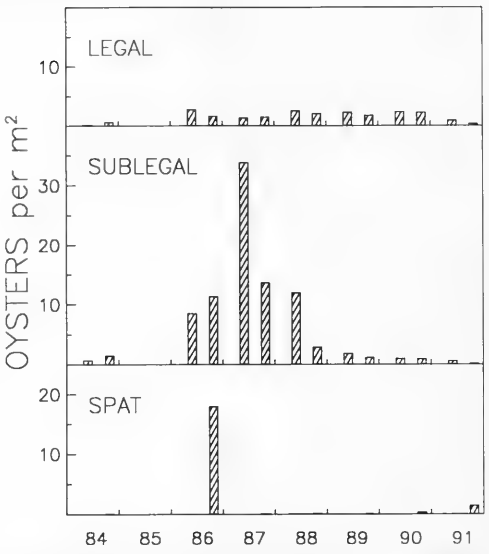

Figure 6. Densities of legal oysters, sublegals, and spat on Governors Run during spring and fall 1984 and 1986–91.

in sublegal density in 1987 (Fig. 5). When examined by season, sublegals did increase slightly from 19.2 m^{-2} in the fall of 1986 to 19.9 m^{-2} in the spring of 1987, but this was substantially less than it could have been. The failure of these two year classes to increase population size was evidence that small oysters (including those less than a year old) were being lost to disease as well as legal oysters. Burreson (1991) also demonstrated that oysters as small as 12–30 mm shell height can be lost to MSX infections. Sublegal densities continued to fall from 18.2 m^{-2} in 1987, to 9.0, 6.6, 4.3, and 3.7 m^{-2} in the four subsequent years as spatfall was very poor during 1988–90 (averaging only 0.8 m^{-2}; Fig. 5).

On Governors Run the sublegal density increased from 1.1 m^{-2} in 1984 to 9.9 m^{-2} in 1986 because of apparent successful 1985 recruitment and survival (although no sampling was conducted that year). Recruitment of spat in 1986 was 18.0 m^{-2} which increased the sublegal density in 1987 to 23.7 m^{-2}, but this was the only good spatfall actually observed during the 7 years sampled. In 1988, sublegals decreased to 7.4 m^{-2} and then declined further to 1.5, 1.0, and 0.4 m^{-2} in 1989, 1990, and 1991, respectively (Fig. 6). The 1991 density was the lowest of all years and reflects the effects of MSX and the lack of spatset as also observed on Flag Pond. The decrease during 1987–88 was actually more severe than indicated by annual means. From spring to fall 1987 to spring and fall 1988 the sublegal density fell from 33 m^{-2} to 13, to 12, to 3 m^{-2}, respectively, a 91% decrease over a 17-month period (Fig. 6).

Spat observed in the fall were generally 10–30 mm in shell height and were probably 1 to 3 months old, although some as small as 3 mm were occasionally seen. Spat densities were highest on Flag Pond during 1986, 1991, and 1985, in that order, and on Governors Run during 1986 (Figs. 5 and 6). Although we did not sample Governors Run in 1985, spat setting had to have been above average, as the density of sublegals increased from 1.4 m^{-2}

in fall 1984 to 8.5 m^{-2} in spring 1986 (Fig. 6). A few spat were collected on Flag Pond in 1987 (6.4 m^{-2}), but Governors Run had only 0.1 m^{-2}. An important factor in successful spat setting was the availability of shells. Area 6, where no shells were ever planted, never had more than a few spat per m^2 even when they were abundant on Flag Pond in general as in 1985 and 1991 (Table 4). During most years, even when setting was poor, Areas 4 and 5 had the highest densities of spat, probably because highest shell densities were found there. The 9-year average shell densities for Areas 2, 4, 5, and 6 were 63, 137, 191, and 28 shells m^{-2}, respectively. Mean spat densities associated with these same areas were 7, 19, 17, and 1 m^{-2}, respectively. Although there were fairly large differences among areas with respect to spat density, the ratios of spat to shells were much more similar. The 9-year mean number of spat per shell in Areas 2, 4, 5, and 6 was 0.11, 0.14, 0.09, and 0.04. Higher shell densities yielded higher spat densities on Flag Pond in contrast to Governors Run. For the 7 years that Governors Run was examined, its 141 ± 21 shells m^{-2} (Mean ± SE) was nearly the same as the 129 ± 16 shells m^{-2} on Area 4 (same 7 years), but it had only 2.9 spat m^{-2} compared to 17.2 m^{-2} on Area 4. Its spat per shell ratio was also worse, averaging only 0.02.

Although it appears that good spat setting is at least partly related to abundant broodstock and the availability of clean shells or other suitable substrate, the 1991 spatfall on Flag Pond was second best (34.6 m^{-2}) after that of 1986 (36.8 m^{-2}), yet broodstock was at much lower levels than in recent years and shells had not been planted on the bar for at least 7 years. Moreover, the highest spat densities were in Area 4 (63.9 m^{-2}) where some individual samples exceeded 300 m^{-2}, yet shells had not been planted since 1982. The same pattern was observed throughout the state where spat setting was the best since at least 1939 with a baywide average of 230 spat bu^{-1} of dredged bottom material despite the fact that broodstock was reduced in most areas and fewer clean shells were available to larvae because fewer were moved under the state's shell planting program (1.8×10^6 bu in 1991 compared to 5–6 $\times$ 10^6 bu in recent years) (Vista 1992; W. Outten, Maryland DNR, pers. comm.). Some seed areas that did receive shells, however, caught up to 3000 spat bu^{-1} (W. Outten, pers. comm.) which only reinforces the value of both the larger shell planting program and the smaller seed oyster program. Thus while broodstock size and shell availability may be important to spat setting, there appear to be other factors involved, perhaps even more critical, which are little understood.

Combining the three size classes, total oyster density on Flag Pond bar decreased from 33.8 m^{-2} in 1983 to 26.1 m^{-2} in 1984, but increased to 41.5 m^{-2} in 1985, and reached 60.1 m^{-2} in 1986. From 1986 to 1989 this combined total fell steadily and rapidly to 8.3 m^{-2} where it remained in 1990. The 1989–90 densities were the lowest totals since the 2.6 m^{-2} of 1979. With the addition of a strong year class in 1991, however, the combined total of all three classes increased to 41.6 m^{-2}, the same as in 1985. Combined totals at Govérnors Run increased from 1.5 m^{-2} in 1984 to 30.0 m^{-2} in 1986, but then declined rapidly to 3.5 m^{-2} in 1989 (Fig. 6). Totals for 1990 and 1991 at Governors Run were similar to those of 1989.

The density of boxes on Flag Pond was highest in 1987 (12.8 m^{-2}) following the outbreak of disease in 1986 (Fig. 7). Boxes on Governors Run were also most abundant in 1987 (Fig. 7), but the density of 4.2 m^{-2} was well below that of Flag Pond. The ratios of 1987 boxes to 1986 live oysters (legals plus sublegals) were

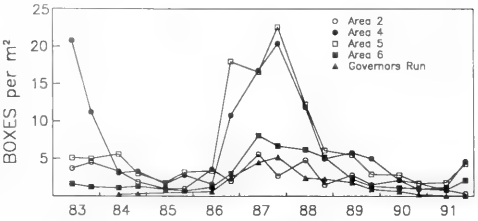

Figure 7. Densities of boxes on individual areas of Flag Pond and on Governors Run during spring and fall 1983 to 1991.

more similar, however, with 55% on Flag Pond and 40% at Governors Run. Although the number of boxes found in a population is not always an accurate indicator of recent mortality, due to the fact that boxes may last for various time periods before breaking apart, the box density in this study did allow us to follow the effects of disease as it swept through the oyster bars and then subsided. Box density was high in Area 4 in 1983 (Fig. 7), and although disease was responsible for mortality on some oyster bars in Maryland in 1981–82 (Krantz and Davis 1983), it appeared that this mortality was caused by siltation and biodeposition in areas where large numbers of oysters had set on planted shell 1 and 2 years earlier rather than by disease (Abbe 1988). Otherwise, box densities ranged from 1 to 5 m^{-2} through the spring of 1986. By fall 1986, however, after MSX had infected the oysters, boxes increased dramatically to nearly 10 m^{-2} (Fig. 7), especially in Areas 4 and 5 where highest densities of live oysters were also found. In the spring of 1987 boxes reached an average 12 m^{-2} with about 17 m^{-2} in Areas 4 and 5. Boxes peaked in fall 1987 at 14 m^{-2} with Areas 4 and 5 reaching 20 and 23 m^{-2}, respectively. A decline began in 1988 while salinity was still high, probably because the density of live oysters that remained (and could die to become boxes) had reached such low levels. Boxes decreased to an average 1.7 m^{-2} in 1990 (similar to that of 1985, Fig. 7) after abundant precipitation in 1989 reduced salinity on Flag Pond bar to less than 8‰ in May and kept it below 10‰ during much of June and July. MSX tolerates 15‰, but is less pathogenic than at salinities of 20‰ or more (Andrews 1964, 1983, Haskin and Ford 1982). Salinity on Flag Pond reached 20‰ during each year from 1985 to 1988 (Fig. 3) after having done this only twice in the previous 16 years. The mean salinity was also above 15‰ (dotted line in Fig. 3) all 4 years after being above 15‰ only once in the previous 16 years. Ford (1985) has shown that oysters infected with MSX at salinities of 20–25‰ that were transferred to salinities of 10‰ or less were free of infection after only 2 weeks when temperatures were 20°C or higher. Thus we suspect that lower salinity during warm summer months in 1989 was the primary factor that ended MSX mortality on both Flag Pond and Governors Run.

Statistical analyses showed that areal differences occurred more often for live oysters than for shells or boxes (Table 1). Mean density values for the four areas of Flag Pond were compared among each other and with Governors Run for legal oysters, sublegals, and spat in Tables 2, 3, and 4, respectively, based on significant results in Table 1; mean values not significantly different are connected. These densities were computed from log values and are therefore somewhat lower than the arithmetic means presented in the text and Figures 5 and 6.

TABLE 1.

Summary of F values testing the ratios of area variances to location variances from an analysis of all five areas of Flag Pond bar and Governors Run bar.

	df	Shells	Boxes	Legal Oysters	Sublegal Oysters	Spat
1983						
Spring	3/49	1.55	1.48	0.77	0.34	—
Fall	3/52	2.65	1.93	1.48	0.33	0.33
1984						
Spring	4/96	3.80*	7.97*	15.58*	13.70*	—
Fall	4/80	2.57	5.67*	9.50*	9.23*	5.03*
1985						
Spring	3/87	4.58*	0.36	4.39*	2.69	—
Fall	3/62	3.15	2.07	0.74	2.34	4.42*
1986						
Spring	4/82	5.47*	1.61	3.65*	3.13	—
Fall	4/64	4.79*	3.27	2.58	1.99	2.87
1987						
Spring	4/70	1.61	1.39	1.79	0.93	—
Fall	4/66	0.91	3.55	3.22	3.26	3.47
1988						
Spring	4/75	1.82	2.68	4.46*	3.00	—
Fall	4/75	0.79	1.77	2.45	4.19*	4.60*
1989						
Spring	4/74	1.05	1.37	3.98*	5.59*	—
Fall	4/81	1.87	2.12	3.00	4.50*	1.21
1990						
Spring	4/83	5.27*	2.16	8.42*	6.46*	—
Fall	4/83	5.88*	1.43	8.99*	5.07*	4.47*
1991						
Spring	4/87	5.74*	2.19	13.59*	6.00*	
Fall	4/73	1.58	8.10*	16.58*	10.32*	11.54*

* F statistic significant at $p < 0.01$.

Governors Run was not sampled in 1983 or 1985 so only four areas were included during those years.

DISCUSSION

Many more significant differences existed between bars (Flag Pond vs Governors Run) than among the four areas of Flag Pond bar with densities of live oysters generally lower on Governors Run than on Flag Pond. Of 22 significant comparisons presented in Tables 2–4 that included all five areas, Governors Run had the fourth or fifth lowest mean values 19 times. Although the density of legal oysters was generally much lower on Governors Run than on Flag Pond, the high mortalities of 1987 reduced Flag Pond densities to less than 1 m^{-2} in 1988 and allowed Governors Run (1.8 m^{-2}) to surpass it for a short time.

Except for 1987, the density of sublegal oysters on Governors Run was also always lower than on Flag Pond, even after the heavy MSX mortalities, probably because spat setting was always lower. During only 2 of 7 years were spat found on Governors Run at densities higher than 0.3 m^{-2}, whereas Flag Pond spat densities exceeded 0.3 m^{-2} during 8 of 10 years (3 of which were above 20 m^{-2}).

The decrease in sublegal oyster density during times of low recruitment levels (1983–85 and 1987–90) resulted from the interaction of several factors. One was the growth of small oysters, which shifted some into the legal size class; another was the mortality caused by siltation and suffocation of small oysters, especially in areas where they had set in high densities in thick shell layers; a third factor was mortality due to predation; and a fourth was disease mortality. In 1986 many of the sublegal oysters of 1985 reached legal size, and this legal portion of the population could have expanded even more had the disease mortality not been so extensive. In 1987 the sublegal population should have increased dramatically considering that the density was 19.2 m^{-2} in the fall 1986 and spat density was 36.8 m^{-2}. Since spat are designated as sublegals the spring after they set, the potential density could have reached 56 m^{-2}. Because it increased to only 19.9 m^{-2}, however, there were obviously heavy losses of both spat and sublegal oysters.

Several factors were responsible for the poor setting during 1987–90, although it is difficult to determine the exact role played by each. Disease had a major effect on reproduction in 1987 and 1988 by killing many adults and thereby reducing the size of the broodstock. The loss of broodstock to MSX infection was suggested by Andrews (1983) and Haven and Fritz (1985) as a major cause of decreased larval setting in the James River, Virginia. Secondly, those adults that did survive infection in 1987 and 1988 may have been in poor physiological condition, so that spawning was reduced during those years (Newell 1985, Barber et al. 1988, Ford and Figueras 1988). Poor condition, however, should not have been a factor in 1989. The loss of broodstock over the past several years is obvious with the density of adults (legal and larger sublegal oysters) declining so dramatically, but the loss of physiological condition in 1989 is not. Spat setting in 1987 and 1988 was light, but oyster shells placed on bottom in 1988 in Area 5 as part of another study were settled by spat at densities many times

TABLE 2.

Results of Student-Newman-Keuls tests applied to significant differences in Table 1 for legal size oysters.

1984 (Spring)	Area 4 3.7	Area 6 2.6	Area 5 1.2	Area 2 1.2	Gov. Run 0.1
1984 (Fall)	Area 4 4.0	Area 6 3.2	Area 5 3.2	Area 2 1.8	Gov. Run 0.4
1985 (Spring)	Area 6 4.0	Area 4 3.1	Area 5 2.1	Area 2 1.0	
1986 (Spring)	Area 4 4.1	Area 5 4.0	Area 6 3.0	Gov. Run 1.8	Area 2 1.4
1988 (Spring)	Gov. Run 1.8	Area 4 0.9	Area 5 0.9	Area 6 0.4	Area 2 0.3
1989 (Spring)	Area 4 1.7	Gov. Run 1.4	Area 6 1.1	Area 5 1.1	Area 2 0.1
1990 (Spring)	Area 4 2.8	Area 6 2.0	Gov. Run 1.5	Area 5 1.0	Area 2 0.3
1990 (Fall)	Area 4 3.8	Area 6 3.0	Area 5 2.3	Gov. Run 1.6	Area 2 0.3
1991 (Spring)	Area 6 3.6	Area 4 3.2	Area 5 1.7	Gov. Run 0.7	Area 2 0.6
1991 (Fall)	Area 4 3.2	Area 6 2.7	Area 5 1.9	Area 2 0.5	Gov. Run 0.3

Densities are per m^2, and areas not significantly different are connected.

greater (approaching 200 m^{-2}) than that of the surrounding area (Abbe, unpublished data). These high densities were on clean shells which had been placed on bottom in early August at the peak of the setting season. The surrounding shells had been on bottom for several years and were at least partially covered with silt and fouling organisms. The difference in cleanliness between these two sets of shells had far more effect on setting density than the physiological condition of the spawning adults, at least in 1988; the effect of shell cleanliness during 1987 is unknown. In 1989 the poor set may have been related to low broodstock density, but below-average salinity (Fig. 3) following above average precipitation probably had a greater influence; salinity was below 10‰ during much of May, June and July. Oysters in 1989 were in good physiological condition, but the gonads of many appeared to develop incompletely, a problem that can occur when water is unusually fresh (Butler 1949, Loosanoff 1953, May 1972). A salinity of 10‰ is low but not rare in this portion of the Chesapeake, but it was a sharp drop from the previous 4 years and was well below that of 1981–82 and 1985–86 (Fig. 3) when major sets occurred (Krantz et al. 1982, Krantz and Davis 1983, Abbe 1988). In 1989, reduced broodstock size may have had little effect on recruitment success if the majority of adult oysters failed to spawn.

When salinity is in a normal range of 12–15‰ and diseases are not widespread, sublegal densities generally increase from fall to spring, as any spat that set enter the sublegal class in the spring of the year following their setting. Even when there is no setting, sublegal densities remain fairly constant from fall to spring. Sublegal densities should decrease from spring to fall as individuals grow and some enter the legal class which should increase from spring to fall. The legal class should also decrease in size from fall

TABLE 3.

Results of Student-Newman-Keuls tests applied to significant differences in Table 1 for sublegal size oysters.

1984 (Spring)	Area 5	Area 4	Area 2	Area 6	Gov. Run
	12.7	6.0	4.4	2.7	0.4
1984 (Fall)	Area 5	Area 4	Area 2	Area 6	Gov. Run
	12.3	5.9	2.2	2.2	0.9
1988 (Fall)	Area 4	Area 6	Area 5	Gov. Run	Area 2
	4.7	3.6	2.9	1.8	0.3
1989 (Spring)	Area 4	Area 6	Area 5	Gov. Run	Area 2
	5.7	5.1	3.6	1.1	0.7
1989 (Fall)	Area 4	Area 5	Area 6	Area 2	Gov. Run
	5.0	3.6	3.4	1.2	0.8
1990 (Spring)	Area 5	Area 4	Area 6	Gov. Run	Area 2
	3.8	3.2	1.7	0.7	0.6
1990 (Fall)	Area 4	Area 5	Area 6	Area 2	Gov. Run
	3.3	3.3	2.3	1.1	0.7
1991 (Spring)	Area 5	Area 4	Area 6	Area 2	Gov. Run
	4.0	3.1	1.8	1.2	0.4
1991 (Fall)	Area 4	Area 5	Area 6	Area 2	Gov. Run
	2.8	2.5	1.5	0.4	0.2

Densities are per m^2, and areas not significantly different are connected.

to spring if much commercial harvesting takes place. This pattern for legal and sublegal oysters was in evidence during 1983–85, but with disease mortality at such high levels during 1986–88, the pattern broke down (Figs. 5 and 6). Sublegal densities decreased in both spring and fall as did legal densities. With a temporary halt to the high salinities of 1985–88 and the disease problems that resulted therefrom, the population in 1989 began to stabilize. By 1990 MSX had been driven downbay to Tangier Sound on Maryland's lower Eastern Shore by lower salinity water (Krantz 1991); Dermo, however, remained fairly widespread and increased its intensity in many parts of the Bay. In 1991 precipitation was 25 cm below average, salinity approached 18‰ on Flag Pond (Fig. 3), and Dermo began to cause mortality by August among oysters in their second year or older. Newly set spat and oysters less than 2 years old, however, appear to be less susceptible than older oysters (Andrews and Hewatt 1957, Andrews 1966, Burreson 1991, Paynter and Burreson 1991). Continued high prevalence and intensity of Dermo could begin affecting the 1991 year class as early as summer 1992.

Commercial activity on Flag Pond bar was fairly intense from 1983 through 1985 as the number of hydraulic patent-tong vessels working offshore from Camp Conoy often exceeded two dozen by 1985. Estimates based on decreases in legal density indicate that annual harvests ranged from 2000 to 5000 bu during the 1983 to 1985 seasons, but based on the number of vessels working in the area the harvests could have been higher. By 1986 the number of tongers was only half that of 1985, and from 1987 through 1989 almost no commercial activity was seen on Flag Pond. The legal population peaked in the fall of 1985 at 18,500 bu (based on 200 oysters bu^{-1}), but this was reduced to 3000 bu by fall 1988. Some of this decrease resulted from harvesting, but most was due to disease. Although Flag Pond had quite low densities of legal oysters from 1987 through 1989, the fall 1991 population of 9500 bu was the highest level since 1986. Despite the increase in population size, however, commercial activity was limited during 1990 and 1991. With the sublegal density presently at such low levels it is unlikely that major improvement will be seen until the 1991 year class has grown to legal size, and that will take another 2 to 3 years at best. Because of its small size and low oyster density, Governors Run was seldom the site of commercial activity during the time period covered by this paper, and now the possibility of a decent recovery in the short term is reduced even further because

TABLE 4.

Results of Student-Newman-Keuls tests applied to significant differences in Table 1 for oyster spat.

1984 (Fall)	Area 5	Area 4	Area 2	Area 6	Gov. Run
	1.2	0.8	0.2	0.0	0.0
1985 (Fall)	Area 4	Area 2	Area 5	Area 6	
	13.0	10.8	6.9	1.6	
1988 (Fall)	Area 5	Area 4	Area 2	Gov. Run	Area 6
	1.5	1.4	1.2	0.1	0.0
1990 (Fall)	Area 5	Area 4	Area 6	Gov. Run	Area 2
	1.0	0.4	0.4	0.2	0.1
1991 (Fall)	Area 4	Area 5	Area 2	Area 6	Gov. Run
	27.4	15.1	9.3	3.0	0.8

Densities are per m^2, and areas not significantly different are connected.

of extremely low densities of both legal and sublegal oysters and continued poor recruitment.

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AN IMPROVED METHOD FOR MAPPING OYSTER BOTTOM USING A GLOBAL POSITIONING SYSTEM AND AN ACOUSTIC PROFILER

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ABSTRACT A method for rapidly and relatively inexpensively mapping oyster bottom is described. The method uses an acoustic profiler to differentiate substrate type, a fathometer to assess bottom relief and a global positioning system to accurately establish position. The method has the following desirable traits: can be performed from a small research vessel, usable in most weather conditions, requires only a two-person crew, rapidly discriminates bottom type while underway, usable in shallow (<1 m) or deep (>10 m) water, provides accurate and precise navigation. The method has been used successfully to map the oyster reefs and oyster bottom of Galveston Bay, Texas, an area of approximately 1000 km^2.

KEY WORDS: oyster reef, mapping, acoustics, method

INTRODUCTION

In 1990, the Galveston Bay National Estuary Program requested that the oyster reefs and oyster bottoms of the Galveston Bay system, including Trinity, East and West Bays, be surveyed and maps prepared showing the location, areal extent and relief of all significant reefs. This effort involved updating maps produced by the Texas Parks and Wildlife Department (TPWD 1976) over a period of 6 years in the late 1960s and early 1970s for part of the region (Benefield and Hofstetter 1976) and a new survey of the remainder of the bay not mapped previously. The total area to be covered was about 1000 km^2 (Wermund et al. 1988).

Surveys of oyster reefs require precise information on reef location and areal extent. The traditional method of surveying involves poling of estuarine bottoms, marking of the reef perimeter, and the delineation of the reef boundaries with triangulation methods (May 1971, Benefield and Hofstetter 1976). This technique is laborious, time-consuming and suffers from the inherent limitations of all line-of-sight methods (e.g. lack of suitable landmarks, poor visibility).

A number of alternatives to traditional methods have been employed. Haven et al. (1979) and Haven and Whitcomb (1983) successfully used an underwater microphone, combined with an electronic positioning system to acoustically detect shell deposits in Chesapeake Bay. DeAlteris (1988) reported mixed results in the capability of side scan sonar to distinguish mud bottoms from oyster reefs. Soniat (1988), however, found side scan sonar to be useful in locating reefs, estimating reef area, and evaluating sedimentation damage to oyster reefs. The technique is adaptable to shallow water (1.5 m) and data can be combined to produce a mosaic picture of the bottom, but the equipment is relatively expensive and inoperable (in the shallow water application) in even slightly choppy seas.

To accurately map an area as extensive as an entire bay system at moderate cost requires a method (1) that can be used from a small research vessel, (2) that requires only a modestly-sized crew, (3) that can be used in unfavorable weather conditions, (4) which can rapidly discriminate bottom type while underway so that the mapping plan can be continuously updated as new reefs are encountered, (5) that accurately and clearly distinguishes bottom types so that little ground-truthing is required, hence maintaining the boat continuously underway, (6) that permits a relatively fast running speed, (7) that is capable of use in shallow (<1 m) as well as deeper (>10 m) depths, and (8) that permits precise, rapid determinations of position on a scale significantly smaller than the average-sized reef (often <20 m in shortest dimension). Methods used previously did not meet these requirements so an improved technique was developed based on precise electronic navigation and an acoustic profiler to differentiate bottom type while continuously underway. Here we present a description of a technique based on this instrumentation which has proven to meet all the above requirements and which has been used to map the Galveston Bay system.

TECHNIQUE DESCRIPTION

Capabilities for Discrimination of Bottom Type

We chose a dual frequency acoustic setup consisting of a Datasonics Dual Frequency Transceiver (Model DFT-210), a Datasonics towed fish with dual transducers (22 and 300 kHz) and an EPC Multichannel Chart Recorder (Model 4800). Primary identification of oyster reefs relied on the record from the 300 kHz channel. On the chart paper, an oyster reef appears as a dark, dense series of spikes projecting well above the background signature from a mud or sand bottom (Fig. 1). DeAlteris (1988) noticed a similar signature with a 200 kHz transducer, however the reliability of the signature was not satisfactory. He relied on a second echo from the hard bottom. The 300 kHz signature is unambiguous. Although we have not investigated the acoustic phenomena involved, we sur-

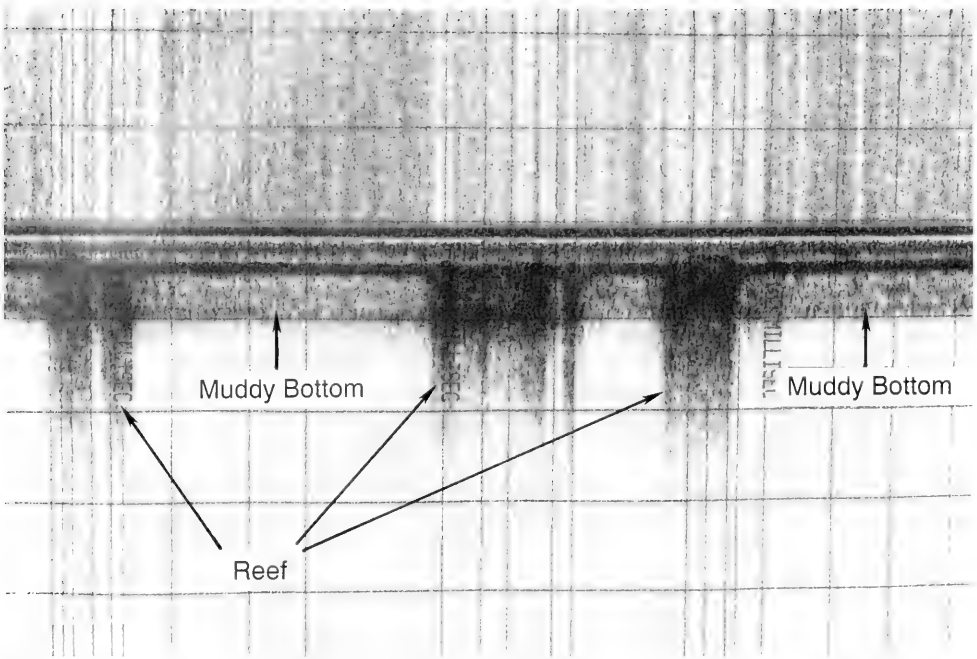

Figure 1. A typical chart record from the 300 kHz channel showing a reef within an area of muddy bottom. The reef is distinguished by a larger return extending well below the more compressed return typical of a muddy bottom.

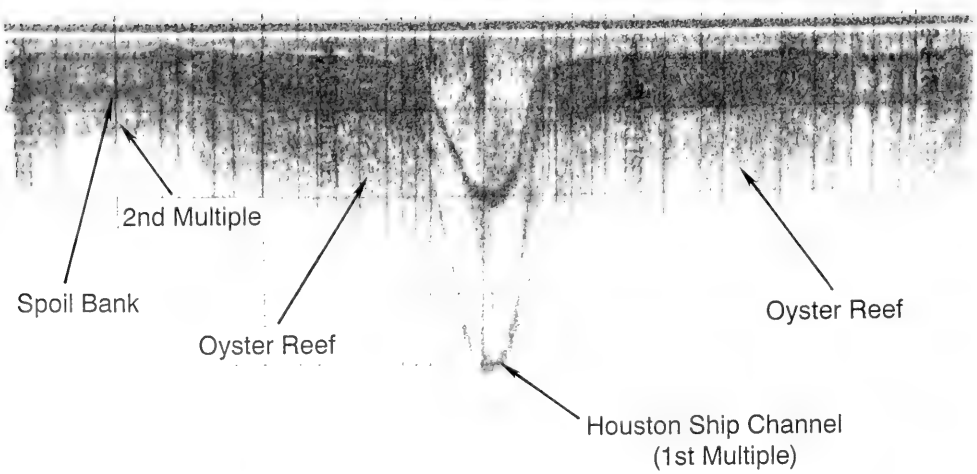

Figure 2. An example of an oyster reef on a spoil bank adjacent to the Houston Ship Channel. The record is from the 300 kHz channel. Reef is identified by the larger denser return extending below the more compressed return. The channel is the deeper V-shaped groove. Note that oyster bottom extends down the channel walls nearly to the bottom, a condition typical of many areas in Galveston Bay.

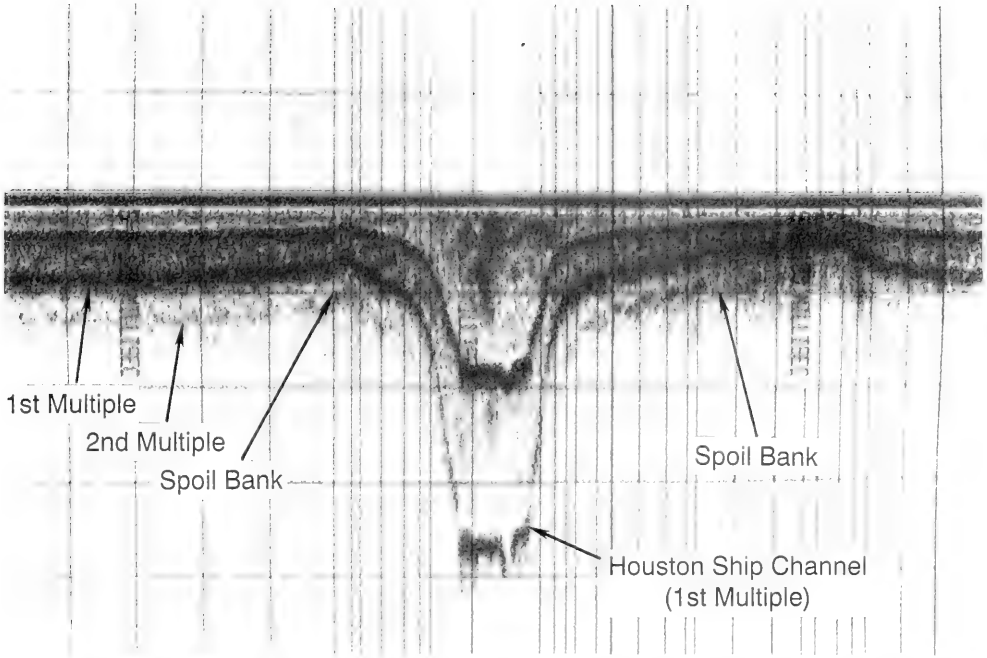

Figure 3. An example of a spoil bank adjacent to the Houston Ship Channel. The record is from the 300 kHz channel. Spoil is identified by the shorter return underlain by a faint halo probably produced by the second multiple. The channel is the deeper V-shaped groove.

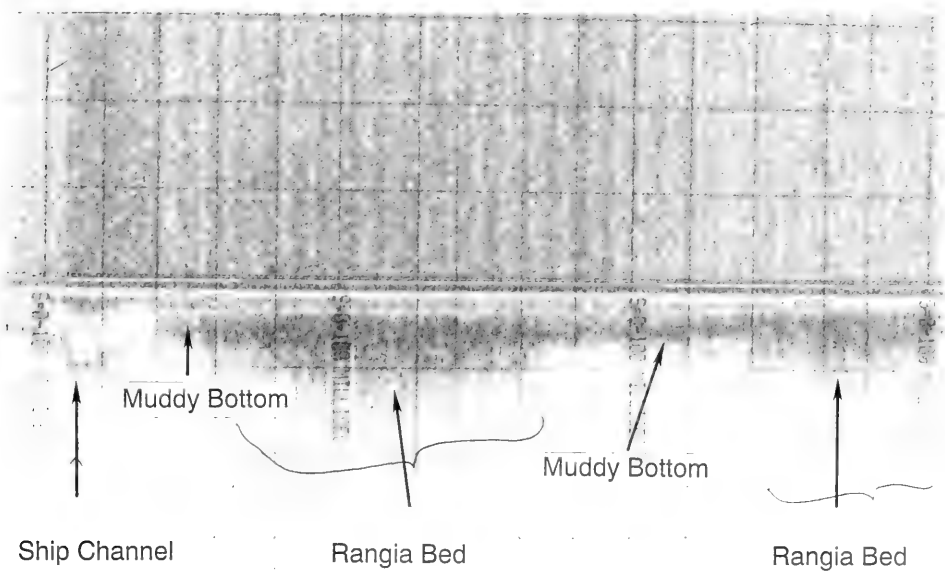

Figure 4. A Rangia bed off Houston Point, Galveston Bay, as recorded by the 300 kHz channel. Clams are identified by the larger denser return below the return typical of muddy bottom. Note how the clam bed fades out at the edges, a condition rarely encountered on reefs. In this case, the 22 kHz channel recorded no distinctive subsurface signal, a characteristic typical of muddy bottom.

mise that the oyster reef signature results from more sound energy bouncing back to the transducer. In the case of a muddy bottom, more of the sound energy is absorbed, thus the signature is reduced. Sand and shell hash give an intermediate, fuzzy signature, still readily distinguishable from reef or other oyster bottom.

In practice, we encountered only two bottom types that required occasional ground-truthing to verify their non-oyster nature: clam beds and coarse shell hash usually associated with points, nearshore sediments and dredge spoil. With experience, coarse shell hash could be discriminated with relative ease and required little ground-truthing. As examples, Figures 2 and 3 show typical returns from a spoil bank adjacent to the Houston Ship Channel with and without an associated reef, respectively. The faint second multiple seen clearly in Figure 3 and just showing through the reef signal in a few spots on Figure 2 is characteristic of dredge spoil, at least in Galveston Bay. With experience, most clam beds could also be distinguished, however dense clam beds required ground-truthing. Figure 4 shows a *Rangia cuneata* bed near Houston Point in Galveston Bay. This technique, then, could be used to identify concentrations of most large epifaunal or semi-epifaunal shellfish, not just oysters.

We used the 22 kHz record to discriminate reefs from oysters on muddy bottom or spoil. In Galveston Bay, oysters occur on true reefs, with a hard basement, on spoil banks next to dredged channels and scattered about on muddy bottom. The latter condition is frequently found (1) on oyster leases used for depurating oysters taken from closed waters for later recapture and sale and (2) on the leeward side of open-bay reefs, presumably where "northers" have blown shell over the reef edge and out onto the surrounding muddy bottom. The 22 kHz record was not always unambiguous, but usually added important information on bottom type. Small reefs and towheads frequently were too small to generate a reef-like subsurface signal although clearly reefal in nature and the substrate under points (e.g. Red Bluff or Dollar Point in Galveston Bay) frequently yielded a strong reef-like return presumably due to the relatively hard basement material forming the point and the meager amount of sediment accumulated upon it. In most cases, however, the three types of oyster bottom could be distinguished accurately using the 22 kHz signal.

Configuration of Acoustic System

In the field, the acoustic system provided reliable data even under unfavorable weather conditions. Signal quality did not deteriorate in 1-m seas, during thunderstorms, or in areas heavily trafficked by boats. Signal quality was satisfactory in depths as shallow as 0.55 m (our minimum running depth) and as deep as 12.5 m (our deepest depths) and at speeds higher than precise navigation would allow (>5.5 knots).

During data collection, the towed fish was lowered from a boom held perpendicular to the boat, well in front of the stern to eliminate the effects of "prop-wash". As many running depths were shallow, we positioned the fish <0.1 m below the water surface to prevent the towed fish from hanging up on underwater obstructions. Signal quality was not affected. To keep the fish from hitting the boat's side during turns and to maintain a proper orientation while underway, the boom was extended 1 m from the boat's rail and a tow rope was run from the fish to the bow to maintain forward aspect during turns and to maintain a vertical downward-facing position for the transducers while underway. The setup is shown in Figure 5.

Figure 5. The setup on the boat as it would appear underway but with the fish raised out of the water. The towed fish extends from a boom to the side of the vessel well forward of the stern. A tow cable to the bow maintains the orientation of the fish while underway.

The settings for the acoustic system will probably need to be adjusted for local conditions to optimize signal quality. As a guide, the transceiver settings we used were:

	300 kHz	22 kHz
Pulse Length	1.0 msec	0.1 msec
Output Attenuation	−10 dB	−3 dB
Band Width	10 kHz	5 kHz
Gain	Left: 0 Right: 3	Left: 0 Right: 13

The chart recorder was set to scan 100 msec with a chart speed of 150 lines in^{-1} and was programmed to key out only once every 5 sweeps of the stylus. The highest sweep rate was used because operating depths were 0.5 to 3 m. Each channel was set as follows: Time varying gain (TVG), none; Threshold, negative stop; Gain, 1.6.

Determination of Position and Relief

Position was determined while underway using a Magellan Global Positioning System (GPS). Loran C proved to be too inaccurate for precise mapping. We emphasize the necessity of using a GPS system for accurate determinations of position. Many reefs were less than 20 m across in shortest dimension and larger reefs had significant variations in relief of a similar scale. In practice,

the precision of our GPS unit was within 0.01 min latitude and longitude on all days. The NOAA-27 datum was used to conform to previous charts of the area.

The frequency at which positions were updated by the GPS unit limited maximum running speed to 5 knots. At speeds greater than 5 knots, the positions of reef details and boundaries could not be accurately recorded. In practice, we used a 4-to-5 knot window for running speed that proved adequate for all applications.

Relief was recorded while underway using an Apelco fathometer. Pictures of the fathometer screen were taken with a 35 mm camera (film speed ≥400 ASA) to record relief of all reefal area because relief changed too quickly to be recorded manually while underway. A chart recorder attached to the fathometer would have been an adequate alternative. Fathometer accuracy declined at depths <0.8 m. We found that a substantial change in running speed affected the depth reading so that maintaining a constant running speed was required throughout a line.

Procedure for Data Collection

Use of an autopilot permitted the pilot to record fathometer data (by taking pictures of the fathometer screen) and positions as well as tend to navigational duties. A second person ran the acoustic profiler. The chart recorder was continually monitored and annotated with position and depth information at least once every minute. When reefs or rapid changes in bathymetry were encountered, positions and depths were recorded at more frequent intervals. Further details of reef relief and position were taken from the pictures of the fathometer screen and calibrated with the chart recorder knowing the speed of the chart paper and the fathometer screen. With a little practice, the entire operation could be easily performed by two people.

For data collection, N-S and E-W lines were run on a 0.125 min grid in areas with reef. An 0.25 min grid was used to map uncharted areas. Subsections having reefal components were then mapped using the 0.125 min grid. The grid choice was a compromise between (1) the detail required to adequately assess reef coverage and the accuracy of positions permitted by the GPS unit and (2) the time required to run the lines. Smaller or larger grids might be used in other applications.

Laboratory Analysis

Because depth changed during the day and from day to day with the tides and wind setup, the bathymetric data must be standardized to a constant datum. To do so, we extended the grid over areas of relatively-deep, flat muddy bottom so that each line and the intersection of several N-S and E-W lines occurred in areas where the depth record was most accurate and where relief changes were minimal. This permitted internal standardization of depth which could then be corrected to some constant datum, such as mean sea level. Army Corps of Engineers tide staffs were used to calibrate the bathymetry to mean sea level.

Once the depth corrections were completed, the data were computerized and processed for use by a Geographic Information System (GIS) to produce the maps. We used Arc/Info software. An example, the lower end of East Bay, is provided as Figure 6.

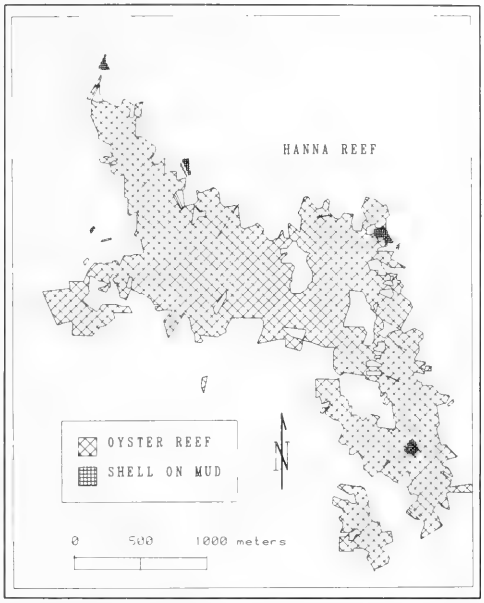

Figure 6. A map of Hanna Reef in East Bay, an arm of Galveston Bay, Texas. Reef is shown by the coarse cross-hatched area; shell on mud by the fine cross-hatched area.

CONCLUSIONS

Our method has been highly successful at differentiating oyster reef and oyster bottom from other substrate types, rapidly and at relatively low cost. The method has also proven useful in locating clam beds and likely could be used for any carbonate-shelled epifaunal or semi-infaunal species that occurs in relatively dense concentrations. The method requires relatively little expertise in acoustics or electronics, but some skill in boat handling. We consider it the technique of choice for most large-scale surveys of oyster resources.

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DIETARY SUPPLEMENTS FOR REPRODUCTIVE CONDITIONING OF *CRASSOSTREA GIGAS KUMAMOTO* (THUNBERG). I. EFFECTS ON GONADAL DEVELOPMENT, QUALITY OF OVA AND LARVAE THROUGH METAMORPHOSIS

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ABSTRACT Kumamoto oysters (*Crassostrea gigas kumamoto*) were conditioned for spawning at 20°C by feeding an algal diet and a supplemental lipid mixture consisting of menhaden oil, egg phosphatidylcholine and partially hydrogenated vegetable oil (Kaomel; Durkee) in the ratio (by weight) 5:2:3. Oysters fed on either algal or lipid mixture gained significantly more weight than non-fed oysters. Supplementary feeding had no effect on the rate of gametogenesis. Oysters fed on supplemental lipid mixtures or algae released higher numbers of eggs compared to those from non-fed broodstock oysters. The percent of straight-hinged larvae from eggs and spat derived from larvae from lipid and algal supplemented broodstock was greater compared to that from non-fed oysters when conditioning was initiated early in the season (March to early April). When conditioning was initiated in May, feeding broodstock oysters with either algal or lipid supplements resulted in no significant increase in larvae or spat produced compared to that from non-fed broodstock oysters. The effects of supplemental feeding on broodstock were similar for oysters held in flow-through and recirculating conditioning systems.

KEY WORDS: Kumamoto oyster, conditioning, aquaculture, supplemental feeding

INTRODUCTION

The normal food used to condition broodstock oysters is a mixture of different algal species; however, culturing various species of algae in the laboratory as food for oysters is labor intensive and costly. Preferably, hatcheries need to supplement broodstock diets with easily manufactured foods. There have been numerous attempts to grow adult oysters, as well as young spat and larvae, on artificial diets (Haven 1965; Turgeon and Haven 1978; Gillespie et al. 1966; Chanley and Normandin 1967; Epifanio 1979; Langdon and Waldock 1981; Langdon and Siegfried 1984). As yet, there is no satisfactory artificial diet, but each investigation adds pertinent information.

C. gigas kumamoto originating from Japan were introduced to the Pacific Northwest at the beginning of the century. The species grows well, but does not reproduce in this environment because of low ambient water temperatures. Kumamoto larvae are produced in hatcheries but conditioning broodstock oysters for spawning is a problem because in the cool waters of the Pacific Northwest gametes form late in the summer. Gametogenesis of this species can be accelerated by elevated water temperatures, and conditioning has been successful when initiated during the period from mid-April through mid-June (Robinson 1992).

In nature the gametogenic cycle of marine bivalves is linked to synthesis of lipid during vitellogenesis at the expense of stored glycogen (Gabbott 1975). Helm et al. (1973) found that survival of *Ostrea edulis* L. larvae was related to lipid content of the larvae at the time of release. Muranaka and Lannan (1984) observed increased fecundity when broodstock oysters (*C. gigas*) were fed on algae compared to that of non-fed broodstock oysters. Bayne (1972) and Bayne et al. (1975) observed reduced growth of larvae obtained from nutritionally stressed *Mytilus edulis*. Gallager and Mann (1986) reported that lipids are an important energy source in the development of ova to normal larvae.

It is hypothesized that Kumamoto broodstock oysters fed on dietary supplements (including lipid and unicellular algae) while conditioned for spawning produce better quality ova than those from non-fed oysters. In this paper this hypothesis is examined.

MATERIALS AND METHODS

Source of Oysters

Kumamoto oysters were purchased from Oregon Oyster Company, Newport, Oregon. The oysters were brought to the Hatfield Marine Science Center, brushed clean, and individuals were separated from clusters without damaging oysters. All of the oysters used in these experiments were from the same stock kept on the commercial oyster grounds in Yaquina Bay.

Flow-Through Conditioning System

The unfiltered seawater from Yaquina Bay was pumped into a large concrete settling tank and then through a second settling tank into a 200-l reservoir, where water was heated to a desired temperature by two 1500 watt immersion heaters controlled by a thermostat. Water in the reservoir was continuously circulated by a pump to maintain a constant temperature, and a float-valve controlled the water level in the reservoir. Water flowed from the reservoir into polyvinyl conditioning containers (30 × 60 × 10 cm). The flow through the conditioning containers was adjusted to 250–300 ml/min, which was the maximum flow supported by the system. Food was introduced by peristaltic pump into the inflowing seawater.

Recirculating Conditioning System

The recirculating system consisted of a 70 liter plexiglass tank used as a water reservoir supplied by an airlift to pump water into polyvinyl conditioning containers (30 × 60 × 10 cm). Water was returned to the reservoir through a short length of tubing. The airlift consisted of a 2 cm diameter PVC pipe, partly submerged in the reservoir and supplied with a steady flow of air at its base. Food was added into the reservoir daily, and seawater in the system was changed once a week. Water temperature was maintained at 20 ± 1°C.

Culture of Algae

Unicellular algae, *Pseudoisochrysis paradoxa,* was cultured in a temperature-controlled room at 18 ± 1°C in continuous light. Sand-filtered seawater was sterilized in 200-l tanks and nutrients were added before inoculation with algae (Matthiessen and Toner 1966). Cultures were harvested for food in log-growth phase at 5–10 million cells/ml. A Coulter Counter, model ZB1, was used to determine cell counts in the cultures.

Preparation of Lipid Microspheres

A lipid mixture of menhaden oil, egg phosphatidylcholine, and "Kaomel" (Durkee; partially hydrogenated vegetable oil) in the ratio (by weight) of 5:2:3 was used as the supplemental lipid source. Menhaden oil was chosen especially for the presence of 20:5 w3 and 22:6 w3 fatty acids which Langdon and Waldock (1981) considered essential. The mixture was homogenized in 2% w/v polyvinyl alcohol warmed to 40°C using a tissue homogenizer, and poured into ice water to form small lipid droplets that were fed to the oysters according to the calculated feeding regimes. Based on results of preliminary feeding experiments, lipid supplementation at a level of 50 percent of the dry weight of the full algal ration was selected as the optimal supplementation level.

Lipid microspheres were initially stained with Sudan Black and fed to oysters to test digestion of the lipid microspheres. Feces were collected two hours after feeding and examined under a microscope for evidence of digested microspheres. Released stain was observed, indicating that oysters were able to digest lipid microspheres.

Experiments

The first conditioning experiment (Exp. I) was initiated April 8, and lasted through May 19, 1987. Oysters were exposed to feeding regimes in flow-through and in recirculating systems. Four conditioning containers with 100 Kumamoto oysters in each were supplied with unfiltered seawater at 20 ± 1°C. In the flow-through system, one container received algae (30,000 cells/ml) and the second container received algae and a supplement of the lipid mixture at a level of 50% of the dry weight of the algal ration. The third container received the 50% lipid supplement alone and the fourth container received seawater with no dietary supplement.

In the recirculating system, 100 Kumamoto oysters were placed into each of three conditioning containers. Reservoirs and conditioning containers were filled with unfiltered seawater at 20 ± 1°C. Two containers received algae (75,000 cells/ml) twice a day, one also received a lipid supplement at 50% of the dry weight of the algal supplement per day. The third batch of oysters received a lipid supplement at 50% of algal ration alone. Fifteen oysters in each container were individually numbered and weighed at the beginning and end of the experiment.

Three additional conditioning experiments, using sand-filtered seawater, were similar in design and were performed from April 6 to June 15, 1988 (Exp. II); from March 29 to July 5, 1989 (Exp. III); and from May 25 to July 27, 1989 (Exp. IV). Feeding regimes were non-fed, algal-fed, and lipid supplements alone at a level of 50 percent of the dry weight of the algal ration. In a flow-through system 30,000 cells of algae/ml were continuously delivered and 75,000 cells algae/ml were delivered twice a day in a recirculating system to oysters.

Oysters from the earlier experiments were used in the fifth conditioning trial (Exp. V). Oysters were spawned and held at 20°C in a flow-through system without supplemental feeding for four months. In efforts to condition nutritionally stressed oysters, supplemental feeding was initiated at 17 ± 1°C instead of 20°C to minimize stress caused by elevated temperatures (Bayne and Thompson 1970; Gabbott 1975; Helm et al. 1973; Bayne et al. 1975). Four groups of 75 oysters each were placed into a flow-through system and each group received a different feeding regime: non-fed, algal-fed (30,000 cells/ml), algae + 50% lipid mixture or algae + 100% lipid mixture. One group of 75 oysters was placed into a recirculating system and conditioned on a ration of 75,000 cells of algae twice a day plus a 50% lipid mixture.

At the beginning and end of the fifth experiment, meats of 20 randomly selected oysters from each treatment were removed, drained for 30 minutes on absorbent toweling, weighed, dried for 48 hours at 60°C, cooled in a desiccator, and re-weighed to obtain dry meat weights.

Histological Examinations

Histological sections of gonadal tissue from 10 conditioned oysters were preserved in Bouin's fixative and sections stained with hematoxylin eosin method every other week or every month during each conditioning experiment. The extent of gonadal development in female oysters was expressed as the percent of ova observed in 200 ova plus oocytes. Male oysters were considered to be in spawning condition when follicles were full of sperm. Maturity for male oysters was expressed as the percent of oysters in spawning condition from the total number of males in the sample.

Larval Culture

Oysters were spawned and percent of straight-hinged larvae were calculated from the number of eggs incubated. Larvae were reared in 25‰ seawater at 26°C through metamorphosis in separate containers to determine variation in the setting success among larvae obtained from broodstock oysters conditioned on each feeding regime (Robinson 1992).

Statistics

Experiment results were analyzed by two-way ANOVA looking at growth, maturity and spawning success as a function of diet and system at 95% significance level. There were no interactions. Before statistical tests were performed, percents were converted to arcsine values. Student-Newman-Keuls multiple range tests were applied on the growth, maturity and spawning success data within both the flow-through and recirculating systems (Sokal and Rohlf 1981).

RESULTS

Experiment I was initiated when no ova from the previous year's gametogenic cycle were present in the gonads. Live weight of oysters increased in all feeding regimes but decreased in non-fed oysters (Table 1). Gametogenesis progressed at approximately the same rate in all groups and after the six week conditioning period oysters responded to spawning stimulus. In contrast, oysters collected from the wild had not developed ova and did not respond to spawning stimulus. The number of eggs collected per female was slightly higher for oysters conditioned in the recirculating system compared to that of oysters from the flow-through system, but the percent fertilized eggs that successfully developed into straight-hinged larvae were approximately the same in oysters from the two systems (Table 1).

TABLE 1.

(Experiment I) Average live weight change (% per week) of conditioned *C. gigas kumamoto* (N = 15).

Regime	Wt. Change %/Week	Maturity		Spawning Success			
		% Ripe Males	% Ova	# Of Males	# Of Females	Eggs/ Female	% D-Larvae
Flow-Through System:							
Ambient (8–12°C)	−0.12*	15	0	0	0	0	0
Non-fed	−0.09*	39	54	2	1	0.3	83
Algal-fed	0.14	54	53	4	2	0.6	91
Algae + 50% lipid	0.15	73	75	5	3	0.97	93
50% Lipid alone	0.10	57	54	4	1	0.5	90
Recirculating System:							
Algal-fed	0.28	36	58	5	4	1.6	94
Algae + 50% lipid	0.25	75	43	6	3	1.4	96
50% Lipid alone	0.22	62	52	5	3	1.6	96

* Change of weight was significant at 95% confidence level.

Lipid supplements were added at a concentration of 50% of the dry weight of the full algal ration.

Maturity for males expressed as percent mature males and the percent oocytes present as ova (N = 10). Spawning success is expressed as the number of oysters spawned (N = 10), number of eggs released per female in millions and percent fertilized eggs that successfully developed into normal D-larvae.

Higher numbers of eggs per female were collected from oysters fed on algal and lipid supplements compared to those from non-fed oysters; however, the differences were not significant at 95% confidence level (Table 1). Also, the percent fertilized eggs that successfully developed into straight-hinged larvae was lowest in the non-fed group, but there were no significant differences in development of fertilized eggs among other treatment groups.

In Experiment II, some ova and sperm were found in oysters from all three feeding regimes after five weeks of conditioning, but only the males responded to spawning stimulus (Table 2). At the end of the ten week conditioning period, female oysters fed on algae possessed the highest percent ova; in both algal- and lipid-fed groups all males were in spawning condition and in non-fed groups, 81 to 83% of males were mature.

Both algal- and lipid-fed treatments resulted in successful induction of spawning of six out of ten oysters (Table 2, Exp. II). Four oysters spawned in the non-fed group, three males and one female. The highest numbers of eggs per female were collected from the algal-fed groups, followed by lipid- and non-fed oysters. Although there was no significant difference among percents straight-hinged larvae obtained from fertilized eggs, there was a significant difference between the percent spat collected from non-fed and fed oysters.

In the recirculating system there was no significant difference in the number of eggs per female between algal- and lipid-fed oysters, but non-fed oysters released half the number of eggs per female compared to oysters from either fed group. The percentage of straight-hinged larvae obtained were approximately the same for all three treatment groups. The highest percent spat yield was collected from algal-fed oysters, followed by lipid-fed and non-fed broodstock oysters.

After a six week period, oysters in Experiment III responded to spawning stimulus but only a few eggs were released (Table 2). After a 14 week period of conditioning, algal-fed oysters produced the highest number of eggs per female in both systems. There were no significant differences between percentages of fertilized eggs which developed into D-larvae among different feeding regimes in the flow-through or recirculating system. There was no significant difference between the number of spat collected from each of the fed oysters in the two systems; however, non-fed oysters produced significantly fewer spat than either of the fed groups.

Experiment IV was started when gametogenesis was underway in oyster populations from the field. At the beginning of the experiment, an average of 2.7% of oocytes present were ova. After a nine week conditioning period in the flow-through system, the percent of ova was over 50 in all feeding regimes (Table 2). In the recirculating system, algal-fed oysters possessed more than 50% of oocytes as ripe ova. Both sexes responded to spawning stimulus and lipid-, algal-, and non-fed oysters yielded 5.6, 4.1, and 2.9 × 10^6 eggs per female respectively and 7.0%, 5.4%, and 6.6% spat yields calculated from straight-hinged larvae used in the experiment. In the recirculating system there were only slight differences in the number of eggs collected per female among feeding regimes. There were no significant differences in percent spat yields from oysters conditioned under the different feeding regimes (Table 2).

At the beginning of Experiment V the mean dry weight of oyster tissue was 110 mg (Table 3). After an 18-week period at 17°C in flowing unfiltered seawater under four different feeding regimes, the oysters had gained weight and formed ripe gametes. Only males responded to spawning stimulus.

Only one feeding regime of algae and supplemental lipids was tested in the recirculating conditioning system (Table 3). Dry weight of tissue increased and both sexes possessed ripe gametes at the end of conditioning period but only two females responded to spawning stimulus.

DISCUSSION

Released stain in fecal pellets of oysters fed on lipid microspheres stained with Sudan Black suggested digestion of lipid microspheres. Furthermore, increases in tissue dry weight during conditioning of oysters fed on algae and lipid supplements indicated the oyster's ability to use the food supplements as a source of energy. Parker and Selivonchick (1986) also reported uptake and metabolism of lipid mixtures by juvenile Pacific oysters. In

TABLE 2.

Maturity of conditioned *C. gigas kumamoto*, expressed as percent mature males and the percent oocytes present as ova (N = 10).

Regime	Maturity		Spawning Success				
	% Ripe Males	% Ova	Males	Females	Eggs/ Female	% D-Larvae	% Spat
EXP. II INITIAL	21	0	—	—	—	—	—
Flow-Through:							
Non-fed	83	65	3	1	0.3	84	0.9*
Algal-fed	100	72	3	3	0.6	96	2.4
50% Lipid alone	100	54	4	2	0.5	97	2.3
Recirculating:							
Non-fed	81	60	7	3	0.8	93	0.8*
Algal-fed	100	86	5	5	1.6	97	2.7
50% Lipid alone	100	72	5	4	1.3	95	2.2
EXP. III INITIAL	30	0	—	—	—	—	—
Flow-Through:							
Non-fed	72	89	4	3	2.0	97	2.2*
Algal-fed	67	79	3	2	3.4	76	3.6
50% Lipid alone	72	88	3	2	1.5	95	3.7
Recirculating:							
Non-fed	100	43	2	2	0.9	83	2.3*
Algal-fed	57	62	3	3	2.2	96	3.9
50% Lipid alone	40	35	1	2	1.8	94	3.4
EXP. IV INITIAL	60	2.7	—	—	—	—	—
Flow-Through:							
Non-fed	86	52	3	3	2.9	92	6.6
Algal-fed	100	56	4	2	4.1	95	5.4
50% Lipid alone	100	57	6	2	5.6	96	7.0
Recirculating:							
Non-fed	83	47	8	2	0.9	94	4.2
Algal-fed	75	57	7	3	1.1	95	5.9
50% Lipid alone	100	46	7	3	1.3	95	5.4

* Significantly different from other means at 95% confidence level.

Lipid supplements were added at a concentration of 50% of the dry weight of the full algal ration.

Spawning success is expressed as the number of oysters spawned (N = 10), number of eggs released per female in millions, percent of fertilized eggs that successfully developed into normal D-larvae and percent of spat.

TABLE 3.

Mean dry tissue weight (g) and standard deviation (SD) of 20 oysters at the beginning and end of Experiment V.

Regime	Dry Weight		Maturity		Spawning Success	
	(g)	(SD)	% Ripe Males	% Ova	# Of Males Spawned	# Of Females Spawned
Flow-Through System:						
Initial	0.11	0.09	0	0	—	—
Non-fed	0.14	0.11	33	6	1	0
Algal-fed	0.21	0.18	42	41	2	0
Algae + 50% lipid	0.26	0.12	27	36	3	0
Algae + 100% lipid	0.29	0.19	54	20	5	0
Recirculating System:						
Algae + 50% lipid	0.27	0.16	29	59	0	2

Lipid supplements were added at concentrations of 50 and 100% of the dry weight of the full algal ration.

Maturity of conditioned Kumamoto oysters, expressed as percent mature males and the percent oocytes present as ova (N = 10). Spawning success is expressed as the number of oysters spawned (N = 10), number of eggs released per female in millions from each feeding regime at 17°C in a flow-through and a recirculating system.

contrast, when sexually mature oysters were kept at 20°C in unfiltered seawater without supplemental feeding for a four month period from April 21, to August 8, 1987, they lost an average of 40% of their live weight and sexes could not be identified. This result agrees with a report by Riley (1976) that during an extended period (176 days) of starvation at 13°C, weight loss of oysters (*Crassostrea gigas*) was equal to 61% of pre-starved weight. Whyte et al. (1990) reported a 65% depletion of total energy in Pacific oysters starved for 405 days at 10°C.

In nature Kumamoto oysters mature once a year and do not spawn in the Pacific Northwest (Robinson 1992). In contrast, when sexually unidentified, nutritionally stressed oysters were fed supplements of algae and lipid at 17°C for a four month period, their average dry tissue weight doubled and they approached sexual maturity for a second time during a calendar year. Supplemental feeding during conditioning did not accelerate the rate of gametogenesis of oysters. Oysters in all feeding regimes matured at approximately the same rate as did non-fed controls.

The benefits of supplemental feeding during conditioning were evident in both increased numbers of eggs released per female and increased percent yields of spat. The numbers of eggs released per female from non-fed broodstock were substantially lower than those from fed oysters. Muranaka and Lannan (1984) found that Pacific oysters fed on algal supplements also showed increased fecundity compared to that of non-fed oysters during conditioning at elevated temperatures.

The percent spat generated from spawning non-fed broodstock oysters was significantly lower than that obtained from oysters conditioned with supplemental feeding. This difference was more pronounced when conditioning was initiated early in the season as was the case in all the experiments started in March and early April. These results agree with those of a previous study where Kumamoto oysters were conditioned for spawning without supplemental feeding in four trials from January to June; percent of spat generated was greater the later in the season conditioning was initiated (Robinson 1992). Lannan et al. (1980) reported that Pacific oysters produced the highest number of spat when conditioned during a "window", a time period determined by the rate at which oysters accumulated glycogen needed to form gametes after the previous spawning season.

Although lipid supplements were not superior to algal supplements for conditioning broodstock oysters, lipid supplements may be useful in hatcheries in the production of higher numbers of larvae and spat early in the summer. Artificial diets like lipid-microspheres are less time consuming to produce than growing algae as food for adult oysters.

The use of the recirculating system resulted in conditioning of Kumamoto oysters that was equally successful as in the flow-through system. The recirculating system requires less seawater than the flow-through system and could save energy and operating costs.

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DIETARY SUPPLEMENTS FOR THE REPRODUCTIVE CONDITIONING OF *CRASSOSTREA GIGAS KUMAMOTO* (THUNBERG): II. EFFECTS ON GLYCOGEN, LIPID AND FATTY ACID CONTENT OF BROODSTOCK OYSTERS AND EGGS

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ABSTRACT Kumamoto oysters, *Crassostrea gigas kumamoto* (Thunberg), were conditioned for spawning at 20 C in either a flow-through or recirculating system and exposed to one of three feeding regimes: non-fed, algae, or lipid microspheres. Sub-samples of oysters from the beginning and end of each experiment, as well as freshly spawned eggs, were analyzed for glycogen, lipid and fatty acid content.

The glycogen level of oyster tissue decreased and the lipid level increased significantly during conditioning in all three treatments. Feeding regimes had no significant effect on glycogen and lipid content of oyster tissue. While feeding regimes had no effect on the fatty acid composition of oyster tissue, they did affect the fatty acid profile of the eggs. The w3 fatty acid content was significantly higher in the eggs released from algal-fed broodstock oysters than that of eggs from the two other treatments. Fatty acid 22:5 w3 was present in the eggs from fed oysters but not in the eggs from non-fed oysters.

KEY WORDS: *Crassostrea gigas kumamoto*, conditioning, supplemental feeding, glycogen, lipid, fatty acids

INTRODUCTION

Biochemical changes are known to accompany gametogenesis in bivalves. Stored glycogen is utilized as energy source by bivalves during gametogenesis (Galtsoff 1964; Gabbott 1975). The importance of lipids in the metabolism of molluscs has been reported by Riley (1976) for Pacific oysters and Millar and Scott (1967) for European oyster species. Lipids are also reported to play a major role in the development of ova into normal larvae (Ansell 1974; Thompson 1977; Gallager and Mann 1981, 1986). Since quantity of lipid in ova is essential for further larval development, it is speculated that quality of eggs may be improved by feeding broodstock oysters on dietary algal or lipid supplements.

A number of unicellular algal species commonly used for bivalve culture have been analyzed for their fatty acid profiles. De Mort et al. (1972) analyzed ten species of estuarine phytoplankton for fatty acid composition and concluded that all tested species had high proportions of palmitic acid, 16:0. Diatoms had high portions of 20:5 and Chlorophyta were rich in 18:3 fatty acids. Chu and Dupuy (1980) and Whyte (1987) analyzed several species of cultured algae for fatty acid composition, algae which are used as food for molluscan bivalve species. Both investigators reported major differences in the fatty acid compositions among different algal species. Undoubtedly, mixing several cultured species of unicellular algae as food for broodstock oysters provides a greater variation of fatty acids than that from only one species of algae; however, it is not cost effective for the oyster industry to produce the large quantities of algae necessary for conditioning broodstock oysters. Based on reports of uptake and digestion of dietary lipids in juvenile oysters and transport from stomach to other tissues (Vassalo 1973; Parker and Selivonchick 1986; Erickson and Selivonchick 1988), broodstock Kumamoto oysters in the current study were fed on lipid microspheres as a source of supplemental lipid. Conditioning oysters with complete or partial replacement of algal diets with lipid dietary supplements may lead to substantially reduced costs.

The Kumamoto oyster *Crassostrea gigas kumamoto* (Thunberg), a subspecies of the Pacific oyster *Crassostrea gigas*, is commercially valuable in the Pacific Northwest of the United States. A significantly higher proportion of larvae successfully metamorphosed into spat from fed Kumamoto oyster broodstock than from non-fed Kumamoto oysters (Robinson 1992a). The objective of this study was to determine the effects of dietary supplements on glycogen and lipid contents and fatty acid composition of Kumamoto oysters during conditioning for spawning at 20 C and spawned eggs.

MATERIALS AND METHODS

Source of oysters, design of conditioning systems, and diets are the same as reported in Robinson (1992a).

Experiments

Three conditioning experiments were conducted in a flow-through and a recirculating system at 20 C. Fifty oysters were placed randomly into each conditioning container. Oysters in one container in each experiment in both the flow-through and recirculating systems were maintained as unfed controls, receiving only sand-filtered seawater. Another container in the flow-through system received 30,000 cells/ml of algae continuously and one was batch-fed at 75,000 cells/ml twice a day in the recirculating system. The third container received lipid supplements consisting of menhaden oil, egg phosphatidylcholine and partially hydrogenated vegetable oil in the ratio (by weight) of 5:2:3 alone at the level of 50% of dry weight of full algal ration in each system.

At the beginning of each experiment, a random sample of five oysters was picked for glycogen and lipid analysis. At the end of each experiment, five females and five males from each feeding regime were sampled for glycogen and lipid analysis. Also at the end of each experiment, ten oysters from each feeding regime were spawned and lipids were extracted from freshly spawned, unfertilized eggs. Fatty acid compositions were determined of oysters at the beginning of each experiment and female and male oysters in spawning condition at the end of the experiment, as well as freshly spawned eggs.

Glycogen Extractions

Oyster meats were shucked, rinsed, and homogenized using a tissue homogenizer. Three samples of two grams each were taken

TABLE 1.

Glycogen content of oyster tissue (% of wet wt.) and standard deviation (SD) at the beginning and the end of conditioning *C. gigas kumamoto* for spawning at 20C in flow-through (F-T) and recirculating (Rec) systems.

		End											
		Non-Fed				Algal-Fed				Lipid-Fed			
Beginning		F-T		Rec		F-T		Rec		F-T		Rec	
%	SD	%	SD	%	SD	%	SD	%	SD	%	SD	%	SD
6.08*	0.14	4.58	0.42	4.80	0.17	4.04	0.79	4.37	0.27	3.51	0.42	4.98	0.28
7.79*	0.48	2.72	0.28	3.53	0.35	2.52	0.63	2.67	0.76	2.85	0.47	2.47	0.58
5.47*	0.56	3.28	0.36	4.15	0.36	3.53	0.23	4.66	0.67	2.15	0.73	4.76	0.39

* Significantly different from final glycogen levels at 95% confidence level.
Three feeding regimes were used: non-fed, algal-fed and lipid-fed.

from each homogenized oyster for glycogen extractions and the remainder reserved for lipid extractions. One ml of 10% trichloroacetic acid was added per gram of tissue homogenate in order to dissolve glycogen. The mixture was centrifuged and supernatant collected. The pellet was washed with one more volume of trichloroacetic acid, centrifuged and the supernatant collected. After two volumes of 95% ethanol were added to one volume of supernatant and stirred, the precipitate was allowed to flocculate and then centrifuged. Supernatant was discarded and the white precipitate was dissolved in 5 ml of water and re-precipitated by the addition of two volumes of 95% ethanol, then centrifuged. The precipitate was washed once more with 95% ethanol, followed by 3 ml of absolute ethanol and 3 ml of diethyl ether; precipitate was then air-dried and weighed (Stetten et al. 1958).

Lipid Extraction

Three sub-samples of two grams each of the homogenized oyster tissues were added into 20 ml of chloroform:methanol (2:1), mixed and filtered through Whatman #1 glass-fiber filter paper. The tissue was scraped off the filter paper and again mixed with 10 ml of chloroform:methanol and re-filtered. This was repeated a third time. The filtrate was poured into a separatory funnel and one volume of water was added to 5 volumes of the extract, mixed and allowed to stand in a refrigerator overnight. The lower phase was transferred into a pre-weighed test tube and evaporated to dryness under N_2 and weighed to determine total lipid content (Folch et al. 1957).

Methyl esters were prepared for fatty acid analysis by gas chromatography. Four ml of 4% H_2SO_4 in methanol were added into the test tube containing lipid extract, flushed with N_2 and closed with a Teflon-lined cap. The mixture was heated at 80–90 C in a water bath for 90 minutes, cooled, neutralized with 5% N_aHCO_3, extracted with 3 ml hexane, transferred into a clean vial and evaporated to dryness under N_2. Methyl esters were dissolved in 1 ml isooctane and 1 µl of solution was injected into a gas-liquid chromatograph for fatty acid determination. Fatty acids were identified by comparing their retention times with those of known standards. Fatty acid composition was also determined for the lipid and algal diets.

Experimental results were analyzed for significant differences by two-way ANOVA and Tuckey's multiple range test at the 95% confidence level. Before statistical tests were performed, percents were converted to arcsine values (Sokal and Rohlf 1981).

RESULTS

By the end of all three conditioning experiments, the glycogen contents of broodstock oysters decreased significantly from the beginning level in all feeding regimes in the flow-through and recirculating systems (Table 1). There were no overall significant differences in glycogen contents among oysters from each conditioning system or among oysters from the different feeding regimes in either conditioning system.

Male oysters in all feeding regimes (both conditioning systems) had approximately 1% more glycogen than female oysters after conditioning; however, differences were not significant at 95% confidence level (Table 2). When lipid contents of female and male oysters were examined, no significant differences were noted. However, female oysters had approximately 1% more lipid than male oysters after conditioning (Table 2).

Lipid contents of Kumamoto oysters were significantly higher

TABLE 2.

Glycogen and lipid content (% of wet wt.) of female and male *C. gigas kumamoto* and standard deviation (SD) after conditioning for spawning at 20C.

	Non-Fed				Algal-Fed				Lipid-Fed			
	Glycogen		Lipid		Glycogen		Lipid		Glycogen		Lipid	
Sex	%	SD	%	SD	%	SD	%	SD	%	SD	%	SD
Female	3.47	0.77	4.37	1.30	3.48	1.78	3.45	1.45	3.51	1.01	3.02	1.01
Male	5.10	1.12	3.64	1.01	4.49	2.22	2.14	1.65	4.31	1.71	2.27	1.05

Three feeding regimens were used: non-fed, algal-fed and lipid-fed.

TABLE 3.

Lipid content of oyster tissue (% of wet wt.) and standard deviation (SD) at the beginning and end of three conditioning experiments.

		End											
		Non-Fed				Algal-Fed				Lipid-Fed			
Beginning		F-T		Rec		F-T		Rec		F-T		Rec	
%	SD	%	SD	%	SD	%	SD	%	SD	%	SD	%	SD
1.19*	0.22	4.12	0.19	4.44	0.26	3.32	0.15	3.50	0.22	2.74	0.31	2.90	0.29
0.64*	0.14	2.03	0.41	2.61	0.16	1.91	0.53	2.30	0.81	2.53	0.26	3.20	0.34
2.63*	0.21	3.89	0.27	2.46	0.41	1.37	0.44	3.04	0.61	2.33	0.42	2.06	0.16

* Significantly different from final lipid levels at 95% confidence level.

C. gigas kumamoto were conditioned for spawning at 20C in a flow-through (F-T) and a recirculating (Rec) system, each with three feeding regimens.

at the end of the conditioning period than at the beginning (Table 3). There was no significant difference between the lipid contents of oysters fed on algal or lipid supplements, but there was a significant difference between those of fed and non-fed groups of oysters.

Since there were no significant differences in the total fatty acid composition in the oysters or the ova from three different experiments, the results from Experiment II were used as an example. There were no significant differences in total fatty acid profiles of oysters from the two systems, neither were there any significant differences between the levels of fatty acids in females or males from the different feeding regimes (Table 4). Significant differences were found, however, in the fatty acid composition among the eggs collected from oysters conditioned in different feeding regimes (Table 5). In both systems 18:0 and 18:3w3 fatty acids were present at >1% level in eggs from the oysters in non-fed treatments compared to levels of <1% for these fatty acids in eggs from algal or lipid-fed oysters. Fatty acid 18:1 was found in the ova from non-fed and lipid-fed oysters but not in algal-fed oysters. Lipid microspheres consisted of 27.2% 18:1 fatty acid compared with only 1.9% in the algal diet, and were therefore noted as a major source of this fatty acid. Fatty acid 22:5w3 was present in

TABLE 4.

Fatty acid composition of dietary algal and lipid supplements as well as oyster tissue at the beginning and end of the conditioning of *C. gigas kumamoto* for spawning in three feeding regimens.

				Ending 7/10/89					
	Dietary Supplement			Females			Males		
Fatty Acid	Algae	Lipid	Beginning 3/29/89	Non-Fed	Algal-Fed	Lipid-Fed	Non-Fed	Algal-Fed	Lipid-Fed
14:0	22.10	3.84	1.60	1.40	1.40	1.60	1.30	1.10	1.10
16:0	15.50	26.40	20.50	18.20	19.50	19.90	17.00	16.00	13.70
16:1w7	—	6.10	3.90	2.00	2.10	1.90	1.30	1.70	1.80
18:0		3.10	3.20	4.30	4.80	4.70	4.60	4.10	3.50
18:1	11.90	26.40	—	—	—	—	—	—	—
18:1w7	—	—	3.00	4.80	6.80	6.10	5.40	5.90	4.90
18:2	1.70	6.70	—	—	—	—	—	—	—
18:2w6	3.20	—	2.50	2.00	2.20	2.00	2.10	2.20	1.60
18:3w6	—	—	0.50	0.50	0.30	0.50	0.20	0.20	0.30
18:3w3	5.30	2.20	4.90	5.80	5.10	5.20	4.80	4.70	3.40
20:1	—	—	1.30	3.10	1.30	1.30	2.20	1.70	1.70
18:4w3	16.20	—	7.80	8.20	8.40	8.90	11.40	17.60	19.40
20:4w6	—	—	1.00	1.10	1.10	1.20	1.00	1.20	1.00
22:1w9	—	—	1.10	1.00	1.20	1.20	1.00	0.90	0.90
20:5w3	0.40	5.20	13.10	12.70	13.30	13.60	11.50	12.30	12.20
22:4w6	—	—	0.80	0.80	0.80	0.80	0.50	0.70	0.50
22:5w3	0.10	—	1.00	0.90	0.90	1.00	0.60	0.90	0.80
22:6w3	6.40	5.00	10.50	11.50	9.80	10.70	9.30	10.60	7.90
Σw3	28.40	12.40	37.30	39.10	37.50	39.40	37.60	46.10	43.70
Σw6	3.20	0.00	4.80	4.40	4.40	4.50	3.80	5.50	3.40
w6/w3	0.11	—	0.13	0.11	0.12	0.11	0.10	0.12	0.08

Algae: *Pseudoisochrysis paradoxa*

Lipid: Mixture of menhaden oil, egg phosphatidylcholine and vegetable oil in the ration (by weight) 5:2:3.

Data for oysters from flow-through and recirculating systems were combined.

TABLE 5.

Fatty acid content in the freshly spawned, unfertilized eggs of *C. gigas kumamoto* conditioned for spawning in three feeding regimes: non-fed, algal-fed and lipid-fed in flow-through and recirculating systems.

	Flow-Through			Recirculating		
Fatty Acid	Non-Fed	Algal-Fed	Lipid-Fed	Non-Fed	Algal-Fed	Lipid-Fed
16:0	28.8	29.3	27.6	22.8	30.1	26.3
18:0	9.4	—	—	6.7	—	—
18:1	7.2	—	11.4	6.1	—	12.6
18:2	3.2	1.3	1.9	3.2	1.2	2.6
18:3w3	6.0	—	—	5.8	—	—
18:4w3	8.9	9.6	9.2	12.0	12.9	11.4
20:5w3	18.4	27.7	22.3	21.3	30.3	24.3
22:5w3	—	2.2	1.6	—	2.6	1.9
22:6w3	13.3	30.0	14.0	15.2	24.3	17.3
Σw3	46.6	69.5	47.1	54.3	70.1	54.9

the ova from algal and lipid-fed oysters but not in the ova from non-fed oysters, however, it was not found in either dietary supplement (Table 4). A significantly higher proportion of w3 fatty acids was found in ova released from algal-fed broodstock oysters compared with that in ova from either non-fed or lipid-fed oysters in both conditioning systems (Table 5). A slightly higher proportion of w3 fatty acids were present in ova from lipid-fed oysters than in those of non-fed broodstock oysters (Table 5).

DISCUSSION

Seasonal changes in glycogen content in oysters coincide with the development of gametes. Investigations on the seasonal fluctuation of glycogen content in *Ostrea edulis* (Holland and Hannant 1974, 1976; Walne 1970; Russell 1923) and *Crassostrea virginica* (Galtsoff 1964) have shown that glycogen levels generally increase during late summer and fall and decrease during winter. Mitchell (1916), Walne (1970), and Gabbott and Walker (1971) have shown that glycogen content in oysters decreases during spawning season. In the present study, glycogen content decreased during conditioning of oysters in all treatments. In the warm water temperature (20 C) of the conditioning systems, the rate of metabolism may have increased to the point where glycogen reserves were used as an energy source by the oysters despite supplemental feeding.

Seasonal changes in the lipid content of oysters and other bivalves have been noted by a number of investigators (Loosanoff 1942; Engle 1950; Giese 1966; Walne 1970; Trider and Castell 1980). Lipid content is reported to increase before spawning and decrease immediately after spawning (Pollero et al. 1979; Barber and Blake 1985; Allen and Conley 1982), probably as a result of losses of lipid in the spawned eggs. Thompson (1977) reported that in female scallops lipid content declines after release of eggs. Trider and Castell (1980) found an increase in the lipid content of American oysters (*C. virginica*) in the spring and a sudden decrease in July, coinciding with spawning.

In the present study, body tissues of female oysters in spawning condition had approximately one percent higher levels of lipid than those of the male tissues, perhaps due to the presence of lipid rich ova. In contrast, conditioned male oysters possessed approximately 1% higher glycogen content than female oysters. Perhaps development of sperm has a lower metabolic cost.

In the present study w3 fatty acids were found in freshly spawned, unfertilized ova at relatively greater levels than in female tissue samples. This agrees with the results of Dawson and Barnes (1966) who have reported high levels of polyunsaturated fatty acids in the eggs of marine invertebrates. It is noteworthy that 22:5w3 fatty acid was found in ova from algal- and lipid-fed oysters but was not found in ova from non-fed oysters in either conditioning system. It is possible that a relatively high level of w3 fatty acids in eggs is required for normal embryonic development. Gallager and Mann (1986) reported that there was a minimum level of lipid required in the ova for the development of normal straight-hinged larvae.

Present findings suggest that w3 fatty acids play a key role in larval development. The greatest percent of larvae that successfully metamorphosed to spat originated from the same algal-fed broodstock oysters followed by percent spat from the same lipid- and non-fed oysters (Robinson 1992a), suggesting that w3-rich eggs result in more successful larval metamorphosis to spat than larvae from w3-poor ova. Furthermore, the fact that 22:5w3 was found only in ova released from fed oysters, ova which also resulted in the greatest percent production of spat, may indicate a significant importance of 22:5w3 in larval development and growth.

Based on this finding and that of Muranaka and Lannan (1984), it is recommended that broodstock oysters be fed dietary supplements for optimal conditioning. Lipid microspheres incorporating various w3 fatty acids could be developed to supply a tool for further studies to determine which fatty acids can be increased in eggs and which fatty acids are the most beneficial for further larval development.

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OBSERVATIONS ON GROWTH RATE AND RESISTANCE TO MSX (*HAPLOSPORIDIUM NELSONI*) AMONG DIPLOID AND TRIPLOID EASTERN OYSTERS (*CRASSOSTREA VIRGINICA* (GMELIN, 1791)) IN NEW ENGLAND

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ABSTRACT Growth rates of triploid and diploid oysters (*Crassostrea virginica*), derived from the same parent stock, were compared during their first and second seasons of growth at three locations in the northeastern United States. Triploid growth rate, as determined by shell height, whole weight and total volume, was significantly greater than diploid growth rate in all categories at all three sites. At the site where the parasite *Haplosporidium nelsoni* (MSX) occurs, the triploids initially suffered lower mortalities than the diploids despite higher prevalence of infection, and their condition index was higher. These results are considered encouraging with respect to the culture of triploid *C. virginica* in New England.

KEY WORDS: *Crassostrea virginica*, oyster, triploidy, growth, resistance, MSX

INTRODUCTION

In recent years, the protozoan parasite *Haplosporidium nelsoni* (MSX), responsible for heavy oyster (*Crassostrea virginica*) mortalities along the Middle Atlantic Coast since 1957 (Haskin et al. 1966), has occurred in many oyster growing areas of New England (Haskin and Andrews 1988). Encouraging results in terms of reducing losses among oysters exposed to this pathogen have resulted through selective breeding programs designed to develop disease-resistant oyster strains (Haskin and Ford 1979, Ford and Haskin 1988). However, even members of highly-selected strains may eventually succumb under prolonged MSX infection (Ford and Haskin 1987).

Experiments in the induction of polyploidy in the Pacific oyster (*C. gigas*) has produced favorable results in that triploid oysters produce few gametes and maintain a high condition index throughout the spawning season, unlike diploids which discharge large quantities of gametes and are in relatively poor physiological condition during the summer months following spawning (Chew 1984, Allen and Downing 1986, Davis, in preparation). Stanley et al. (1984) found that the growth rate among triploid *C. virginica* was superior to that of sibling diploids and attributed this to the likelihood of greater heterozygosity among the triploids.

MSX infection among Eastern oysters in New England appears to occur very shortly after spawning (Leibovitz et al. 1987, Matthiessen et al. 1990), when the oysters are generally in poor condition. It therefore seemed possible that triploids, retaining a relatively high condition index during the infective period, might have a higher level of resistance than diploids. In addition, the possibility that the triploids might grow significantly faster than the diploids could provide them with a distinct advantage in areas infected with MSX by reducing the period of exposure and infection prior to market.

The purpose of this study was to evaluate the growth rate of triploid Eastern oysters at three different culture sites in relation to diploids derived from the same parent stock. One of the selected areas—Cotuit Bay, Massachusetts—has been the site of periodically heavy MSX-induced mortalities since 1985 and provided the opportunity to compare triploid and diploid survival under MSX pressure.

METHODS

Initial efforts to induce triploidy in *C. virginica* at the Ocean Pond Corporation hatchery facility on Fishers Island, New York were made in 1987 and again in 1988, with inconsistent and generally unsatisfactory results. It was supposed that the relatively low salinities and variable temperatures at this facility may have imposed additional stress upon embryos and larvae already exposed to chemical treatment. Therefore the broodstock to be utilized for the present experiment were sent—in January, 1990—to Aquacultural Research Corporation in Dennis, Massachusetts, for conditioning and subsequent spawning. These oysters (1987 year-class) were members of the seventh generation of a strain originally obtained from Long Island Sound and bred for MSX resistance at the Rutgers Shellfish Research Laboratory (Haskin and Ford 1979) with the Rutgers code designation R-BLA. This particular group of 50 oysters was selected on the basis of superior growth rate.

Eggs and sperm were obtained from six males and three females that were induced to spawn on April 9. The embryos were treated in a manner generally similar to the procedures described by Stanley et al. (1981) for *C. virginica* and by Downing and Allen (1987) for *C. gigas*, using the antibiotic cytochalasin B to prevent polar body extrusion. Treatment consisted of adding 1 mg of cytochalasin dissolved in 1 ml of DMSO (dimethylsulfoxide) to each 1-liter container holding the embryos. In this case, however, treatment with cytochalasin occurred 20 minutes after fertilization for a period of 20 minutes, followed by a 15-minute rinse in 0.1% DMSO at 25°C. The embryos were then transferred in two groups of 7.5 million each to individual culture containers, and an additional group of embryos, derived from the same spawning but not treated with cytochalasin B, were retained as diploid controls.

Unfortunately, the control group was accidentally discarded, and two males and two females from the same group of LISR parents were induced to spawn on 11 April. The resulting larvae were retained as diploid controls for the treatment group deriving from the 9 April spawning. Samples from both groups of larvae were collected and sent to the University of Washington for ploidy analysis.

The two groups of juvenile oysters were shipped to Ocean Pond

Corporation on 24 May, when approximately 45 days old. At this time, oysters in both groups ranged from 3 to 7 mm in shell height. Each group contained approximately 100,000 oysters. They were placed in a series of 0.6 × 0.6 m floating fly-screen mesh trays, at a density of approximately 8,000 oysters per tray, and held in the brackish water pond utilized by Ocean Pond Corporation for rearing seed oysters. Twice each week, each tray was flipped over in order to rid the screen on the underside of fouling.

On 18 June, when the oysters were slightly over two months old, both groups were screened through a 6-mm wire mesh sieve. Those large enough to be retained were transferred to 6-mm mesh pearl nets at densities of 500 per net and suspended from buoyed lines in the pond.

On 3 July, the oysters in pearl nets were considered large enough for initiation of the growth and disease resistance experiments. Oysters in both groups were sieved through a 12-mm mesh screen. A random sample of 400 individual oysters was drawn from both the control and treatment groups retained in the sieve. Each of these samples of 400 were divided into groups of 200 and placed in separate 6-mm mesh plastic bags measuring 0.6 m × 0.9 m, which in turn were placed in 25-mm mesh vinyl-coated wire trays. As a further control on disease-related mortality, a sample of 200 1989 year-class oysters with no history of genetic resistance to MSX were obtained from Ocean Pond and placed in a similar tray. These trays were then placed on the bottom in Cotuit Bay. At this time the water temperature in Cotuit Bay was 22.0°, and the salinity was 30‰.

Two additional random samples of 100 oysters each were drawn from both the treatment and control groups that were retained in the 12-mm mesh screen as described above. These were transferred to 6-mm mesh pearl nets, which were suspended from buoyed long-lines in Fishers Island Sound and in Ocean Pond on 5 July. In the Sound, water temperature on this date was 20.0°; the salinity was 30‰. The pond temperature and salinity were 25.0° and 16‰, respectively.

At this time, samples of 50 oysters each were drawn from the treatment and control populations held in Ocean Pond since 24 May and were sent to the University of Washington for ploidy analyses. Near the conclusion of the growing season, on 26 October, samples of 25 oysters each were again taken from both groups, this time from those that had been placed in Cotuit Bay, and sent to the University of Washington for ploidy verification. Prior to ploidy analyses, the condition indices (C.I. = [dry weight (gm)/cavity volume (cc)] × 100) of these oysters were determined.

At approximately monthly intervals between 3 July and 5 December, random samples of 30 oysters were drawn from the populations of treatment and diploid control groups in Fishers Island Sound and Ocean Pond and measured for shell height, whole weight and total volume. Similar measurements were made for the treatment and control groups in Cotuit Bay, but, in addition, samples of 25 oysters each were drawn from the treatment, control and 1989 year-class groups on 15 August, 21 September and 26 October and sent to Battelle Laboratories in Duxbury, Massachusetts, for histological examination. All oyster groups were examined for evidence of mortality when these samples were taken.

Shell height, whole weight and survival rate determinations for both groups at all three sites were made on three occasions during the 1991 growing season, i.e. in April, July and September. At the termination of observations in September, 53 oysters from the treatment group and 49 from the control group at the Cotuit Bay site were identified by individual number marked on the shell, measured for shell height and weighed, and sent to the University of Washington for ploidy analyses and condition index determination. Tissue sections were obtained from 25 oysters from each group for subsequent histological analysis at the Battelle Laboratories.

RESULTS

Cytochalasin treatment of the embryos obtained from the 9 April (1990) spawning resulted in a high percentage of triploids. Out of three samples (total of 125 individuals) taken from the treatment group in April, July and October of 1990 and analyzed for ploidy, only one individual—from the October sampling—was found to be diploid, or less than 1 percent. Analyses of the control group confirmed all were diploid.

Of approximately 15 million fertilized eggs deriving from the 9 April spawning that were treated with cytocholasin B, 1.3 million, or slightly less than 10%, survived to setting size 15 days later. The great majority of the losses occurred during the first week. Survival of the 11 April control group to setting size, which occurred 13 days after spawning, was slightly higher, i.e. 14%.

During the initial month in the pond, there was no evidence of mortality among the diploid group. However, approximately 50% of the triploids died during the initial few weeks, for reasons unknown, although it is possible that the stress of being transferred from relatively high (30‰) to the much lower (16.5‰) salinity of the pond environment was lethal to individuals already weakened by the cytochalasin treatment.

The diploids initially showed better growth than the triploids. On 3 July, the diploid oysters retained in the 12-mm mesh screen screen averaged slightly larger than the triploids with respect to shell height, whole weight and total volume. (These differences were found by t-test to be significant ($P < 0.05$)). However, the triploids grew faster at all three locations during the remainder of their first growing season (Figs. 1–6; 95% confidence limits indicated by vertical bars). The results of height and weight measurements at the end of October (Table 1a) were analyzed by two-factor ANOVA, with the results given in Table 1b. (Volume measurements closely paralleled weight measurements and are not included in the Figures or Table 1).

The superior rate of growth of triploids persisted at all three

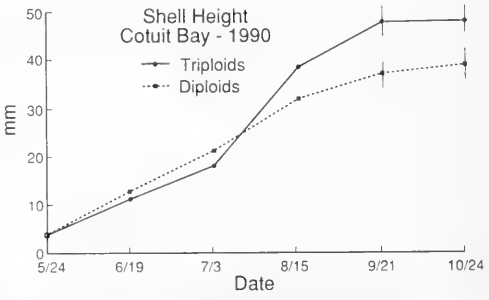

Figure 1. Shell height, Cotuit Bay, 1990.

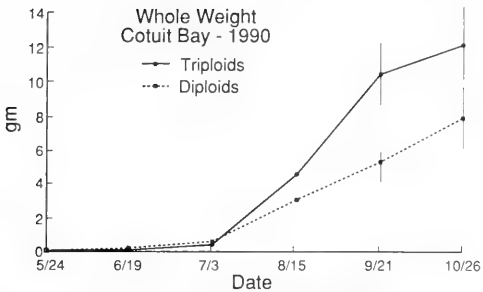

Figure 2. Whole weight, Cotuit Bay, 1990.

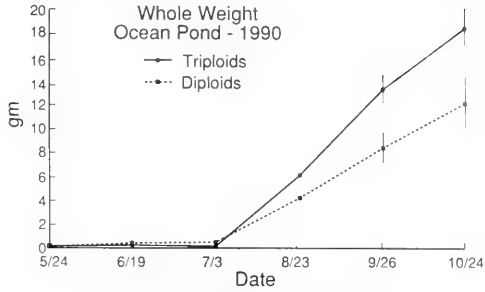

Figure 4. Whole weight, Ocean Pond, 1990.

sites during the 1991 growing season. Analysis of the results of final growth measurements in September, 1991, are given in Tables 2a and 2b. The significant interaction between ploidy and location is attributed to the exceptional growth rate of the triploids at the Fishers Island Sound site, particularly with respect to shell weight. (One-way ANOVA of shell height and of total weight showed significant ($P < .001$) differences in both categories among the different groups; the significant ($P < .001$) disparity of the Fishers Island Sound triploid group from the other groups was shown by Scheffe's test (Zar 1974)). It is believed that the combination of relatively high salinity (30‰), moderate to strong tidal currents, and off-bottom suspension of the oysters at this site contributed significantly to the exceptional shell growth.

A significant difference in condition index between the two groups at Cotuit was found at the end of their first growing season (October, 1990) (t-test; $P < 0.01$). The mean for the diploid sample was 5.1 ($N = 27$, $s = 1.0$) as compared with 7.0 ($N = 28$, $s = 0.8$) for the triploid group. However, comparison of mean condition indices for the two groups in September, 1991—6.4 and 6.2 for the diploid and triploid groups respectively—revealed no significant difference.

Histological examinations during August, September and October, 1990, indicated gonad development in slightly more than half the oysters sampled from both groups. Only three diploids, however, contained follicles with lumina at least half filled with gametes.

Ploidy analysis in September, 1991, of 53 oysters in the triploid group that had been held in Cotuit Bay during the two growing seasons revealed a much higher percentage of diploids—15%—than in previous samples. The 45 oysters in this group identified as triploids were considerably larger than the 8 siblings found to be diploids (Table 3). Analysis of height and weight for the three groups—triploids, sibling diploids, and diploid control group—by one-way ANOVA indicated significant ($P < .001$) differences between groups in both categories. Only the triploids were found by Scheffe's test to be significantly different from the other two groups.

Histological examinations of oysters collected from Cotuit Bay on 15 August, 1990 revealed the presence of MSX in all three groups: the 1990 year-class diploids and triploids and the 1989 year-class oysters from Ocean Pond. The incidence of infection was highest in the 1989 group (92%), followed by the 1990 triploids (60%), with the 1990 diploids having the lowest incidence of infection (24%) (Table 4). This last group had the highest mortality (6%) of the three, although there was no evidence of predation.

By 21 September, 1990, the 1989 Pond oysters had suffered heavy mortalities (44%) (Fig. 7), and 76% of the survivors sampled were infected. The 1990 diploids continued to show the lowest incidence of infection, i.e. 36% as compared with 60% for the triploids. Their survival rate, based upon the mean of replicate trays, was 85%, considerably lower than for the triploids (98%). Comparisons of mean total volumes of infected and uninfected oysters from the triploid group showed the infected oysters to be significantly (t-test; $P < .05$) smaller: 6.2 cc vrs 9.3 cc. Mean volume comparisons of the diploids also showed the infected oysters to be slightly smaller, although not significantly so.

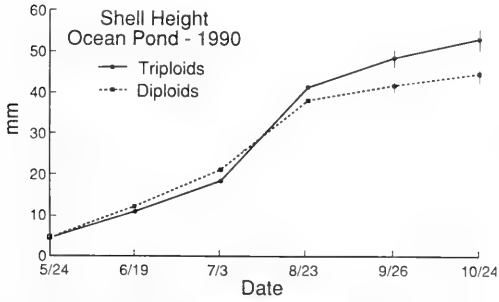

Figure 3. Shell height, Ocean Pond, 1990.

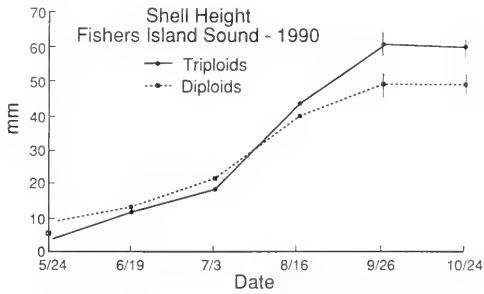

Figure 5. Shell height, Fishers Island Sound, 1990.

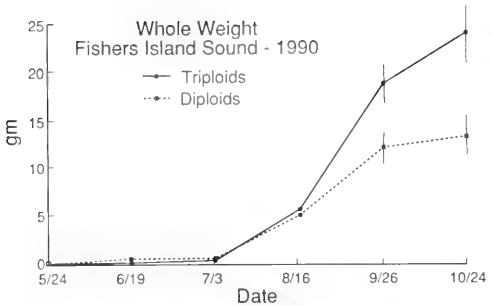

Figure 6. Whole weight, Fishers Island Sound, 1990.

Observations on 26 October revealed that only 21% of the 1989 Pond oysters were still alive, having suffered nearly 80% mortality since mid-August. Of the remaining survivors, 68% were infected. Mortalities had suddenly increased markedly among the triploid group, where survival was now reduced to 72% since 3 July. However, the triploids still showed higher survival than the diploids, the population of which had fallen to 62% by this date. Mortality rates among both groups at the other two sites were negligible (<10%).

By September of 1991, percent survival among the two groups of diploid oysters at the Cotuit Bay site was again lower than for the two triploid groups (31 and 34% for the diploid groups as compared with 37 and 43% for the triploids). Seven of 26 members of the triploid group were infected with MSX; two infections were systemic, five were localized to the gill tissues. Of 22 diploids analysed, three were infected, two of these systemically (Table 4).

During 1991, 20% mortality occurred among the diploid group at Ocean Pond during the latter part of the summer. No pathogens were found in histological analyses of this group.

TABLE 1A.

Means ($\bar{x}$), standard deviations (s) and coefficients of variation (CV) in shell height (mm) and while weight (gm) at the three experimental sites at end of first growing season.

Site		Triploids mm	Triploids gm	Diploids mm	Diploids gm
Cotuit Bay (10/26/90)	$\bar{x}$	48.4	11.8	38.8	7.5
	s	11.0	6.3	10.0	6.0
	CV	22.7	53.4	25.7	79.4
Fishers Island Sound (10/24/90)	$\bar{x}$	60.5	24.2	49.5	13.5
	s	8.2	7.2	8.3	5.6
	CV	13.6	29.8	16.8	41.5
Ocean Pond (10/24/90)	$\bar{x}$	53.8	18.9	45.6	12.4
	s	4.4	4.0	8.1	5.8
	CV	8.2	21.2	17.8	46.8

TABLE 1B.

Results of analysis of variance for effects of location and ploidy upon growth rate (shell height and whole weight) at conclusion of first growing season.

Sources of Variation	DF	Shell Height F-ratio	Shell Height Prob	Whole Weight F-ratio	Whole Weight Prob
Location	2	29.61	<0.0001	49.45	<0.0001
Ploidy	1	63.80	<0.0001	103.58	<0.0001
Interaction	2	1.33	0.2665	9.22	0.0002

TABLE 2A.

Means ($\bar{x}$), standard deviations (s) and coefficients of variation (CV) in shell height (mm) and whole weight (gm) at the three experimental sites near end of second growing season.

Site		Triploids mm	Triploids gm	Diploids mm	Diploids gm
Cotuit Bay (9/20/91)	$\bar{x}$	72.8	49.7	60.1	29.8
	s	15.3	26.5	12.3	17.3
	CV	21.0	53.3	20.5	58.1
Fishers Island Sound (9/18/91)	$\bar{x}$	95.4	92.9	72.0	40.9
	s	7.4	17.0	9.4	14.5
	CV	7.8	18.3	13.1	35.4
Ocean Pond (9/18/91)	$\bar{x}$	65.8	37.1	57.4	23.1
	s	5.7	8.6	8.9	11.2
	CV	8.7	23.3	15.5	48.3

TABLE 2B.

Results of ANOVA for effects of location and ploidy upon growth rate during second growing season.

Sources of Variation	DF	Shell Height F-ratio	Shell Height Prob	Whole Weight F-ratio	Whole Weight Prob
Location	2	70.81	<0.0001	73.48	<0.0001
Ploidy	1	70.11	<0.0001	105.19	<0.0001
Interaction	2	8.77	0.0002	22.50	<0.0001

DISCUSSION

The rate of growth observed in the triploid group was exceptionally high. At the Fishers Island Sound site, the triploids averaged 60 mm in shell height and nearly 25 gm in weight by the end

TABLE 3.

Comparisons in shell height and total weight between triploids, sibling diploids of the triploid group, and non-sibling diploids.

Group	N	Height x	Height 95% CL	Weight x	Weight 95% CL
Triploids	45	72.8	68.1–77.4	49.7	41.7–57.6
Sibling Diploids	8	53.2	50.1–56.4	22.4	14.3–30.6
Diploids	49	60.1	56.5–63.6	29.8	24.8–34.7

TABLE 4.

Percent prevalence of MSX infection (percent systemic in parentheses)

Date	Triploid Group	Diploid Group	Control Group
8/15/90	60	24	92
9/21/90	60 (20)	36 (4)	76 (60)
10/25/90	36 (20)	28 (20)	68 (88)
9/20/91	27 (8)	14 (9)	—

of their first growing season. From previous experience at this site (Matthiessen 1989), this growth rate would yield a marketable oyster (75 mm in height, 50 gm in weight) less than 18 months old. In fact, 87% of the oysters in this group were marketable by mid September, 1991.

Although we have no previous data for first season growth at the Fishers Island Sound site, growth rate of the diploid group at the Ocean Pond site was equal or superior to that of diploids of the same strain in previous years. There is no indication, therefore, that the diploid oysters used in this investigation were inferior growers.

Stanley et al. (1984) also reported superior growth rate of triploid *C. virginica* over diploid siblings in Maine, but not until the end of the second growing season. Lack of detectable difference in growth between the two groups at eight months was attributed to the fact that they had not yet reached sexual maturity. Therefore the triploids would have had no energetic advantage over their diploid siblings (Stanley et al. 1981). However, in the investigation by Stanley et al. (1984), 3-year old triploids averaged only 40.4 mm in shell height, as compared with averages of up to 60.5 mm (Fishers Island Sound site, Table 1) for oysters less than 7 months of age reported here.

It is possible that the significant differences in growth rate between the two groups were due to genetic differences resulting from different parents, even though both derived from the same parental group selected over several generations for rapid growth. However, the pronounced difference in size between the 8 diploids in the triploid group and their 45 triploid siblings suggests ploidy as a significant factor.

It is of interest that, although members of the triploid group appeared more prone to MSX infection than the diploids, the former showed higher survival and, after completion of the first season at least, a higher condition index. (However, analysis of the survival data over two seasons by logrank test (Pyke and Thompson, 1986) did not indicate a significant difference between the two groups). It seems possible that the higher condition indices among the triploids may have conferred energetic advantages to this group, resulting in superior growth and initial survival rates.

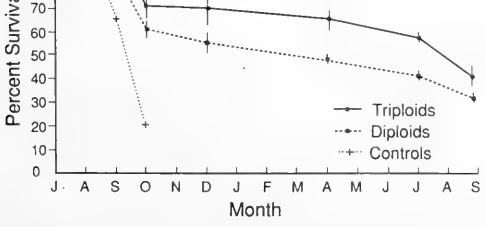

Figure 7. Comparative survival (mean of replicate trays; range =) – 1990–91.

The results in comparative growth rate and condition indices observed at Cotuit Bay are complicated by the heavy MSX pressure that occurred in this area during the summer of 1990. (This would not be the case for the groups held in Fishers Island Sound or in Ocean Pond, where mortality during the summer of 1990 was negligible and no MSX was found). During the summer of 1988, a sharp reduction in growth rate was noted among heavily infected diploids in this area (Matthiessen et al., 1990). During 1990, growth rate discrepancies between triploid and diploid groups in Cotuit Bay became clearly defined between early July and mid-August, when infection first began to occur. However, differences in growth rate were also apparent at the other two sites by mid-August.

The impact of infection upon growth is also reflected in the coefficient of variation, CV (Table 1). With the onset of infection, the growth of many of the infected individuals apparently declined or ceased entirely, resulting in a wider discrepancy in size. (The fact that infection by MSX has a negative effect upon clearance rate and condition index of *C. virginica* has been reported by Newell (1985) and Barber et al. (1988)). It is evident from Table 1a that, for both diploid and triploid groups, variation in size by October, 1990 was considerably greater at the Cotuit Bay site than at the other two sites.

In our experience, results of triploidy inducement in *C. virginica* have been irregular and unpredictable. (Reasons for variable results in the treatment of *Crassostrea gigas* embryos with cytochalasin have been discussed by Downing and Allen, 1987). Initial attempts in 1987–1989 were frustrated by an excessively low survival rate among the treated embryos and larvae. Also, results of ploidy analyses of triploid juveniles produced in 1988 were inconsistent, making interpretation of juvenile growth and survival comparisons impossible. Before 1990, the triploid groups were cultured at relatively low (<20‰) salinities following treatment with cytochalasin B. In 1990, the larvae and juveniles were held at a higher salinity (30‰) until more than a month old, with improved results; however, significant mortalities occurred among the triploids following transfer from the hatchery to the brackish water pond environment. There is therefore the possibility of an inverse relationship between mortality rate and salinity in triploid inducement.

Despite these uncertainties, the results described here seem encouraging, and triploid production may serve a useful purpose in the Eastern oyster industry.

ACKNOWLEDGMENTS

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TROPICAL MANGROVE OYSTER PRODUCTION FROM HATCHERY-RAISED SEED IN CUBA

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ABSTRACT Seed production of *Crassostrea rhizophorae* Guilding 1828 in Cuba began in 1986 at an experimental hatchery in Cayo Libertad, Matanzas Province. In July 1990, the first commercial scale hatchery was built in Cabo Cruz, Granma Province. From August 1990 to March 1991, 40×10^6 eyed larvae, 5×10^6 cultch seed, and 0.1×10^6 free spat were produced. Main constraints to seed production have been asynchronous gametogenic cycles of tropical oysters and high larval mortality after 10 days of culture. Oysters cultivated on rafts, with 24 hour air exposure each week to prevent fouling, reached commercial size (40 mm) in 4 to 8 months, depending on localities. Current work includes grow-out of cultchless spat in trays at controlled densities.

KEY WORDS: tropical mangrove oyster, *Crassostrea rhizophorae*, hatchery, raft system, tray culture, fouling control

INTRODUCTION

The tropical mangrove oyster, *Crassostrea rhizophorae* Guilding 1828, has been commercially cultured in Cuba since 1975 using natural seed collected on mangrove branches used as cultch material (Nikolic and Alfonzo 1968, Nikolic et al. 1976, Bosch and Frías 1977). Although nearly 50% of oyster production is still obtained by this practice, availability of natural seed, together with interannual spatfall variability, have been serious constraints to increasing oyster production. This problem has stimulated development of a technology for seed production on a commercial scale.

The project began in 1986 with the building of a small experimental hatchery in Varadero (Matanzas Province) in order to test the technology for seed production of *C. rhizophorae* under tropical condition (Guerrero 1987).

Once main technological problems were identified (Rodríguez et al. 1990) the first commercial scale hatchery was built on the Southeast coast of Cuba at Cabo Cruz (Granma Province). The hatchery began operation in August 1990. The aim of this paper is to report the main results and problems faced during the first 8 months of operation, including the grow-out of seed in shell collectors on raft systems with control of fouling.

First results of the current work with free seed in trays are also included.

On the basis of this experience the Ministry of Fisheries Industry formulated a program to build three additional hatcheries in the next 4 years.

HATCHERY TECHNOLOGY

Hatchery Design

The Hatchery (Fig. 1) has a main building (36 × 30 m) in which broodstock, larval rearing tanks, setting tanks, and laboratory facilities are located. Besides living quarters and office areas, the building also has cultch preparation, warehouse and maintenance rooms.

Behind the main building, there is a smaller one (30 × 12 m) for phytoplankton culture. Beside these two buildings, there is a pump house, storage tanks for fresh and salt water, an electricity generator house, and a platform for massive culture of phytoplankton.

The hatchery utilizes very clear,low-productivity oceanic water. The site is free from industrial pollution, and reasonably close to the main grow-out areas.

The hatchery is designed with a large capacity for phytoplankton culture and larval rearing, and with setting facilities large enough to produce 100×10^6 2–3 mm seed set on old *C. rhizophorae* shell.

Broodstock Conditioning

Perhaps one of the main problems faced in a hatchery design for tropical *Crassostrea* species is the asynchronous behavior of gametogenic cycles in natural populations, making it difficult to obtain adequate quantities of mature oysters for spawning.

To solve this problem, a protocol was developed to condition adult oysters for spawning (Helm 1990 and 1991). The method consists of a recirculating sea water tank in which oysters are placed at densities of 4–5 g/l, and the water is changed every second day. The oysters are fed a mixture of *Tetraselmis tetratele*, *Chaetoceros gracilis* and *Isochrysis galbana* at a daily rate of 6% of the dry meat weight. Temperature and salinity are carefully controlled at 20° ± 1°C, and 28‰ to 30‰.

Spawning

Oyster spawning was achieved by thermal stimulation, raising the temperature from 20°C to 28–30°C in a 3–4 hour period. Oysters which did not spawn during the first attempt were removed from the water for several hours and the treatment was repeated. Sperm or H_2O_2 can be added in order to facilitate spawning.

Average number of eggs per female was 1.36×10^6, with a fertilization rate of 86% (Table 1).

Larval Production

At the beginning of the hatchery operation, technology for larval rearing was similar to that used on the West Coast of the United States, employing large volume tanks (10 m^3), low larval densities (2–3 larvae/ml), and low algal concentration (10–30 × 10^4 cells/ml). Because of the low fecundity of *C. rhizophorae* (compared to *C. gigas*), it was difficult to maintain larval densities. Also, shape of the 10 m^3 tanks proved to be inefficient for larva culture being (shallow and of very large diameter).

In November 1991, the system was changed to small tanks (1 m^3), higher larval densities (5–30 × 10^6 larvae/ml) and algal

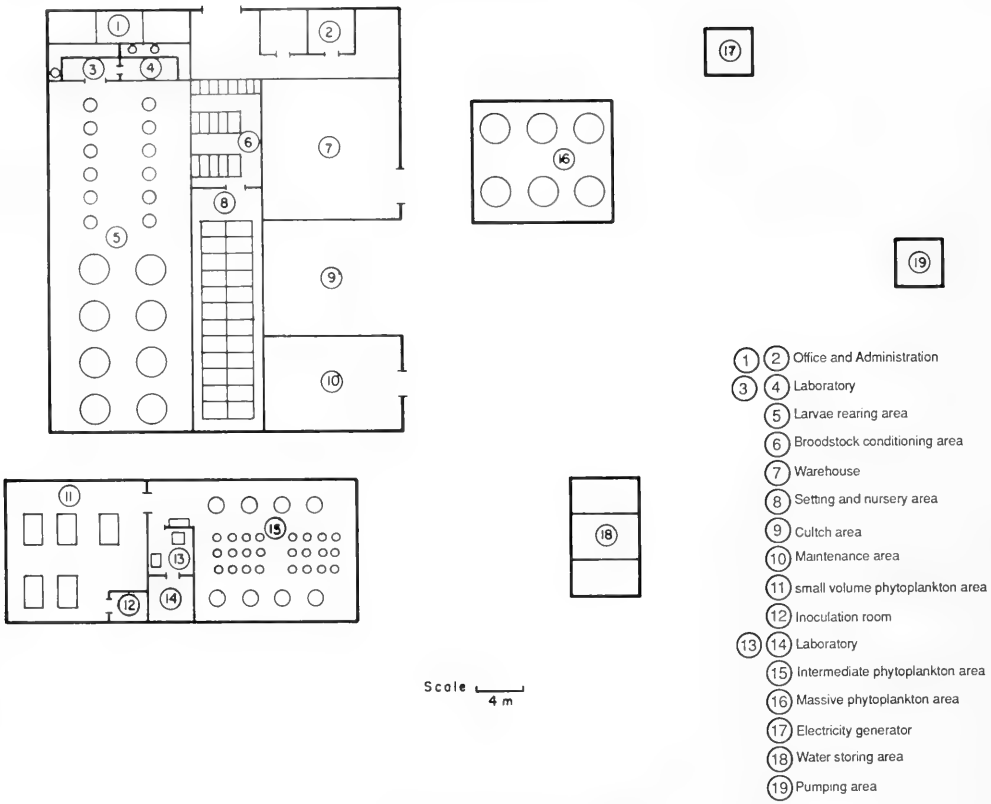


Figure 1. Cabo Cruz Hatchery design.

concentrations adjusted to the new larval densities and sizes (Helm, 1991). Larvae of different developmental stages produced (Table 2), showed a very small survival rate from the "D" shape to the "eyed" larvae (about 2.6%).

Growth curves of larvae from two different broodstocks, coming from Marea del Portillo, and the other from Manzanillo, are presented in Figure 2. The condition index of 25 oysters from Manzanillo (dry meat weight relative to the shell cavity) was a mean value of 74%, while the index in the Marea del Portillo stock ($n = 25$) was 103.6%.

Larval Setting and Nursering

Once eyed larvae were produced, they set on old cultch oyster shell.

Rectangular concrete tanks (1.5 × 1.2 × 0.7 m), 1 m^3 working volume, were painted inside with bee wax to avoid spat setting on the concrete surfaces. Densities were about 100 eyed larvae per each oyster valve, maintained undisturbed for 3 days until complete settlement had occurred.

Nursering is also performed in the same tanks keeping algal densities about 10^4 cells/ml (*T. tetrathele* equivalent). These densities were maintained by feeding the tanks 3 to 4 times a day. Water was exchanged every second day in order to maintain good water quality.

After 21 days, survival rate of larvae was about 12% (Table 2) with spat distribution which averaged 17.9 spat per valve. At that time, shell with seed which measured between 3 and 5 mm were selected, and collector strings were made using nylon thread.

Each collector consisted of 40 oyster valves fitted in four nylon branches of 10 oysters each, 7 cm between shells.

In order to make available a better quality product for the recent tourist market, some free oyster seed are also produced. Eyed larvae set on PVC sheets, and 24 hours later, we separated them from the sheets using a razor or turkey feather.

Culture of free seed was performed in a recirculating up-welling system, described by Helm (1991) in which seed were fed according to their biomass. Water was exchanged every other day, and seed were harvested weekly at 3 to 5 mm.

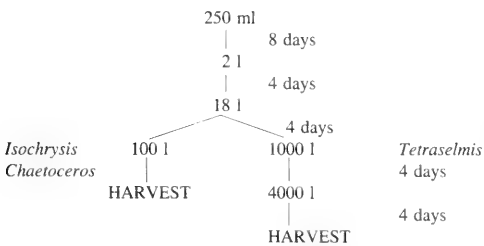
Phytoplankton Culture

Three species of phytoplankton were cultured in the hatchery (*I. galbana, C. gracilis* and *T. tetrathele*) using the following volume progression:

TABLE 1.

Results of spawnings made since the beginning of the hatchery operation on August 1, 1990 to March 31, 1991.

Date	No. Oysters Spawned	Female Percentage	No. Eggs Produced ($\times 10^6$)	Fertilization Percentage
1–15 Aug	291	82.5	261.0	85.0
16–31 Aug	372	71.0	397.0	81.0
1–15 Sept	396	57.6	251.0	86.0
16–30 Sept	460	30.0	94.0	96.0
1–15 Oct	508	68.0	480.0	70.0
16–31 Oct	618	71.2	237.0	83.0
1–15 Nov	743	56.0	176.0	83.0
16–30 Nov	190	54.0	61.0	90.0
1–15 Dec	93	29.0	60.0	80.0
16–31 Dec	275	66.0	320.0	91.0
1–15 Jan	795	80.0	720.0	82.0
16–31 Jan	690	70.0	679.0	89.0
1–15 Feb	333	50.0	737.0	93.0
16–28 Feb	364	58.0	555.0	89.0
1–15 Mar	241	67.0	223.0	87.0
16–31 Mar	290	74.7	550.0	88.0
TOTAL	6659	57.3	5801.0	86.0



Volumes up to 18 l were cultured indoors using artificial fluorescent light and Walne enrichment media (Walne, 1970). Volumes higher than 18 l were cultured outdoors. The 100 l tanks are fertilized with a modified Walne media (Perera and Rodriguez, 1990), and the 1000 l and 4000 l tanks with Miquel-Matue media (Hudinaga, 1942).

Average cell densities after four days of culture are showed in Table 3.

GROW-OUT TECHNOLOGY

Description of Working Area

Seed produced at the Cabo Cruz Hatchery, have been grown at the Marea del Portillo Lagoon, 80 km east of Cabo Cruz (Fig. 3). This lagoon has an area of about 25 ha and is surrounded by a

TABLE 2.

Number of larvae, spat and free seed produced since the beginning of the hatchery operation in August to March 31.

Date	"D" Shape Larvae ($\times 10^6$)	Eyed ($\times 10^6$)	Spats on Cultch ($\times 10^6$)	Free Seeds ($\times 10^6$)	Water Temperature (°C)
1–15 Aug	21.1	1.4	—	—	28.0 ± 0.4
16–31 Aug	49.3	3.3	—	—	28.4 ± 0.4
1–15 Sept	62.7	8.0	0.70	—	28.2 ± 0.5
16–30 Sept	25.2	4.8	0.36	—	28.5 ± 0.3
1–15 Oct	22.3	5.0	0.63	—	27.8 ± 0.2
16–31 Oct	25.5	4.2	0.90	—	26.4 ± 0.6
1–15 Nov	54.2	3.4	0.90	—	25.8 ± 0.5
16–30 Nov	82.2	2.4	0.30	—	26.0 ± 0.4
1–15 Dec	45.2	1.1	0.40	—	24.6 ± 0.9
16–31 Dec	33.8	0.2	0.60	—	25.3 ± 0.6
1–15 Jan	99.2	4.2	—	—	24.4 ± 0.3
16–31 Jan	266.0	0.7	—	0.08	23.8 ± 0.8
1–15 Feb	286.8	0.4	—	—	24.9 ± 0.6
16–28 Feb	110.0	0.1	0.16	0.02	24.3 ± 0.8
1–15 Mar	135.0	0.1	—	0.01	25.5 ± 0.8
16–31 Mar	242.9	0.7	—	—	26.0 ± 0.3
TOTAL	1561.9	40.0	4.95	0.11	26.1 ± 0.5

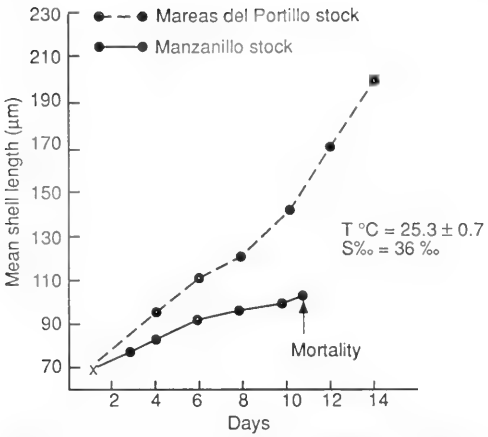


Figure 2. Growth rate larvae coming from two different broodstocks.

narrow fringe of red mangroves (*Rhizophora mangle* L.). Mean depth of the lagoon is 2.4 m and it communicates with the open sea through a wide, short channel, allowing good water exchange.

Studies carried out by Montalvo and Perigó (unpublished) showed small water temperature fluctuations (27.6°C to 30.6°C). Dissolved oxygen values ranged from 5.1 to 7.3 mg/l, salinity from 21 to 38‰, and pH between 7.55 and 8.46.

Primary production in the lagoon is higher than that in other oyster growing areas, with a mean value of 294.0 mg/m^3/light hour (González, unpublished).

Raft System

Reasons for the use of rafts instead of the traditional intertidal line fixed at the bottom, are as follow:

Rafts permit the use of all the lagoon surface and water column, oyster growth is higher and more uniform in rafts, permanent immersion allows oysters a longer feeding period and results in faster growth and control of fouling is more effective and less expensive than on the bottom.

Each raft (Fig. 4) can hold 280 collectors placed on 28 wooden rods with 10 units each. Each raft structure is constructed of galvanized steel pipes (19 mm in diameter) and can be dismounted, into its three main parts (passages, railings and cross-pieces) in order to facilitate transportation.

The effective working area of each raft is 18 m^2 (6 × 3 m) and the design allows the joining of two or more rafts. Normally, up to five units are used.

Floats consist of two blocks of polyfoam (density 40) 5 × 1 × 0.5 m (2.5 m^3), attached to each raft and wrapped in polyethylene to allow cleaning without damaging the blocks.

Rafts are also provided with railings which raise the rods out of the water for cleaning.

To prevent fouling, collectors are exposed to the air for 24 hours each week. A turbine is used to pump water to remove mud from the oysters as soon as they are pulled from the rafts.

This method of preventing fouling came from experiments with artificial collectors developed to obtain greater uniformity in size and higher growth rates than those used in the traditional system operating in Cuba since 1975 (Zayas and Frías 1989).

Rafts are anchored at the four corners, positioned so that dominant currents pass directly through the collectors.

The growth curve of oysters growing with this system in Marea del Portillo lagoon is showed in Figure 5.

Tray System

For free seed produced at the Cabo Cruz Hatchery, small scale culture with a tray system was developed as an alternative to raft.

Oysters grown in the system result in more uniform shell shapes and better yields ((meat weight/total weight) × 100) than on the raft.

Previous experiments performed with *C. gigas* in trays were not successful, probably because of the particular requirement of

TABLE 3.

Cell density (cell/ml × 10^6) produced since the beginning of the hatchery operation up to February 2.

	Isochrysis			Tetraselmis				Chaetoceros		
Date	2 l	18 l	100 l	2 l	18 l	1000 l	4000 l	2 l	18 l	100 l
1–15 Aug	15.2	5.6	—	2.2	0.6	0.4	0.2	—	—	—
16–31 Aug	18.4	5.7	—	1.7	0.8	0.3	0.3	—	—	—
1–15 Sep	17.8	5.0	2.5	2.6	0.8	0.3	0.3	—	—	—
16–30 Sep	15.1	4.0	2.2	1.4	0.7	0.3	0.2	—	—	—
1–15 Oct	15.8	6.7	2.0	2.2	0.9	0.3	0.2	—	—	—
16–31 Oct	16.9	8.3	2.2	2.7	1.2	0.2	0.2	—	—	—
1–15 Nov	15.5	2.5	2.6	2.5	0.9	0.3	0.2	—	—	—
16–30 Nov	15.5	4.2	2.2	2.8	0.7	0.3	0.2	—	—	—
1–15 Dec	14.7	4.7	1.7	1.7	0.7	0.3	0.2	—	—	—
16–31 Dec	16.5	7.0	2.2	2.9	0.7	0.2	0.2	—	—	—
1–15 Jan	20.2	4.7	—	3.3	0.9	0.2	0.2	16.0	4.6	—
16–31 Jan	21.1	6.0	1.9	2.2	0.3	0.3	0.2	17.1	4.8	—
1–15 Feb	18.6	4.5	2.2	2.8	1.4	0.3	0.2	15.1	3.9	2.0
16–28 Feb	17.3	5.8	2.2	3.2	0.8	0.2	0.2	10.0	1.7	2.1
TOTAL	17.0	5.3	2.2	2.4	0.8	0.3	0.2	14.6	3.8	2.1

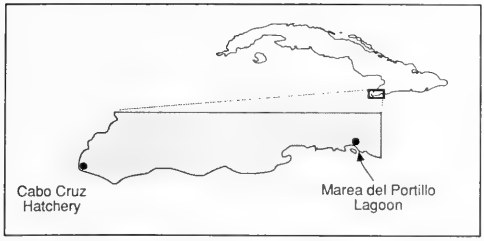


Figure 3. Geographic location of Cabo Cruz Hatchery and Marea del Portillo Lagoon.

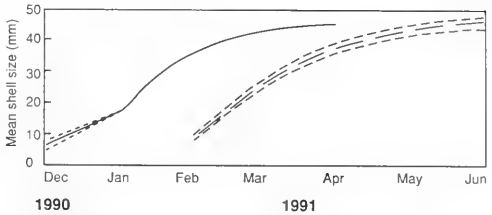


Figure 5. Monthly means sizes and confidence intervals of oysters grown on raft (dotted lines) and in trays (continuous lines).

this species and because trays were insufficiently cleaned (Frías and Lorente, unpublished).

In experiments carried out with *C. rhizophorae* below commercial size, oysters accidentally detached during harvesting from mangrove collectors and transfer to wooden trays with galvanized bottom mesh (Nikolic and Alfonzo, 1968), resulted in mortalities attributed to piling up of oysters caused by waves and currents.

Currently in use is a modification of the Mexican-made, NESTIER type tray. Each is a square (56 cm each side and 7.3 cm high), providing a useful surface of 3136 cm^2, divided into four compartments with a partition 32 mm high. Holes are 6 mm in diameter (0.94 holes/cm^2) in the lateral walls and 8 mm in diameter (0.48 holes/cm^2) in the bottom.

These trays, like the NESTIER, fit into another to form the top. Six trays are used, five containing oysters and the top one with a polyfoam block (55 × 55 × 5 cm) for flotation. The tray package is tied with a kapron rope (8 mm thick) and fixed to a long-line with stainless steel loops, and placed one meter apart along the long line, which is placed across the current flow.

In this first phase of the work, the trays have been planted with a density of 0.2 g of oysters per cm^2 (Helm, per. com.). Currently, experiments using two different densities (0.4 and 0.6 g of oysters/cm^2) are well underway.

Trays are cleaned weekly with a water jet while in the long line and every fortnight are pulled out of the water for 6 to 8 hours to adjust density of oysters.

The growth curve of oysters growing with this system in the Marea del Portillo lagoon is showed in Figure 5. Average yield was 23.20% (Table 4); this value is higher than that obtained with traditional systems, which are strongly dependent on the season and oscillate between 5 and 8%.

This tray system is not ready for commercial use because control of fouling must be improved and optimal densities of oysters per tray under various ecological conditions must be determined.

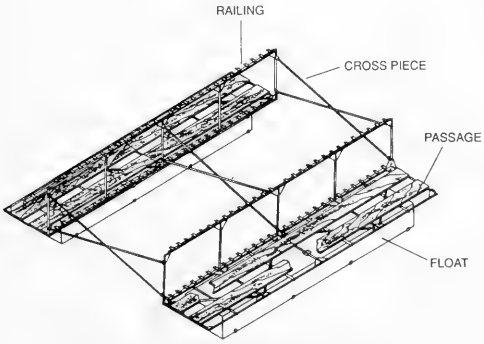


Figure 4. Raft for growing oysters in Cuba.

DISCUSSION AND CONCLUSIONS

According to the results previously reported, the hatchery technology for oyster production in tropical countries is feasible, especially when natural spatfall has been reduced by industrial pollution or other causes.

A comparison with temperate species (Loosanoff and Davies 1963, Bresse and Malouf 1975 and Dupuy et al. 1977) shows that the main problem faced with *C. rhizophorae* is the asynchronous behavior in gonad maturation, the low fecundity of female and the relatively low quality of eggs in terms of polysaturated fatty acids contents, specially when the broodstocks come from a low productive area. For these reasons some modifications which were specified throughout this paper are needed in the usual hatchery technology.

Phytoplankton production could be improved with CO_2 enrichment, better illumination for indoor cultures and better designs for outdoor tanks.

The raft system described here for grow-out of *C. rhizophorae* increases considerably the extent of suitable surface for oyster farming, giving a good solution to the needs of new areas for the higher seed production due to the new hatchery technology. In addition, the method has proved to be efficient by improving oyster growth rates and avoiding fouling; nevertheless, it is considered that this system of fouling control could be varied in re-

TABLE 4.

Yield of oysters grown in a tray system (n = 150), harvested in Apr. 1991.

Class Size (mm)	Means Total Weight (g)	Meat (g)	Industrial Yield (%)
40–50	13,64	3,11	22,80
50–60	14,72	3,49	23,70
TOTAL	14,05	3,26	23,20

spect to duration and periodicity depending on the localities and seasons.

The free seeds proved also to be a good option particularly for the specialized market which needs bigger, better-shaped and fatter oysters.

The main problem with free seeds is the fouling control. We have observed that as immersion time of oysters increases, fouling also increases (mainly ascidians) in spite of weekly cleaning and air exposure every fortnight. Fouling causes obstruction of holes, limiting water flow, making cleaning more difficult and probably increasing natural mortality.

Unfortunately, more information is needed for an economical evaluation of the production system.

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APALACHICOLA BAY'S PROCLIVITY FOR SEDIMENT EXPORT DURING HURRICANES AND ITS IMPACT ON OYSTER PRODUCTION FROM 1960–1985

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ABSTRACT Chesapeake Bay's response to Tropical Storm Agnes in 1972 included mass sediment import and a concominant degradation of estuarine health. Apalachicola Bay's response to Hurricane Elena in 1985 included mass sediment export and improved estuarine health. The depositional event that occurred in Chesapeake Bay in 1972 resulted from the flooding associated with Tropical Storm Agnes while the erosional event that occurred in Apalachicola Bay in 1985 resulted from the high winds associated with Hurricane Elena. These two events represent the extremes of episodically driven estuarine sediment deposition and erosion. Chesapeake Bay's response to Tropical Storm Agnes was well documented and is well known within estuarine science circles. Apalachicola Bay's response to Hurricane Elena is documented but is not well known within estuarine science circles. As a result, this paper reviews Apalachicola Bay's proclivity for sediment export during hurricanes and its impact on oyster landings (1960–1985) as a surrogate indicator of ecosystem perturbation.

KEY WORDS: oysters, Apalachicola Bay, hurricanes, Hurricane Elena, estuarine sediments, ecosystem perturbations

INTRODUCTION

In 1972, Tropical Storm Agnes' deposition of 50 million metric tons of sediment into Chesapeake Bay was well documented and is frequently referred to whenever episodic weather events are discussed in terms of impacts on estuaries (Andersen et al. 1973, Davis 1974, Schubel 1974, Zabawa and Schubel 1974, Davis 1975, Dyer 1986). Chesapeake Bay's "estuarine health" (Nixon 1983, Biggs 1986) was negatively impacted by this massive importation of sediment and sediment associated toxins (Andersen et al. 1973, Davis 1974, Schubel 1974, Zabawa and Schubel 1974, Davis 1975, Dyer 1986, Orth and Penhale 1988). This paper's intent is to present observations that are on the other end of the depositional spectrum concerning estuaries.

Hurricanes have removed decades worth of estuarine sediment (e.g., in 1985, Hurricane Elena removed 80 million metric tons of bottom sediment from Apalachicola Bay, Florida, and in 1979, Hurricane Frederic removed 263 million metric tons of bottom sediment from Mobile Bay, Alabama) (Isphording et al. 1987, Isphording and Imsand 1991). Episodic weather events have prolonged the life of estuaries by deepening them (e.g., Apalachicola Bay deepened 0.18 m (average depth) during Elena, and Mobile Bay deepened 0.46 m (average depth) during Frederic) (Isphording et al. 1987, Isphording and Imsand 1991). In addition, episodic weather events have improved estuarine health via winnowing clay from bottom sediments and concomitantly reducing the heavy metal content of the remaining bottom sediments (e.g., Apalachicola Bay's response to Elena, and Mobile Bay's response to Frederic) (Isphording et al. 1987, Isphording and Imsand 1991).

Due to the availability of relevant information on Apalachicola Bay, this paper focuses on Apalachicola Bay's responses to hurricanes in order to illustrate episodically driven erosional impacts. Historic series of bathymetric and sedimentologic surveys indicate that Apalachicola Bay has a proclivity for sediment export and clay winnowing during hurricanes (Isphording et al. 1987, Isphording and Imsand 1991). An evaluation of hurricane impacts on Apalachicola Bay's ecosystem is carried out using the bay's oyster harvest as a surrogate indicator of perturbation. Droughts, hurricanes, and floods are major factors influencing oyster production. In order to evaluate the impacts of hurricanes on oyster production, the influences of droughts and floods are concurrently evaluated. The alignment of the above information presents a unique opportunity to evaluate hurricane impacts on an estuary from a historical perspective.

METHODS

As is often the case in estuarine science, perfectly aligned data (temporally, spatially, and volumetrically) are rarely available at the scales and historical time frames needed to address estuarine processes (Nixon 1983). As a result, if any progress is to be made in our understanding of estuaries, estuarine researchers must use the available data, albeit imperfect, to best advantage. With the above in mind, an applied science approach is employed in the alignment of drought, flood, and hurricane influences on Apalachicola Bay's oyster production from 1960–1985.

Past droughts and floods that have influenced Apalachicola Bay's salinity regime (Dawson 1955) and annual oyster landings (1960–1985) (Berrigan 1991) were identified according to the following. United States Geologic Survey (USGS) stream flows for Apalachicola River (near its terminus at Blounstown, Florida from 1956–1988) were used to identify extremely low and high river flow periods (Perry 1988). Dawson (1955) was used to relate Apalachicola River's mean monthly stream flows to Apalachicola Bay's monthly mean surface salinity. Approximately two years is required for oysters to grow from larvae to harvestable adults in Apalachicola Bay (Berrigan 1991, Cake 1983). In general, oysters harvested in Apalachicola Bay are mostly two year olds (Berrigan 1991, Cake 1983). As a result, a two year time-lag was applied to the USGS stream flows (Perry 1988) versus annual oyster landings (Berrigan 1991) in order to evaluate the influence of stream flow on oyster year class success as indicated by oyster landings (Fig. 3). Applying the two year lag to the USGS stream flow aligns the stream flow's influence with the most vulnerable oyster life stages (larvae, spat, juveniles) of the year class being harvested. Based on the above, the following stream flow (drought) conditions were associated with poor oyster year class successes (Fig. 3). Mean monthly stream flows $<254{,}844$ liter sec^{-1} in combination with

minimum monthly stream flows <184,054 liter sec^{-1} identified years with periods of extremely low river flow (drought conditions) and concomitantly high salinities in Apalachicola Bay (Dawson 1955; Perry 1988). In addition to the above, USGS stream flows were evaluated versus annual oyster landings (without the two year time-lag) in order to assess the influence of stream flow on adult oyster stocks. The following stream flow (flood) conditions were associated with instantaneous reductions in adult oyster stocks (Fig. 3). Mean monthly stream flows >1,132,642 liter sec^{-1} during the summer (June, July, August) identified years with periods of extremely high river flow (flood conditions) and concomitantly low salinities (Dawson 1955, Perry 1988) during warm water conditions when oysters are most vulnerable to low salinities (Cake 1983). A more detailed discussion of the impact of salinity on oyster stocks is presented in the "Ecosystem perturbation as indicated by oyster production" subsection in the "Results and Discussion" section.

Past hurricanes that may have influenced Apalachicola Bay's annual oyster landings (1960–1985) (Berrigan 1991) were identified according to the following. Based on the hurricane impact accounts listed in Isphording (1985) it was determined that the stronger hurricanes (Saffir-Simpson Scale Class 4) could significantly impact Apalachicola Bay from a distance (hurricane's eye to Apalachicola Bay) of approximately 280 km and less. As a result, a distance of 280 km (eye's track to Apalachicola Bay) was used as the furthermost distance for consideration of hurricanes that may have significantly impacted Apalachicola Bay. Neumann et al. (1987) was used to identify hurricane tracks within the Gulf of Mexico that passed within 280 km of Apalachicola Bay from 1956–1985. At the time of the writing of this paper, there were no standard means of assessing hurricane impacts on estuaries. In lieu of being able to develop estuarine specific hurricane impact assessments, the following standard hurricane parameters are presented (Table 1). The distances between the hurricane tracks and Apalachicola Bay were placed into the following distance brackets: 1) 280 > x > 240 km, 2) 240 > x > 160 km, 3) 160 > x > 80 km, and 4) less than 80 km. The duration of time that the hurricane tracks remained within its closest distance bracket was approximated based on the time and date information provided on the track diagrams. Saffir-Simpson Scale categories for the hurricanes during their closest distance category were provided by the track diagrams.

RESULTS AND DISCUSSION

Apalachicola Bay's Proclivity for Episodically Driven Sediment Export

Apalachicola Bay's geomorphology and geographic orientation contributes to its sediment exporting tendencies during hurricanes. Apalachicola Bay (Fig. 1) is a shallow coastal plain lagoon-estuary system. The estuary's basin encompasses 554.3 km^2 and averages 2.74 m in depth [National Oceanic and Atmospheric Administration (NOAA), Strategic Assessments Branch (SAB), National Estuarine Inventory (NEI): 1988 data base—unpublished]. The bay is 40.1 km^2 in length and averages 11.6 km^2 in width (Ehler and Basta 1985, p. 3.06). The primary orientation of the estuary is along an ENE axis. The bay is open to the east, bound by the mainland to the north and by barrier islands to the south and west. Four passes connect the estuary to the Gulf of Mexico. The western portion of the estuary connects via two narrow and deep passes: 1) Indian Pass—305 m wide by 15.5 m deep; and 2) West Pass—640 m wide by 7.6 m deep. The eastern portion of the estuary connects via two relatively wide passes: 1) St. George Sound—11,988 m wide and averages 3.7 m in depth; and 2) East Pass—2,743 m wide and averages 5.5 m in depth.

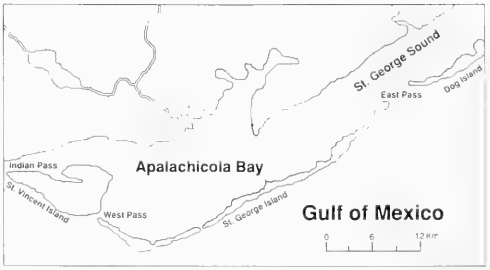

Figure 1. Reference map of Apalachicola Bay. Note orientation of basin and longer fetches along ENE-WSW vectors.

Apalachicola Bay serves as the terminus for the Apalachicola River which is the largest river system in Florida (Livingston 1983, Isphording 1985). The estuary's watershed covers 53,094.8 km^2 (Isphording 1985). This watershed supplies 824,020 m^3 sec^{-1} freshwater inflow (long term daily average) (Livingston 1983) and 1.4 million metric tons of sediment (annually) to Apalachicola Bay (Isphording 1987). In general, the Apalachicola River supplies sand, silt, and clay to the middle and western portions of the estuary while longshore transport processes supply sand to the eastern portion of the estuary via St. George Sound and East Pass (Isphording 1985).

Historic series of bathymetric mappings of Apalachicola Bay show the bathymetry of the estuary fills and erodes on a periodic basis (Isphording 1985, Isphording et al. 1987). Historic series of bottom sediment mappings reveal stability in the eastern portion of the estuary (sand dominated constituents) and a recurring alternation in the middle and western portions of the estuary (either clay/sand dominated or silt/sand dominated constituents) (Livingston 1983, Isphording 1985, Kofoed and Gorsline 1963). The occurrences of the silt/sand dominated bottom sediment conditions have been attributed to episodic weather events such as the passage of hurricanes and tropical storms (Isphording 1985, Isphording et al. 1987).

In general, as hurricanes and tropical storms approach an estuary they drive storm surges into the estuary's basin (Hayes 1978, Harris 1983) increasing the amount of water present in the basin. Within the estuary, the hurricanes set up wind generated wave fields and surface currents that increase with fetch, wind duration and velocity (Hayes 1978). Prolonged strong winds blowing down an estuary's longest fetch set up strong currents in that direction (Conner et al. 1982). The duration and strength of these currents depends to a large degree on the volume of water present in the estuary. As a result, storm surge height and duration heavily influence the currents strength and duration.

Hurricane Elena's course (Fig. 2) was unusual in that it remained 80–160 km off Apalachicola Bay for approximately 36 hours (August 31 to September 1, 1985) (Hine et al. 1987). During that time, the winds over Apalachicola Bay averaged 128–145 km hr^{-1} (Isphording et al. 1987). These winds were predominantly from easterly vectors (Hine et al. 1987) which set up a storm surge in Apalachicola Bay of 3.1 m above mean sea level during this period (Isphording 1987). The duration of the winds in combina-

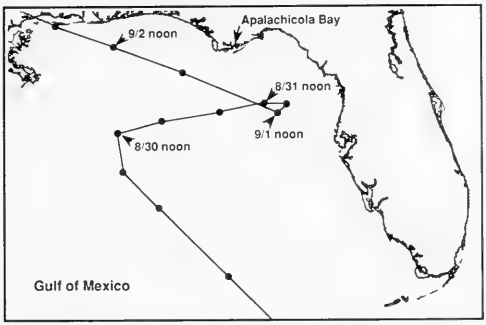

Figure 2. Track of Hurricane Elena in relation to eastern portion of the Gulf of Mexico. Note Hurricane Elena's prolonged proximity to Apalachicola Bay.

tion with the high storm surge contributed to the development of strong currents of prolonged duration. Currents in Apalachicola Bay average less than 0.31 m s^{-1} under normal conditions (moderate to low wind conditions) (Swift 1897). Current velocities of 1.5–2.4 m s^{-1} have been observed in Apalachicola Bay during gales (wind speeds 51–101 km h^{-1}) (Swift 1897). These gale associated current velocities are capable of transporting bottom sediments with nominal diameters as large as oyster shells (Dyer 1986, Wright 1990).

Apalachicola Bay's morphology and geographic orientation contributed to the development of strong currents during Elena. As Elena approached from the Gulf, the winds were predominantly easterly which blew down the bay's longest fetch. St. George Sound and East Pass allowed the storm surge to enter the estuary freely (Fig. 1), driving the storm surge through St. George Sound westward down the length of the basin, and exiting the estuary via the western passes (West Pass and Indian Pass). Prior to Elena the estuary averaged 2.56 m in depth (Ehler and Basta 1985). After Elena, the estuary averaged 2.74 m in depth (NOAA, SAB, NEI: 1988 data base—unpublished) and an estimated 93.4 million m^3 of bottom sediment had been removed (net) from the bay (Isphording et al. 1987).

Sediment analyses indicate a pattern of clay winnowing from the bottom sediments of Apalachicola Bay (Isphording 1985, Isphording et al. 1987). Prior to Hurricane Elena an extensive core sampling of Apalachicola Bay was carried out in 1984 (Isphording et al. 1987). Several sediment layers were identified by referencing the depth of the sediments (in the cores) to historic bathymetry (Isphording et al. 1987). The following sediment constituent percentages (per layer) were developed from the core analyses: 1) 1984—40% clay, 50% sand, 10% silt; 2) 1900—27% clay, 57% sand, 16% silt; and 3) 1825—26% clay, 58% sand, 16% silt (Isphording et al. 1987). After Elena the 1986 survey of Apalachicola Bay found the surficial sediment layer constituents closely matched the 1900 and 1825 percentages (Isphording et al. 1987). The most notable change being the reduction of clay content in the surficial sediments from 40% to approximately 26% (Isphording 1985, Isphording et al. 1987).

Pre-hurricane/post-hurricane sedimentologic surveys for Mobile Bay, Alabama have also been carried out (Isphording and Imsand 1991). According to Isphording (personal communication), the Mobile Bay surveys show the same clay winnowing as was seen in Apalachicola Bay. More interestingly, the heavy metals content of the bottom sediments dropped considerably after Hurricane Frederic's passage over Mobile Bay in 1979 and Elena's passage over Apalachicola Bay. Since clays and organics bind toxins, the prevalence of clays and organics in the sediments results in their retention of toxins. Prior to Frederic, Mobile Bay was one of the most heavily polluted (in terms of heavy metals) estuaries on the Gulf Coast (Isphording and Flowers 1987), and after Frederic, Mobile Bay was one of the least polluted (in terms of heavy metals) (W. Isphording, personal communication). The clay winnowing and sediment removal may improve conditions within the estuaries abiotically. Conversely, estuaries may experience short term defaunations and stock reductions as the result of hurricanes (Berrigan 1988, Berrigan 1990, Knott and Matone 1991).

Ecosystem Perturbation as Indicated by Oyster Production

In evaluating annual oyster harvests there is a premium on understanding the factors controlling oyster stock growth. Along the Gulf Coast, oyster production is primarily controlled by 1) availability of clean calcium carbonate surfaces for oyster larvae (spat) attachment (Cake 1983), 2) predation associated with periods of high salinities (>20 ppt) during droughts (Cake 1983), 3) stock depletions due to *dermo* outbreaks (an oyster disease caused by the protozoan *Perkinsus marinus* Mackin, Owen, and Collier) (Cake 1983), 4) hurricane related stock depletions (Berrigan 1988, 1990, 1991), and 5) prolonged periods of low salinities (<5 ppt) which kill adult oysters (Cake 1983). Oysters grow rapidly after attachment, reaching harvestable size within approximately 2 years (Cake 1983). As a result, oyster harvest for a given year usually reflects estuarine conditions two years prior to the actual harvest (Cake 1983). Droughts increase salinities over oyster reefs and contribute to increased abundance of predators from organisms preferring higher salinities (e.g., oyster leech, oyster drill, stone crab) (Cake 1983). Freshets and non-drought inputs lower salinities over the reefs and subsequently lower the numbers of stenohaline predators on the reefs (Cake 1983). Some of these predators become *P. marinus* vectors as they feed on "*dermo*-infected" oysters and migrate about the reef(s) (Cake 1983). *P. marinus* outbreaks are most severe during periods of high salinity and warm water temperatures (Cake 1983). *P. marinus* primarily affects adult oysters and can wipe out a reef's adult population within weeks (Cake 1983). Flood related mass mortalities of oysters occur as a result of prolonged exposure (months during winter and weeks during summer) to salinities less than 5 ppt (Cake 1983).

Apalachicola Bay's oyster fishery suffered a substantial stock depletion during Elena (Berrigan 1988, Berrigan 1990). Large quantities of oysters were removed from their pre-Elena locations and scattered downstream into deeper areas known not to support oysters (Berrigan 1988). This prompted protection of the remaining stock and implementation of management strategies designed to augment oyster stock recovery (Berrigan 1988). Unfortunately, the post-hurricane field surveys that generated the above information have not been carried out for earlier hurricanes. However, Apalachicola Bay is a major producer of oysters, traditionally accounting for 80–90 percent of Florida's oyster harvests (Livingston, 1983). Berrigan (1991) provides Apalachicola Bay (Franklin County, Florida) oyster landings from 1960 to 1987. As a result, historic landings records are available which can be used to provide information on ecosystem perturbations (hurricanes, droughts, floods) affecting oyster harvests.

TABLE 1.

Hurricanes that have impacted Apalachicola Bay from 1956–1985 (Neumann 1987).

Year	Month	Hurricane Name	Saffir-Simpson Category	Distance from Bay (km)	Duration of Influence (hours)
1960	September	Donna	4	$280 > \times > 260$	less than 8
1964	September	Ethel	1	$160 > \times > 80$	less than 8
1966	June	Alma	2	less than 80	approx. 12
1968	October	Gladys	2	$260 > \times > 160$	approx. 12
1972	June	Agnes	1	less than 80	approx. 36
1975	September	Eloise	3	$160 > \times > 80$	less than 8
1979	September	Frederic	3	$240 > \times > 160$	approx. 12
1985	September	Elena	3	$160 > \times > 80$	approx. 36
1985	November	Kate	2	less than 80	approx. 12

As previously mentioned in the methods section, there are no standard means for assessing the impacts of hurricanes on Apalachicola Bay. However, some insight is afforded by noting 1) duration of hurricane's impact at its nearest distance from Apalachicola Bay (Neumann, et al. 1987), 2) hurricane's strength (as indicated by the National Weather Service's assignment of the Saffir-Simpson scale) at time nearest to Apalachicola Bay (Neumann et al. 1987), and 3) past observations on hurricane impacts (Isphording 1985, Isphording et al. 1987). Based on the above criteria, the hurricanes listed in Table 1 have impacted Apalachicola Bay and have likely affected oyster production (Fig. 3).

As mentioned in the methods section, it is possible to hindcast the impacts of droughts and floods on Apalachicola Bay's salinity regime. Based on mean and minimum monthly USGS steamflow records (Apalachicola River near Blountstown, FL) (Perry 1988) and their relationship to Apalachicola Bay's salinity regime (Dawson 1955), the following droughts and summer floods have significantly impacted salinity: 1) 1962 drought, 2) 1968 drought, 3) 1973 summer flood, 4) 1981 drought, 5) 1986 drought, and 6) 1987 drought (Fig. 3).

Given the disparate nature of the above data, an applied approach is employed in identifying ecosystem level perturbations relating to oyster harvests. The interpretations of hurricane, drought, and flood impacts on Apalachicola Bay's oyster harvests are offered with the caveat and explicit understanding that they serve to provide gross evaluations. However, these evaluations sufficiently illustrate the impact of these events from a historical perspective (Fig. 3).

From 1960 to 1987, Apalachicola Bay's oyster production has been heavily influenced by droughts, hurricanes, and floods (Fig. 3). The droughts have been followed by two years of reduced oyster harvests. The post-drought oyster harvests decreased 20% (average) in the first year and 42% (average) in the second year (excluding 1986–1987, due to harvest restrictions, hurricane induced oyster stock depletion, and consecutive droughts). The oyster harvest following the 1973 flood was reduced 26% from the previous year's landings. The hurricanes have apparently affected oyster harvests in two ways 1) oyster harvest reductions as in Elena, Agnes, and Eloise, and 2) post-hurricane oyster harvest increases as in Donna, Ethel, Alma, Eloise, and Frederic. The post-hurricane (Donna, Ethel, Alma, Eloise, Frederic) oyster harvests (not affected by droughts or floods) increased 24% (average) in the first year and 70% (average) in the second year. The oyster harvests from 1970 to 1975 are interesting in that recovery from the 1968 drought is apparently thwarted by 1) Agnes, 2) the 1973 flood, and 3) Eloise.

The historical evaluation of oyster landings indicates that Apalachicola Bay has been regularly impacted on the ecosystem level by hurricanes. Hurricane impacts on Apalachicola Bay's oyster stocks include 1) short term depletions during hurricanes, 2) improved conditions favoring post-hurricane spatfall and subsequent growth, and 3) post-hurricane stock increases with maturation of subsequent year classes. Marine resources managers along the Gulf Coast have recently recognized that post-hurricane oyster harvests tend to be good for several years, barring droughts and floods (E. Slaughter, NOAA's SAB Shellfish Monitoring Program, personal communication). Post-hurricane oyster stock

Figure 3. Apalachicola Bay annual oyster landings (meats) in relation to droughts (left), floods (left), hurricanes (right), and limited harvest (right). Limited harvest imposed to protect post-Elena stock and enhance stock recovery. As a result, the 1986 oyster harvest is not directly comparable to the previous (1960–1985) landings.

growth may be enhanced by 1) overall cleansing of the bay's surficial sediments (clay winnowing reducing toxins), 2) scouring of extant oyster shell surfaces increasing the area available for spat attachment, 3) unburying of fossil oyster shells increasing the area available for spat attachment, and 4) short term defaunations of oyster predators, parasites, and disease organisms. In support of the above, the following observations are offered. Post-hurricane reductions of heavy metals in the bottom sediments have been observed in Apalachicola Bay after Elena and in Mobile Bay after Frederic (W. Isphording personal communication). Scouring of extant oyster shells, unburying of fossil oyster shells, and removal of oyster parasites and predators have been observed in Apalachicola Bay after Elena (Berrigan 1988).

IMPLICATIONS

In conclusion, the observed hurricane induced bathymetric maintenance of Apalachicola Bay suggests that estuaries may not be as bathymetrically ephemeral as once thought (Nichols and Biggs 1985, p. 77) and that sediment export may dominate during some episodic weather events (Isphording and Imsand 1991). Additionally, hurricanes can induce an abiotic cathartic cleansing and removal of estuarine bottom sediments that improves biotic conditions within estuaries (Isphording and Imsand 1991). As indicated by the historical evaluation of Apalachicola Bay's oyster landings, hurricanes have heavily influenced oyster production and been significant perturbators of the bay's ecosystem. Apalachicola Bay is not unique in its responses to hurricanes and other estuaries are likewise perturbed by hurricanes. As a result, historic data series concerning estuaries (especially sessile shellfish landings and sedimentological surveys) should be evaluated in light of these episodic weather events. Further investigation and re-evaluation of episodic weather event impacts on estuarine processes (biotic and abiotic) in relation to estuarine ecology (Meeder and Meeder 1989) and health (Nixon 1983, Biggs 1986) is warranted.

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THE USE OF ALGAL SUBSTITUTES AND THE REQUIREMENT FOR LIVE ALGAE IN THE HATCHERY AND NURSERY REARING OF BIVALVE MOLLUSCS: AN INTERNATIONAL SURVEY

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ABSTRACT The mass-production of micro-algae has been recognized by several authors as the main bottle-neck for the culture of bivalve seed. This has prompted a search for alternatives to on-site algal production, such as dried heterotrophically-grown algae, preserved algal pastes, micro-encapsulated diets, and yeasts. However, the extent to which these products have been tried, and rejected or retained by hatchery operators is poorly documented. Also, the actual algal requirement and production cost of the bivalve seed industry is difficult to estimate.

The present inquiry allowed the collection of data concerning the requirement for live algae and its associated costs encountered in 50 commercial and experimental hatcheries from all over the world. Furthermore, the hatchery operators were questioned about their experience with alternatives for live algae, the quality and quantity of hatchery produced algae and bivalve seed, and the employment in this sector of aquaculture.

The capacity of the algal production facilities ranged between 1 m^3 for a few research laboratories to nearly 500 m^3 for one commercial hatchery. The total algal production capacity reported by 37 hatcheries amounted to about 500 m^3 algal culture day^{-1}, which is equivalent to about 50 kg of dry biomass. The total cost of algal production in 1990 reported by 20 hatcheries approximated U.S. $700,000 and averaged about 30% of the total seed production cost. The estimates for the algal production cost ranged from U.S. $50 to 400 per kg dry weight.

About a third of the questioned operators considered algal production as a limiting factor in the rearing of bivalve seed, whereas over 50% planned an expansion of the algal cultures and more than 90% was interested in the use of a suitable artificial diet.

The large interest for alternatives for on-site algal production was further demonstrated by the fact that more than 50% of the operators claimed to have experimented with artificial diets. Despite the extensive research efforts, artificial diets are rarely applied in the routine process of bivalve seed production and are mostly considered as a useful backup diet.

KEY WORDS: micro-algae, bivalve, algal substitute/artificial, diet, hatchery, nursery

INTRODUCTION

In the early stages of research in the field of intensive bivalve rearing, the mass culture of micro-algae was identified as the main constraint. An extreme illustration of this can be found in the earlier literature, where it was estimated that one oyster, during its growth from egg to market size, will consume approximately 1.28 10^{12} cells of the alga *Thalassiosira pseudonana* (Pruder et al. 1976), which is equivalent to about 250 liter of dense algal culture. At present, the requirement for live algae in the intensive culture of bivalves is strongly reduced by the transfer of the small spat (1–2 mm) as soon as possible from the hatchery to the nursery (Claus 1981, Manzi 1985, Helm 1990). From the latter stage onwards they are fed partially, or in some cases exclusively, natural phytoplankton. Once the seed attains planting size (5–10 mm), they are transferred to grow-out areas, where they reach market size feeding solely on natural food. Nevertheless, several authors have recognized the production of large volumes of micro-algae, which is labor-intensive and requires specialized facilities, as the main bottle-neck for the culture of bivalve seed (Persoone and Claus 1980, Urban and Langdon 1984, De Pauw and Persoone 1988, Jones et al. 1991). This has resulted in the development of several alternatives to on-site algal production such as dried heterotrophically-grown algae (Laing et al. 1990, Gladue 1991, Laing and Verdugo 1991, Laing and Millican 1992), preserved algal pastes (Donaldson 1991, O'Connor and Nell 1991), microencapsulated diets (Jones et al. 1984, Langdon et al. 1985, Southgate et al. 1991), yeast-based diets (Epifanio 1979, Urban and Langdon 1984, Coutteau et al. 1990, 1991). Except for the sporadic reports at international meetings (Helm and Hancock 1990), the extent to which these products have been tried, and rejected or retained, by the hatchery operators is poorly documented. Furthermore, in order to direct future research efforts, it is essential to know the selection criteria of the farmer for an algal substitute which is eventually to be used in the daily practice of bivalve seed production. In this way, depending on the bivalve species and the applied production technology, either a cheap bulk feed or a more complete, high quality diet may be preferred.

The actual algal requirement and production cost of the bivalve seed industry is difficult to estimate due to the nearly complete lack of information concerning the quantity of seed or algae produced. Also, the requirement for live algae greatly varies between

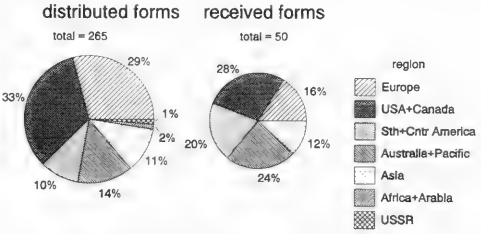

Figure 1. Contribution of the various regions in the world to the distributed and received questionnaires.

hatcheries, as it depends on the availability of natural phytoplankton, the size at which the spat leaves the hatchery, and the bivalve species cultured. The great diversity in the algal culture technology hinders the estimation of a standard algal production cost, since the latter varies with the yield and efficiency of the culture system. As a result, extrapolations based on a case study of one hatchery are of limited value. For this reason, the present survey aimed at the collection of data concerning the requirement for live algae and the associated costs encountered in several commercial as well as academic hatcheries. Furthermore, the hatchery operators were questioned about their knowledge of and experience with alternatives for live algae, and their intention and requirements to use artificial diets. Finally, this survey offered the opportunity to collect some unique data on the quality and quantity of hatchery-produced bivalve seed, and the employment in this relatively small sector of aquaculture.

DESIGN, DISTRIBUTION, AND RETURN RATE OF THE QUESTIONNAIRE

The questionnaire consisted of six multiple questions. The first question offered the possibility to protect confidential data that

TABLE 1.

Hatchery and nursery production of various bivalve species in 50 commercial and academic hatcheries in 1990.

	Hatchery (larvae + spat < 2 mm)				Nursery (spat ≥ 2 mm)			
Species	Total Production (10^6 units)	% C	n_C	n_A	Total Production (10^6 units)	% C	n_C	n_A
Oysters								
Crassostrea gigas	29661.0	99	10	3	183.0	98	7	2
Crassostrea virginica	336.5	96	4	2	64.5	98	3	2
Saccostrea commercialis	6.0	50	1	2	6.3	79	1	2
Pinctata maxima	11.0	100	2	0	5.2	100	2	0
Crassostrea belcheri	20.3	0	0	3	2.4	0	0	3
Ostrea edulis	53.2	100	4	1	1.1	100	3	0
Crassostrea iredalei	0.5	0	0	1	0.5	0	0	1
Pinctada fucata	0.4	0	0	1	0.3	0	0	1
Crassostrea lugubris	0.5	0	0	1	0.050	0	0	1
Pinctada margaritifera	0.060	0	0	1	0.050	0	0	1
Tiostrea lutaria	0.017	0	0	1	0.007	0	0	1
Saccostrea echinata	2.0	100	1	0	—	—	—	—
Number of species	12				11			
Total production (10^6 units)	30092				263			
Clams, cockles, and arkshells								
Tapes philippinarum	1982.0	85	7	2	155.0	96	6	2
Mercenaria mercenaria	211.0	74	4	3	63.4	84	4	3
Tapes decussata	103.8	100	4	1	25.7	100	4	1
Panopea abrupta	150.0	0	0	1	7.0	0	0	1
Tapes pullastra	1.6	100	1	0	7.0	100	1	0
Spisula solidissima	5.3	57	1	1	3.1	97	1	1
Mya arenaria	7.0	71	1	1	2.5	20	1	1
Mulinia lateralis	5.0	0	0	1	1.0	0	0	1
Tridacna gigas	59.1	0	1	2	0.3	7	1	2
Anomalocardia brasiliana	0.5	0	0	1	0.3	0	0	1
Anadara brouhtoni	0.4	0	0	1	0.3	0	0	1
Tridacna derasa	0.110	0	0	1	0.110	0	0	1
Hippopus hippopus	25.3	0	0	2	0.095	0	0	2
Tridacna maxima	15.0	0	0	1	0.040	0	0	1
Codakia orbicularis	3.0	0	0	1	—	—	—	—
Number of species	15				14			
Total production (10^6 units)	2569				266			
Scallops								
Argopecten purpuratus	110.0	100	3	1	22.5	100	2	1
Patinopecten yessoensis	172.0	99	1	1	0.1	0	0	1
Argopecten irradians	14.0	71	1	1	5.5	27	1	1
Argopecten circularis	202.0	0	0	1	1.0	0	0	1
Pecten ziczac	1.0	0	0	1	0.5	0	0	1
Crassadoma gigantea	0.5	0	0	1	0.025	0	0	1
Number of species	6				6			
Total production (10^6 units)	500				30			

Species were ranked according to the nursery production. The percentage contribution of commercial operations in the production of each species (% C), and the number of commercial (n_C) and academic (n_A) hatcheries involved are indicated.

were possibly communicated in the questionnaire. The second question aimed at an evaluation of the profile of the hatchery on the basis of its productivity and the number of employees in 1990. The third and fourth question offered the possibility to detail, respectively, the knowledge of and experience with algal replacement diets. The capacity and nature of the algal production facilities of the hatchery were queried in question five. Finally, the last question consisted of various subquestions concerning the algal production cost, the extent to which the algal production capacity is a limiting factor for the hatchery and may be expanded in the future, the intention to use artificial diets and the most important characteristics these should comply with.

The survey was announced in several aquaculture magazines and newsletters and through a poster presentation at two international aquaculture meetings (Coutteau and Sorgeloos 1991a,b). In total, 265 forms were distributed over 43 countries. Over 90 people responded to the survey and 50 questionnaires were retained for evaluation. The efficiency with which the distributed forms were returned ranged between 10% (Europe) and 38% (South and Central America). In this way, the contributions of the various regions in the world were well balanced in the survey, with the exception of the exclusion of the USSR, and Arabic and African countries (Fig. 1). For the analysis of the 50 completed forms, a distinction was made between 25 private hatcheries (further referred to as "commercial") and 25 facilities run by research institutes and governmental agencies ("academic").

RESULTS

1. *Secrecy Clause*

Since about 50% of the commercial and 30% of the academic hatcheries demanded secrecy, all data were treated anonymously.

2. *Profile of the Farm*

2.1 *Production Data for 1990*

In the present investigation, hatchery production included the rearing of eyed larvae (300–500 μm) for remote setting as well as small postset (1–2 mm; 1 cm for giant clams). Nursery production consisted of the rearing of juveniles from 2 mm to planting size (4–15 mm: clams; 5–30 mm: oysters and scallops; 15–20 cm: giant clams).

The total hatchery and nursery production reported for 33 different bivalve species, and the relative contribution of the academic and commercial hatcheries is presented in Table 1. The production figures and the number of hatcheries producing each species demonstrated that the commercial hatcheries focus on the production of a few species of oysters (*C. gigas, C. virginica, S. commercialis, O. edulis*), clams (*T. philippinarum, M. mercenaria, T. decussata*) and scallops (*A. purpuratus, P. yessoensis, A. irradians*), representing over 98% of the total seed production. The remaining bivalve species were primarily reared in research and state owned facilities in relatively low numbers. Furthermore, the hatchery production was dominated by the large amounts of eyed larvae and small postset (<1 mm) of *Crassostrea gigas*, produced primarily in hatcheries along the West Coast of the United States. As a result, oysters represented 90% of the recorded hatchery production. Interesting was that more than 70% of the larval production of the pacific oyster was due to the efforts of one company. The recorded production of larger clam and oyster seed was equally important, whereas scallops represented only 5% of the nursery production.

2.2 *Number of Employees*

The total number of people employed in about 30 bivalve rearing facilities, including hatchery, nursery, and grow-out operations, was less than 500 (Table 2). Most of the private companies engaged two to four people in the hatchery and about the same number in the nursery, whereas a larger staff was involved in the more labor-intensive grow-out operations (Fig. 2).

3. *Inventory of Algal Substitutes*

The limited number of algal substitute diets reported in this study was classified either as dried algae, algal pastes, microencapsulated diets, yeast-based diets, or miscellanea (Table 3).

4. *Experience with Algal Substitutes for the Hatchery and Nursery Culture of Bivalves*

34 out of the 50 questioned people had knowledge of artificial diets for bivalves, while 28 (15 academic and 13 commercial) operators had experimented with at least one of them (Fig. 3). Nearly 60% of the interrogated people knew the dried *Tetraselmis suecica* product and more than half of the latter had evaluated its nutritional value experimentally. The other alternatives to live algae were relatively less well-known (Fig. 3).

It should be emphasized that the experimental results reported in this survey could not be verified concerning the dependability and profundity of the applied methodology, and should thus be regarded as preliminary. It was tried to reproduce the data as they were mentioned by the experimenters in the questionnaire. The experience recorded for the various bivalve species, culture phases and substitute diets is summarized in Table 4. The routine application of algal substitutes was reported by only three interviewees.

TABLE 2.

Total number of employees in the various stages of bivalve culture operations.

	Hatchery		Hatchery + Nursery		Hatchery + Nursery + Grow-out	
	Total Employment	n†	Total Employment	n†	Total Employment	n†
Commercial hatcheries	77	19	127	20	427	14
Academic hatcheries	30	12	95	16	61	14
Total	107	31	222	36	488	28

† Number of replies received from 50 returned forms.

Live algae were routinely replaced for up to 75% by algal paste (Coast oyster Co., USA) in the rearing of spat and broodstock of *C. gigas* and up to 25% by spray-dried *T. suecica* (Cell Systems Ltd., UK) in the culture of spat. Furthermore, algal culture was absent in five of the six hatcheries producing giant clam larvae, which were fed dried yeast, dried *T. suecica*, the Frippak micro-encapsulated diet, or a mixture of the latter two.

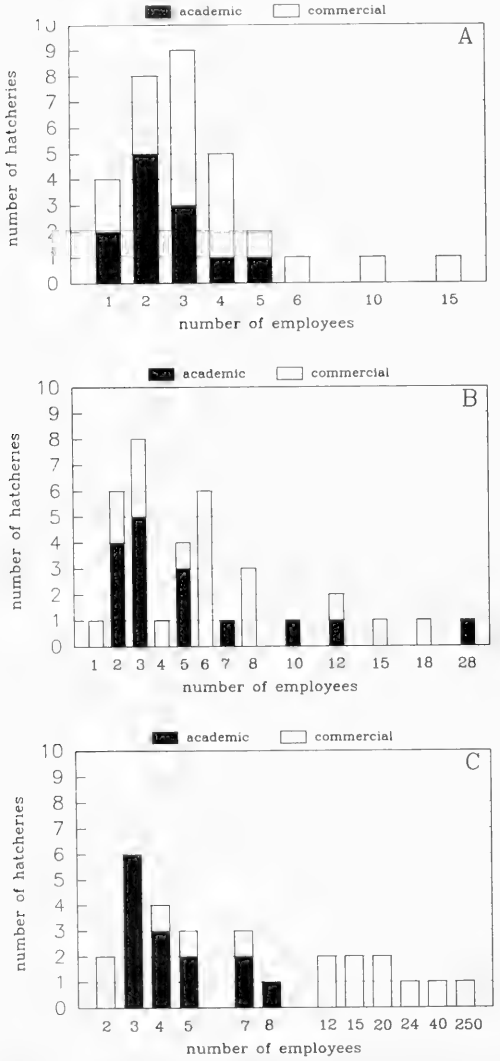

Figure 2. Employment per operation for hatchery (A, n = 31), hatchery + nursery (B, n = 36) and hatchery + nursery + grow-out (C, n = 28) rearing of bivalves.

TABLE 3.

Substitute diets for live algae in bivalve culture.

Classification	Diet (C = commercially available, E = experimental)
Dried algae	—*Tetraselmis suecica* (C, Cell Systems Ltd., Cambridge, UK)
	—*Nitzschia* sp. (E, Martek Corp., Maryland, USA)
	—*Spirulina* (C, Earthrise Farms, California, USA)
Algal pastes	—Coast oyster diet 1 (C, Coast Oyster Co., Washington, USA)
	—algal paste (E, SeaAg Inc., Florida, USA)
	—algal paste (C, Innovative Aquaculture, British Columbia, Canada)
	—algal paste (refrigerated, centrifuged from excess production)
Microcapsules	—Frippak Booster (C, Frippak Feeds, Sanofi, Paris, F)
	—micro-encapsulated diet (E, James Cook University, Townsville, Australia)
Yeast-based diets	—Topal (C, Artemia Systems N.V.-S.A., Gent, Belgium)
	—manipulated yeast diets (E, University of Ghent, Gent, Belgium)
	—various brands of dried baker's yeast (*e.g.* Mauri, Nauplius)
Miscellanea	—cornflour (maizena)
	—corn starch (source not specified)
	—fry food (C, Biokyowa, Montana, USA)

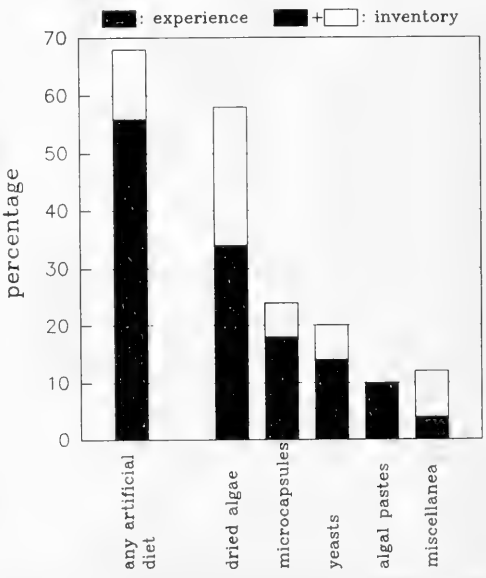

Figure 3. Percentage of hatchery operators that claimed to have knowledge of (total bar) and/or experience with (filled bar) various classes of artificial diets as reported in 50 questionnaires.

TABLE 4.

Reported experience with the use of various algal substitutes in the culture of different species and stages of bivalves (B = broodstock, L = larvae, S = spat).

Bivalve Species	Artificial Diets				
	Dried Algae	Algal Pastes	Yeasts	Microcapsules	Miscellanea
Oysters					
Crassostrea gigas	BS † ‡	BS ‡	S	LS	B
Crassostrea virginica	BS †	BS †			
Ostrea edulis	LS				
Saccostrea commercialis				L	
Pinctada margaritifera				L	
Clams					
Tapes philippinarum	BLS †		BS	S	
Tapes decussata			BS		
Mercenaria mercenaria	BLS †	BS †	S		
Panopea abrupta	S				
Dosinia dunkerii			L		
Tridacna gigas	LS		LS	LS ‡	
Tridacna maxima	LS ‡		LS ‡	LS ‡	
Tridacna derasa	L ‡		LS ‡	LS ‡	
Tridacna squamosa	L ‡		LS ‡	LS ‡	
Hippopus hippopus	LS			LS ‡	
Scallops					
Patinopecten yessoensis	BS				

The results obtained with the various diets experimentally, as backup (†) or in routine culture (‡) are given in the text.

Several experiments indicated that substitute diets may be used to supplement insufficient rations of live algae. The spray-dried *T. suecica* and algal paste were found to be useful as a backup diet to replace 50% of the live algae in the diet of broodstock and spat of *C. virginica* and *M. mercenaria*. For spat of *T. philippinarum*, a replacement of 20–30% of the algae by dried *T. suecica* was applied in the absence of sufficient amounts of live algae. Dried *T. suecica* was found to be a satisfactory diet for feeding *O. edulis* in the size range of 2–10 cm during disease experiments, although growth was inferior to that in nature.

Despite the extensive efforts to evaluate various diets, the use of artificial diets appeared to be mostly restricted to the experimental stage. Contrary to the previous reports on the use of dried *T. suecica* as a partial algal substitute, various operators found it unsatisfactory either because of its high price (i.e. U.S. $170 per kg) or its poor performance (reported for larvae of *M. mercenaria, O. edulis;* spat of *T. philippinarum, C. gigas, P. yessoensis;* broodstock of *M. mercenaria*). The latter was mostly associated with difficulties to resuspend the dried algal cells without disintegrating them, and the fast settling of the food particles in the bivalve cultures. In this regard, dried *T. suecica* was found to be valuable in the culture of pedal feeders, such as *P. abrupta*, when it was introduced in the substrate.

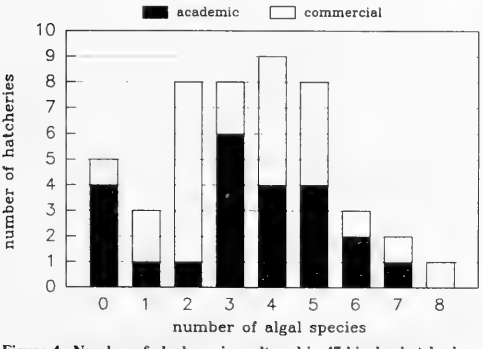

Figure 4. Number of algal species cultured in 47 bivalve hatcheries.

Dried yeast (source not specified), was reported as being of low value as food for juvenile *C. gigas* at substitution levels ranging from 25 to 100%. Also, feeding Topal (Artemia Systems N.V.-S.A., Belgium) resulted in poor growth and high mortality for larvae of *Dosinia dunkerii* and yielded poor growth in spat of *C. gigas* and *T. philippinarum*. Manipulated yeasts (University of Ghent, Belgium) gave satisfactory results as an 80% algal substitute for spat of *M. mercenaria, T. philippinarum,* and *C. gigas*. Preliminary tests with broodstock of *T. decussata* and *T. philippinarum* indicated an acceptable conditioning index, but a retarded maturation in clams fed a 20/80% mixture of algae and manipulated yeasts.

The replacement of the algal diet fed to spat of *C. gigas* and *T. philippinarum* by microcapsules (Frippak Booster, Sanofi, France) yielded poor growth. However, the combined feeding of the experimental micro-encapsulated diet (James Cook University, Australia) with dissolved yeast extract resulted in a better growth of giant clam larvae (*T. gigas, H. hippopus*) than controls fed either *I. galbana* or *Pavlova salina*. Feeding these microcapsules to larvae of *S. commercialis* yielded up to 81% of the shell growth and similar ash free dry weight growth compared to algal-fed controls during a one-week experiment. By contrast, poor growth was re-

TABLE 5.

Frequency of use and total daily production of various algal species.

Algal Species	Frequency of Use†	Total Daily Production	
		n‡	Volume (m^3)
Isochrysis galbana, clone T-Iso	31	18	23.8
Chaetoceros gracilis	23	11	14.1
Chaetoceros calcitrans	16	10	6.0
Tetraselmis suecica	15	10	39.1
Thalassiosira pseudonana, clone 3H	14	9	112.0
Pavlova lutheri	11	7	11.7
Isochrysis galbana	8	5	9.1
Skeletonema costatum	6	3	58.8
Chroomonas salina	5	3	0.76
Dunaliella tertiolecta	4	2	2.2
Chaetoceros simplex	3	3	1.76
Chaetoceros muelleri	3	2	5.0
Nannochloropsis sp.	3	2	0.20
Cyclotella sp.	2	1	0.36
Phaeodactylum tricornutum	2	1	2.0
Tetraselmis chui	2	0	—
Pavlova salina	1	1	3.18
Dicrateria sp.	1	1	4.07
Tetraselmis levis	1	0	—
Dunaliella perva	1	1	0.012
Thalassiosira weissfloggii	1	1	0.12
Chlamydomonas sp.	1	1	0.52
Chlorella sp.	1	1	0.36
TOTAL	43	23	295

Species are ranked according to decreasing frequency of use.

† Number of hatcheries growing each algal species (from 43 completed forms).

‡ Number of hatcheries providing data which allowed to calculate daily production per algal species (from 23 completed forms).

ported when the same diet was fed to larvae of pearl oysters (*P. margaritifera*).

Cornflour may serve as a 20% algal supplement for broodstock of the pacific oyster, although increased bacterial growth was observed when fed to spat cultures.

5. *Total Algal Production in 1990*

Most hatcheries cultured between two and five different algal species (Fig. 4). Five of the six hatcheries in which giant clam larvae were reared did not maintain any algal culture. An inventory of the algal species recorded in this study is presented in Table 5. Eight algal species (*I. galbana*, clone T-Iso; *C. gracilis; C. calcitrans; T. suecica; T. pseudonana*, clone 3H; *P. lutheri; I. galbana; S. costatum*) were widely used and represented over 90% of the volume of algal culture produced in 23 facilities. The other species were used less frequently and about a third was reported only once.

The total capacity of the algal culture showed a wide range from less than 1 m^3 for a few research laboratories up to nearly 500

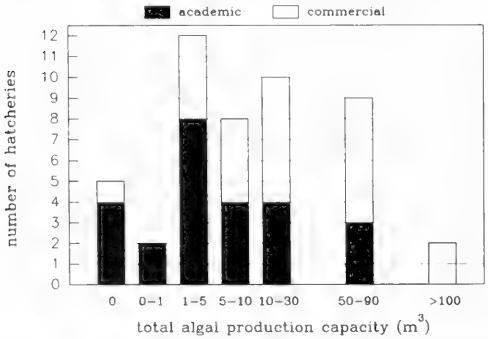

Figure 5. Total container volume available for algal culture in 48 hatcheries.

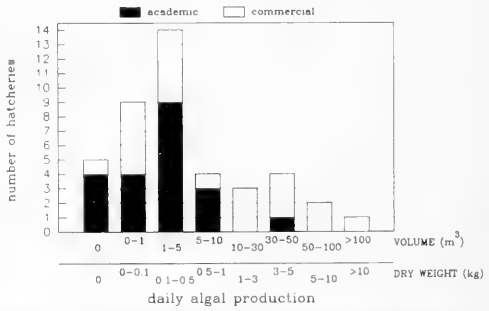

Figure 6. Daily algal production in 42 hatcheries expressed either as volume of algal culture or as dry algal biomass.

TABLE 6.

Total production capacity and daily production of small and large scale algal culture systems in 21 hatcheries.

Scale Algal Culture	Average Yield (g/m^3)†	Daily Algal Production			
		m^3	%	kg DW§	%
$<1\ m^3$	248	31	12	7.7	28
$\geq 1\ m^3$	89	228	88	20.3	77
TOTAL		259	100	28.0	100

† Derived from data on algal culture densities from 19 hatcheries and conversion to dry weight based on data from Brown (1991).
§ Estimated from average yield × daily production (m^3).

m^3 for one commercial hatchery (Fig. 5). Because the yield of the algal culture greatly varies according to the culture and climatological conditions, the volume available for algal culture is a poor estimate for the productivity. The latter was computed as the daily volume of algae produced and converted to dry weight assuming an average culture density of 100 g/m^3 (Gladue 1991). Apparently, about 50% and 60% of, respectively, the commercial and academic hatcheries produced daily less than 5 m^3 of algal culture, i.e. about 0.5 kg dry biomass (Fig. 6). A quarter of the commercial hatcheries produced between 10 and 50 m^3 algal culture per day, and six out of 21 companies reported a daily production between 30 and 110 m^3. The total volume of algal culture produced per day by 37 hatcheries amounted to 500 m^3, i.e. about 50 kg dry biomass.

Detailed data, obtained from 21 hatcheries, showed that most of the algae (i.e. 88% and 72% in terms of volume and dry weight, respectively) are produced in systems larger than 1 m^3 (Table 6). It is interesting to note that the average production yields of large and small scale cultures (respectively, 89 and 248 g/m^3; weighed average: 108 g/m^3) approximated the estimate of Gladue (1991). The majority of the hatcheries applied batch cultures (Table 7). Only 3 hatcheries, which were all located in Europe, grew algae solely in continuous cultures.

6. *Algal Production Cost in 1990 and Requirement for Artificial Diets*

The total cost of algal production in 1990 reported by 20 hatcheries amounted to nearly U.S. $700,000 (Table 8). The cost of live food production per hatchery greatly varied as could be expected from the large differences in production capacity, and averaged about U.S. $40,000 and 24,000 for commercial and academic hatcheries, respectively. The algal production cost averaged about 30% of the total seed production cost. Although few data were collected concerning the cost of live algae per unit dry weight, six out of nine estimates were in the range of U.S. $300–400.

Algal production was considered as a limiting factor by only a third of the interviewees (14 out of 43), whereas over 50% (24 out of 43) planned an enlargement of the algal culture units and nearly everybody (37 out of 40) considered the use of an artificial diet. The relative importance of the various characteristics of an artificial diet was estimated by summation of the quotations (between 1 and 5, 5 = most important) which were given to the features listed in each questionnaire. Food value, price, ease of use, and shelf-life were recognized as the most significant parameters that determine the value of an artificial diet (Fig. 7).

TABLE 7.

Frequency of use of batch, semicontinuous, and continuous algal culture systems in 42 hatcheries.

Culture Method	Applied	Solely Applied
Batch	38	27
Semicontinuous	5	1
Continuous	10	3

DISCUSSION

The success of the questionnaire allowed the compilation of data from a significant number of companies, and research and demonstration centra involved in bivalve seed production. However, it is difficult to estimate the fraction which is represented by the latter in comparison with the total existing number of hatcheries. Also, the contributing hatcheries may not be a representative sample as a few important countries (e.g. France, China) did not participate in the survey. Therefore, the quantitative data concerning the production of algae and seed should not be used as a basis for straight extrapolation to production on a world scale, but rather indicate the order of magnitude. Also, because algal production is often varying according to the season, the data expressing the daily algal production estimated the maximal rather than the yearly average production.

Although the total catches of the various commercially important bivalve species are well documented (FAO Yearbook of Fisheries Statistics), literature data estimating seed production are completely lacking. The reported total production for 1990 of *Crassostrea gigas* seed of planting size (i.e. 183 million oysters) is comparable with about 3% of the total world catches for this species in 1987 (i.e. 620,000 metric ton, FAO Yearbook of Fisheries Statistics, assuming a market size of 100 g). The pacific oyster harvest on the West Coast of North America amounted to 25,000 mt in 1989 (Chew 1990), i.e. approximately 250 million oysters. The production of over 25 billion eyed larvae of *C. gigas* for remote setting, reported by the hatcheries along the Pacific coast of the United States, confirms that this technique is providing the main part of the seed to the farmers in this region (Chew 1990) and is in agreement with the value of 27 billion recently reported by Le Borgne and Bodoy (1992).

Walne (cited in Persoone and Claus 1980) composed in 1978 a preference list of algal species suitable for hatchery rearing of bivalves on the basis of a survey held among ten institutes active in intensive bivalve culture in Europe and North America (Table 9). It is clear that the relative importance of several algal species has significantly changed over the last decade, in particular with

TABLE 8.

Algal production cost in bivalve hatcheries. Replies obtained from n hatcheries.

	ALGAL PRODUCTION COST (C = commercial, A = academic hatchery)					
	Total Per Hatchery (US $)		Fraction of Total Seed Cost (%)		Cost Per Unit Weight (US $ (kg DW)$^{-1}$)	
	C	A	C	A	C	A
total	442,000	212,000				
n	11	9	8	15	4	5
average	40,000	24,000	33	27	400/338/150/ 50–100	365/322/318/ 300/75
min	5,000	4,000	5	6		
max	160,000	74,000	60	60		

the recognition of *I. galbana*, clone T-Iso (Helm and Laing 1987) and *C. gracilis* (Enright et al. 1986) as valuable species.

The algal production capacity recorded for the various hatcheries, varying from 1 to over 100 m^3 day^{-1}, can be related to the estimates of Helm (1990) for the quantities of algae required in a hatchery operation. The latter author calculated that one million juvenile clams or oysters of 3 mm shell length will consume about 1.4 m^3 of dense algal culture each day at the optimum rearing temperature of 24°C, while one million of larvae require only 15 liter of algal culture.

The algal production cost per unit dry weight appeared to be known (or released) by few hatchery operators. The few values, reported by hatcheries producing less than 5 m^3 algal culture per day mostly exceeded U.S. $300 (kg DW)$^{-1}$, which is higher than most data in the literature (Table 10). Lower estimates, ranging between U.S. $50 and 100, were reported by hatcheries producing relatively large quantities of algae (Fig. 8). Obviously, large scale culture systems, which provide the bulk of the algal biomass grown in bivalve hatcheries, yield lower production costs per unit of dry weight.

The survey revealed that more than half of the questioned operators had experimented with algal substitutes in their hatchery. Despite these efforts, artificial diets are included in the routine production process of only a few hatcheries and more often considered as a useful backup diet. In either case, the live algae could only be partially replaced by dried *Tetraselmis suecica* (up to 25–50%) or a preserved algal paste (up to 75%). The hatcheries rearing giant clams appeared to be an exceptional case as they mostly lack algal culture facilities, and the feeding regime of the larvae and early postset consists solely of artificial diets. This may be ascribed to the lower food requirements of giant clams compared to other bivalves due to the establishment of a symbiosis with dinoflagellates shortly after metamorphosis (Fitt et al. 1984) and to the relatively high costs associated with maintaining algal cultures on the often remote sites where giant clam farms are located (Munro, pers. comm. 1991).

It is interesting to note that the results obtained with the same artificial diet greatly vary between experimenters and are often inferior to those reported in scientific papers. In this way, the unsatisfactory results, reported for the micro-encapsulated Frippak diet fed to spat of the Manila clam and the Pacific oyster, conflict with the successful experiments performed by Laing (1987). The

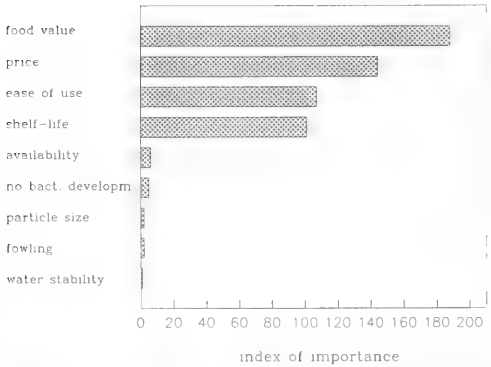

Figure 7. Index of importance of various parameters of an artificial diet for bivalve rearing, based on 50 completed questionnaires. The index was calculated by summation of the quotations given to each parameter (between 1 and 5, 5 = most important, maximal index = 250).

TABLE 9.

Relative importance of various algal species in bivalve hatcheries as reported in the survey of Walne (1978, in Persoone & Claus, 1980) and in the present study.

Algal Species	Frequency of Use	
	Walne (out of 10)	Present Study (out of 43)
Isochrysis galbana	8	8
Pavlova lutheri	7	11
Tetraselmis suecica	6	15
Phaeodactylum tricornutum	5	2
Pseudoisochrysis paradoxa	5	—
Thalassiosira pseudonana	4	14
Chaetoceros calcitrans	4	16
Skeletonema costatum	2	6
Isochryis galbana, clone T-Iso	2	31
Chlamydomonas sp.	1	1
Pyramimonas obovata	1	—
Tetraselmis chui	1	2
Rhodomonas sp.	1	—

TABLE 10.

Production cost of marine micro-algae.

Production Cost (US $/kg dry weight)	Remarks	Source
300	*Tetraselmis suecica*, 200 l batch culture	Calculated from Helm et al. (1979)
77		Pruder (1981; in Urban & Langdon, 1984)
167	Various diatoms, continuous flow cultures (240 m^3)†	Calculated from Walsh et al. (1987)
4–20	Outdoor culture	De Pauw & Persoone (1988)
160–200	Indoor culture	
23–115	Summer-winter production, continuous flow cultures in bags (8 m^3) and tanks (150 m^3)†	Dravers (pers. comm., 1990)
50	Tank culture (450 m^3)†	Donaldson (1991)

† Total volume available for algal production.

latter author obtained for the same bivalve species a similar growth as the algal-fed controls when substituting up to 60–85% of the algal diet by microcapsules. Also, the limited replacement of live algae by dried *T. suecica* is in contrast with reports of successful substitution of up to 75% and 90% of the live algae in the spat rearing of, respectively, *Crassostrea virginica* (Helm and Hancock 1990) and *Tapes philippinarum* (Laing & Millican 1992). Although various authors have demonstrated through laboratory experiments that live algae could be substituted for up to 50% by dried yeast (*Candida utilis*) in the juvenile rearing of several bivalve species (Epifanio 1979, Alatalo 1980, Urban and Langdon 1984), no confirmation of this was revealed in the survey. The inconsistent performance of artificial diets may have several explanations. Certain products, such as the dried algae, appeared to be difficult to use and may not always have been presented in the optimal form to the animals. Alternatively, the experimental conditions, including quality and quantity of the algal control diet, stocking density, water quality, and scale of the experiment, may affect the performance of the artificial diet. In this regard, the specific conditions of laboratory experiments can be expected to differ from those encountered in a hatchery.

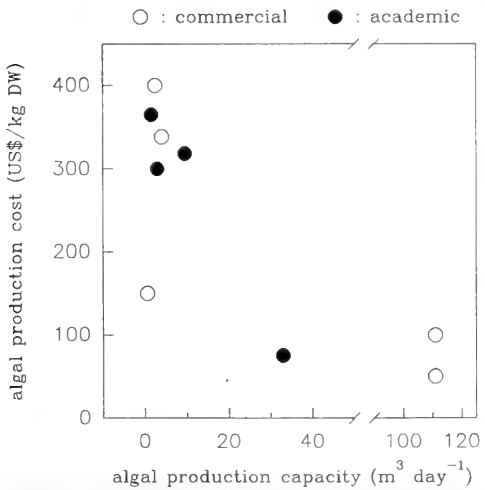

Figure 8. Algal production cost as a function of the production capacity for 8 bivalve hatcheries. Dotted line connects estimates from one company.

ACKNOWLEDGMENTS

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GYMNODINIUM NAGASAKIENSE RED TIDE OFF SOMESHWAR, WEST COAST OF INDIA AND MUSSEL TOXICITY

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ABSTRACT A dinoflagellate bloom of *Gymnodinium nagasakiense*, caused a "red tide" off Someshwar, West coast of India during September–October 1989. Mortality of a number of fish species accompanied the red tide. Mussels *Perna viridus* exposed to the red tide and *G. nagasakiense* cells showed some fat soluble toxins active against mice.

KEY WORDS: *Gymnodinium nagasakiense*, mussel toxicity, red tide

The dinoflagellate, *Gymnodinium nagasakiense* has been implicated in massive kills of marine fauna in Northern European, Japanese and possibly Gulf of Maine waters (Tangen 1977, Boalch 1979, Potts and Edwards 1987, Matsuoka et al. 1989, Heinig and Campbell 1992). Taylor (1985) reported that *Gyrodinium aureolum* Herbert and *Gymnodinium nagasakiense* Takayama and Adachi are the same organisms recorded from the Atlantic and Western Pacific respectively. Several species of wild and farmed fishes as well as many species of benthic invertebrates have been reported to be killed during blooms of *G. nagasakiense* (Matsuoka et al. 1989, Okaichi 1989, Wong 1989). To our knowledge, so far, there has been no report of *G. nagasakiense* blooms in tropical waters. In this communication we report a bloom and consequent fish kills off Someshwar near Mangalore, South west coast of India.

On September 8, 1989, fishermen reported massive fish kills along the 2 KM coastline between Someshwar and Uchila, south of Mangalore. The fish species killed were *Lutianus johni* (Moses perch), *Thysanophrus indicus* (Indian flat head), *Lutianus argentimaculatus* (red snapper), *Otolithus argenticus* (croaker), *Tachysurus* sp. (marine catfish), *Scylla serrata* (sole) and *Portunus pelagicus* (crab). Plankton analyses were carried out on surface water samples near the shore line collected in 5 litre polythene carbuoys and brought to the laboratory within one hour. 1 ml of the water sample was placed in the plankton counting chamber and average values of ten samples computed. The results revealed a monospecific bloom of *G. nagasakiense* (Fig. 1) at a concentration of 10^8 cells l^{-1}. Fish kills also occurred on the following day and the loss is estimated to be about 8–10 tons for all species combined. On September 11, the red tide persisted but the counts had dropped to 10^7 l^{-1}. Subsequently, counts could not be taken for nearly a month due to heavy rains and rough seas. When the weather cleared and boats were launched again on October 10, 1989, *G. nagasakiense* at concentrations of 10^8 l^{-1} were observed and the counts slowly decreased over the next fortnight (Fig. 2).

Though *G. nagasakiense* blooms have been previously reported to cause fish kills (Okaichi 1989, Wong 1989), no effects on man have been reported following consumption of fish and shellfish from bloom areas. Even during the present red tide, fishermen admitted eating fish which were alive while being washed ashore, without any ill effects. In the case of *Gymnodinium* (*Pty-*

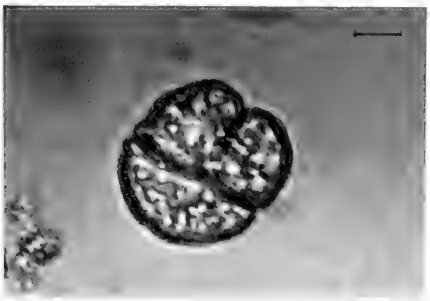

Figure 1. ***Gymnodinium nagasakiense*** **cell - Bar 15 µm.**

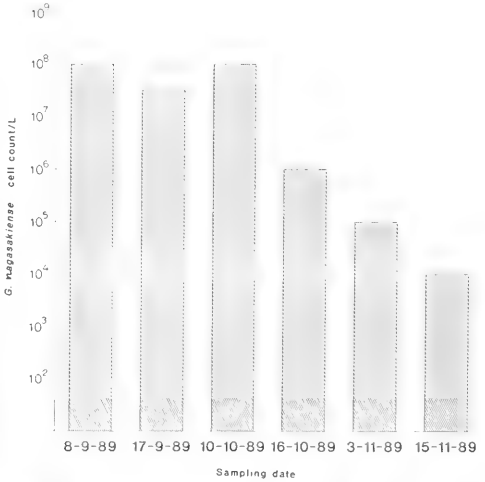

Figure 2. ***Gymnodinium nagasakiense*** **cell concentration in waters off Someshwar.**

chodiscus) *breve* on the other hand, human toxicities have been reported (Steidinger, 1983).

We tested mussel beds exposed to the *G. nagasakiense* red tide on October 10, 1989 for presence of brevetoxin-like toxins by the method described by APHA (1970). The mussels (*Perna viridis*) contained toxin equivalent to 10 Mouse Units (MU) per 100 g shellfish meat. The mussels were small (less than 10 g meat per mussel) and therefore were not eaten by the people. To determine the toxicity of *G. nagasakiense*, sea water containing high counts of this organism was centrifuged and the cells extracted with diethyl ether, which was evaporated and the residue was suspended in cotton seed oil. This extract caused death in mice indicating toxicity. Partensky et al. (1989) reported cytotoxicity of cultured cells of *G. nagasakiense* on human KB cells. Our attempts to culture the bloom species did not succeed; however, our studies suggest that *G. nagasakiense* produces toxic compounds. The toxin was present at a level of 10 MU per 100 g and even in the case of brevetoxin, human illness has been reported only when the concentration exceeds 50 MU per 100 g (Steidinger, 1983).

This study suggests that blooms of *G. nagasakiense* and fish kills also occur in tropical environments. *G. nagasakiense* has been observed over wide temperatures of 13°C to 31 °C and its favourable temperature is reported to be 24°C–29°C (Matsuoka et al. 1989). The temperature in Someshwar during the red tide was 23–29°C thus creating conditions favourable for the bloom. This bloom occurred immediately following the monsoon (June–September) in this area. Pre-monsoon phosphate concentration in sea water in this area ranged from traces to 0.4 $\mu g\ l^{-1}$ and during the bloom, the level ranged from 1.1 μg–2.6 $\mu g\ l^{-1}$. When the *G. nagasakiense* counts declined in late October, phosphate concentration ranged from 0.1 μg to 0.26 $\mu g\ l^{-1}$ thus suggesting that phosphate concentration might have been another factor contributing to the bloom.

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PHYTOPLANKTON PIGMENTS ACCUMULATED BY THE ARCTIC SURFCLAM, *MACTROMERIS POLYNYMA* (STIMPSON, 1860)

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ABSTRACT The Arctic surfclam, *Mactromeris polynyma* (previously Stimpson's surfclam, *Spisula polynyma*), was investigated for the presence of phytoplankton pigments in selected tissues. Dissection of clams, commercially landed in Gloucester, MA, revealed that pigment-containing cells were restricted to the epithelium of the foot, siphon, and mantle edge. These organs were analyzed for pigments using UV/visible spectrometry and HPLC. Initial analyses revealed that α- and β-carotene represented only minor components in *M. polynyma* foot, mantle, and siphon tissues; whereas, xanthophyll pigments were present in higher concentrations. Several xanthophylls were identified in *M. polynyma* tissues, based upon comparisons of clam extracts with those of cultured phytoplankton having known pigment profiles. Foot tissue contained fucoxanthin, diadinoxanthin, and chlorophyll *a*; mantle tissue contained peridinin, fucoxanthin, neoxanthin, violaxanthin, and lutein; and siphon tissue contained peridinin, fucoxanthin, neoxanthin, violaxanthin, and an unidentified pigment which was also found in *Tetraselmis maculata* and *Dunaliella tertiolecta*.

KEY WORDS: carotenoid, HPLC, *Mactromeris*, microalgae, red-necked clam, Stimpson's surfclam, xanthophyll

INTRODUCTION

The Arctic surfclam, *Mactromeris polynyma* Stimpson 1860, previously known as *Spisula polynyma* Stimpson 1860 and commonly called Stimpson's surfclam in the Northeast and the pink- or red-neck clam in the Pacific northwestern United States (Turgeon et al. 1988), is an infaunal bivalve of interest to American, Canadian, and Japanese fisheries. Foot, siphon, and mantle edge epithelial tissues are deep, purplish-red in 95% of live samples (John Monroe, personal communication). When cooked, typically steam shucked, these tissues turn bright red. Sushi chefs sometimes carve intricate designs in the foot through the red epithelium into the white muscle beneath. Canadian and American fisheries agencies have conducted resource-abundance surveys for *M. polynyma* (Hughes and Bourne 1981). Canadian fisheries authorities have also undertaken experimental programs to investigate the economic feasibility of commercial fishing for this species (Amaratunga and Rowell 1986, Roddick and Kenchington 1990). Most studies have assumed that *M. polynyma* would be used to bolster the strip- or fried-clam market which normally uses the Atlantic surfclam, *Spisula solidissima* Dillwyn 1817. This is considered a low-value market. Within the last four years, several fishing companies on the Atlantic coast have begun directed fishing for *M. polynyma* in response to the opening of a high-value market in Japan, where the foot of *M. polynyma* is prized for use in sushi dishes.

The nature and source of pigments in *M. polynyma* have not been investigated. We hypothesized that pigments in clam tissues could be carotenoid pigments derived from the microalgal diet of *M. polynyma*. Other marine and aquatic animals, including invertebrates and finfish, possess pigments derived from plants (D'Abramo et al. 1988, Liaaen-Jensen 1977, Sommer et al. 1991). Several forms of the xanthophyll astaxanthin have been isolated from aquatic organisms (Matsuno et al. 1984, Renstrom et al. 1981). The xanthophylls present in the tissues of *M. polynyma* may prove to be a signature of the microalgal taxa ingested by this animal. Each taxonomic class of microalgae, with some variations, produces characteristic chlorophylls, carotenes, and xanthophylls. By determining the major carotenoids present in clam tissues we may be able to illuminate the source of these pigments.

For valid comparisons, equivalent methods for the extraction and analysis of photosynthetic pigments in both plant and animal tissues must be used. A review of the literature, and methods employed in preliminary work, revealed several methods for plant pigment extraction (National Institute of Standards and Technology 1990, Paerl and Millie 1991, Reuter personal communication, Wilhelm et al. in press, Wright et al. 1991). Extraction of plant pigments from animal tissues has been achieved, but there is little information as to the comparability of these methods with those used on plants. Identification of xanthophyll pigments extracted from plant tissues, using high performance liquid chromatography (HPLC), has been undertaken and reported by several researchers (Gieskes and Kraay 1986, Rivas et al. 1989, Zapata 1989, Wright et al. 1991); indeed, plant pigment separations can be traced back to some of the first chromatography experiments (Ettre 1992). The purpose of this study was to determine if the dominant pigments, that are representative of the major microalgal classes and are likely to be consumed by marine bivalves, are present in *M. polynyma*. Tissues from *M. polynyma* were analyzed for carotenoid pigments using UV/Vis spectroscopy and HPLC with UV/Vis detection.

MATERIALS AND METHODS

HPLC

The HPLC system used was a Perkin-Elmer* model 250 binary LC pump with a model LC-75 UV-VIS detector. Data were collected, by computer, using Omega version 2.5 software. Maximum pressure was set at 2000 psi and the detector wavelength at 430 nm. At the start of each day, the system was equilibrated for 15 minutes with methanol at a flow rate of 1.0 ml/min.

Pigments were separated on a VYDAC #201TP54 4.6 mm ×

*Mention of trade names does not imply endorsement by the NMFS.

15 cm 5 μm particle size C-18 column with a Perkin-Elmer C-18 guard column kit, flushed previously with 96% methanol:water (v/v), for 4 min at 1.5 ml/min. Between 20 and 500 μl samples were injected with a Rheodyne model 7125 Syringe Loading Sample Injector. Sample runs used 1.5 ml/min of 96% methanol for 15 min.

One xanthophyll standard, lutein, was obtained commercially. Standards of two additional xanthophylls, fucoxanthin and diadinoxanthin, purified from algal biomass, were obtained from Dan Repeta, Woods Hole Oceanographic Institution, Woods Hole, MA. It was therefore necessary to analyze several axenic isolates of microalgae from different taxonomic classes to establish chromatograms for the other major xanthophylls of each class (Dawson 1966, Goodwin 1974, Whittle and Casselton 1975, Wilhelm et al. in press, Wright et al. 1991).

Sample Preparation

The axenic microalgal isolates used were from the Milford Microalgal Culture Collection. Cultures were grown axenically for 11 d at 15°C and 115 $\mu E/M^2s$ in 80 ml E medium (Ukeles 1973) and harvested in the log phase. Two strains were analyzed in stationary phase and were not axenic: EXUV and Sticho-GSB. The microalgal strains analyzed and their taxonomic classes are listed in Table 1.

The extraction procedure was modified from the method of Strickland and Parsons (1968). Microalgae were vacuum-filtered onto Whatman 25 mm glass-fiber filters (GF/F). Prior to filtration, both filters and housings were washed with sterile E medium. Algae and filters were then washed with 10 ml of 14 ppt NaCl, which is isotonic to the growth medium. Samples were put in 25 ml Pyrex, Oak Ridge centrifuge tubes with 5 ml of 90% acetone: 10% double glass-distilled water (v/v). Algal cells were disrupted by sonicating with a Tekmar 500 W sonic disrupter on a pulsed setting and 80% power for 2 min using ice to cool the sample. Screw caps were sealed securely by wrapping with Parafilm and then pigments were extracted for 20 h in the dark at 3°C. Samples were mixed thoroughly, drawn into a 10 ml syringe through a 20 ga 5.1 cm hypodermic needle, and then passed through a 0.45 μm Millex-HV filter unit. Filtered samples were deposited into Pyrex 15 ml centrifuge tubes with ground glass stoppers and maintained at 3°C in the dark pending HPLC analysis within 4 h.

Live clams, shipped on ice, were obtained from New England Clam Corporation, Gloucester, MA. Upon receipt, clams were dissected and gut contents inspected for algal cells using light microscopy. Nine samples each of foot, mantle, and siphon tissues were removed and stored at −80°C. Samples were then thawed partially and placed in one half of a 5 cm glass petri dish. The sample was held with stainless steel tweezers and minced with a razor knife, and then homogenized in a fritted glass tissue grinder with a minimal amount of 90% acetone. The whole sample was returned to a 25 ml centrifuge tube and henceforth treated in the same method as the algae—beginning with the sonication step.

RESULTS

Spectrophotometric analysis of 90% acetone extracts of *M. polynyma* foot tissue showed an absorbance peak at 430 nm (Wikfors unpublished data). Many photosynthetic pigments show absorbance at this wavelength. Preliminary analyses of clam tissues were conducted at the Laboratory of Epidemiology and Public Health (Yale University, New Haven, CT) for carotenoid pigments using HPLC. Analysis of foot, mantle, and siphon tissue indicated only small amounts of α- and β-carotene, but relatively high concentrations of xanthophylls, which eluted too quickly to identify with the method employed initially (S. T. Mayne and C. S. Kim, unpublished data).

Our chromatographic results of algal extracts (Table 2) agree with published reference chromatograms (Wright et al. 1991). Standards of lutein, fucoxanthin, and diadinoxanthin confirmed retention times found for these pigments in algae possessing them. One peak which eluted early in *Tetraselmis maculata* and *Dunaliella tertolecta* could not be identified. It is possible that this "unknown" green algal pigment is a degradation product or isomer of neoxanthin. The elution times of Wright et al. (1991) loosely support this hypothesis.

The EXUV strain of *Prorocentrum minimum* did not have enough dinoxanthin to yield a clear peak and had very little diadinoxanthin. In addition, a peak with a retention time corresponding to violaxanthin was found consistently in this strain. Dinophyceae have been thus far considered not to possess violaxanthin. Plant pigments are not always consistent throughout a given taxonomic class (Bjornland et al. 1988, Fawley 1991, 1992). There is even some evidence that constituent pigment variation can be dependent on nutrient conditions (Lee and Soh 1991) and genetic variability within genus (Buma et al. 1991). It is possible that the peak we labelled as violaxanthin in *P. minimum* is, instead, an isomer or degradation product of peridinin. In addition, the peak we identified as fucoxanthin in the dinoflagellate also may be a degradation product of peridinin. To our knowledge, fucoxanthin has never been identified in *Prorocentrum* spp.

The pigments found in clam tissues, according to our method,

TABLE 1.

Strains of microalgae, from the Milford Microalgal Culture Collection, analyzed for photosynthetic pigments.

Clone Name	Algal Species	Taxonomic Class
DE	*Dunaliella tertiolecta* (Butcher)	Chlorophyceae
EXUV	*Prorocentrum minimum* (Pavillard) Schiller	Dinophyceae
F-3C	*Rhodomonas salina* (Wislouch) Hill et Wetherbee	Cryptophyceae
MONO	*Pavlova lutheri* (Droop) Hibberd	Prymnesiophyceae
PORPH	*Porphyridium cruentum* (Gray) Nägeli	Rhodophyceae
PHAEO	*Phaeodactylum tricornutum* (Bohlin)	Bacillariophyceae
Sticho-GSB	*Nannochloropsis salina* (Droop) Hibberd	Eustigmatophyceae
SYN	*Synecococcus bacillaris* (Butcher)	Cyanophyceae
TTM	*Tetraselmis maculata* (Butcher)	Prasinophyceae

TABLE 2.

Plant pigments identified by HPLC in strains of microalgae analyzed in this study (retention times, in minutes, are in parentheses).

Pigment (R_t):	Algal Strain: TTM	DE	MONO	PORPH	SYN	PHAEO	F-3C	STICHO-GSB	EXUV
Peridinin (2.31)									*
Fucoxanthin (3.00)			*			*			*[1]
Unknown (3.50)	*	*							
Neoxanthin (4.08)	*	*							
Violaxanthin (5.00)	*	*						*	*[1]
Antheraxanthin (7.29)	*	*							
Diadinoxanthin (7.80)			*			*			*
Lutein (10.10)	*	*							
Alloxanthin (11.14)							*		
Myxoxanthophyll (11.20)					*				
Diatoxanthin (11.70)			*						
Zeaxanthin (12.30)	*[2]	*[2]		*	*				
Chlorophyll a (13.70)	*	*	*	*	*	*	*	*	*

[1] See text for discussion.

[2] There is a possibility that chlorophyll b co-eluted with zeaxanthin in these two strains.

are listed in Table 3. Fucoxanthin, chlorophyll *a*, and diadinoxanthin all were found consistently in foot tissue (see also Figure 1). The undefined peak in the mantle tissue was very small and difficult to identify. The mantle was the only tissue, of the three analyzed, that contained lutein (see also Figure 2). The presence of chlorophyllides in the siphon tissue is supported by the elution order published by Wright et al. (1991) and the unknown pigment appears to be the same as found in *T. maculata* and *D. tertiolecta* (see also Figure 3). Both the mantle and siphon tissues contained peridinin, fucoxanthin, neoxanthin, and violaxanthin.

DISCUSSION

Basic information on *M. polynyma* has appeared in the scientific literature for about 80 years. As with many bivalve mollusks,

TABLE 3.

Presence or absence of plant pigments in the colored tissues of *Mactromeris polynyma* (retention times, in minutes, are in parentheses).

Pigment (R_t):	Foot	Mantle	Siphon
Possible Chlorophyllides (<2.3)			*
Peridinin (2.31)		*	*
Fucoxanthin (3.00)	*	*	*
Unknown (3.50)			*
Neoxanthin (4.08)		*	*
Violaxanthin (5.00)		*	*
Antheraxanthin (7.29)#			
Diadinoxanthin (7.80)	*		
Undefined Peak (8.41)		*	
Lutein (10.10)		*	
Alloxanthin (11.14)#			
Myxoxanthophyll (11.20)#			
Diatoxanthin (11.70)#			
Zeaxanthin (12.30)#			
Chlorophyll a (13.70)	*		

* The pigment was found in this tissue.

The pigment was not present in these tissues.

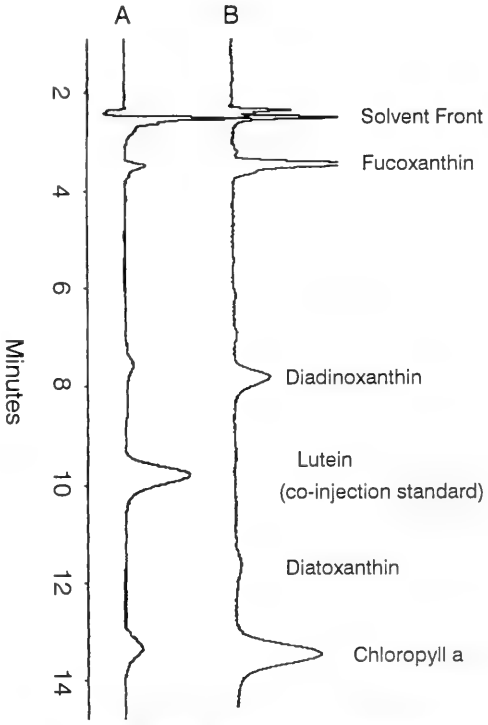

Figure 1. The major pigments of *Mactromeris polynyma* foot tissue (A) compared to the pigments found in *Pavlova lutheri* ((B). Horizontal axes are absorbance units.

particularly those considered "off-shore" species, little is known about their diets. By determining the pigmentation of the Arctic surfclam, the microalgae constituting the organisms's diet may be deduced. This can provide a better understanding of the phytoplankton assemblage contributing to the benthic food chain in the location that these clams were caught. Use of relative pigment ratios to deduce phytoplankton community structure is currently being developed by other researchers. Oldham et al. (1985) are using fluorescence spectroscopy excitation-emission matrices to characterize phytoplankton assemblages; still others are continuing to refine methods of pigment detection in water samples (Everitt et al. 1990, Yacobi et al. 1990). Analysis of phytoplankton pigments in sessile, filter-feeding organisms may prove to be another tool for phytoplankton research.

M. polynyma appears to be selective in the anatomical locations in which it harbors different plant pigments. This may involve the reactivities of the pigment molecules or may be a result of the recent dietary history of the clam. Clam samples analyzed in the present study were taken from Stellwagen Bank in the Gulf of Maine in early November 1991; this coincides with an annual bloom of coccolithophorids, mainly *Emiliania huxleyi* (Lohm.) Hay et Mohler, in that area (Marshall 1984). Coccolithophorids are in the same class, Prymnesiophyceae, as *Pavlova lutheri*, strain MONO; therefore these algae should have major pigments in common, although there are some differences. *E. huxleyi* possesses 19′-hexanoyloxyfucoxanthin (Bjornland 1988), and none was detected in *P. lutheri*. The major prymnesiophyte pigments, such as those found in *P. lutheri*, account for the pigments found only in the foot tissue of *M. polynyma*.

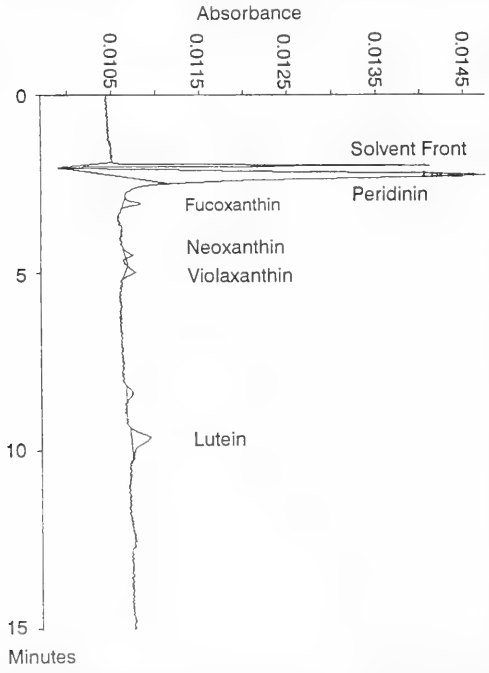

Figure 2. The pigments identified in *Mactromeris polynyma* mantle tissue. The unlabelled peak eluded identification. It is listed in table 3 as "undefined peak."

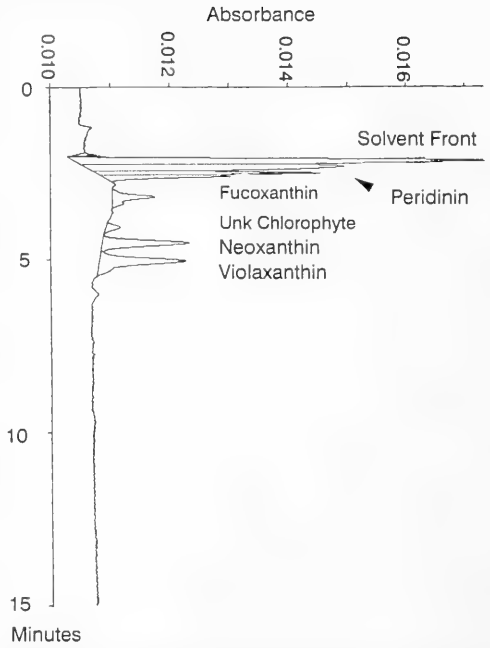

Figure 3. The pigments identified in *Mactromeris polynyma* siphon tissue. There is a peak before and one after peridinin. They are probably chlorophyllides or degradation products of peridinin and can not be resolved with the method used.

Gut analysis of clams examined in this study revealed the presence of only a few undigested *Prorocentrum sp.* cells (class Dinophyceae). These dinoflagellates are potential contributors of the peridinin found in the mantle and siphon tissues and also of the diadinoxanthin and chlorophyll *a* present in the clam tissues. Dinoflagellates such as *Prorocentrum spp.* are also sizable constituents of the fall phytoplankton assemblage in Stellwagen Bank (Marshall 1984). The contributions of neoxanthin, violaxanthin, the "unknown" peak, and lutein in the tissues are most likely from chlorophytes. It is not known how long these pigments take to accumulate in the tissues nor how long they remain. Only when these questions are answered will it be possible to apply information on pigment composition of field-collected clams to food-web research. Laboratory studies in which *M. polynyma* are fed selected algal species will be needed to "calibrate" pigment uptake and loss dynamics.

The highest market value for *M. polynyma* is obtained only from those retaining a rich pigmentation when cooked. With knowledge of the phytoplankton pigments responsible for this coloration, it may be possible to maximize the market value of these clams using current aquaculture technology (Raymond 1990). Commercial fishermen could use a "finishing-tank" treatment in which harvested *M. polynyma* are fed cultured phytoplankton rich in the desired pigments. *P. lutheri*, strain MONO, possesses such pigments and, as such, is one candidate for enhancement of clam

foot pigmentation. This is analogous to efforts of some aquaculturists who enhance the color of finfish flesh by using food organisms rich in plant pigments such as astaxanthin (Saito and Regier 1971, Spinelli et al. 1974).

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PROCEEDINGS OF THE SPECIAL SYMPOSIUM: MOLLUSCAN INTRODUCTIONS AND TRANSFERS: RISK CONSIDERATIONS AND IMPLICATIONS

Presented at the 82nd Annual Meeting

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AND
SHELLFISH INSTITUTE OF NORTH AMERICA

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Convened and edited by

James T. Carlton and Aaron Rosenfield

CONTENTS

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INTRODUCTION TO THE SPECIAL SYMPOSIUM ON MOLLUSCAN INTRODUCTIONS AND TRANSFERS: RISK CONSIDERATIONS AND IMPLICATIONS

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The collection of papers that appears within these proceedings is the outcome of a Symposium entitled "Introductions and Transfers of Mollusks: Risk Considerations and Implications". The Symposium was held as part of the 82nd Annual Meeting of the National Shellfisheries Association in April 1990 in Williamsburg, Virginia. One paper from this Symposium was published earlier in this *Journal* (Mann et al. 1991). In addition to those papers that appear here, six other papers were presented orally during the Symposium. Unfortunately these latter papers were not completed in time to be included in this issue of the *Journal of Shellfish Research*. Although the editors would like to allow additional time, it was concluded that further delays would risk outdating the papers submitted by the rest of the Symposium participants.

The Mollusca occur world wide in extraordinary diversity, abundance, and distribution both in aquatic and terrestrial habitats. They are readily available and harvestable; with common sense and a little knowledge and care they are among the easiest of the invertebrates to collect, manipulate, transport, and maintain for extended periods using relatively uncomplicated conditions and inexpensive holding facilities. It is no wonder their exploitation for food, ornamentation, dye materials, tools, construction material, music, toys, utensils, money, and shell collections has been practiced for millennia. More recent imaginative and remarkable advances have been made in the use of mollusks for aquaculture, genetic engineering (including the development of transgenics), toxicology, and biomedicine. The use and application of mollusks in fields such as these necessarily involve the shipment and importation, that is, the translocation of mollusks from one location to another. As can be deduced from the title of this Symposium considerable apprehension and concern exists today over the risks or dangers associated with the potential movement of molluscan species from one ecosystem to another. These movements create a strong potential for the introduction of new species or the infusion of new genetic material into regions where they may have profound impacts on native species.

In very recent years the subject of the natural and human mediated invasions of nuisance species into ecosystems where they have not been resident before has and continues to be the subject of a great deal of attention. This attention is particularly strong among individuals and groups associated with intentional movements of molluscan species, not only for aquaculture purposes but also for scientific study, aquarium use, new product development and depuration. In addition others are interested in the unplanned, accidental translocation of exotic mollusc species and transfers or indigenous species, either of which when released into new environments may become nuisances themselves or act as carriers for other plants or animals that become pests, parasites, pathogens, or competitors with resident organisms. There are always risks associated with translocation of animals and plants resulting in impacts that could be either positive or negative from the viewpoints of environmental and resource sustainability. Careful consideration must be given to the ecological, genetic, sociological, economic, aesthetic and political impacts that may result from undesirable introductions regardless if they are deliberate or accidental. On the other hand, the use of mollusks for purposes of aquaculture, stock enhancement and improvement, sanitation, recreation, science and technology, education, and food production could bring enormous benefits. However, such programs must be well thought out and carefully designed, and must be considerate of maintaining environmental integrity and ecological balance.

This Symposium thus considers some of these risks and benefits involved with both known and anticipated introductions and transfers of mollusks, and discusses the potential implications, past, present, and future, of these movements.

We are most grateful to the National Marine Fisheries Service Office of Research and Environmental Information for providing funding for this Symposium, and particularly to Dr. Glenn A. Flittner and Dr. Carolyn Brown for their generous support and help in planning and conducting the Symposium.

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INTRODUCED MARINE AND ESTUARINE MOLLUSKS OF NORTH AMERICA: AN END-OF-THE-20TH-CENTURY PERSPECTIVE

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ABSTRACT A review of the introduced marine and estuarine (brackish water) bivalves and prosobranch and pulmonate gastropods of the Atlantic, Gulf and Pacific coasts of North America reveals an established fauna of 36 non-indigenous species. Sixteen species are native to temperate or tropical coasts of North America, and have been transported to regions of the continent where they did not occur in historical time; the remaining 20 species are from Europe, the Mediterranean, South America, the Indo-Pacific, and the northwestern Pacific. The movement of Pacific (Japanese) and Atlantic commercial oysters to the Pacific coast, and ship fouling, boring, and ballast water releases, have been the primary human-mediated dispersal mechanisms. Regional patterns are striking: 30 species are established on the Pacific coast, 8 on the Atlantic coast, and 1 on the Gulf coast (three species occur on both coasts); 19 (63%) of the Pacific species occur in San Francisco Bay alone. These patterns may be linked to a combination of human-mediated dispersal mechanisms and regional geological-biological Pleistocene history: at least 27 species of Japanese and Atlantic coast mollusks were introduced to the American Pacific coast by the oyster industry, in large part into geologically young regions with low native molluscan diversity. With the exception of a few species, there is little experimental elucidation of the ecological impact of the introduced marine mollusks in North America. Negative effects by introduced gastropods on native gastropods have been demonstrated on both the Atlantic and Pacific coasts; for one species, the Atlantic pulmonate marsh snail *Ovatella* on the Pacific coast, experimental evidence suggests that its establishment did not arise at the expense of native species. No introduced marine mollusk in North America has had a greater ecological impact than the periwinkle *Littorina littorea*, which colonized the Atlantic coast from Nova Scotia to New Jersey in the 30 year period between 1860 and 1890, and subsequently altered the diversity, abundance, and distribution, of many animal and plant species on rocky as well as soft bottom shores. Future marine invasions, through ballast water release and perhaps through aquaculture activities, can be expected with confidence.

KEY WORDS: mollusks, introductions, invasions, nonindigenous, exotics

INTRODUCTION

> "A good deal of chess play has also been done with clams. . . ."
>
> —Charles S. Elton (1958)

At the close of the 20th century we are witnessing rapidly growing interest in the phenomenon of biological invasions of coastal waters. As a result of an increasing number of unintentional invasions of marine organisms due to the release of ballast water through international shipping activities, and of increasing pursuit of the intentional use and release of marine organisms for mariculture purposes and for open sea fisheries enhancement, concern is growing relative to the potential ecological, genetic, economic, and social risks that may be associated with future invasions.

I review here the diversity, distribution, regional invasion patterns, and ecological impacts of the introduced marine and estuarine (brackish water) bivalves and prosobranch and pulmonate gastropods of the Atlantic, Gulf, and Pacific coasts of North America. Introduced species (exotic, non-indigenous, alien, or invader species) are those taxa transported by human activity to regions where they did not éxist in historical time (Carlton 1987). While there has been no previous continent-wide review of the introduced mollusks, Quayle (1964), Hanna (1966) and Carlton (1975, 1979a, 1979b) have provided regional lists and treatments for the Pacific coast. Abbott (1974), Bernard (1983) and Turgeon (1988) list many of the species discussed here. I include all species which have been recorded as free-living outside of mariculture operations. One species, the Japanese sea scallop *Patinopecten yessoensis*, is included because of its current mariculture use and potential to become naturally established. I have excluded opisthobranch mollusks (sacoglossans, nudibranchs and pyramidellids), pending a global and/or continental review of the candidate species. There are no introduced polyplacophorans (chitons) or scaphopods (tusk shells) in North America. I also exclude most records of single specimens of living mollusks whose anomalous presence outside their recorded ranges appears to be due to discarding through hobby (aquarium) or fishing activities.

Mechanisms of introduction of non-indigenous marine organisms to North American waters have been reviewed by Carlton (1985, 1987, 1989, 1992a). The most important human activities have been or are the following: (1) the transportation of organisms on the outside (fouling species) or on the inside (boring species) of ships, (2) the transportation of organisms inside vessels in solid ballast, such as rocks, sand, and detritus, (3) the movement of oysters, and the concomitant movement of organisms on the oyster shells or in associated sediments and detritus, (4) the intentional release of species for fisheries purposes, and (5) the release of larvae, juveniles, or adults of marine organisms in the ballast water of coastal, transoceanic, and interoceanic vessels. I review below the relative importance of each of these mechanisms to the established introduced mollusks in North America.

METHODS

Field, museum, and literature work from 1962 to 1979 are summarized by Carlton (1979a). Field work during that period was conducted from Vancouver Island to southern California; 18 museums or private collections on the west and east coasts of the United States and Canada were studied. From 1979 to 1992 field work was conducted from Newfoundland to Virginia, as well as on

the Pacific coast, and museum collections were revisited to examine additional species. Throughout both periods I corresponded with malacologists and other biologists and undertook continual literature reviews. The records and dates recorded here are thus based upon field work, museum collections, personal communications, and the literature, and form the basis of a monograph now in preparation. I present here an abstract of this work.

RESULTS

Regional Patterns of Invasion

Table 1 is a comprehensive synthesis of the introduced marine and estuarine mollusks reported since the early 19th century in North America. The introduced mollusks can be placed into 4 categories (Table 2): established (naturally reproducing populations are known), establishment not certain (no recent records, but the species may still be present), not established (not found in recent surveys or, if present, naturally reproducing populations are not known), and cryptogenic (Carlton 1987; status as introduced or native is not known).

Thirty-six species of non-indigenous marine and estuarine mollusks are established on the Pacific, Atlantic, and Gulf coasts of North America (Table 3). Sixteen species are native to temperate or tropical coasts of North America, and have been transported to regions of the continent where they did not occur in historical time. Thus, 14 species (Table 2) native to the Atlantic coast have been transported to the Pacific coast (Table 3 indicates 15 species on this route; this includes the European *Ovatella*, established on the American Atlantic coast). At least 3 species (*Rangia cuneata, Mytilopsis leucophaeata* and *Teredo bartschi*) have been transported from their apparently native southern ranges to more northern localities (shown in Table 3 as 1 species from the Gulf of Mexico and 2 species from the northwest Atlantic, respectively). The remaining 20 species include 4 from Europe, 1 questionably from Europe (the shipworm *Teredo navalis*), 1 from the Mediterranean (the mussel *Mytilus galloprovincialis*), 1 from South America (the mussel *Perna perna*), 1 questionably originating in the Indo-Pacific (the shipworm *Lyrodus pedicellatus*), and 12 from the northwestern Pacific.

Four species (Table 2) are questionably established; field work has not been focused on locating these species in recent years, and they may still be present. Seven species have not become regionally established: the Atlantic periwinkles *Littorina littorea* and *Tectarius muricatus*, once found living in California and the Gulf of California respectively; the European snail *Truncatella subcylindrica*, found in 1880 to be common at Newport, Rhode Island; the Asian clam *Laternula limicola*, found over a period of several years in Coos Bay, Oregon in the 1960s; the European oyster *Ostrea edulis*, widely released on the American Pacific coast, and the South American mytilid *Mytella charruana* which appeared in numbers in Jacksonville, Florida in 1986. Of these, *Littorina littorea* and *Ostrea edulis* have become established on the Atlantic coast. The Japanese sea scallop *Patinopecten yessoensis* while present in mariculture operations in British Columbia has not been reported in natural sets.

Cryptogenic species include (Table 1) the pulmonate limpet *Siphonaria pectinata* and the shipworm *Teredo navalis*. Nineteenth century or earlier shipping has been implicated in creating the modern distributions of both species, but details of their historical biogeography in the north Atlantic Ocean remain uninvestigated.

Regional patterns (Table 3) are striking: 30 species are established on the Pacific coast, 8 on the Atlantic coast, and 1 on the Gulf coast (3 species, the snail *Ovatella*, the clam *Corbicula*, and the shipworm *Teredo bartschi* occur on both the Atlantic and Pacific coasts). Most (27 species) of the introduced mollusks on the Pacific coast originate either from Asia or the Atlantic coast of North America. Of the Pacific species, 5 are recorded from only 1 locality: the Atlantic whelk *Busycotypus* and the Asian clam *Potamocorbula* occur only in San Francisco Bay, the Atlantic clam *Mercenaria* occurs only in Colorado Lagoon, Alamitos Bay, the Atlantic oyster *Crassostrea virginica* now survives only in the Serpentine and Nicomekl Rivers of the Boundary Bay region, British Columbia, and the shipworm *Lyrodus takanoshimensis* has been reported only from Ladysmith Harbor, British Columbia. I do not include here the clam *Macoma* "*balthica*," whose San Francisco Bay population appears to arise from an Atlantic coast stock, as this genotype may in fact be widespread in central California embayments.

Four species are restricted to the Pacific Northwest (Washington and British Columbia): the Japanese snails *Cecina manchurica* and *Nassarius fraterculus*, the Japanese clam *Trapezium liratum* and the Pacific oyster (*Crassostrea gigas* (which rarely reproduces south of Willapa Bay, WA). Two additional species reported only from British Columbia are the questionably established *Clanculus ater* and *Sabia conica*. Four Atlantic species are well established in a few restricted localities: the slipper limpet *Crepidula convexa* occurs only in San Francisco and Boundary Bays (newly recognized in British Columbia by Robert Forsyth); the mudsnail *Ilyanassa obsoleta* occurs only in San Francisco, Willapa, and Boundary Bays; the angelwing clam *Petricola pholadiformis* occurs only in San Francisco, Newport, and Boundary Bays, and the gem clam *Gemma gemma* is restricted to 5 bays in central California (Bodega Harbor (not Bodega Bay), Tomales Bay, Bolinas Lagoon, San Francisco Bay, and Elkhorn Slough). Seven oyster-associated introductions occur in British Columbia/Washington and in California, but for reasons that remain unclear do not occur "naturally" in Oregon bays and estuaries: these are the Japanese snail *Batillaria attramentaria* and the Atlantic gastropods *Ilyanassa obsoleta, Crepidula convexa, C. fornicata, C. plana,* and *Urosalpinx cinerea;* the fifth species, the Japanese clam *Venerupis philippinarum*, occurs in Netarts Bay, Oregon only by virtue of an intensive planting program (the only bay in Oregon where the Japanese oyster drill *Ceratostoma inornatum* is also established).

The Asian clam *Theora lubrica* and the Atlantic mussel *Geukensia demissa* occur disjunctly in San Francisco Bay and again in southern California bays. The abundant and widespread freshwater clam *Corbicula fluminea* appears occasionally in estuarine situations in Oregon and California. The tropical Atlantic shipworm *Teredo bartschi* has been introduced to at least 2 sites in western Mexico, and is probably more widespread than these records indicate.

Of the 30 introduced species on the Pacific coast, then, only 12 are relatively widespread. These are the gastropods *Crepidula fornicata, Crepidula plana, Batillaria attramentaria, Urosalpinx cinerea, Ceratostoma inornatum,* and *Ovatella myosotis*, and the bivalves *Mytilus galloprovincialis, Musculista senhousia, Venerupis philippinarum, Myra arenaria, Teredo navalis,* and *Lyrodus pedicellatus*.

TABLE 1.

Introduced marine and estuarine mollusks of North America (exclusive of opisthobranch gastropods). *Common names* after Turgeon 1988; (*) species listed without common name in Turgeon 1988; (+) species not listed in Turgeon 1988.

Species	NATIVE TO/Introduced To (date of collection)/MECHANISM (M) (see keys, below)	References and Remarks
GASTROPODA: Prosobranchia		
Trochidae		
Clanculus ater Pilsbry, 1911 (+topsnail)	NW PACIFIC/NE Pacific: BC: Queen Charlotte Sound (1964). M: BW?	Clarke, 1972. Not reported since 1964; status not known.
Pomatiopsidae		
Cecina manchurica A. Adams, 1861 (+Manchurian cecina)	NW PACIFIC/NE Pacific: BC (date?); WA: Whatcom Co. (1961); Willapa Bay (1963). M: COI	Morrison, 1963a, Duggan, 1963, Carlton, 1979a, Kozloff and Price, 1987:210. High intertidal, common, co-occurring with *Ovatella myosotis*, found by digging down inside piles of old oyster shells in damp, rich organic debris (Willapa Bay, 1977, JTC), a microhabitat similar to the one in its native Japan (Davis, 1979:117). Also at base of salt marsh plant *Salicornia*.
Littorinidae		
Littorina littorea (Linnaeus, 1758) (common periwinkle)	NE ATLANTIC/NW Atlantic: (<1840) Canada to VA/NW ATLANTIC/NE Pacific: see remarks. M: Atlantic: SB or IR; Pacific: DA	Carlton, 1982, Carlton et al. 1982, Vermeij, 1982a,b, Lubchenco, 1978, 1983, 1986, Brenchley, 1982, Brenchley and Carlton, 1983, Kemp and Bertness, 1984, Bertness, 1984, Blackstone, 1986, Yamada and Mansour, 1987, Petraitis, 1989. Became extinct in North America in precontact times; reestablished through either intentional release (for food) or accidentally with ballast rocks. Collected in San Francisco Bay in 1968–1970 and again in 1977 (Carlton, 1969, 1979a), but not found since despite sporadic searches throughout the bay (JTC, personal observations). Now one of the most predominant mollusks of the Atlantic rocky shore, and in some regions the marshes and mudflats, from Newfoundland to New Jersey.
Tectarius muricatus (Linnaeus, 1758) (beaded periwinkle)	NW ATLANTIC/Mexico: Gulf of California (1986, 1988). M: ?	Bishop, 1992, Chaney, 1992. No records since 1988.
Truncatellidae		
Truncatella subcylindrica (Linnaeus, 1767) (+)	NE ATLANTIC/NW Atlantic: RI: Newport (1880). M: SB?	Burch (1962) is the most recent to repeat this early record of Verrill (1880), who found this species to be common; it has not been collected since.
Potamididae		
Batillaria attramentaria (Sowerby, 1855) (=*Batillaria zonalis* auctt.) (Japanese false cerith)	NW PACIFIC/NE Pacific: BC (1959) to WA (1920s), but not Grays Harbor or Willapa Bay; CA: Tomales Bay (1941); Monterey Bay: Elkhorn Slough (1951). M: COI	Hanna, 1966, MacDonald, 1969a, 1969b, Whitlatch, 1974, Carlton, 1979a, Whitlatch and Obrebski, 1980, Yamada, 1982. Abundant locally on mudflats.
Hipponicidae		
Sabia conica (Schumacher, 1817) (*hoofsnail)	NW PACIFIC/NE Pacific: BC: Queen Charlotte Sound: Table Island (1940); Vancouver Island (1963). M: BW?	Cowan, 1974, Carlton, 1979a, Kay, 1979. Current status not known.
Calyptraeidae		
Crepidula convexa Say, 1822 (convex slippersnail)	NW ATLANTIC/NE Pacific: BC: Boundary Bay (R. Forsyth, personal communication, 1991); CA: San Francisco Bay (1898); M: COI	Hanna, 1966, Carlton and Roth, 1975, Carlton, 1979a. Very common on snail shells on mudflats along shores of San Francisco Bay.

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TABLE 1.

continued

Species	NATIVE TO/Introduced To (date of collection)/MECHANISM (M) (see keys, below)	References and Remarks
Crepidula fornicata (Linnaeus, 1758) (common Atlantic slippersnail)	NW ATLANTIC/NE Pacific: WA: Puget Sound (1905?); Grays Harbor (1970s); Willapa Bay (1937); CA: Humboldt Bay (S. Larned, collector, 1989); Tomales Bay?; San Francisco Bay (1898). M: COI	Hanna, 1966, Carlton, 1979a
Crepidula plana Say, 1822 (eastern white slippersnail)	NW ATLANTIC/NE Pacific: WA?: Puget Sound?; Willapa Bay (1937); CA: San Francisco Bay (1901). M: COI	Carlton, 1979a, Wicksten, 1978 (as *Crepidula perforans*)
Muricidae		
Ceratostoma inornatum (Recluz, 1851) (=*Ocenebra japonica* (Dunker, 1860)) (+Japanese oyster drill)	NW PACIFIC/NE PACIFIC: BC (1931); WA: south to Puget Sound (1924); Willapa Bay (present populations since 1960s?); OR: Netarts Bay (1930–34); CA: Tomales Bay (1941); Morro Bay?; M: COI.	Chew, 1960, Hanna, 1966, Squire, 1973, Radwin and D'Attilio, 1976, Carlton, 1979a. Locally common on oyster beds in the Pacific Northwest.
Urosalpinx cinerea (Say, 1822) (Atlantic oyster drill)	NW ATLANTIC/NE Pacific (1890 and later years): BC: Boundary Bay; WA: Puget Sound and Willapa Bay; CA: Humboldt, San Francisco, Tomales, and Newport Bays. M: COI	Carlton, 1979a; populations last reported in Humboldt Bay in 1950 are still present (S. Larned, collector, 1989). Locally common on oysters and rocks.
Melongenidae		
Busycotypus canaliculatus (Linnaeus, 1758) (channeled whelk) Nassariidae	NW ATLANTIC/NE Pacific: CA: San Francisco Bay (1938). M: COI?	Stohler, 1962, Carlton, 1979a (who reviews evidence for retention of 1938 date).
Nassariidae		
Ilyanassa obsoleta (Say, 1822) (=*Nassarius obsoletus*) (eastern mudsnail)	NW ATLANTIC/NE Pacific: BC: Boundary Bay (1952); WA: Willapa Bay (1945); CA: San Francisco Bay (1907). M: COI	Hanna, 1966, Carlton, 1979a, Race, 1982 Astronomically abundant in San Francisco Bay.
Nassarius fraterculus (Dunker, 1860) (Japanese nassa)	NW PACIFIC/NE Pacific: BC: Boundary Bay (1959); WA: Puget Sound region (1960). M: COI	Hanna, 1966, Carlton, 1979a, Cernohorsky, 1984:184–185
Pulmonata		
Melampodidae		
Ovatella myosotis (Draparnaud, 1801) (=*Phytia setifer* (Cooper, 1872)) (*European ovatella)	NE ATLANTIC/NW Atlantic: Nova Scotia to West Indies; Bermuda; NW ATLANTIC/NE Pacific: BC: Boundary Bay (1965) to Mexico: Scammons Lagoon (1972). M: Atlantic: SB; Pacific: COI	Stimpson, 1851, Morrison, 1963a, Abbott, 1974, Carlton, 1979a, Berman and Carlton, 1991. Earliest Pacific coast record is 1871 (San Francisco Bay); earliest record on Atlantic coast is 1841 (Massachusetts). Very common in high salt marsh and drift habitats.
Siphonariidae		
Siphonaria pectinata (Linnaeus, 1758) (striped falselimpet)	MEDITERRANEAN?/NW Atlantic (19th century or earlier): FL to Mexico, Caribbean Cuba, and northern South America. M: S	Morrison, 1963b, 1972. Morrison believed this species to be introduced from the Mediterranean on ships R. T. Abbott (personal communication, 1990) concurs. G. Vermeij (personal communication, 1990) questions this conclusion based on habitat and broad Western Atlantic distribution. Cryptogenic (see text).
BIVALVIA		
Mytilidae		
Mytilus galloprovincialis Lamarck, 1819 (=*M. edulis* auctt). (+Mediterranean mussel)	MEDITERRANEAN/NE Pacific: Northern CA (date?) to southern CA (1880s?), Mexico M: S	McDonald and Koehn, 1988, Koehn, 1991, Seed, 1992. Late twentieth century distribution probably enhanced by ballast water transport as well as ship fouling. An abundant fouling mussel.

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TABLE 1.

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Species	NATIVE TO/Introduced To (date of collection)/MECHANISM (M) (see keys, below)	References and Remarks
Musculista senhousia (Benson, 1842)	NW PACIFIC/NE Pacific: BC: Boundary Bay (R. Forsyth, personal communication, 1991); Puget Sound (1959); northern CA: Bodega Harbor (1971) to Elkhorn Slough (1965), earliest record for Pacific coast is 1941 (San Francisco Bay); southern CA: Newport Bay (1977) to San Diego Bay (1976); Mexico: Papilote Bay, south of Ensenada (1970). M: Pacific NW, northern CA: COI; southern CA-Mexico: BW?	Hanna, 1966, Morton, 1974, Carlton, 1979a. Abundant locally in dense mats over soft bottoms.
Geukensia demissa (Dillwyn, 1817) (ribbed mussel)	NW ATLANTIC/NE Pacific: CA: San Francisco Bay (1894), southern CA: Alamitos (1957), Anaheim (1972) and Newport (1940) Bays, Bolsa Chica Lagoon, Orange Co. (M. Wicksten, personal communication, 1979). M: San Francisco Bay: COI; southern California: S?/COI?	Hanna, 1966, Carlton, 1979a, Sarver et al., 1992. Juvenile *Geukensia* occur in fouling on ships, suggesting a mechanism for intracoastal transport from San Francisco Bay to southern California. Abundant in marshes, mudflats, and at bases of retaining walls in San Francisco Bay.
Perna perna (Linnaeus, 1758) (+edible brown mussel)	EASTERN SOUTH AMERICA/Gulf of Mexico: TX: Port Aransas (1990) to Port Mansfield (1991). M: BW/S	Hicks and Tunnell, 1993. Also recorded from Namibia to Mozambique (Kennelly, 1969).
Mytella charruana (d'Orbigny, 1846) (+, charru mussel)	EASTERN SOUTH AMERICA/NW Atlantic: FL: Jacksonville (1986). M: BW?/S?	Lee, 1986. Appeared briefly in large numbers in seawater intake of power plant in 1986, but disappeared by 1987 (H. Lee, personal communication, 1992). Perhaps released in ballast water of oil tankers from Venezuela.
Pectinidae		
Patinopecten yessoensis (Jay, 1856) (+Japanese sea scallop)	NW PACIFIC/NE Pacific: BC (1984–85), see remarks. M: IR	Raised in open sea aquaculture operations in BC (T. Carey, personal communication, 1990), but naturally reproducing populations not reported as of 1992.
Anomiidae		
Anomia chinensis Philippi, 1849 (=*Anomia lischkei* Dautzenberg and Fischer, 1907) (+Chinese jingle)	NW PACIFIC/NE Pacific: WA: Samish Bay (1924), Willapa Bay (1952); OR: Tillamook Bay (<1970s). M: COI	Carlton, 1979a. Current status not known. May be established (Hanna, 1966, Abbott, 1974), although Bernard (1983) believed otherwise.
Ostreidae		
Crassostrea gigas (Thunberg, 1793) (Pacific oyster)	NW PACIFIC/NE Pacific: Cultured from AK to Mexico; well established in BC, WA, sporadically reproducing south to CA: Tomales Bay. NW Atlantic: Sporadic plantings along Atlantic and Gulf coasts since 1930s. No established populations reported as of 1992, despite reported unauthorized private plantings of 1000s of bushels in Chesapeake Bay about 1988–90. M: IR.	Pacific: Galstoff, 1932, Barrett, 1963, Hanna, 1966, Quayle, 1969, Carlton, 1979a, Bourne, 1979, Chew, 1979, Ketchen et al. 1983, Foster, 1991:41. Atlantic: Galtsoff, 1932, Nelson, 1946; Turner, 1949, 1950, Mann, 1979, Mann et al. 1991. Experimental introductions in 1875 in WA (Barrett, 1963:48–49) were followed by regular attempts throughout the Pacific Northwest starting in 1902; CA plantings began in 1928.
Crassostrea virginica (Gmelin, 1791) (eastern oyster)	NW ATLANTIC/NE Pacific: BC: Boundary Bay only (since 1917–1918). Population in Willapa Bay WA is now extinct (K. Sayce, personal communication, 1990)	Elsey, 1933, Barrett, 1963, Hanna, 1966, Carlton, 1979a, Bourne, 1979. Plantings began in 1869–1870 in San Francisco Bay with completion of Transcontinental Railroad, and continued along entire Pacific coast in subsequent years.

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TABLE 1.

continued

Species	NATIVE TO/Introduced To (date of collection)/MECHANISM (M) (see keys, below)	References and Remarks
Ostrea edulis Linnaeus, 1758 (edible oyster)	NE ATLANTIC/NW Atlantic: ME (1949) and RI (1991). NE Pacific: See remarks. M: Maine: IR; Rhode Island: ?	Loosanoff, 1962, Welch, 1966, Hidu and Lavoie, 1991. May be established in bays and harbors of Rhode Island (J. D. Karlsson, collector, 1991). Raised in aquaculture facilities on the Pacific coast, but not known to be naturally established (rare natural settlement has occurred in Tomales Bay CA (Davis and Calabrese, 1969)). Raised along NW Atlantic coast with small natural sets north to Halifax County, Nova Scotia (M. Helm, personal communication, 1990).
Mactriade		
Rangia cuneata (Sowerby, 1831) (Atlantic rangia)	GULF OF MEXICO/NW Atlantic: FL east coast to Chesapeake Bay (1955); NY: Hudson River (1988, C. Letts, collector). M: to Chesapeake Bay: COI?/BW?, to Hudson River: BW	Hopkins and Andrews, 1970. Newly established in lower Hudson River perhaps due to release as larvae in ballast water from Atlantic or Gulf coasts
Tellinidae		
Macoma "balthica" (Linnaeus, 1758) (Baltic macoma)	NW ATLANTIC/NE Pacific: San Francisco Bay. M: COI	Meehan et al. 1989. The genetic similarity of San Francisco Bay populations to NW Atlantic populations (as opposed to specimens from Europe or further north on the Pacific coast) suggest that the San Francisco *M. "balthica"* were probably introduced in the 19th century. Very common.
Semelidae		
Theora lubrica Gould, 1861 (Asian semele)	NW PACIFIC/NE Pacific: CA: Los Angeles Harbor, Anaheim Bay, Newport Bay (earliest southern CA record, 1968); San Francisco Bay (1982). M: BW	Seapy, 1974, Carlton et al. 1990. It is of interest to note the increase of this species in 1978–79 in polluted environments in the Inland Sea of Japan (Sanukida et al. 1981), the source of much ballast water carried to the NW Pacific, and its appearance in the early 1980s in San Francisco Bay. Intracoastal movement to San Francisco Bay from southern CA is also possible.
Dreissenidae		
Dreissena polymorpha (Pallas, 1771) (+ zebra mussel)	NE ATLANTIC/NW Atlantic: estuarine populations in NY: Hudson River (summer 1992, up to 5/oo, W. Walton, personal communication, 1992). M: from Europe to the Great Lakes (1988), BW; within North America: see Carlton, 1992b	Griffiths et al. 1991, Strayer, 1991, Hebert et al. 1991, Carlton, 1992b, Nalepa and Schloesser, 1992. Ballast water in coastal vessels and ballast, bilge, or incidental water in small sailing vessels could transport zebra mussels between estuaries along the Atlantic coast. Usually in low densities in brackish water (W. Walton, personal communication, 1992).
Mytilopsis leucophaeata (Conrad, 1831) (dark falsemussel)	NW ATLANTIC-GULF OF MEXICO/NW Atlantic: NY: Hudson River (1937); MA: no locality (Marelli and Gray, 1985:118), perhaps Boston: Charles River? M: S/BW	Rehder, 1937, Jacobson, 1953. Specimens are believed to have been collected from the lower Charles River, near Boston (R. T. Abbott, personal communication, 1990; R. Turner, personal communication, 1992). Native (?) from Chesapeake Bay south.

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TABLE 1.

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Species	NATIVE TO/Introduced To (date of collection)/MECHANISM (M) (see keys, below)	References and Remarks
Trapeziidae		
Trapezium liratum (Reeve, 1843) (+Japanese trapezium)	NW PACIFIC/NE Pacific: BC: Ladysmith Harbor (1949?); WA: Willapa Bay? (1947?). M: COI	Carlton, 1979a. Populations are present in BC (R. Forsyth, personal communication, 1991). Status in WA not known. Never established in CA; report in Abbott (1974) of appearance "prior to 1935" based upon interceptions in Pacific oyster shipments. Nestling in fouling communities.
Corbiculidae		
Corbicula fluminea (Muller, 1774) (=*C. manilensis* auctt.) (Asian clam)	NW PACIFIC/NE Pacific: estuarine populations in OR: Siuslaw River; CA: Smith River, San Francisco Bay; NORTH AMERICA/NW Atlantic: estuarine populations in Chesapeake Bay: James River. Freshwater populations throughout the United States, northern Mexico. M: from Asia to N. America (1920s–1930s), IR; within North America: see Counts, 1986	Counts, 1986, 1991; estuarine populations: Diaz, 1974, Carlton, 1979a, Nichols et al. 1990, Counts, 1991:105. Abundant locally, but in lower densities in brackish water.
Veneridae		
Venerupis philippinarum (A. Adams and Reeve, 1850) (=*Tapes semidecussata* Reeve, 1864; = *T. japonica* Deshayes, 1853; also placed in subgenus *Ruditapes*). (Japanese littleneck)	NW PACIFIC/NE Pacific: BC (1936) to CA: Monterey Bay: Elkhorn Slough (1949). OR: Netarts Bay (see remarks). M: COI except for OR: IR	Fisher-Piette and Metivier, 1971 (specific taxonomy and synonymy), Bourne, 1982, Anderson et al. 1982, Bernard, 1983, Ketchen et al. 1983. Generic placement follows E. Coan and P. Scott (personal communication, 1992). Intentional plantings in OR: Netarts Bay sporadically from 1960s–1980s resulted in a naturally reproducing population (Gaumer and Farthing, 1990); also planted in other OR bays, where specimens should be expected. Common to abundant in coarser sediments.
Gemma gemma (Totten, 1834) (amethyst gemclam)	NW ATLANTIC/NE Pacific: CA: Bodega Harbor (1974) to Elkhorn Slough (1965); earliest record 1893, San Francisco Bay. M: COI	Carlton, 1979a. Records from north of Bodega or south of Monterey Bay are based upon misidentifications. Abundant in soft sediments.
Mercenaria mercenaria (Linnaeus, 1758) (northern quahog)	NW ATLANTIC/NE Pacific: CA: Alamitos Bay (1967). M: IR	Crane et al. 1975, Murphy, 1985a, 1985b. The only established population on the Pacific coast of this common Atlantic species is in this small CA bay. Hertz and Hertz (1992) report a single live specimen from Mission Bay, San Diego, probably from discarded bait or food.
Petricolidae		
Petricola pholadiformis (Lamarck, 1818 (false angelwing)	NW ATLANTIC/NE Pacific: WA: Willapa Bay (1943); CA: San Francisco Bay (1927), Newport Bay (1972). M: COI	Hanna, 1966, Carlton, 1979a. In higher shore hard shale, clay, mud substrates.
Myidae		
Mya arenaria Linnaeus, 1758 (softshell)	NW ATLANTIC/NE Pacific: AK (1946) to Monterey Bay: Elkhorn Slough (<1911). M: COI	Carlton, 1979a, Bernard, 1979. Became extinct on Pacific coast from southern AK south in late Tertiary; reestablished (earliest record 1874, San Francisco Bay) through accidental introduction with Atlantic oysters. Now one of the most common upper bay clams from WA to San Francisco Bay.

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TABLE 1.

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Species	NATIVE TO/Introduced To (date of collection)/MECHANISM (M) (see keys, below)	References and Remarks
Corbulidae		
Potamocorbula amurensis (Schrenck, 1861) (+ Amur river corbula)	NW PACIFIC/NE Pacific: CA: San Francisco Bay (1986). M: BW	Carlton et al. 1990, Nichols et al. 1990. In densities of tens of thousands per square meter in estuarine reaches of San Francisco; to be expected in other CA bays through intracoastal transport of larvae in ballast water.
Teredinidae		
Lyrodus pedicellatus (de Quatrefages, 1849) (=*Teredo diegensis* Bartsch, 1927) (blacktip shipworm)	INDO-PACIFIC?/NE Pacific: CA: San Francisco Bay (1920); Monterey Bay (1935); Santa Barbara to San Diego Bay (earliest southern CA record 1871). M: S	Kofoid and Miller, 1927, Turner, 1966, Ecklebarger and Reish, 1972, Carlton, 1979a
Lyrodus takanoshimensis Roch, 1929 (+)	NW PACIFIC/NE Pacific: BC: Ladysmith Harbor (1981). M: COI (in wooden oyster boxes)	Popham 1983.
Teredo bartschi W. Clapp, 1923 (Bartsch shipworm)	NW ATLANTIC/NW Atlantic: NJ: Barnegat Bay (1974), CT: Long Island Sound: Waterford (1975); NE Pacific: Gulf of California: La Paz (<1971); Mexico: Sinalao (1978–79). M: S	NW Atlantic: Hoagland and Turner, 1980, Hoagland, 1981, 1986, Richards et al. 1984. Gulf of California: R. Turner in Keen, 1971:282, Hendrickx, 1980. Reported by Abbott (1974) as introduced to CA, a record based upon specimens from San Diego Bay in the 1920s (Kofoid and Miller, 1927). May no longer be present in Barnegat Bay in thermal effluents, but still established in Long Island Sound heated power plant effluents at Millstone.
Teredo navalis Linnaeus, 1758 (naval shipworm)	NE ATLANTIC?/NE Pacific: BC: Pendrell Sound (1963); WA: Willapa Bay (1957); OR: Coos Bay (1988); CA: San Francisco Bay (1913); southern CA? NW ATLANTIC: see remarks. M: S	Turner, 1966, Carlton, 1979a. Coos Bay record: JTC, field records. Cryptogenic in NW Atlantic: early American records include reports both from visting vessels (Russell, 1839, MA) and from established populations (DeKay, 1843, NY). Grave (1928) enigmatically noted, "The date of its first appearance in [Woods Hole] is not known," noting records as early as 1871. If introduced, it may have arrived centuries ago with visits of earliest European vessels.
Teredo furcifera von Martens in Semon, 1894 (+)	NW ATLANTIC (Caribbean north to FL)/NW Atlantic: NJ Barnegat Bay (1974). M: S	Hoagland and Turner, 1980, Richards et al., 1984. Probably only temporarily established in Barnegat Bay in thermal effluents of power plant (K. E. Hoagland, personal communication, 1992) and may no longer be present there. Turner (1966) records an earlier nonestablished population in NC.
Laternulidae		
Laternula limicola (Reeve, 1863) (=*L. japonica* auctt.) (+)	NW PACIFIC/NE Pacific: OR: Coos Bay (1963). M: BW	Keen, 1969. Not recorded in Coos Bay since 1965, and not re-discovered there despite intensive searching from 1986–1989 (JTC and students, field records).

Mechanisms of introduction

S	= Ships (fouling and boring)
SB	= Ships (solid ballast: rocks, sand)
BW	= Ships (ballast water)
COI	= Fisheries: Accidental release with commercial oyster industry
IR	= Fisheries: Intentional release
DA	Fisheries: Accidental release with discarded algae (seaweed) in shellfish packing

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TABLE 1.

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Species	NATIVE TO/Introduced To (date of collection)/MECHANISM (M) (see keys, below)	References and Remarks

Regions (as used here:)
Northwest (NW) Pacific = Asia: China, Japan, Korea
Northeast (NE) Pacific = Pacific coast of North America: Alaska to Mexico
Northwest (NW) Atlantic = Atlantic coast of North America: Canada to Florida
Northeast (NE) Atlantic = Europe: northern and western

AK	Alaska	NJ	New Jersey
BC	British Columbia	NW	Northwest
CA	California	NY	New York
CT	Connecticut	OR	Oregon
FL	Florida	RI	Rhode Island
MA	Massachusetts	TX	Texas
ME	Maine	VA	Virginia
NC	North Carolina	WA	Washington
NE	Northeast		

It is of interest to note that 19 (63%) of the 30 species occur in San Francisco Bay. Only the embayments of the Pacific Northwest approach this number of established species, with Willapa Bay having 12 species, Puget Sound 11 species, and Boundary Bay 13 species. These numbers will increase with further exploration (for example, *Trapezium liratum, Crepidula convexa* and *Crepidula plana* should be expected more widely than now reported in Washington and British Columbia) and with new introductions.

Four of the introduced mollusks on the Atlantic coast are from Europe and 3 (as noted above) are southern species now established in northern localities. Only 2 species are widespread, the European periwinkle *Littorina littorea,* and the Gulf of Mexico clam *Rangia cuneata.* The European oyster *Ostrea edulis,* long restricted to Maine, now occurs in Rhode Island as well, although the means of introduction of this population (whether by transport from Maine as a ship-fouling organisms, or by intentional release, or by escape from aquaculture facilities) is not yet known. The shipworm *Teredo bartschi* occurs within the thermal plume of a nuclear power plant in Long Island Sound; the status of the population of this species, and of another southern teredinid, in New Jersey is not clear. Estuarine populations of 2 typically freshwater bivalves, the Asian clam *Corbicula fluminea* and the European zebra mussel *Dreissena polymorpha,* are known from limited locations.

The sole clearly introduced marine mollusk in the Gulf of Mexico, *Perna perna,* is from South America. Were it not for this recent report, there would be no certain records of introduced mollusks in the Gulf fauna.

Regional Patterns of Mechanisms of Introduction

The human-mediated dispersal mechanisms that have led to the introduction of non-indigenous mollusks to North American coasts have played strikingly different regional roles (Table 4). Far exceeding all other mechanisms in terms of number of species successfully transported and introduced is the now largely historical movement of the Atlantic oyster *Crassostrea virginica* and the Pacific (Japanese) oyster *Crassostrea gigas* to the bays and estuaries of the Pacific coast of North America from the 1870s to the 1930s, and from the 1900s to the 1970s, respectively (Table 1). Atlantic oyster importation ceased due to lack of breeding success and because of competition with the increasing importation and culture of the Pacific oyster. Pacific oyster importations stopped after sufficient natural sets and regional aquaculture operations were able to supply adequate amounts of seed.

These industries led to the introduction of at least 22 mollusks to the Pacific coast (Table 4: the 20 species shown for COI plus the 2 species of oysters); 9 are from Japan and 13 are from the Atlantic. Intentional fishery releases added another 2 species (the Asian clam *Corbicula fluminea* and the Atlantic quahog *Mercenaria mercenaria,* which curiously did not become established through the oyster industry) to the Pacific coast fauna.

Prior to these industries and releases, only a few species of mollusks had been transported to or within North America. The earliest introduction may have been the cryptogenic shipworm *Teredo navalis* to the New England coast. The European snail *Littorina littorea,* prehistorically present in the northwestern Atlantic, was returned to North America before 1840 either intentionally (released by European settlers in eastern Canada to establish a periwinkle fishery) or accidentally (with ballast stones). A late 18th century—early 19th century introduction to the Atlantic coast with ballast stones may have been the European marsh snail *Ovatella myosotis* (subsequently then transported with oysters to the Pacific coast). On the Pacific coast, mid-19th to early 20th century ship-mediated introductions included the shipworms *Teredo navalis* and *Lyrodus pedicellatus,* as well as the Mediterranean mussel *Mytilus galloprovincialis,* whose introduced status was long overlooked in California due to its previous identification as the "native" *Mytilus edulis.*

Ballast water has played a small role in terms of the numbers of introduced species, although at least 2 of the species introduced by this means are ecologically and/or economically significant invasions. For a number of species, the role of ballast water as a mechanism is submerged among a number of other mechanisms that are not easily distinguished from each other. Thus, ballast water or ship fouling may have led to the 20th century movement of the North American native dreissenid *Mytilopsis leucophaeata* to the Hudson River. Either mechanism may also have played a role in the appearances of the South American bivalves *Mytella*

TABLE 2.

Introduced marine and estuarine mollusks of North America: Established and other species arranged by donor region. *Regions:* See Table 1, footnote.

	Donor Region	Receiver Region
ESTABLISHED		
Cecina manchurica	NW Pacific	NE Pacific
Batillaria attramentaria	NW Pacific	NE Pacific
Ceratostoma inornatum	NW Pacific	NE Pacific
Nassarius fraterculus	NW Pacific	NE Pacific
Musculista senhousia	NW Pacific	NE Pacific
Crassostrea gigas	NW Pacific	NE Pacific
Theora lubrica	NW Pacific	NE Pacific
Trapezium liratum	NW Pacific	NE Pacific
Corbicula fluminea	NW Pacific	NE Pacific
Venerupis philippinarum	N America	NW Atlantic
Potamocorbula amurensis	NW Pacific	NE Pacific
Lyrodus takanoshimensis	NW Pacific	NE Pacific
Lyrodus pedicellatus	NW Pacific	NE Pacific
Littorina littorea	Indo-Pacific?	NE Pacific
Ovatella myosotis	NE Atlantic	NW Atlantic
Ostrea edulis	NE Atlantic	NW Atlantic
Dreissena polymorpha	NE Atlantic	NW Atlantic
Mytilus galloprovincialis	NE Atlantic	NW Atlantic
Teredo navalis	Mediterranean	NE Pacific
Crepidula convexa	NE Atlantic?	NE Pacific
Crepidula fornicata	NW Atlantic	NE Pacific
Crepidula plana	NW Atlantic	NE Pacific
Urosalpinx cinerea	NW Atlantic	NE Pacific
Busycotypus canaliculatus	NW Atlantic	NE Pacific
Ilyanassa obsoleta	NW Atlantic	NE Pacific
Ovatella myosotis	NW Atlantic	NE Pacific
Geukensia demissa	NW Atlantic	NE Pacific
Crassostrea virginica	NW Atlantic	NE Pacific
Macoma "balthica"	NW Atlantic	NE Pacific
Gemma gemma	NW Atlantic	NE Pacific
Mercenaria mercenaria	NW Atlantic	NE Pacific
Petricola pholadiformis	NW Atlantic	NE Pacific
Mya arenaria	NW Atlantic	NE Pacific
Perna perna	South America	Gulf of Mexico
Rangia cuneata	Gulf of Mexico	NW Atlantic
Teredo bartschi	NW Atlantic	CT: Long Island Sound
Mytilopsis leucophaeata	NW Atlantic	NE Pacific
	NW Atlantic	NY: Hudson River
ESTABLISHMENT NOT CERTAIN		
Clanculus ater	NW Pacific	NE Pacific
Sabia conica	NW Pacific	NE Pacific
Anomia chinensis	NW Pacific	NE Pacific
Teredo furcifera	NW Atlantic	NJ: Barnegat Bay
NOT ESTABLISHED		
Littorina littorea	NW Atlantic	NE Pacific
Ostrea edulis	NE Atlantic	NE Pacific
Tectarius muricatus	NW Atlantic	NE Pacific
Truncatella subcylindrica	NE Atlantic	NW Atlantic
Mytella charruana	South America	NW Atlantic
Patinopecten yessoensis	NW Pacific	NE Pacific
Laternula limicola	NW Pacific	NE Pacific
CRYPTOGENIC		
Siphonaria pectinata	Mediterranean?	NW Atlantic?
Teredo navalis	NE Atlantic?	NW Atlantic?

charruana in Florida and *Perna perna* in Texas. Ballast water or the movement of commercial oysters may have transported the clam *Rangia cuneata* from the Gulf of Mexico to Chesapeake Bay, from where it may have spread down the coast to Florida, and from where it may have been carried in ballast water to the Hudson River.

On the California coast, a complex mixture of ballast water, ship fouling, or the movements of shellfish may have led to the

TABLE 3.

Summary of introduced marine and estuarine mollusks (excluding opisthobranchs) of North America.

	Established	Establishment Not Certain	Not Established	Cryptogenic
To Pacific coast (Northeast Pacific) from:				
Northwest Pacific	12	3	2	
Indo-Pacific?	1			
Northwest Atlantic	15		2	
Northeast Atlantic?	1			
Northeast Atlantic			1	
Mediterranean	1			
Subtotal	30	3	5	
To Atlantic coast (Northwest Atlantic) from:				
Northeast Atlantic	4		1	1?
Gulf of Mexico	1			
Northwest Atlantic	2	1		
South America			1	
North America	1			
Subtotal	8	1	2	1
To Gulf of Mexico from:				
Mediterranean				1?
South America	1			
Subtotal	1			1
Total	39(*)	4	7	2

(*) Total of 36 species; *Ovatella, Corbicula,* and *Teredo bartschi* are each scored twice (see Table 2), because they originate from different donor regions depending upon the recipient regions.

transportation of the Atlantic mussel *Geukensia demissa* from central California to southern California and of the Japanese mussel *Musculista* senhousia from the northern Pacific coast to southern California. Superimposed upon these potential intracoastal mechanisms and routes is the probability that Asian mollusks have been introduced more than once to the Pacific coast; early introductions of the mussel *Musculista* are linked to the commercial Pacific oyster industry, while its appearance in the 1970s in southern California may be due to ballast water release directly from Asian ports. Similarly, the Asian clam *Theora lubrica* may have been introduced in separate incidents from Asia to both central and southern California; nearly 15 years separate its initial discovery in southern California bays (to where it was probably introduced in the ballast water of ships returning from Indonesia and southeast Asia during the Vietnam War) from its later discovery in San Francisco Bay. The latter invasion may be linked (Table 1, remarks) to an increase in *Theora's* population in regions which now supply large amounts of ballast water to the Bay.

In contrast to these complex dispersal histories, 2 bivalves have appeared in North America whose introduction is clearly linked to ballast water release. These are the Asian corbulid clam *Potamocorbula amurensis* and the Eurasian zebra mussel *Dreissena polymorpha*. *Potamocorbula* established large populations in San Francisco Bay in the 1980s (Carlton et al. 1990, Nichols et al. 1990), at the same time *Dreissena* was establishing large populations in the Great Lakes (Griffiths et al. 1991). *Dreissena* is included here by virtue of its spread into brackish (oligohaline) waters (Table 1). A second species of *Dreissena* (May and Marsden 1992), whose specific name remains unclear, also introduced by ballast water into the Great Lakes, has not appeared (as of November 1992) in estuarine environments in North America.

DISCUSSION

Regional Patterns and Mechanisms of Introduction

The striking differences between the number of molluscan invasions on the Atlantic, Pacific, and Gulf coasts of North America (Table 3) may be due to a combination of human-mediated dispersal events and regional geological and biological Pleistocene history. The two are difficult to separate.

A global mechanism for the potential introduction of nonindigenous mollusks to all shores is shipping. With the ebb and flow of human colonization and commerce, shipping has had a differential impact upon different regions at different times. Societal changes (the colonization of new lands, the opening and closing of ports due to political changes, the birth of new or the demise of old commodities, regional and world wars) and shipping changes (the replacement of wood with iron ships, increased vessel speed, the development of more effective antifouling paints, the advent of ballast water in the 1880s) have led to new invasions in largely unpredictable manners. Colonization and commercial shipping have occurred on a regular basis between Europe and Atlantic America since the early 17th century (or for about four centuries). While contact between Europe and Pacific America is just as old, regular shipping did not commence until the early 19th century, or about two centuries later (Carlton 1987). Despite this two century dichotomy, shipping does not contribute significantly to the regional differences in invasions between the Atlantic and Pacific coasts (Table 4).

A major mechanistic distinction occurs, however, in the history of commercial oyster movements to the two coastlines. Massive inoculation of the Pacific coast of North America for 60 years between 1870 and the 1930s with millions of tons of living oysters

TABLE 4.

Introduced marine and estuarine mollusks: Mechanisms of introduction of established species (M) in parentheses indicates one of two possible transport mechanisms; see key, Table 1 footnote.

	To:		
	Atlantic Coast	**Pacific Coast**	**Gulf Coast**
MECHANISM			
Shipping:			
Fouling/Boring	*Mytilopsis* (BW)	*Mytilus*	*Perna* (BW)
	Teredo	*Geukensia* (COI)	
		Lyrodus pedicellatus	
		Teredo (2 spp.)	
Shipping:			
Solid Ballast	*Littorina* (IR)		
	Ovatella		
Shipping:			
Water Ballast	*Rangia* (**)	*Theora*	*Perna* (S)
	Mytilopsis (S)	*Potamocorbula*	
	Dreissena (*)	*Musculista* (COI)	
Commercial Oyster	*Rangia* (**)	*Cecina*	
Industry		*Batillaria*	
		Crepidula (3 spp.)	
		Ceratostoma	
		Urosalpinx	
		Busycotypus	
		Ilyanassa	
		Nassarius	
		Ovatella	
		Geukensia (S)	
		Musculista (BW)	
		Macoma	
		Trapezium	
		Venerupis	
		Gemma	
		Petricola	
		Mya	
		Lyrodus takanoshimensis	
Intentional	*Littorina* (SB)	*Crassostrea* (2 spp.)	
Release	*Ostrea*	*Venerupis* (Oregon)	
	Corbicula (**)	*Mercenaria*	
		Corbicula (***)	

* *Dreissena* was transported to North America in ballast water from Europe (Carlton, 1992b), but its occurrence in the oligohaline zone of the lower Hudson River is probably due to natural transport as larvae or as juveniles on floating materials from the upper River basin.

** *Rangia* may owe its reappearance on the Atlantic coast in Holocene times either to the transportation of oysters from the Gulf of Mexico to Chesapeake Bay or to its transportation as larvae in ballast water from the Gulf. Ballast water is the probable mechanism of its recent introduction to the oligohaline portions of the Hudson River. Genetic analyses would be of interest to establish whether the Hudson River population originates from the Atlantic coast (such as Chesapeake Bay) or the Gulf coast, if indeed these potential parental populations are genetically distinct.

*** *Corbicula* was probably transported and released intentionally in Western North America no later than the 1920s–1930s (perhaps in more than one incident); subsequent dispersal from western to eastern America has been both through anthropogenic means (the use of the clam as bait, for example), and by natural dispersal along water corridors.

from Japan and from the Atlantic coast led to the simultaneous unintentional inoculation of scores if not hundreds of species of associated protists, invertebrates, algae, seagrasses, and perhaps fish. No such introductions of exotic oysters on this scale occurred on the Atlantic coast of North America.

As a result, 27 species of Asian and Atlantic mollusks have become established on Pacific shores. The bays and estuaries of the Pacific coast where these species are established are geologically young (recently flooded, $<10{,}000$ years old) and do not have a diverse native biota, suggesting that these systems were relatively susceptible to invasion (Carlton 1975, Carlton 1979b, Nichols and Thompson 1985). Only one introduced species, the Mediterranean mussel *Mytilus galloprovincialis,* occurs in open coast, high energy environments on the Pacific coast; all remaining species are restricted to bays and estuaries. While the extraordinarily diverse molluscan fauna of these open coast rocky shores may thus, in turn, resist invasion, few human-mediated mechanisms serve to transport rocky shores species, and it may be that few if any non-indigenous species from comparable habitats around the world been released into these communities. Thus, on the Pacific coast, there was an apparently coincidental combination of biotically depauperate regions subjected to invasions by a transport mechanism that served to bring species appropriate to those habitats from other regions of the world.

It is of interest to note that in a parallel sense the most significant molluscan invasion of the Atlantic shore also occurred in a geologically young (recently deglaciated, <10,000 years old), biotically depauperate environment. The European periwinkle *Littorina littorea* invaded hard and some soft bottom intertidal communities of the Atlantic coast in the presence of relatively few native herbivorous or omnivorous gastropods. Why, however, other western European rocky shore gastropods failed to colonize American Atlantic shores during centuries of intensive shipping is not clear. It may be that European populations of the common periwinkle *Littorina saxatilis* have been mixed in with aboriginal populations and thus gone undetected. However, it is clear that a variety of other small to medium size European snails (such as trochids and patellid limpets) either were not introduced or were not successful. Here again transport mechanisms may have been rare, with little solid (rock) ballast originating from these habitats (which may suggest that ballast rocks may not have been the means of introduction of *Littorina littorea* to America).

The near absence of recorded introduced mollusks in the Gulf of Mexico may be linked, as with the Atlantic coast, to the absence of large scale importations of commercial oysters or other shellfish from other regions. Pre-ballast water shipping contributed few or no clear introductions, although a detailed biogeographic analysis of the shipworms of the Gulf of Mexico would be of interest. The recent appearances of the South American fouling bivalves *Mytella* and *Perna* in Florida and Texas may suggest that the global increase in ballast water-mediated invasions (Carlton 1985, 1987) may be an active mechanism that will add to the non-indigenous mollusks of the Gulf. The movement of the zebra mussel *Dreissena polymorpha* down the Mississippi River and its arrival (perhaps by 1993) in the oligohaline waters of that delta will add a second species to the list of Gulf marine and estuarine invasions.

Ecological Impacts

With the exception of a few species, there is little experimental elucidation of the ecological impact of the introduced marine mollusks in North America. Carlton (1979b) reviews general ecological considerations, including a remarkable, albeit anecdotal, early account of the interactions between the introduced Atlantic marsh mussel *Geukensia demissa* and the California clapper rail. Nichols and Thompson (1985) document the persistence of an "introduced mudflat community" in San Francisco Bay, where all of the mollusks are introduced (*Macoma "balthica,"* indicated as native in their paper, was later shown to be a probable introduction to the Bay (Meehan et al. 1989)).

Remaining largely uninvestigated is the alteration of benthic community dynamics by the abundant introduced bivalves on the Pacific coast, such as *Mytilus galloprovincialis, Geukensia demissa, Musculista senhousia, Mya arenaria, Crassostrea virginica, Venerupis philippinarum,* and *Gemma gemma*. All of these species can occur in great densities. Certain community-level interactions for some of these species (such as *Geukensia, Mya,* and *Gemma*) are known in their donor regions, but are applied with difficulty to the Pacific coast where different suites of potentially interacting species occur. Only the most recent bivalve introduction, the Asian clam *Potamocorbula amurensis,* has been the subject of intensive observational studies relative to its rapid predominance in certain parts of San Francisco Bay, reaching densities of >10,000 per square meter at sites where the former biota has become rare or absent (Nichols et al. 1990). *Potamocorbula* thus joins *Mya, Musculista,* and *Gemma* as species potentially critically important in regulating phytoplankton dynamics in the Bay (Carlton et al. 1990).

On the Pacific coast and Atlantic coasts, interactions between several pairs of native and introduced gastropods have been examined. Interactions between the introduced European periwinkle *Littorina littorea* and native gastropods on the Atlantic coast have been studied by a number of workers. In experimental studies, Petraitis (1989) found that *Littorina littorea* negatively affected the growth of the native limpet *Tectura testudinalis*. Yamada and Mansour (1987) also experimentally demonstrated that *Littorina littorea* can depress the growth rate of the native rocky shore snail *Littorina saxatilis*. Brenchley (1982) documented that *Littorina littorea* was the most abundant consumer of eggs of the native mudsnail *Ilyanassa obsoleta* in mid-intertidal habitats on the Atlantic coast. Brenchley and Carlton (1983) further demonstrated that there has been a historical change in the distribution of *Ilyanassa* due to competitive exclusion by *Littorina littorea,* with microhabitat displacement in the mid intertidal zone of 70% of *Ilyanassa,* calculated from littorinid removal experiments. *Littorina* also limits both the upper and lower distribution of *Ilyanassa*.

On the other hand, Race (1982) found that the Atlantic *Ilyanassa obsoleta,* introduced to San Francisco Bay, in turn limits the distribution of the native mudsnail *Cerithidea californica,* by means of competitive interactions and by predation on *Cerithidea*'s egg capsules. Whitlatch and Obrebski (1980) found that while the introduced Japanese snail *Batillaria* and the native Pacific coast snail *Cerithidea* can be sympatric in Tomales Bay, CA, similar-sized individuals exclude each other when feeding on the same size diatoms.

Berman and Carlton (1991) examined the potential interactions between the introduced Atlantic marsh snail *Ovatella myosotis* and the native Pacific coast marsh snails *Assiminea californica* and *Littorina subrotundata*. No observational or experimental evidence of competitive superiority by *Ovatella* could be found, and they concluded that the establishment of the introduced species in high shore, semiterrestrial environments did not arise at the expense of the native species.

While the introduced freshwater bivalves *Corbicula fluminea* and *Dreissena polymorpha* have had and are having profound impacts on the communities in which they have invaded (references in Table 1), ecological interactions of these species in brackish water remain largely uninvestigated.

Perhaps no introduced marine mollusk in North America has had a greater impact than the periwinkle *Littorina littorea,* which colonized most of the Atlantic coast from Nova Scotia to New Jersey in only 30 years, between 1860 and 1890 (references in Table 1). Perhaps because little or no economic impact has been associated with this invasion, it has attracted relatively little notice globally as a classic example of an invasion, aquatic or terrestrial. *Littorina* has fundamentally altered the distribution and abundance of algae on rocky shores (references in Table 1), altered hard-bottom, soft-bottom, and salt marsh habitat dynamics (Bertness 1984) negatively interacted with native gastropods (reviewed above), dramatically altered the hermit crab shell resource (providing an abundant larger shell) and modified shell utilization and preference patterns of the native hermit crab *Pagurus longicarpus* (Blackstone 1986), and as grazing herbivores and vacuuming omnivores, may have important impacts on a wide variety of small invertebrates, such as barnacles, whose newly settled larvae are consumed in large numbers (see "Life Habit" review in Brenchley and Carlton 1983).

In summary, all but the snail *Ovatella* of the abundant species

of introduced mollusks that have been studied have been shown to have dramatic impacts on the pre-existing structure of the communities in which they have invaded. These results would suggest that the extensive populations of those species not yet studied may also have had, or are having, substantial impacts on population dynamics and interactions among co-occurring species, both native and introduced. Numerous fruitful investigations remain to be undertaken.

Future Invasions

Predictions of what species will invade, and where and when invasions will occur, remain one of the more elusive aspects of biological invasion science (Mooney and Drake 1986; Drake et al. 1989). Thousands of species of marine and estuarine mollusks that occur in Europe, Africa, South America, Asia, and Australia overlap in basic environmental requirements with habitats that occur in North America. Selecting probable invasion candidates from this vast fauna, and predicting competitive, predatory, or other interactions with previously established molluscan species or ecological equivalents as potential mediators of successful establishment, is a frustrating task. It is doubtful, for example, if an examination of the Asian biota would have identified the clam *Potamocorbula amurensis,* among a background of scores of other estuarine taxa, as a high profile potential invader.

Nevertheless certain limited projections may be made. The New Zealand fresh and brackish water snail *Potamopyrgus antipodarum,* established in western Europe, and occurring in densities of up to 800,000 snails per square meter, is a probable future invader of eastern North American fresh and oligohaline habitats (JTC, C. L. Secor, and E. L. Mills, in preparation). Abundant fouling bivalves in India and Asia, such as the mussels *Modiolus striatulus* and *Limnoperna fortunei* (Morton 1977), may yet reach North America. If large scale inoculations of the Pacific oyster *Crassostrea gigas* on the Atlantic coast commence in the 1990s (as opposed to the many smaller previous releases), successful establishment may take place (presumably the species will be raised on the Atlantic coast from larvae or clean seed, and the introduction of associated organisms with large stocks of adult oysters will not take place).

Also predictable are the eventual detection of natural sets of the Japanese sea scallop *Patinopecten yessoensis* in British Columbia, the spreading of the European edible oyster *Ostrea edulis* from Rhode Island south and west into Long Island Sound, the establishment of the periwinkle *Littorina littorea* in San Francisco Bay if not elsewhere on the Pacific coast, the establishment of the New Zealand green lipped mussel *Perna canaliculus* (Carlton 1992a: 16) in California (to where it is now imported daily in large numbers for direct human consumption) and the spreading of the Asian clam *Potamocorbula amurensis* from San Francisco Bay to other bays on the Pacific coast.

Broadly, the recent appearances of *Rangia cuneata* in the Hudson River, of *Perna perna* in Texas, of two species of the zebra mussel *Dreissena* in the Great Lakes and thus much of the rest of North America, and of *Potamocorbula amurensis* in San Francisco Bay, argue strongly that future, ballast-water mediated invasions will continue to be a regular phenomenon in North America. On any day, perhaps any hour, it is likely that the larvae of dozens of species of mollusks are released into coastal waters of North America by ballast water. Similarly, steadily increasing local, national, and global pressures to expand mariculture industries through the importation of new candidate species will almost certainly mean the accidental (or intentional) release of novel species.

These predictions arise from the projection that the basic mechanisms of human-mediated transport of non-native species outlined at the beginning of this paper will remain in place for many years to come. This forecast is despite the existence of a number of international guidelines (including those of the International Council for the Exploration of the Sea, Carlton, 1989) that exist to prevent the release of detrimental species through fisheries and mariculture activities, and despite growing international awareness of the role of ballast water in transporting exotic species transoceanically and interoceanically. While our inability to always distinguish between certain mechanisms of introduction of exotic species may make full control difficult, identifying and quantifying the role of such mechanisms, followed by cooperative management efforts, are the necessary precursors to eventually modifying the rate of "chess play" of new invasions.

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INTRODUCTIONS AND TRANSFERS OF MOLLUSCS: RISK CONSIDERATIONS AND IMPLICATIONS

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Writings on ecological biogeography provide a global historical perspective for presentations to be given at this symposium (Ekman 1953, Briggs 1974, Vermeij 1978, Pielou 1979, Cox and Moore 1985, Mooney and Drake 1986). In the course of geologic epochs, floral and faunal populations of the world have become naturally distributed into generally defined geographic areas whose boundaries have expanded or retreated over the centuries. In quite recent geologic history, however, humans have been altering this pattern critically, wittingly and sometimes unwittingly, manipulating artificially the redistribution of many species populations. Molluscs have been no exception. These introductions and transfers have occurred, sometimes beneficially, more often in muddled uncontrolled ways, and occasionally with "disastrous backlash consequences" to the receiving communities (Odum 1971, Rosenfield and Kern 1979, Mooney and Drake 1986). Elton is quoted as writing ". . . about this spate of invasions . . . make no mistake: we are seeing one of the great historical convulsions in the world's fauna and flora" (Dobson and May 1986)!

In this overview I introduce the symposium, consider the significance of artificial dispersal of marine molluscs, and whether the reportedly worrisome problems of these invasions are exaggerated or real. Speakers in the symposium will no doubt set me straight, and bring us all up-to-date on the problems, advantages, and safety practices related to human-directed introductions and transfers of commercial and potentially commercial marine molluscs.

That dispersal has been occurring with increasing intensity, is confirmed by many biological surveys. Results of these show that marine molluscan biota, especially commercial estuarine and coastal populations, continue to be moved about widely (for example, Korringa 1942, Allen 1953, Carriker 1955, Hanna 1966, Ansell 1968, Mann 1979, Counts 1983). Large scale global intermingling, fueled by an increasing commercial market for edible molluscs, will undoubtedly accelerate its pace.

But why the flap over the fact that several molluscan species populations are becoming geographically homogeneous? Why not adopt the noninterference attitude of "let nature take its course"? Some would suggest that, anyway, little can be done about the problem, and besides some invasions can be beneficial. Take for example, the case of the early, little-controlled importation of *Crassostrea gigas* to the West Coast of the United States, which reaped a valuable commercial industry, a recreational fishery, and a seed-producing operation (Bourne 1979, Chew 1979).

But alas! because an introduction has been profitable in one venture does not guarantee that others will be also. Courtney and Robins (1989) put it this way: "What is happening is at best a lottery in which an occasional lucky or even well thought-out success is replayed, only to result in losses in the form of noncorrectable environmental mistakes of varying severity."

If invasions do constitute a gamble, we should next explore the consequences of uncontrolled introductions and transfers. Three major sequels come to mind: a) a wide spectrum of other organisms can piggyback on or in the invaders, b) potential genetic changes can occur in both invaders and residents, and c) physical alteration of the invaded habitat can result (Sindermann 1970, 1977, Vermeij 1978, Bourne 1979, Rosenfield and Kern 1979, Courtney and Taylor 1986, Ward 1986, Fisher 1988). Let's consider these consequences in more detail:

a) Organisms carried on, or within invaders, for example, could include:

- Disease microorganisms (viruses, bacteria, fungi, yeasts, sporozoans, ciliates, dinoflagellates),
- Multicellular parasites (copepods, trematodes, cestodes, odostomid snails, pinnotherid crabs),
- Predators, especially their larvae and young (muricid and naticid snails, conchs, octopuses, crabs),
- Competitors contending for food and space (barnacles, bryozoans, sea squirts, chitons, limpets, other commercial bivalves).

In this lengthy list, disease microorganisms and parasites with a single host and with direct waterborne transmission and short generation times, are potentially the most pernicious in cultivated molluscan populations (Sindermann 1970, 1977, Dobson and May 1986). The likelihood of introductions of disease microorganisms is very high (Fisher 1988); and because checks and balances in the new habitat are rarely the same as in the original environment, invading microorganisms are less apt to be restrained. Because of their long association with, and natural immunity to their hosts, pathogens carried by invaders can have deleterious effects on unprotected resident species. Unquestionably, diseases will continue a significant problem in mariculture; the limiting factor in their control is the meager knowledge available about them (Sindermann 1970). Nonetheless, Sindermann (1970) is optimistic about their eventual control. Little, also, is known about multicellular parasites: how they become established in new hosts remains essentially unexplored (Fisher 1988).

b) The genetic consequences of introductions and transfers of molluscan species can be examined instructively with reference to how readily they will hybridize. In this context, closely related invading and native species will produce hybrids differing in fitness from that of the natives. If survival and reproduction of these hybrids is greater than that of the natives, it is probable that invaders carry genes, which in combination with natives genes, are advantageous. The rapid introgression of favorable genes will likely decrease the distinctiveness of the native species—with unpredictable consequences. If fitness of hybrids is inferior to that of the natives, then introgression of alien genes will probably decrease the fitness of hybrids in the short term; whether reduced fitness persists, will be determined by whether or not it is eliminated by natural selection. As to whether distantly related species will hybridize, is probably not possible to predict. If they should,

little genetic interaction can be expected. It is thus quite clear that it is possible to assess only short term genetic interactions between invaders and natives; those occurring after acclimation of invaders to the invaded habitat are not foreseeable (S. Allen, personal communication). It is also evident that monitoring of these hybrid species, if they occur, is difficult—if not impossible (Andrews 1979, Newkirk 1979, Courtney and Taylor 1986, Regal 1986, Pimentel et al. 1989, Tiedje et al. 1989).

c) A third consequence of uncontrolled molluscan invasions could include alteration of the ecosystem by invaders as well as by their genetically modified descendents. Changes could take place in the physical structure of the habitat, redistribution of populations, or trophic interactions, resulting in a modified ecological balance not necessarily commercially beneficial. Unfortunately, it is not yet possible accurately to predict the ecological impact of molluscan invaders (Courtney and Taylor 1986, Pimentel et al. 1989).

Indisputably, then, intentional and accidental spreading of molluscan species about the rim of the world-ocean can be dangerously risky. But why some species are extremely successful invaders, while close relatives may not be (Ehrlich 1986), and some habitats are colonized while others are not, is still a puzzle (Cox and Moore 1985). Ekman (1953) observed almost four decades ago that organisms become distributed in conformity with their genetic nature, which is adapted to specific environmental conditions.

It follows, consequently, that successful geographic dispersal is the product of an interaction between physiological properties of the organism and the quality of the environment. A case in point is estuarine species, which though broadly tolerant to a widely fluctuating complex of ecological factors (Hedgpeth 1957, Carriker 1967), only rarely invade oceanic habitats; and conversely, oceanic species seldom successfully move into sharp estuarine gradients. On the other hand, successful invasion by estuarine species into other brackish waters, especially at similar latitudes does occur—not only undesignedly on bottoms of ships and in their holds, but also through intentional human ventures (Allen 1953). Natural barriers to dispersal are also imposed by latitudinal thermal zones along coasts, as well as by differences in aerial exposure on intertidal-subtidal reaches. Human enterprises, ironically enough, have aided the insidious, highly successful spread of some species by inadvertently making available ecologically "open" habitats (Mooney et al. 1986); deplorable examples are the catastrophic invasion of human-made waterways by Asian clams of the genus *Corbicula* (Counts 1983, Mooney et al. 1986), and the devastating infestation of the Great Lakes by the zebra mussel *Dreissena polymorpha* (Garton and Haag 1989) called "an ecological disaster of oil-spill proportions" (D. Israelson, Toronto Star, Canada, March 12, 1990). Most recently word has come (Williams 1990) that there are at least three projects in the greater Caribbean region raising Pacific giant clams (*Tridacna* sp.); whether these molluscs have been properly screened for potential pathogens has yet to be determined.

Can biological (morphological, physiological, reproductive, genetic, behavioral, etc.) characteristics of successful invaders be identified with any degree of reliability? Probably not. Nonetheless, Ehrlich (1986) has come up with the following possible attributes of potentially successful invaders: abundance in the native habitat, polyphagous, short reproductive cycles, high genetic variability, fertilized females able to colonize alone, larger in size than most relatives, associated with *Homo sapiens*, and able to function well in a wide range of physical-chemical environmental factors. At this stage in the advancement of biology, identification of even a few of these attributes would not be easy, if indeed possible. Hence, it is no surprise that prediction with certainty of successful invasions is not yet within our grasp (Mooney and Drake 1986).

In view of the serious risks of introductions and transfers, concerned biologists and managers in many countries have been developing strict policies and procedures to control them. The latest revision of guidelines for control encompasses a worldwide program. The guidelines are summarized in the ICES "Codes of practice and manual of procedures for consideration of introductions and transfers of marine and freshwater organisms" (Turner 1988). A section on molluscs is included. A rigorous procedure for limiting risks of introductions of shellfish diseases has also been prepared by Sindermann (1970, 1977), who cautions that even with safeguards a disease in the enzootic phase could escape detection.

As might be anticipated, not all aspects of the "Codes" are acceptable to everyone. Some sections are controversial, others are difficult to implement, and some aspects of control have not been addressed. With references to the latter, Mann (1979) noted that the document deals almost exclusively with the limiting of adverse biological effects of introductions, and does not speak to supportive socioeconomic and political pressures that may favor introductions. As he emphasizes, the guidelines should be implemented in a practical way and in a realistic time scale, or they will be ignored. Notwithstanding its deficiencies, the "Codes" is an important guide and must continue to evolve and fine-tune to international needs a) as new knowledge on invading species, their diseases, parasites, predators, and competitors becomes available, and b) as the "Codes" program is more widely adopted and tested across international boundaries.

The biological characteristics of many marine molluscs, especially bivalves and gastropods, simplify the arduous task of control of introductions and transfers. For one thing, although they create the same range of inherent ecologic, pathologic, and genetic problems as other organisms, most commercial adult bivalves and gastropods are capable of no, or only localized movement on their own; thus risks attending their handling can be controlled more effectively than those of more motile species (Turner 1988). For another, many species of molluscs can now be raised in hatcheries to the F2 and F3 generation, shelled species can be disinfected upon arrival at their destination, fertilized eggs can be disinfected before shipment, and hatchery-raised shelled pediveligers can be transported for setting in tanks near planting grounds. It goes without saying, that all steps in introductions and transfers should be computer-recorded so that original sources and history of movements can be traced readily.

As already stated, attention on introductions and transfers has been focused primarily on the biological aspects, and little on the socioeconomic and political considerations (Mann 1979). Several writers have touched on the latter; some of their thoughts follow: Managers, when considering the introduction of a foreign species, should seriously question why a local native species would not be commercially adequate (Courtney and Robins 1989). Foreign species should probably be considered only if there is a demonstrated scientific need or a high potential for commercial success (Mann 1979, Rosenfield and Kern 1979). Approval of introductions should be based only on biological decisions—not on management or political mandates alone. Federal and state agencies should support, more than now done, research on the biology of potential introductions, making available a biological base for management

and control (Courtney and Robins 1989). Coastal universities should be urged to expand basic interdisciplinary graduate training on commercial and potentially commercial species (including such subjects as culture, nutrition, behavior, physiological pollution, ecology, genetics, microbiology, parasitology, and predation) (Regal 1986, Fisher 1988, Courtney and Robins 1989, Tiedje et al. 1989). Integrated resource management, which incudes a multidisciplinary, integrated approach at all involved levels of government and industry (Tiedje et al. 1989), not only enhances multiple uses of resources, but also reduces sociopolitical conflicts (Cairns 1988). This approach, it should be noted, finds immediate application in the chaotic zebra-mussel dilemma in the Great Lakes.

Persons knowledgeable in the subject of introductions and transfers suggest that requests for them should be examined with extreme care by a single national body (perhaps an interjurisdictional and interagency council with peer reviews) to insure, insofar as possible, that exotic species will be beneficial (Bourne 1979, Courtney and Taylor 1986, Fisher 1988, Turner 1988, Courtney and Robins 1989).

In a provocative suggestion, Cairns (1988) points out that inasmuch as employment of rigorous procedures in control of introductions and transfers would avoid exceedingly expensive litigation problems that could result from movements of these organisms, funds thus freed could be redirected to constructive research, training, and control activities. The idea merits discussion, but its implementation might be difficult!

With reference to my opening question in this overview, I answer that the intrusive problems of invasions are unequivocally real and challenging. Nevertheless, I close optimistically, and affirm that through international goodwill and by creative cooperation (Wooster 1969) the frustrating, complex problem of human-coupled movements of molluscan populations can be controlled, and done so beneficially and minimally disruptively: biologically, socioeconomically, and politically . . . an appropriate goal for proponents of controlled malacological zoogeography!

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ECONOMICS OF MOLLUSCAN INTRODUCTIONS AND TRANSFERS: THE CHESAPEAKE BAY DILEMMA

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INTRODUCTION

The major reason for introduction or transfer of molluscan species is economic gain. As Mann (1979) states, the economic incentive increases when an existing fishery becomes depleted or devastated due to overfishing, degradation of environmental quality, or disease. Also, even if there is no existing native fishery, great demand for a product may provide enough economic incentive for an introduction. Whether the introduction is intended to benefit a public or private fishery, the public sector's role is paramount in the decision to allow or disallow introductions. Economists have two interrelated roles in the public decision process regarding molluscan introductions. First, estimates of the net benefits (benefits minus costs) to the various groups affected by the introduction should be provided. This will involve estimating the net benefits to harvesters, processors and consumers but also might include benefits and costs external to these groups. An example is where introduction of filter feeders provides benefits of improved water quality (Newell 1988). The economists' role does not end at the provision of benefit-cost information, but includes, interpreting this information within the context of policy setting. This is particularly important in that exotic introductions have many uncertainties surrounding the benefits and costs of the action.

Our paper discusses both roles in the context of potential molluscan introductions and transfers. To illustrate, we use the potential introduction of *Crassostrea gigas* into the Chesapeake Bay to replace the devastated native *Crassostrea virginica*. To place the event in context, recent events in the Maryland oyster industry are reviewed. A review of molluscan introductions is then presented to provide a qualitative range of benefits and risks likely to be encountered in the Chesapeake Bay. Brief descriptions are provided of the effects of molluscan introductions into North America and other parts of the world. Although we made every attempt to document the market value of these introductions, these statistics are hard to come by, particularly when harvests are small relative to indigenous populations of fish and shellfish. These descriptions are followed by a theoretical discussion of measuring costs, benefits and associated risks of the contemplated introduction.

THE MARYLAND EXPERIENCE

Several useful and insightful histories of the Maryland oyster industry have been offered over the years (e.g. Power 1970, Wennersten 1978). The most recent and comprehensive is the Kennedy and Breisch (1983) work in which the dichotomy of the politics surrounding the oyster management and the science of oyster propagation is explained. The results of the mismanagement can be seen in the precipitous drop in Maryland oyster production between the late 1800's and late 1920's (Fig. 1). Landings apparently stabilized over the next 30 years to 1950. Although there are lessons to be learned from the early period, we focus on the post-war events.

In order to understand the current situation, the period from 1950 through 1989 is divided, from the point of view of landings, into an apparently stable period (1950–1980) and a declining period (1981–1989).

The oyster seasons from 1950[1] through 1981, while giving the impression of an unusually stable period, contains a major structural change. The 1950 season began the period with harvests of 2.16 million bushels and the 1981 season completed the period with landings of 2.10 million bushels (see Fig. 2). However, Maryland's oyster harvest declined by 50% from 1950 to 1962 and then exhibited an extraordinary revival. The resurgence in Maryland (1962–1981) is represented by an increase in production from 1.24 million bushels in 1962 to a period high of 3.01 million bushels in 1966. Events in the Maryland industry are best understood if we consider the complete East Coast oyster market. In 1950, there were three East Coast areas each with production in excess of 2 million bushels: the Mid-Atlantic (New York, New Jersey and Delaware), Maryland and Virginia. Possibly as a result of eutrophication, production from New York's waters dropped dramatically between 1950 and 1954. The decline in the Mid-Atlantic was exacerbated when MSX invaded the water of Delaware Bay in 1957. The effect of MSX on oysters is well-known, inflicting mortalities in adult oysters in the range of 50–90% (Haskins and Andrews, 1988). Total production in the Mid-Atlantic dropped from nearly three million bushels to 0.2 million during the decade. There was a corresponding drop in nominal

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[1]The oyster season is referred to in terms of the year in which it began. Thus, the oyster season lasting from September of 1980 to March of 1981 is denoted as the 1980 oyster season.

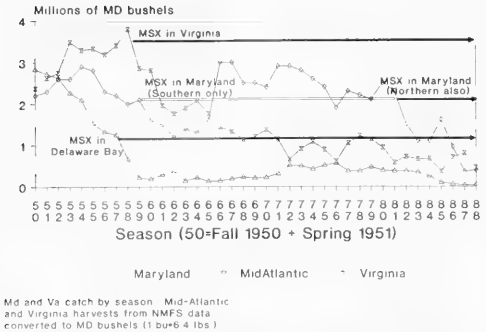

Figure 1. East coast oyster landings by region, 1950–1988.

value from $9.6 million in 1950 to $1.3 million in 1959. Production has remained at that magnitude until recently.

It is instructive to observe the effects in Maryland and Virginia from the decreases in Mid-Atlantic harvests. Ex-vessel prices rose nearly 15% in Maryland and 10% in Virginia. In response, the Chesapeake harvest in 1954 rose by nearly 50%. Maryland, which relies primarily on harvest from public grounds, had a spurt in production for approximately three years (1954–57), followed by a gradual decline in the harvest. At this point in time, the decision was implicitly made by the state not to increase expenditures to expand the industry. Budgetary constraints both at the state and private harvester level prevented it.

Virginia's production, on the other hand, was principally from grounds leased by private interests. The increase in price signalled greater profits to the private growers and they increased purchases of seed from Virginia's vast seed beds on the James River. Production rose from under three million bushels in 1950–1952 to around 3.5 million after 1954. The peak occurred in the 1959 season when nearly four million bushels were harvested in Virginia.

Whether the private growers and Virginia's seed resources could have sustained this production into the future became a moot point when MSX began to affect Virginia production around 1960. By 1964, Virginia production was one-half of the 1959 peak harvest. Growing areas in the southern portion of the Chesapeake Bay were devastated because, to a degree, the disease is confined to the higher salinity areas of the Bay. However, some sections of Maryland's Tangier Sound, Pocomoke and Fishing Bay were also affected.

Maryland was fortunate, however, as the disease did not move further north in Chesapeake Bay until much later, a fact which permitted actions which temporarily reversed the declining harvest. The key to the reversal was oyster shell. The nature of oyster reproduction is such that young larvae require a hard substrate on which to attach. The oyster shell provides such a substrate. However if the harvested shell is not replaced in the Bay by a suitable substrate, there is a strong likelihood that the future availability of oysters will be reduced. This relationship was recognized long ago and the Maryland legislature in 1927 passed a law providing funds for state shell-planting activity (Kennedy and Breisch). That legislation also required processors to make 10% of their shucked shell available for purchase by the state. These efforts were at least partially responsible for the upswing in oyster production from around 2 million bushels in the 1928–29 season to over 3 million a decade later. As the years passed, however, it became more expensive to use the shucked shell for the repletion program.

However, the discovery of pre-historic fossil shell sources and the development of a dredge to extract it provided a cheap[2] alternative to freshly shucked shell and fueled the resurgence observed in the 1962 to 1967 period. The use of inexpensive dredged shell momentarily changed the philosophy of oyster management from trying to sustain a collapsing industry to a philosophy of revitalizing a potentially valuable industry. In the process, the fundamentals of oyster production also changed. No longer would the watermen be solely dependent on the "recycling" of processed oyster shell, they would have a partial reprieve from the constraints of nature. Assuring a strong market with high prices was the new focus of attention.

In 1960, Maryland devoted substantial resources to the use of dredged shell for repletion of beds and enhancement of oyster production. There were 1.2 million bushels of fresh shell planted and 3.3 million bushels of dredged shell planted in that year. By 1966, fresh shell plantings had fallen to .5 million bushels whereas dredged shell plantings had risen to nearly 6 million bushels.

The results of the increased enhancement activity on Maryland's production are evident in Figure 2. In the period from 1960–1966, Maryland oyster production doubled, from around 1.5 million bushels to around 3 million, and nominal value increased from $7 million to $13 million. While the production stayed high through the 1967 season, it began to wane in the late 1960's and continued the trend throughout the 1970's.

Despite the trend, Maryland oyster production remained over 2 million bushels until 1981. The increase in importance of the repletion program relative to natural set transformed the oyster fishery from traditional natural resource gathering into a "put-and-take" state fishery. The constraining feature was no longer the natural reproduction but rather a belief that the market could not absorb, at an acceptable price, more than about 2.5 million bushels. The repletion program used this level of harvest as a target for its programs.

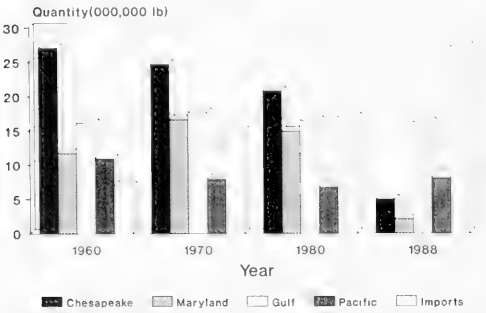

Figure 2. Oyster production and imports by region and selected years.

[2]Reasons for the shift relate both to costs of acquiring the shell and the relative productivity of the two types of shell. Although cost/bushel data for fresh shell does not extend back to 1960, there are records in 1970 showing that the cost of dredged shell was about $.15/bushel whereas the cost of fresh shell was around $.25/bushel. It has also been shown that a bushel of dredged shell has potentially greater effect on future oyster production than a bushel of fresh shell (Cabraal, 1978).

There was a reason for concern over the price in this period. The real price[3] obtained by Maryland watermen was greatest in the 1962 season when both Maryland and Virginia harvests were low. The near doubling of Maryland production in the 1966 season caused real prices to drop by nearly 20% in the short-run and by about 40% in the longer run. The lowest real price received by Maryland watermen occurred in the 1974 season.

The importance of the period is in the change in the role of the state in "managing" the industry. At the beginning of the period, the role was primarily to make it difficult for the industry to deplete the natural oyster beds. The discovery of an inexpensive alternative to provide seed created a different role for the State. The choice was made to increase production, rather than build up natural beds. Rather than being regulators, the State became the source of growth. However, the production was constrained by the market—production was not to surpass 2.5 million bushels.

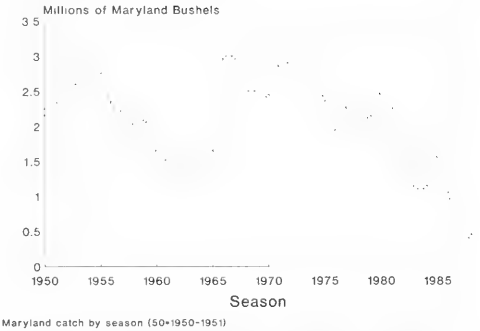

Figure 3. Maryland oyster harvest, by season, 1950–1988.

Diseased Waters 1981–1988

The dominant factor in the Maryland oyster industry after 1980 was the reappearance of the disease MSX and greater outbreaks of Dermo (*Perkinsus marinus*). Unlike the previous invasion in the 1960's which was limited to Maryland's portion of Tangier Sound, this invasion affected most of Maryland's major oyster bars from 1981–1983. There was a brief reprieve in disease-related mortality in 1984 and 1985, but a return of MSX in previously infected areas and an expansion into more areas followed in 1986–1988.

The trend in oyster harvests during the period parallels the course of MSX infection. Harvest declined from over 2.5 million bushels during the 1980–1981 season to just over a million bushels in the 1983 season. In the next two seasons, the catch increased to almost 1.6 million bushels. This brief revival did not last and production fell to around 0.4 million bushels in the 1987 and 1988 seasons.

The more than doubling of the real ex-vessel price of oysters from the 1980 ($8 per bushel) season to the 1987 ($20 per bushel) season did not offset the effect on watermen income of the decline in oyster harvest. As a result, gross revenues fell from over $20 million dollars to less than $8 million. The higher prices, however, did act to keep the level of effort (as measured in mandays) relatively constant even though the landings were declining. The number of individuals commercially harvesting and selling oysters declined by around 40%. The low resource abundance had the effect of removing most of the part-time fishermen and raising the level of effort of full-time fishermen.

The sporadic nature of the MSX infection made it difficult to develop a comprehensive strategy for the oyster repletion program. The amount and location of shell and seed plantings depended more on availability of seed and the location of disease than on any other factor. Initially during the period, seed oyster plantings closely followed the index of spat set in the previous year. With the 1983 and 1984 season being particularly poor for spatfall, seed plantings in 1984 and 1985 were extremely small even though they included several year classes of submarket oysters. Seed plantings increased steadily from 1985 and peaked in 1988, assisted by relatively good spat sets during the drought years from 1985 through 1987. In 1989, seed plantings were down 37% from 1988 but were still the second highest of the decade.

The pattern of decline in Maryland oyster landings over the period differs slightly from the pattern observed in other regions[4] (Fig. 3). Over the entire period (1980–1988), Maryland's decline in landings was 82%, while other regions declined only 37%. The Gulf states' oyster production increased to a record 29 million pounds of meats by 1983, but harvests declined continually to 16 million pounds in 1988. Only Pacific oyster production was greater in 1988 than it was in 1982, and that increase was only 600 thousand pounds.

Imports, which are principally low value canned or smoked oysters, increased from 27 million pounds of meats to a record 52 million pounds in 1987. Imports declined to 27.5 million pounds in 1990.

Interestingly, the source of production for the increased imports and West Coast production is the species, *Crassostrea gigas*. The reason is it has not been exposed to the levels of infestation of either MSX or Dermo that occur in Maryland, Virginia, Delaware and New Jersey. Thus, the obvious interest in importing it into Maryland, and Chesapeake Bay waters to test its hardiness against those diseases.

It is within the context of a devastated East Coast oyster industry that the introduction of *C. gigas* is being contemplated. In Maryland, harvesters have turned to an alternative resource, the softshell clam (*Mya arenaria*) for some economic relief. In Virginia harvest and culture of the hardshell clam (*Mercenaria mercenaria*) have helped cushion the impact of a declining oyster industry for some watermen. If *C. gigas* could be introduced, another possible source of income could make the difference between continuing to fish or having to leave fishing altogether.

EFFECTS OF RECENT MOLLUSCAN INTRODUCTIONS

Introductions of many aquatic species have taken place over the centuries. However, many attempts to establish populations have not been reported. The establishment of a reproducing population of the species is, presumably, the goal of an introduction. However, this goal is not always realized. As such, the examples we have are somewhat self-selecting; that is, many of the failures are not documented in the literature because researchers have little to

[3]Real prices are actual prices adjusted for the general level of inflation.

[4]Comparisons are made on a calendar year basis and in meat-weight rather than bushels because the available data are given in these terms.

report. Thus, our examples of introductions are those that have been mostly successful to date. France is the notable exception, the victim of many unfavorable experiences.

British Columbia

More than twelve known exotic species have been accidentally introduced in British Columbia (Quayle 1964). The introductions of *Crassostrea virginica* and *Crassostrea gigas,* however, were intentional

Ostrea lurida (the Olympia oyster) is the sole native oyster of British Columbia, and was the basis of the early oyster industry. Overfishing, and later, competition from introduced species led to its decline as a major fishery (Ketchen et al. 1983). Its diminutive size, slow growth, and high labor cost caused the industry to seek other species for commercial use (Bourne 1979).

Crassostrea virginica was first introduced to British Columbian waters in significant numbers in 1906, although minor introductions had occurred previously. Attempts ceased in 1936 due to limited natural spatfall and high mortality rates (Quayle 1964). Currently only a small population exists in Boundary Bay. Along with *C. virginica, Urosalpinx cinerea* (Eastern oyster drill), and *Nassarius obsoletus* (Eastern mud snail) were accidentally introduced. *U. cinerea* has the potential to cause severe damage to commercial shellfish populations. However, its population is apparently small and declining due to the limit of a suitable environment. *N. obsoletus* is well established in some areas, but apparently has not created any ecological harm.

Mya arenaria was planted in Puget Sound from Willapa Harbor, Washington, and water movement patterns distributed the species northward along the U.S. and British Columbian coasts (Quayle 1964). To date, the British Columbian and U.S. West Coast market for softshell clams has not developed to provide the incentive for significant commercial exploitation of the established *M. arenaria* population. A significant recreational fishery has developed. The lack of high fishing mortality has probably contributed to the successful establishment of the *M. arenaria* population.

Crassostrea gigas has been introduced to British Columbia on numerous occasions. The initial introductions are believed to have taken place in 1912 or 1913. The first significant official introduction occurred in 1926, with oysters from both Japan and the state of Washington (Quayle 1969). Reliable seed sources were a problem at the start of the industry as no local seed was available, and the imported seed had low survival (Im and Langmo 1977). It is too cold in most British Columbian waters for regular breeding, but Pendrall and Hotham Sounds have good breeding conditions, and now serve as the main sources of seed oysters. Imported seed has been completely displaced due to the natural set and the supply of domestic hatchery seed. *C. gigas* now constitutes the entire commercial oyster industry in British Columbia, at a catch of nearly 1800 tons in 1980 (Ketchen et al. 1983). Although the *C. gigas* introduction resulted in numerous accidental imports, as a whole, the introduction is viewed as a success.

Venerupis japonica (Manila clam) was accidently introduced with *C. gigas* (Quayle and Bourne 1972, Bourne 1982, Ketchen et al. 1983, Chew 1989). It is believed to have been included with oyster introductions on more than one occasion, establishing a strong population in the southern areas of the Province. Later intentional plantings in 1962 and 1969 in Northern British Columbia were unsuccessful. The *V. japonica* catch fluctuates greatly, and in good years comprises a significant portion of the local clam fishery. The landings have increased dramatically of late, from 700 metric tons in 1982 to approximately 1400 metric tons in 1989 (Chew 1990). Production figures demonstrate that although a species may require little effort to become established, its long-term success as a fishery requires good management.

Another accidental introduction, *Ceratostoma inornatum* (the Japanese oyster drill) was potentially threatening to the local oyster stocks. Fortunately, its lack of a pelagic larval stage resulted in negligible initial dispersal. When first observed, regulations were immediately enacted to prevent further spread. However, its presence has caused closing of some oyster beds (Quayle 1984). Another introduced oyster drill species, *Purpura clavigera,* does have a pelagic larval stage. Distribution appeared unavoidable, but a prompt, aggressive eradication program removed adults and egg capsules in the initial location area, and no others have been detected (Quayle 1964).

Other organisms accidentally introduced with the Japanese oyster include:

Batillaria zonalis—a somewhat common but innocuous gastropod.

Mytilicola orientalis—a parasitic copepod, also reasonably common in areas. Apparently the copepod does not cause harm to oysters, although it may pose a problem with mussels.

Limnoria tripunctata—the marine wood borer, responsible for significant damage to wood pilings in Southern British Columbia.

Pseudostylochus ostreophagus—a flatworm predator of small oysters, has not posed a serious problem in British Columbia, although it is a problem with oyster spat in Japan.

Sargassum muticum—a seaweed which has not posed any problems except as a nuisance to people using the shores for recreation.

Mikrocytos mackini—the Denman disease, an oyster ailment, was discovered after the introduction of the Japanese oyster. However, there is not conclusive evidence linking it to the Japanese oyster. The industry is said to have "learned to live with it" (Bourne 1979).

As previously noted, the introduction of *C. gigas* into British Columbia is considered successful, even though it was accompanied by accidental imports. *C. gigas* has prospered where the native oyster (*O. lurida*) failed. The accidentally introduced *V. japonica* is viewed as an important resource. Although serious biological and economic damage could have resulted from a few of the other accidental introductions, quick action and good fortune denied them any significance.

U.S. West Coast

California, Oregon, and Washington have followed similar routes in mollusc introductions. *O. lurida* is the native oyster species, currently comprising a small percentage of the commercial oyster catch. *C. virginica* was introduced in the late 1800's to northern California and Washington. As in British Columbia, its establishment as a commercial fishery failed, but its import was responsible for the introduction of *Nassarius obsoletus* and *Mya arenaria* (Quayle 1964). *Nassarius obsoletus,* although abundant, is apparently innocuous, as aforementioned. The success of *M. arenaria,* as in British Columbia, is limited by the lack of a strong market.

Crassostrea gigas was first introduced to the Northwestern states of the U.S. unsuccessfully in 1902 (Chew 1979). The first marginally successful introduction to Washington occurred in

1919. With subsequent introductions, commercial cultivation of *C. gigas* was realized by 1928. As in British Columbia, the *C. gigas* industry of the U.S. west coast at first relied upon seed imported from Japan. For some time, the Washington State Department of Fisheries monitored the concentration of oyster larvae in natural spawning areas (such as Dabob and Willapa Bays) and notified culturers, who then collected the pelagic larvae on cultch for their own leased beds. However, spat was not abundant enough to allow the industry to become independent from Japanese seed. Currently, culturists rely on purchasing larvae from hatcheries to sustain the industry and the dependence upon Japan for seed has lessened (Burrell 1985; Chew, personal communication). *C. gigas* does best in Washington; prolonged periods of relatively high water temperatures may be the reason for its limited success in California (Chew 1979). Regardless of the limitations of the natural set, *C. gigas* is the basis for the oyster industry in both states. Oregon harvests have never been significant. The 1988 commercial catch for the U.S. west coast totaled 7.97 million pounds, of which 6.6 million pounds originated in Washington. The ex-vessel value of the harvest in 1988 was $14.5 million. Although such a harvest may appear impressive, they represent a decline from the record 1946 harvest of 13.4 million pounds of meats. Expansion of the industry has probably been limited by the size of the market, competition from low cost imported oysters, and until recently, the availability of East and Gulf Coast oysters.

Other recognized accidental introductions with *C. gigas* to these states are *O. japonica, V. japonica,* and *P. ostreophagus. C. inornatum,* as in British Columbia, has limited distribution due to its lack of a pelagic larval stage. With regulations, further distribution has been avoided.

Venerupis japonica grows well in these areas, particularly in the slightly warmer waters. Harvests fluctuated somewhat in the early years, possibly due to the erratic nature of reproduction in small populations, but has increased greatly since 1975, and may be stabilizing. The 1980 harvest was second in pounds landed and economic value (at $1.1 million) to *Panopea generosa* (geoduck clam). In Washington, approximately 1.5 million pounds were landed in 1981, while the commercial harvests in California and Oregon are negligible. Ninety-five percent of the commercial catch is from natural set, but culturists are beginning to use hatchery seed on leased beds.

Ostrea edulis was introduced to northern California and Washington beginning in 1951. The oysters originated from the newly developing *O. edulis* population of Maine (Loosanoff 1955). There is no natural spawning in the west coast waters, and although a few hatcheries produce *O. edulis* seed, interest in culturing the European oyster in these areas is limited—the majority of interest remaining with *C. gigas* (Hulbrock, Chew, personal communication). There is no evidence of accidental introductions with the European oyster. There is a protozoan parasite (*Bonamia ostrea*) discovered in 1965, but it may be native to California and Washington waters (Katkansky et al. 1969). It attacks *O. edulis*' immune system, but apparently does not harm *C. gigas* or *C. virginica. O. edulis* populations in Washington, having been exposed to the disease, have been found to harbor the parasite while resisting damage (Elston et al. 1987).

Hawaii

Hawaiian molluscan shellfish introductions are unique in that according to state law, non-native organisms are prohibited from introduction to open waters. Therefore, all culturing of exotic species is done in landbased pond operations (Fassler, personal communication). The practice of purely landbased operations is costly. Consequently, only a smattering of exotic molluscan introductions have ensued. One (now bankrupt) oyster farm cultured *C. gigas, C. virginica,* and *O. edulis,* which all did very well from a biological standpoint. There was a slight problem with the mud worm (*Polydora* sp.), but placing the oyster in warmer water killed the worm. Although achieving biological success, the high costs of the operation precluded the possibility of economic success.

A recently opened oyster farm is anticipating its first harvest this year, 1991 (Archibald, personal communication). At present, only *O. edulis* is cultured for harvest. In order to approximate the oyster's natural habitat, salmon and kelp are also maintained in the ponds. Sea urchins and abalone are present to control the environment in the main kelp growing ponds. To minimize the possibility of introducing disease and other organisms, all eyed larvae are purchased from a single Maine hatchery. Each shipment is then kept in separate growing tanks. The company is optimistic about the economic success of the harvest; for there is promise of great demand for the product. It is now left to the market to determine if the introduction and culture of *O. edulis* in Hawaii under such costly conditions is economically feasible.

Maine

The native oyster of Maine is *C. virginica*. The present stock is sparse, with successful spawnings in only the warmest of years (Lewis, personal communication). As a result, Atlantic oyster production is erratic, yet it remains a significant component of the oyster industry. Reported commercial harvests of *C. virginica* were 2,510 bushels in 1988, and 3,715 bushels in 1989, worth $277 thousand.

Ostrea edulis was introduced in Maine in 1949 (Loosanoff 1955). Although oysters from Holland were brought to Connecticut for research purposes, a few bushels were held in reserve at the Boothbay Harbor Maine Laboratory where spawning occurred. Some spat survived and later reproduced, forming the foundations of a resident population. Thoughts turned to further introduction of the species in order to replace a then-failing softshell clam industry. Later introductions were made at various points along the coast from Boothbay Harbor to Merepoint Bay (Welch 1963). Although *O. edulis* is fairly well distributed, the populations are not very large, with the industry having just attained commercial significance in 1984. The 1988 harvest was 6,346 bushels, and in 1989: 14,435 bushels. No accidental introductions accompanying the *O. edulis* introduction have been discovered. The oyster is harvested from both natural and leased cultivation beds; leased beds utilizing both naturally produced spat and hatchery seed (Lewis, personal communication). The hope is for *O. edulis* to fill a market niche in the domestic market for gourmet oysters, and for possible export to Europe.

France

Ostrea edulis is the sole native oyster species of France. The oyster, considered an important component of French culture, has always been in high demand. The fishery, however, has a volatile history. Two distinct diseases caused production to fall in 1920 and 1950 (Goulletquer undated). In 1968, the protozoan parasite *Marteilia refringens* further reduced stocks. The origin of these

diseases and the protozoan is uncertain. *M. refringens* is limited to estuarine areas, between which it was transferred with the movement of oysters. (Goulletquer undated). In 1979 *Bonamia ostrea,* a protozoan that generates microcell disease, was introduced. The protozoan was introduced with *O. edulis* adults from Washington state, the progeny of infected Californian oyster stocks (Elston et al. 1987, Mann 1983). These oysters were intended to supplement the low French stocks. *B. ostrea* is considered by many the final blow to the industry, causing the flat oyster fishery in Brittany to fall from a harvest of 4,000 tons in 1978 to 2,000 tons in 1987.

Other oysters were introduced over the years to meet the high French demand and later, to compensate for the falling stocks of *O. edulis. Crassostrea angulata* (Portuguese oyster) was introduced in the 1860's without official regulation. The oysters thrived, with harvests reaching 85,000 mt in 1960 (Goulletquer and Héral 1991). However, *C. angulata* experienced high mortalities from an iridovirus damaging the labia and gills in 1964 and 1965 (Farley 1991), and again from 1970 to 1972 from damage to the blood by yet another iridovirus (Grizel and Héral 1991). The latter outbreak was estimated to cost the industry $90 million a year in revenues (Goulletquer and Héral 1991). The present *C. angulata* population is negligible.

Unofficial importations of *C. gigas* began in 1966 because of the oystermen's frustrations with the declines in *O. edulis* and *C. angulata*. Officials, alarmed by an increase in *C. angulata* mortalities, prevented further *C. gigas* introductions until studies in Japan cleared *C. gigas* of any responsibility for the *C. angulata* deaths. Official introductions of *C. gigas* from both Japanese and British Columbian waters ensued in 1971. *C. gigas,* as previously mentioned, is resistant to *Bonamia ostrea*. The resistance of the Pacific oyster to the diseases of the Portuguese oyster as well allowed the expansion of an otherwise failing oyster industry in France (Grizel and Héral 1991). Presently, *C. gigas* is the principal species in the French oyster industry, accounting for 92% of the 1990 landings which were a record 150,000 metric tons valued at $210 million (Goulletquer and Héral 1991). It will not reproduce in the northern waters of France, however, reproducing best in the warmer waters of southern France.

Accidental introductions did occur with the importation of *C. gigas,* although precautions were taken in the official introductions. A few of these species are still present, although in low numbers. These species include: *Balanus amphitrite* and *B. albicostatus, Aiptasia pulchella, Anomia chinensis* (Grizel and Héral). The low numbers render the organisms of relatively little concern to the French, although the significance of any accidental introduction, harmful or not, should not be denied.

Currently, research is underway to seek out other oyster species with a resistance to *B. ostrea*. As mentioned, a strain of *O. edulis* in Washington state was found to carry, but not be highly damaged by the parasite. Also, breeding the immune *C. gigas* at the same time as *O. edulis* reduces the severity of the protozoan in *O. edulis*. Other species that have been studied in labs include *O. chiliensis* from Chile in quarantined system in 1981. Studies were abandoned due to lack of success (Mann 1983). *O. puelchana,* of Argentina, however, appeared insusceptible to the parasite in hatchery lab studies and was subsequently planted in northern Patagonia waters in 1988, the success of which is still to be determined (Goulletquer undated).

The French oyster industry has experienced what may be the most severe problem encountered thus far with the introduction of species, when *O. edulis* of America's West Coast brought new disease to a declining industry. The utilization of *C. gigas* has helped overcome that failure. *C. gigas* has eventually gained market acceptance as an alternative to *C. angulata,* although both are considered inferior to the native *O. edulis*. The French have presumably decided that an inferior oyster is better than no oyster. Further research on both of these species and others bring hope to the industry, which apparently has decided that one tragedy should not preclude further development.

Australia

The experience of Australia in regards to mollusk introductions depends on the state involved. In Tasmania and South Australia, *C. gigas* has been successfully introduced and is forming the basis for a cultured oyster industry. In 1989–1990, 3.5 million dozen oysters worth 13.7 M$Aus were harvested (Ayres 1991). In both these states there is no extant native oyster to compete with. However, in New South Wales there is an existing fishery based on the native rock oyster (*Saccostrea commercialis*). An unofficial or accidental introduction of *C. gigas* occurred in the seed production area of Port Stephens sometime prior to 1985. After several years of trying to eradicate *C. gigas,* because it interferes with the setting of *S. commercialis,* the government finally decided to allow the cultivation and sale of *C. gigas* from Port Stephens (Ayres 1991).

New Zealand

The native oyster in New Zealand is the rock oyster (*Saccostrea glomerata*) which was the basis of an oyster culture industry. *C. gigas* was accidently introduced to New Zealand waters in the 1960's and 1970's (Dinamani 1991). Distribution was aided by the traditional movement of *S. glomerata* seed in which was mixed *C. gigas* seed. In the course of about a decade, *C. gigas* went from a density on spat collectors of 1/1000 to 4/5. Harvests in 1985 reached 2000 mt. *C. gigas* is now the basis of the New Zealand cultured oyster industry.

NET BENEFITS OF MOLLUSCAN INTRODUCTIONS

Estimating Direct Net Benefits

In some ways, the estimation of net benefits for molluscan introductions is easier than most cost-benefit analysis. Many net benefits from environmental improvements arise from consumers' use of goods not sold in the marketplace. These non-market goods pose special difficulties in measurement. For example, improvements in water quality may improve recreational fishing opportunities, but because there is not a market with corresponding prices and quantities of fishing, there are unique problems in measuring the change in benefits to sportfishermen (Bockstael, Hanemann and Strand 1986). Fortunately, molluscs are market goods for which we can observe changes in prices and quantities, and thus, estimate supply, demand and corresponding welfare changes from introductions.

Although detailed data on the distribution of oysters in the marketplace are not available, it is common knowledge that the flow of West Coast *C. gigas* to the East Coast has increased since the collapse of the Chesapeake oyster fishery. It is not known to what extent consumers are aware or care about what oyster species they are consuming. In cases where a species is introduced to replace a depleted local species, and the two species are considered by the consumer to be close substitutes, demand studies based on a time series of prices and quantities of the depleted species may serve to estimate demand for the introduced species. Thus, in

France where there was an industry based on *C. angulata*, it is not surprising to expect similar demand for *C. gigas*. Of course, the reliability of forecasts from historical data are diminished the further out in time those forecasts must be made. Traditional consumer welfare measures (i.e., consumer surplus) can be made once the demand for the introduced oyster is determined.

The measurement of producer benefits, as in the case of consumers, must be measured net of costs. Total value of the harvest, probably the most often cited figure of success of an introduction, is not a measure of producer welfare unless culturing and harvesting are costless activities. Cost estimates can be made from current data on culturing, harvesting and processing costs to the extent these are available. Bosch and Shabman (1989) have developed such cost estimates for Virginia oyster growers, and these could be appropriately modified for the different species. In cases where data is not available, an economic-engineering approach can prove useful (Park and Jackson 1984). The opportunity cost of the producer's labor (i.e., what he could earn in the next best employment opportunity) should be included in the cost estimate. In areas where there are few alternative opportunities, the opportunity cost of labor tends to be low and results in higher producer benefits. Thus, in France, where there was a large oyster industry with few alternative opportunities, the benefits of the introduction of *C. gigas* are higher than in an area where there are several alternative fishing and culturing alternatives.

If the introduction is for purposes of restoring a public fishery, the net benefit to producers will depend on how the resource is managed. If an open access management regime is maintained, then net benefits to producers will be less than if a bottom leasing program or limited entry program on public grounds are producers will be less than if a bottom leasing program or limited entry program on public grounds are instituted. This is the well-known result of rent dissipation in common property fisheries (c.f., Gordon 1954, Copes 1972). Simply replacing one species with another does not necessarily eliminate the man-induced factors that caused the decline of the native species. One must still deal with the problem of overfishing, potential disease, and a decline in water quality.

Measuring "External" Costs of Mollusk Introductions

Although the direct net benefits of mollusk introductions may be many years off, the costs of these potential introductions are being incurred today mainly in the form of research dollars. Costs of general research on mollusk introductions that is applicable to a variety of species and variety of areas cannot fairly be assigned totally to the cost of introduction of one species in one area. However, as a specific introduction is contemplated, more of the research dollars are focused on determining the impact and likelihood of success for that given area.

The actual cost of performing the introduction or transfer, and monitoring and maintenance may be substantial. However, once it is determined what functions have to be performed, predicting the costs would not be an overwhelming task. For example, the magnitude of costs will be much greater when introducing a non-reproducing organism into an area for yearly harvest, as compared to an introduction of an organism that can successfully reproduce.

The most contentious issue regarding mollusk introductions and their costs is the potential that an introduction may be accompanied by deleterious effects to other resources in an area. These can include the case of an otherwise successful introduction of an organism that outcompetes native resources and causes a population decline of the native resource such as occurred in New Zealand. The introduction may also inadvertently introduce other undesirable species, disease organisms or parasites that can disrupt the ecosystem. The end result may simply be a nuisance or considered a disaster. The results may be either reversible or irreversible.

Uncertainty and the Use of Benefit-Cost Analysis

The fundamental issue surrounding introduction of molluscan species is the uncertainty of the effects. Even though the history of molluscan introductions, reviewed above, shows few disastrous external effects, the evidence is clear that molluscan introduction have resulted in inadvertent species being introduced with the mollusks. Some might say that it was a stroke of luck that no disasters occurred. A finite probability exists that an ecological and economic disaster can occur with an introduction of *C. gigas* into the Chesapeake Bay. How does one consider uncertainty within the benefit cost framework?

There are two primary ways it has been considered- through the use of expected net benefits and through a game-theoretic approach. When using expected net benefits, the distributions about the costs and benefits are used and the expected value of net benefits is calculated. In concept, this is a straightforward procedure but the distributions about net benefits are not easy to determine, especially the ones concerning future events. Often, higher discount is given to more risky choices.

The uncertainty involved in the decision on whether to allow an introduction can also be approached through game theory. Bishop (1978) applied this approach when examining extinction of a potentially valuable species due to building a dam. The game is depicted as follows:

	States		
Action	No Disaster	Disaster	Maximum Losses
Introduction	0	b	b
No introduction	a	a–b	a

Man has two choices, to allow or not allow an introduction. If he does not allow the introduction, the net benefits foregone are denoted as a. If the introduction is allowed and causes a disaster in existing populations this is denoted by b. The last column indicates the maximum losses under the introduction and no introduction scenario.

One strategy in playing this game is to adopt the minimax principle—chose the strategy that minimizes maximum possible losses (Ciriacy-Wantrup 1968). Thus, if we feel the damages from a potential disaster exceed the benefits from an introduction with no disaster, then under the minimax principle the decision would be not to allow the introduction.

Clearly, before any strategy is chosen, measures of the consequences of introductions and damages must be made. As discussed earlier, measuring a, the foregone benefits of not allowing the introduction has difficulties, but they are not insurmountable. Two issues will accompany this estimate: how should the stream of net benefits be discounted over time; and what are the characteristics of the uncertainty of these measurements.

It is entirely possible that the introduction of *C. gigas* into Chesapeake Bay will have negative net benefits. Given the nega-

tive publicity surrounding the health and safety aspects of eating molluscan shellfish, it is possible the demand for the product is highly inelastic so that a slight increase in the available quantity will be accompanied by a large decline in price. It may also be that the Chesapeake Bay has a comparative disadvantage relative to other areas for producing *gigas*. This may be due to natural environmental differences as well as production costs in this region.

Measuring b, the potential damages is much more problematic. Although it is probably not possible to predict all the potential consequences of introductions into an area, it may be possible to narrow the field of potential damages, and provide an estimate of maximum loss from this subset of damages. For example, at present in Maryland it would only take the destruction of three species, the blue crab, native oyster and soft clam, to virtually eliminate the Maryland bay fishery. These two species with an exvessel value of $31.2 million in 1988 make up approximately 60% of Maryland's Chesapeake Bay landings. The net loss to harvesters from a $31.2 million a year fishery would be significantly less because of the costs of harvesting. If this loss was irreversible or occurred over a long period, discounting would again be an important issue. The timing of when the disaster occurred would also be important, particularly when it is coupled with discounting.

In the case of uncertainty surrounding the benefit estimates, we probably have some intuition about what the probability distribution of net benefits looks like. In the case of the disaster our intuition about probabilities is severely diminished. If the probability distributions are known, it is possible to play the game with other standards. For example, one could compare the expected value of the introduction and no introduction scenarios, and choose the action with the greater expected value. Clearly, this is a much less conservative approach than the minimax principle. Policymakers may want to look at other moments of the probability distributions such as the variance to help in the decision process.

Finkel (1990) offers an excellent guide on how to represent the uncertainty present in an analysis, and how policymakers (risk managers) should use that information in making a decision. It will be necessary to assume some probability distribution for damages from an introduction. Monte carlo techniques are particularly useful in analyzing these types of problems when a number of different probability distributions must be combined.

CONCLUSIONS

Economics offers no perfect prescription for making decisions about molluscan introductions and similar types of environmental decisions. It, however, can aid, as Finkel (1990) states in "narrowing the riff between good decision processes and good outcomes". That is, one can ignore economic and risk analysis in the decision to make a species introduction, and by chance have a positive outcome anyway. This, however, does not validate the decision process. Our summary of the information on molluscan introductions to date seem to fall into the category of poor decision processes and good outcomes. Most of the introductions were done unofficially or unintentionally. Fortunately, the diseases and organisms that were introduced, for the most part have had minimal effects on the local ecology. The major exceptions are the introduction of oyster diseases in France, and the demise of the native rock oyster in New Zealand.

For the Chesapeake Bay, the magnitude of the potential benefits from an introduction of *C. gigas* will depend on the availability of alternative native species that will allow watermen and processors to continue to operate in their professions. For example, increases in striped bass populations, hard and soft clams, and other species would reduce the need for a renewed oyster fishery. Benefits will also depend greatly on the consumer perception of *C. gigas* as an alternative for *C. virginica*. If they are not considered substitutes, Chesapeake Bay production of *C. gigas* along with west coast production and imports will result in a substantially lower price, requiring fewer watermen, culturists and processors to handle larger quantities of product at low profit margins, in order to maintain profit levels. The potential cost from an introduction of *C. gigas* will depend on what is at risk. In terms of native oyster populations, there currently appears to be much less at risk in Virginia as compared to Maryland, because of the distribution of the oyster diseases MSX and Dermo. The economic magnitude of an ecological disaster resulting from the introduction would rise if other commercially important species were involved, such as blue crabs. Another possibility which would raise the impacts would be the introduction of a nuisance organism, such as a fouling organism like the zebra mussel. The good decision process requires the resource manager to weigh these factors in the decision of whether to allow an introduction.

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PUBLIC HEALTH ASPECTS OF TRANSFERRING MOLLUSKS

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ABSTRACT This paper discusses microorganisms associated with molluscan shellfish borne illness, their growth after harvesting, transportation and storage, and their response to depuration and relaying. Organisms of public health concern are categorized as to whether they originated in the natural environment or are present as the result of pollution. The organisms of concern and their significance were determined by examining the North East Technical Services Unit of the Food and Drug Administration and Centers for Disease Control data bases over a 15–17 year period. Enteric viruses accounted for most of the illness, followed by naturally occurring marine vibrios. Other microorganisms accounted relative few incidences of illness. Vibrios and certain indicator bacteria will increase in number during storage and transportation. Furthermore, vibrios are resistant to depuration. Relaying will cause reduction in enteric bacteria and viruses but not marine vibrios.

KEY WORDS: pathogens, shellfish, public health

INTRODUCTION

This paper will discuss microorganisms associated with molluscan shellfish borne illness and their growth after harvesting and during transportation and storage. Also, their response to depuration and relaying will be discussed. Organisms of public health concern can be divided by their source. They may originate in the natural environment or be present as the result of pollution. The organisms of concern and their significance can be estimated by examining the data bases of the North East Technical Services Unit of the U.S. Food and Drug Administration (NETSU) and the Centers for Disease Control (CDC) over a 15–17 year period (Tables 1 and 2). These data bases do not agree because of their nature. The CDC data base is a summary of foodborne outbreaks reported by the states. It is possible that an outbreak will be published in the CDC publication Mortality and Morbidity Weekly and yet, not appear in the data base because the outbreaks form was not submitted by the state. On the other hand, the NETSU data base is a summary of outbreaks and cases reported in the literature and includes personal communications. Thus, it is more complete but at the same time less precise. The NETSU data includes individual cases that were not reported as outbreaks. The CDC defines an outbreak as two or more persons becoming ill after consuming a common food at the same time. Illness that only affects specific individuals will not be reported in the CDC data base. For example, the NETSU data base lists several cases of *Vibrio vulnificus*, but because this bacterium only affects individuals in high risk categories, no outbreaks (two or more individuals having a similar illness after consuming the same food), have been reported. Thus, illnesses from this organism do not appear in the CDC data base. Finally, the definition of shellfish used by the data bases is different. The NETSU data base includes only bi-valve mollusks, whereas CDC defines shellfish to include bi-valves, uni-valves and crustacea.

The information in tables 1 and 2 is useful for estimating the risk from microbial contaminants. Most of the illnesses associated with molluscan shellfish have been associated with either enteric viruses or naturally occurring marine organisms of the family Vibrionaceae. Other microorganisms account for far fewer illnesses. The first section will deal with agents associated with pollution followed by a section on pathogens associated with the environment.

The effect of depuration (controlled purification) and relaying on various microorganisms will be discussed. Controlled purification and relaying is a process whereby shellfish are allowed to purge themselves of contaminants, either in a natural setting or in land based facilities (Richards 1988). Controlled purification is usually a land based process, where the shellfish are put into tanks with purification systems for the water. Relaying is the process of transferring the mollusks from polluted water to areas approved for shellfish harvesting. The process of controlled purification is based on reduction of indicator (fecal coliforms) counts, whereas relaying depends upon a specified time. The time for controlled purification is usually far shorter than that of relaying, 2–3 days versus 14 days. It is important to have an understanding of the relationship between indicator microorganisms and the various types of pathogens in these systems. For example, the time required for depuration of indicator bacteria and enteric bacterial pathogens is similar. However, rates vary greatly between indicator bacteria and some enteric viruses. In addition, the depuration of naturally occurring vibrios is quite different than that of indicator bacteria (Richards 1988). With respect to relaying, the numbers of naturally occurring bacteria such as vibrios are increased or at least stay the same. This is because *Vibrios* are indigenous to the

TABLE 1.

Illness Associated with Naturally Occurring Pathogens in Shellfish: Summary of CDC and NETSU Data, 1973–1987.[1,2]

	Reported By		
	CDC[3]		NETSU[3]
	Cases	Outbreaks	Cases
	No. %	No. %	No. %
Pathogens			
Aeromonas	0	0	7 (0.1)
Bacillus cereus	6 (0.7)	2 (4.3)	—
Plesiomonas	0	0	18 (0.3)
Vibrio cholerae 01	16 (1.8)	3 (6.4)	7 (0.1)
V. cholerae non-01	11 (1.2)	2 (4.3)	125 (2.3)
V. fluvialis	0	0	3 (0.1)
V. hollisae	0	0	5 (0.1)
V. mimicus	0	0	6 (0.1)
V. parahaemolyticus	298 (32.9)	18 (38.3)	98 (1.8)
V. vulnificus	0	0	104 (1.9)
Total	331	25	373

[1] No illnesses associated with parasites or *C. botulinum*, were reported in these data bases.

[2] The number in parentheses is the % of total illness of Tables 1 and 2 combined.

[3] The term shellfish in the CDC data base includes all molluscan and crustacean shellfish. In the NETSU data base only bivalve shellfish are considered.

marine environment and their numbers are not affected by pollution. Of course waters used for relaying are classified by pollution, not by absence of naturally occurring pathogens.

AGENTS ASSOCIATED WITH POLLUTION

Agents associated with pollution include certain enteric viruses and bacteria. As mentioned earlier, enteric viruses are agents most often associated with shellfish borne illness. Of the enteric bacteria only salmonellae, *Campylobacter jejuni* and *Shigella* have been associated with illness from molluscan shellfish in the United States. Pathogenic *Escherichia coli* has been implicated with shellfish borne illness in other countries, such as Japan. Many other pathogens have been isolated from molluscan shellfish but have not been implicated in illnesses associated with mollusks. These include *Yersinia enterocolitica* and *Listeria monocytogenes*. In this discussion, only organisms that have caused documented illness are discussed.

Human Enteric Viruses

Human enteric viruses are of concern in seafood products, especially in raw molluscan shellfish that may have been harvested from fecally polluted waters. Viruses are inert in food systems and are only active inside the host; therefore, they will not multiply during storage after harvesting. Only a few viruses can be transmitted through food. These are usually transmitted by the fecal oral route which includes contamination from human sewage. Enteric virus infections are limited mostly to the intestine. However, when the infection goes past the intestine, a more serious illness such as hepatitis may result. When a person becomes infected they shed viruses in their feces which may in turn contaminate seafood through pollution or poor personal hygiene habits. Most of the reported outbreaks of viral illness associated with seafood have involved fecally contaminated bi-valve shellfish; however, viruses have the potential to contaminate seafood during processing. This has happened with other food products.

More than 100 enteric viruses can be found in human feces. Picornaviruses make up the largest of all virus families with nearly 200 host-specific picornaviruses having been identified in man. Of these, 69 enteroviruses inhabit the enteric tract (White and Fenner 1986, Gerba 1988). These viruses have a naked icosahedral capsid 25–30 nm in diameter, appear as smooth and round in outline, are constructed from 60 protomers, and replicate in the cytoplasm. Each protomer is comprised of a single molecule of four polypeptides, VP 1, 2, 3 and 4, or 1D, 1B, 1C and 1A respectively. The genome is a single stranded RNA linear molecule of positive polarity with a M.W. of 2.5×10^6. The molecule is polyadenylated at its 3′ end with the protein VP_g covalently linked to its 5′ end.

Enteroviruses have been subdivided into the species group Polioviruses (PV), Coxsackie viruses, Echoviruses and Enteroviruses. While polioviruses are frequently isolated from bi-valve shellfish, they are mostly vaccine strains and are not a cause of concern with respect to public health. Hepatitis type A (HAV) is the picornavirus of most concern in shellfish.

Enteric viruses are obligate parasites and of course do not multiply in shellfish. They do however, survive quite well. For example, in oysters polioviruses survive more than 30 days in shucked product stored under refrigeration.

Hepatitis Type A (HAV) (Enterovirus Type 72)

The onset of HAV is associated with the clinical symptoms of fever, malaise, anorexia, nausea and lethargy. Symptoms also include dark urine, jaundice and an enlarged, tender, palpable liver. In children, most infections are anicteric; however, the se-

TABLE 2.

Illness Associated with Contamination of Shellfish by Fecal Pollution: Summary of CDC and NETSU Data, 1973–1987.[1,2,3]

	Reported By		
	CDC[3]		NETSU[3]
	Cases	Outbreaks	Cases
	No. %	No. %	No. %
Salmonellae (non-typhi)	80 (8.8)	3 (6.4)	—
Salmonella typhi	—		—
Hepatitis A	335 (36.9)	9 (19.2)	356 (6.6)
Hepatitis (unspecified)			4479 (82.9)
Norwalk and similar viruses	42 (4.6)	2 (4.3)	82 (1.5)
Shigella	77 (8.5)	4 (8.5)	93 (1.7)
Staphylococcus aureus	14 (1.5)	2 (4.3)	5 (0.1)
Campylobacter	—	—	16 (0.3)
Clostridium perfringens	28 (3.1)	2 (4.3)	—
Total	907 (100)	47 (100)	5404 (100)

[1] No illnesses associated with parasites, *C. botulinum*, enterococci, or *S. typhi* were reported in these data bases.

[2] The number in parentheses is the % of total illness of Tables 1 and 2 combined.

[3] The term shellfish in the CDC data base includes all molluscan and crustacean shellfish. In the NETSU data base only bivalve molluscan shellfish are considered.

verity of the disease increases with age (Overby et al. 1983, Bryan 1986, White and Fenner 1986, Cliver 1988).

When HAV is ingested it multiplies primarily in the intestinal epithelium. Secondary infection of the parenchymal cells of the liver is through the blood stream. The virus is found in the feces approximately one week prior to the clinical signs. It may also be found in the blood approximately one week prior to the appearance of the main clinical sign of dark urine. It disappears after serum transaminase levels reach their peak. The onset time for symptoms is normally four weeks, but may range from 2–6 weeks. Infection with HAV results in permanent immunity.

HAV is spread by the fecal-oral route. It is hyperendemic in countries which are overcrowded, have inadequate sanitation and poor hygiene. Most infections in these communities occur in childhood and are subclinical. In more developed countries the disease is seen most often between the ages of 15 and 30.

Contaminated food and water and person to person contact are the main routes of transmission of HAV. Each year 20,000 to 30,000 cases are reported to the Centers for Disease Control (CDC). Of these cases, approximately 140 are due to foods (0.5% of the total). Most of these food-borne outbreaks are due to mishandling of foods by infected individuals (Cliver 1988). Outbreaks can also occur due to inadequate cooking of contaminated foods and by human sewage contamination of drinking water supplies, swimming waters and shellfish growing waters.

In the 1950's the first documented case of shellfish-associated HAV occurred in Sweden. The first case was documented in the U.S. in the 1960's (Richards 1985, Cliver 1988, Gerba 1988). Richards (1985) reported approximately 1400 cases of molluscan shellfish-associated HAV since 1961. The Centers for Disease Control reported 335 cases of shellfish-related HAV from 1973 to 1987. The North East Technical Services Unit of the U.S. Food and Drug Administration reported approximately 438 cases of shellfish-associated HAV from 1973 to 1990 (CDC 1973–1986, Rippey 1991).

Prevention and control of HAV can be accomplished at several levels. Municipal sewage systems should be properly functioning to prevent contamination of public water supplies and shellfish producing waters. Also, proper classification of shellfish growing areas and restricting shellfish harvest only to approved areas is important in preventing HAV contamination from untreated human sewage.

Considerable research has been conducted on the fate of enteric viruses, including HAV, during depuration and relaying. This research was hampered until recently because cell cultures were not available to propagate HAV virus. HAV viruses seems to be resistant to depuration in comparison to indicator bacteria and many other enteric viruses (Richards 1991). Sobsey (cited by Richards 1991) examined the depuration of poliovirus, *E. coli,* enterococci, the bacteriophage MS-2 and HAV. The organisms were taken up naturally by feeding in contaminated water in laboratory tanks. Each organism depurated at a different rate, with poliovirus being depurated the quickest, followed by *E. coli,* enterococci, the bacteriophage MS-2 and HAV. HAV remained in the oyster for more than five days after being exposed to clean water at various temperatures and salinities. This implies that commercial depuration would not eliminate HAV from shellfish.

Non-A, Non-B Enteral Hepatitis; Hepatitis E

The disease caused by Hepatitis E Virus (HEV) is called hepatitis E, or enterically-transmitted non-A non-B hepatitis (ET-NANBH). Other names include fecal-oral non-A non-B hepatitis, and A-like non-A non-B hepatitis. It should not be confused with hepatitis C, also called parenterally transmitted non-A non-B hepatitis (PT-NANBH), or B-like non-Anon-B hepatitis, which is a common cause of hepatitis in the U.S. (Gouvea 1991).

HEV has a particle diameter of 32–34 nm, a buoyant density of 1.29 g/ml in KTar/Gly gradient, and is very labile. Serologically related smaller (27–30 nm) particles are often found in feces of patients with Hepatitis E and are presumed to represent degraded viral particles. HEV has a single stranded polyadenylated RNA genome of approximately 8 kb (Gouvea 1991). Enteral HEV can be more severe than HAV with a high incidence of cholestasis. The incubation period for hepatitis E varies from 2 to 9 weeks. Disease usually is mild and resolved in 2 weeks leaving no sequelae. The fatality rate is 0.1–1% except in pregnant women. This group is reported to have a fatality rate approaching 20%. The highest attack rate is in young adults, especially pregnant women in the third trimester (Gouvea 1991). The incidence of chronic active hepatitis is extremely low in HEV (Overby et al. 1983, White and Fenner 1986).

Enteral HEV is transmitted mainly by sewage contaminated water in epidemics. It is also transmitted sporadically by person to person contact. In the middle East and Africa, it appears to be endemic (Overby et al. 1983). Cliver (1988) noted water-associated outbreaks have been reported for years from India, Africa, the USSR, and most recently, Mexico. Cases have been associated with consuming raw shellfish (Rippey 1990). No research has been conducted on the fate of HEV in shellfish during depuration or relaying. This work is needed as the potential for spread in the U.S. is great.

Unclassified Viruses

These include the non-specific agents of gastroenteritis including Norwalk and Norwalk-like agents, Snow Mountain agent, and Small Round Viruses (SRV's). The Norwalk group is 25–32 nm in diameter, while the SRV's are 27–40 nm. The SRV's have been identified in the feces of infants with diarrhea using Immune Electron Microscopy (IEM). The incubation period for the Norwalk agent is from 24 to 72 hours. Infection results in the sloughing of intestinal villi followed by rapid regeneration. Clinical symptoms include diarrhea, nausea, vomiting, abdominal cramps, and in some cases, headache, myalgia and low grade fever. Symptoms are more serious in adults. Immunity following an infection with Norwalk virus is only temporary, lasting approximately one year. This may be one of the reasons for the very high attack rate in at risk individuals of 50–90% (Cliver 1988).

Outbreaks of viral gastroenteritis due to the Norwalk agent has been associated with swimming in waters contaminated with human sewage, fecal contamination of food or drinking water and consumption of uncooked or partially cooked shellfish harvested from estuaries contaminated with human sewage. The first documented shellfish-associated outbreak of gastroenteritis involving Norwalk virus was in 1979 in Australia, where more than 2000 people were involved. Since this time, there have been many documented outbreaks in the U.S. Norwalk virus illness associated with shellfish is a continuing problem and has increased with the last decade while HAV infections have decreased. Between 1973 and 1990, the USFDA NETSU reported 176 shellfish-related outbreaks of Norwalk gastroenteritis, 71 outbreaks of Snow Mountain agent, and 5924 cases of gastroenteritis of unknown etiology,

many of which may have been caused by Norwalk like viruses (Rippey 1991). The Centers for Disease Control reported 3524 shellfish-related cases of unknown etiology from 1973 to 1987 and 42 cases from Norwalk virus (CDC 1973–1987). Richards (1985) reported over 6,000 cases of shellfish-associated gastroenteritis over the past 50 years. It is presumed that many of these are of viral etiology, possibly Norwalk or Norwalk-related agents. Over 75% of these cases have been reported since 1980, which shows increased awareness and reporting practices in regards to shellfish illnesses.

Good personal hygiene and good manufacturing practices, proper classification of recreational and shellfishing waters and prevention of sewage contamination in drinking, swimming, or shellfish growing waters are the most effective preventive measures for the Norwalk and related gastroenteritis viruses since they are found only in human sewage.

Because these agents do not grow in tissue culture, very little information is available on their fate during transfer. Outbreaks have been associated with depurated clams imported to the U.S. from the United Kingdom. However, these clams were most likely depurated in contaminated water.

ENTERIC BACTERIA ASSOCIATED WITH POLLUTION

Salmonella

From a historical perspective, *S. typhi* is a bacterium of concern; however, no cases have been associated with shellfish since the 1950s. The food poisoning type has been associated with shellfish. The food poisoning syndrome develops after ingestion of a food that contains a sufficient number of Salmonella cells to cause infection, usually between 100,000 and 100,000,000 cells. (The infective dose can be much lower in certain high fat foods such as cheese or chocolate.) The symptoms usually develop 12–14 hours after ingestion of the food, although incubation times of greater than 24 hours are not uncommon. Symptoms consist of mainly diarrhea along with nausea, vomiting, abdominal pain, headaches and chills. The symptoms are often accompanied by prostration, muscle weakness, moderate fever and drowsiness. Symptoms usually last only 2–3 days. The death rate is less than 0.2% (Jay 1987).

Raw foods, particularly those of animal origin, are the major vehicles of salmonellosis (Cox and Bailey 1987, Allred et al. 1967). The five most common food vehicles for *Salmonella* in the United States are beef, turkey, homemade ice cream (containing eggs), pork and chicken (Jay 1987, Cox and Bailey 1987). Turkey is the most common vehicle in Canada. However, many other foods have been involved in salmonellosis. For example, in 1985, the largest outbreak ever reported (18,000 cases) was traced to pasteurized milk produced in Illinois.

In the United States most outbreaks of salmonellosis are traced to contaminated products of terrestrial animals. However, vehicles for sporadic salmonellosis are rarely identified. While CDC and NETSU foodborne surveillance data indicate that seafood is a much less common vehicle for *Salmonella* than are other foods such as chicken and red meat, fish and shellfish may be responsible for at least a small proportion of the total number of *Salmonella* cases that occur each year in the United States. However, current data are inadequate to make any attempt at estimating attributable risk. Seafood has been infrequently incriminated as a vehicle of foodborne salmonellosis.

When examining the importance of salmonellae in seafood, it is useful to examine the overall incidence reported to CDC. CDC tracks disease incidence by several mechanisms, including laboratory-based *Salmonella* Surveillance system and the Morbidity and Mortality Weekly Report (MMWR). These systems do not agree and data in one system often is not included in the other systems. When examining the annual foodborne disease incidence data for the 14 year period from 1973 to 1986, an average of 55 foodborne outbreaks of non-typhoidal *Salmonella* infections affecting a total of 3944 persons were reported each year to CDC. During this same time frame, only 6 seafood borne outbreaks involving 147 cases were reported. Two of these outbreaks involving 40 cases were shellfish-associated (Chapter 8). Examining the other surveillance systems; during the 14 year period from 1973 to 1987, an annual average of 32,957 and 35,490 *Salmonella* cases were reported through the laboratory-based *Salmonella* Surveillance system and the Morbidity and Mortality Weekly Report (MMWR), respectively.

The NETSU data base, which attempts to document all cases of shellfish borne disease outbreaks from 1894 to 1990, reported only two shellfish associated outbreaks of confirmed non-typhoidal salmonellosis between 1894 and 1973 (Rippey 1991). A 100-case outbreak that occurred in Florida in 1947 was traced to contaminated oysters. The other outbreak occurred in New York in 1967 and involved 22 cases. This outbreak was associated with oysters imported from England (Rippey and Verber 1988). No cases of *Salmonella* infections from shellfish were reported to the NETSU between 1973 and 1988. However, several sporadic cases of salmonellosis associated with shellfish occurred in 1989 and 1990 (Rippey 1991). In September, 1989, three cases of salmonellosis were associated with mussels harvested in Maine and consumed in Connecticut. *S. infantis* was isolated in two of the cases. In October and December, 1989 oyster associated cases were reported in Florida. In 1990, four separate oyster associated cases were reported in Florida (Rippey 1991).

In other countries outbreaks of salmonellosis have been associated with shellfish. For example, an outbreak of salmonellosis associated with clams (*Venus verrucosa*) was reported in Italy (Cantoni et al. 1985). In this outbreak fifty people were affected. The causative agents were *S. typhimurium* and *S. mbandaka*. The estimated count per clam was 400–800 cells which implies that the infective dose was low. The NETSU data base on shellfish associated outbreaks—Foreign Reports, did not report outbreaks due to *Salmonella* during the period from 1973–1990.

Isolations of salmonellae from shellfish is not uncommon. Fraiser and Koburger (1984) examined various seafoods including clams and oysters from the east and west coasts of Florida for the presence of *Salmonella*. The highest incidence of *Salmonella* was from clams harvested from the Gulf (west) coast of Florida. The shellfish were analyzed very quickly after harvest and the authors felt that quick analysis greatly increased recovery of salmonellae. In addition, individual animals were analyzed instead of using composite samples. The authors felt this increased the probability of isolating different sero-types of *Salmonella*. In this study 43% of the clams tested were positive for *Salmonella*. This is one of the few studies that report the numbers of *Salmonella* present in the samples. In analysis of oyster meats the levels of *Salmonella* isolated was 2.2 per 100 grams of tissue. It was noted that this level of salmonellae would be unlikely to cause illness in most consumers. Eleven different sero-types of *Salmonella* were isolated, with as many as six sero-types being isolated from the same group of samples. The authors went on to theorize that salmonellae might be part of the free living micro-flora of shellfish.

Andrews et al. (1974) examined the coliforms as indicators of

Salmonella in oysters and clams. Over an 18 month period 263 oyster and 96 clam samples were tested for coliforms, fecal coliforms and the presence of *Salmonella*. Thirty-nine of the oyster and 5 of the clam samples were positive for *Salmonella*. It was observed that the indicators did give an indication of the presence of *Salmonella*. However, high numbers of indicators did not necessarily mean that the pathogen was present.

In later work this same group examined the comparative validity of members of the total and fecal coliforms groups for indicating the presence of *Salmonella* in the eastern oyster (*Crassostrea virginica*). In this study 539 oyster samples and corresponding harvest water samples were analyzed. Occurrence of *Salmonella* more closely paralleled increases in fecal coliform counts compared to total coliform counts. More *Salmonella* was isolated from water, meeting the total coliform standard compared to the fecal coliform standard. *Salmonella* was not isolated from samples that met both the sanitary survey and fecal coliform standard. This study points out the importance of using both the sanitary survey in conjunction with microbial analysis to insure safety.

Andrews et al. (1976) studied the validity of members of the total coliforms and fecal coliform groups for indicating the presence of *Salmonella* in hard clam (also called quahaug). In this study 214 samples were tested over a two year period. The harvesting waters were tested for coliforms and fecal coliforms and classified as to whether it met either the total coliform standard of less than or equal to 70 coliforms per 100 mLs or less than or equal to 14 fecal coliforms per 100 mLs. The clams were further classified as to whether they met the market guideline of 230 fecal coliforms per 100 grams of tissues. None of the clams harvested from waters meeting either standard contained *Salmonella*. Furthermore, *Salmonella* was not isolated from any meat sample meeting the market guideline. *Salmonella* was isolated from some of the samples which exceeded the National Shellfish Sanitation Program's standards. From this work the investigators concluded that fecal coliforms were adequate indicators of shellfish safety, with respect to *Salmonella*.

Timoney and Abston (1984), studied the contamination and elimination of *E. coli* and *S. typhimurium* in the hard clam, *Mercenaria mercenaria*. The bacteria were eliminated at similar rates; however, *E. coli* levels declined more rapidly than salmonellae. The organisms were eliminated from the clams becoming associated (non-ionicly bound) with feces and pseudo-feces particulate matter. Most of the test organisms were eliminated between six and twenty-four hours. This study indicates that *E. coli* is a good indicator with respect to salmonellae.

Hood et al. (1983), examined the relationship among fecal coliforms, *E. coli* and *Salmonella* species in freshly harvested, Gulf of Mexico coast oysters and clams. *Salmonella* was only found in samples which exceeded the National Shellfish Sanitation Program's market guideline of 230 fecal coliforms per 100 grams of product. These investigators reported that low levels of fecal coliforms and *E. coli* were good indicators of the absence of *Salmonella*. However, high levels of these indicators did not necessarily indicate the presence of *Salmonella*.

Elimination of Salmonellae and *E. coli* from Shellfish

It is interesting that there are species differences in depuration rates of species of *Salmonella*. For example, Cook and Ellender (1986) examined the depuration of *S. typhimurium*, *S. montevideo* and poliovirus in Gulf oysters. *S. montevideo* persisted longer than *S. typhimurium*.

Matev et al. compared the depuration of *S. typhimurium* and *S. enteritidis* to that of *E. coli* and *Staphylococcus aureus* in artificially contaminated Black Sea mussels. *E. coli* was recovered for six days compared to four days for the *Salmonella* and two days for *S. aureus*. Rowse and Fleet (1982) observed that both *Salmonella* and *E. coli* survived in oyster feces and could be released in the overlying waters. Survival depended on water temperature. In later studies Rowse and Fleet (1984a,b) studied the effects of water temperature and salinity on the depuration of *S. charity* and *E. coli* from the Sidney Rock oyster. In this study the organism was eliminated at similar rates. For this species, elimination was most rapid at 18–22°C and salinities of 3.2–4.7%. Higher or lower temperatures and lower salinities slowed depuration. Eyles and Davey (1988) observed that isolation of *Salmonella* from the Sidney rock oyster was correlated to rainfall and to a lesser extent low salinity waters. The presence of salmonellae in this study was related to high *E. coli* counts.

Campylobacter jejuni and Other Species

Campylobacter are curved, spiral Gram-negative rods that are nonsporeforming and microaerophilic (Simbert 1984). Campylobacters grow between 25 and 43°C, are motile, oxidase positive and do not ferment or oxidize carbohydrates (Stein et al. 1992, Franco 1988). The campylobacters can be broadly placed into two groups on the basis of the catalase test. The catalase-positive campylobacters are most frequently associated with human disease.

Campylobacteriosis may be the first or second leading cause of food poisoning in Western countries including the United States (Seattle-King County Depart. Pub. Hlth. 1984, Totten 1987, Franco 1988). Only recently has its importance been realized because methodology to detect the organism in food and feces was not available (Doyle 1981).

Campylobacter species were once thought to be primarily important to veterinary medicine. Prior to 1974 these bacteria were placed in the genus *Vibrio* because of their shape (Blaser 1981, Doyle 1981). The organism, now known as *C. jejuni* was grouped with *V. fetus*. In the 1974 edition of Bergey's Manual the genus *Campylobacter* was created. The genus *Campylobacter* currently consists of at least 18 species, subspecies, and biovars, with 17 names officially recognized by the International Committee on Systematic Bacteriology (Franco 1988, Stern et al. 1992).

Human illness is associated with three species of *Campylobacter, C. jejuni, C. coli* and *C. laridis*. These organisms are carried in the intestinal tract of animals and therefore, may contaminate foods of animal origin. In addition, fecal contamination of harvesting waters may allow shellfish to be a vehicle for the pathogens (Rippey 1991). *C. jejuni* is recognized as a leading cause of acute bacterial gastroenteritis. It is recognized as both a food and water borne pathogen. Foodborne illness is usually associated with the consumption of products of animal origin. In addition, *C. coli* and *C. laridis* are also recognized causes of gastroenteritis, but less frequently than *C. jejuni*. These three species are collectively referred to as the *C. jejuni* group.

Campylobacteriosis Associated with Shellfish Consumption

NETSU reported 1 domestic shellfish-associated outbreak of campylobacteriosis between 1894 and 1988, an outbreak of 16 cases due to contaminated hard clams that occurred in New Jersey in 1980. In addition, *Campylobacter* was suspected in several outbreaks reported to the NETSU where the etiological agent was

listed as unknown (Rippey and Verber 1988). In the 1991 update of the NETSU data base several outbreaks and cases of *Campylobacter* associated with shellfish were reported. Most of these illnesses were reported in the state of Florida. In one outbreak in 1989 two people became ill three days after consuming oysters. *C. jejuni, Vibrio paraheamolyticus* and *V. vulnificus* were all isolated from the individuals. In another incident, a single case of confirmed *Campylobacter* infection was reported in Lee County, Florida. That same year in December, four separate cases of confirmed *Campylobacter* were reported (Rippey 1991). In 1990 six separate cases of illness from *Campylobacter* were reported in Florida. Five of the incidents involved oysters and clams were implicated in the other case. The age of the victims ranged from 23 to 70 years of age. In addition another case of *Campylobacter* illness from oysters was suspected in Alabama in 1989 (Rippey 1991).

Isolation from Shellfish

Arumugaswamy and Proudford (1987) reported the isolation of *C. jejuni* and *C. coli* from the Sidney Rock oyster. These investigators were able to detect these organisms in 17 of 79 samples. This work is interesting, because the Sidney Rock oyster is usually harvested from water of fairly high salinity. *Campylobacter* species are reported to be very sensitive to environmental conditions; however, in the Sidney Rock oyster, survival was reported during refrigeration and freezing. Arumugaswamy et al. (1988) allowed the oysters to feed in waters containing approximately 10,000 cells of *C. jejuni* and *C. coli* per mL. The oysters were then subjected to depuration. They were depurated within the 48 hour period usually allowed for depuration systems. These investigators also investigated survival of the organisms during storage as shellstock at 20 and 30°C, on the half shell during refrigeration, shucked and bottled, stored refrigerated and frozen. The organisms failed to multiply during room temperature storage, but did survive for 2–9 days. At 3 or 10°C the organism survived 8–14 days. Survival was better at the lower temperature and in the shucked product. The organisms survive for months during frozen storage at −20–24°C. Another *Campylobacter* species linked to illness is *C. laridis*. This organism has been isolated from mussels (Owen et al. 1988).

Shigella

Shigella are Gram-negative, non-motile, non-sporeforming rods-shaped bacteria. The illness caused by *Shigella* (shigellosis) accounts for less than 10% of the reported outbreaks of foodborne illness in this country. *Shigella* rarely occurs in animals; principally, a disease of humans except other primates such as monkeys and chimpanzees. The organism is frequently found in water polluted with human feces.

Symptoms of the illness include: abdominal pain; cramps; diarrhea; fever; vomiting; blood, pus, or mucus in stools; tenesmus. The on-set time is 12 to 50 hours. The infective dose is very low and can be as few as 10 cells depending on age and condition of host. The disease is caused when virulent *Shigella* organisms attach to, and penetrate, epithelial cells of the intestinal mucosa. After invasion, they multiply intracellularly, and spread to contiguous epithelial cells resulting in tissue destruction.

Association with Shellfish

Sewage pollution has been associated with outbreaks of shigellosis from shellfish. The number of cases are limited. The organism does not survive well and illness is most often the result of contamination by a handler. Cantori et al. (1980) reported an outbreak of shigellosis from mussels (*Mytilus galloprovincialis*). The report was written in Italian and only the abstract was in English. The outbreak occurred in Milan in 1978 and approximately 100 people were affected. Studies of the mussels revealed the presence of *S. dysenteriae* and *S. boydii*.

Taylor and Nakamura (1964) reported that *S. sonnei* and *S. flexneri* could survive at 25°C in clams for more than 50 days and in oysters for more than 30 days.

As is the case for the salmonellae, 3 surveillance systems for shigellosis exist at CDC. For the years 1978–1987, an average of 7 foodborne outbreaks affecting a total of 573 persons were reported each year to the foodborne disease surveillance system (CDC 1989). Seven outbreaks involving 137 cases were seafood borne. Four of the 7 outbreaks, involving 77 cases, were shellfish associated (Chapter 8). During the same period an annual average of 14,460 and 18,498 total foodborne cases were reported through the laboratory-based *Shigella* Surveillance system and the MMWR, respectively. NETSU (Rippey and Verber 1988) reported 4 shellfish-associated outbreaks involving a total of 93 cases of shigellosis in the United States between 1894 and 1988. Nine persons were reported ill in Massachusetts in 1977, 11 in California and 26 in Arizona in 1979, and 47 in Texas in 1986. Between 1978–1987 NETSU reported 84 cases of shellfish-associated shigellosis (Rippey and Verba 1989).

In 1989 and 1990 additional cases of shigellosis from the consumption of oysters were confirmed or suspected (Rippey 1991). The cases were for the most part sporadic and only two outbreaks occurred. All the cases were reported in the state of Florida. The two outbreaks where *Shigella* was suspected as the causative agent occurred in October 1989. In both incidences, four people became ill after consuming oysters. In the first outbreak four of nine people became ill one day after eating the oysters. *S. sonnei* and/or *Vibrio parahaemolyticus* and *V. fuluvialis* were suspected. In the other suspected outbreak four of four people became ill. Again either *Shigella* or a *Vibrio* was suspected. One case of *Shigella* in a two year old girl was reported in November of the same year. In 1990, four separate cases of shigellosis were reported. Three of the victims were female and the other was a male. Oysters were the vehicle in all cases.

Pathogenic Escherichia coli

E. coli is often thought of as an indicator of fecal pollution. In 1887, Escherich observed the ubiquity of what we now designate as *Escherichia coli* in human stools. Shardinger, in 1892, suggested that members of this species be used as an index of fecal pollution because they could be recovered more easily than *Salmonella* species (Kator and Rhodes 1991, Banwart 1989). Pathogenic strains fall into four categories; enterotoxigenic, enteropathogenic, enteroinvasive and hemorrhagic (Medallion 1987, Mehlman 1982, Frank 1988). The first three are usually associated with human fecal contamination, whereas, hemorrhagic strains are most often associated with farm animals.

Association with Shellfish

Much of the research on isolation and incidence of pathogenic *E. coli* in shellfish has been done in Japan. Sato (1971) was perhaps the first person to report on the isolation of enteropathogenic *E. coli* from oysters. From July 1969 to February 1971, this author examined 160 commercial samples of foods including 66 ground

pork samples, 34 chicken samples and 60 oyster samples. Nineteen of the 60 oyster samples were positive for enteropathogenic *E. coli*. The author lists the serotypes of the strains isolated; however, only the abstract was in English and this reviewer could not determine which serotypes were specifically associated with the oyster isolates. Other work published in Japan include an article by Kokubo (1978) that describes a study where 405 oyster samples were examined for the presence of *E. coli* and a portion of the isolates were tested for pathogenic strains of *E. coli*. Only four pathogenic strains were isolated. One strain produced only heat labile enterotoxin, while the other three strains produced only heat stable enterotoxin. Ogawa et al. (1980) studied the incidence of enteropathogenic *E. coli* in sea water, oysters, river water, and sediment samples over a 10 year period. In this extensive study enteropathogenic *E. coli* was isolated from 14.4% of the sea water samples, 14% of the oyster samples, 15.3% of the river water samples and 3.7% of the sediment samples. The relationship between *E. coli* levels and enteropathogenic *E. coli* levels was excellent. As the numbers of *E. coli* increased, the frequency of isolation of the pathogenic strains increased. Perez Martinez et al. (1981) investigated the incidence of enteropathogenic *E. coli* in raw oysters obtained from supermarkets in Mexico using inoculation of suckling mice to evaluate for toxin. Only 3.7% of the isolates produced heat-stable toxin. Stephen et al. (1975) reported the isolation of both enteropathogenic and enterotoxigenic *E. coli* from mussels in India.

There are currently no data to indicate that any seafood, including shellfish, is an important source for diarrheagenic *E. coli* infections in this country. Neither the CDC annual summaries (from 1973 through 1987) or the NETSU data base (Rippey 1991) report any shellfish borne illness associated with pathogenic *E. coli*.

NATURALLY OCCURRING BACTERIA

Vibrionacae

The members of the family vibrionacae of concern include *V. cholerae* 01 and non 01, *V. parahaemolyticus, V. vulnificus, V. mimicus, V. hollisae, V. fluvalis, Pleisomonas shigelloides,* and *Aeromonas*. A brief description of these organisms is presented below. For the most part, these organisms are associated with the marine environment, show a definite seasonal variation, and are easily killed during heating.

V. cholerae is usually divided into two groups, serotype 01 and non-01 *V. cholerae*. Those groups can be further subdivided as toxigenic and non-toxigenic. Toxigenic strains are capable of producing cholera toxin or a very similar toxin.

Toxigenic *V. cholerae* 01 is the causative agent of endemic or asiatic cholera. The 01 serotype contains two biotypes; classical and El tor, both of which may contain toxigenic and non-toxigenic strains. The biotypes are differentiated by sensitivity to polymyxin B and Murkee's group four phage and by the ability to agglutinate chicken red blood cells (Sakazaki 1979). The classical biotype predominated worldwide until the 1960's. The El tor biotype is currently predominant world wide and is the biotype associated with recent cases in the U.S. and South America (Blake et al. 1980, Levine 1981, Morris and Black 1985, CDC 1986).

Symptoms of *V. cholerae* 01 infection can range from asymptomatic or mild diarrhea to severe cases (cholera gravis). In severe cases, *V. cholerae* 01 can cause profuse watery diarrhea, dehydration and death if not promptly treated. The incubation period varies from 6 hours to five days. Initially, the stool is brown with fecal material but it quickly assumes the classic "rice water" appearance. Enormous amounts of fluids are passed effortlessly, resulting in dehydration and circulatory collapse. The stool is rich in potassium and bicarbonate. Renal function is suppressed and the patient suffers from severe thirst, leg cramps, hoarse speech, weakness and rapid pulse (Morris and Black 1985, Blake et al. 1980, Sakazaki 1979). Fortunately, cholera gravis is relatively uncommon. Cholera gravis results in only 1 in 25–100 infections from the El tor biotype and in 1 in 5–10 infections by the classical biotype. People with type O blood are more susceptible to the severe disease (Sakazaki 1979).

The infective dose for *V. cholerae* is estimated to be approximately one billion cells; however, consumption of antacids or medication to lower gastric acidity markedly lowers the infective dose (Blake 1987). *V. cholerae* 01 induces illness by elaborating cholera toxin which stimulates the production of cyclic AMP (Holmgien 1981). Therefore, only toxigenic strains can cause cholera. Non-toxigenic strains of *V. cholerae* can cause diarrhea but not cholera and have also been implicated in wound infections.

Cholera in the United States is relatively rare. The U.S. has been spared any identified cholera outbreak from 1911 until 1973, then a single unexplained case occurred in Texas. A second cholera outbreak occurred during August, September and October of 1978 when 11 people were infected with *V. cholerae* 01 El Tor from recontaminated cooked crabs (Blake et al. 1980). In 1981, there were two cases of cholera involving residents of the Texas Gulf Coast and 17 additional cases on an oil rig in the Gulf (Morris and Blake 1985). Thirteen cases of domestically acquired cholera occurred in 1986; 12 in Louisiana and one in Florida (CDC 1986). Inadequate cooking or improper handling of crustaceans seems to have been the vehicle in this outbreak. Ten of the patients had severe diarrhea and 7 required hospitalization. The *V. cholerae* 01 was of the El Tor biotype. Of course, an epidemic of cholera is currently under way in certain South American countries. Poor sanitation and consumption of raw fecally contaminated seafood is responsible for many of the cases. It is not believed that this outbreak is a threat to the U.S. because of better sanitation and sewage disposal.

V. cholerae 01 is widely distributed and is probably part of the indigenous bacterial flora in estuarine waters (APHA 1985, Colwell 1984). There is evidence of seasonal variation and most cases of domestically acquired cholera have occurred during the late summer and fall; with August being the primary month for infection (Madden et al. 1982).

Non 01 V. cholerae

At least 70 other groups of *V. cholerae* are known to exist. They are referred collectively as non-01 *V. cholerae* or non-agglutinable (NAG) *V. cholerae*. The majority of the strains isolated from seafood and patients are non-toxigenic strains; less than 5% of the non-01 strains from human sources in the United States produce cholera toxin. The non-toxigenic strains are principally associated with gastrointestinal illness; but in the U.S. about ⅓ of the human isolates are from extra-intestinal sources, including wound infection, ear infection and primary and secondary septicemia (Morris and Black 1985). Associated symptoms of gastroenteritis have included diarrhea (100% of cases; 25% have bloody stools), abdominal cramps (93%) and fever (71%). Nausea and vomiting occurs in 21% of the victims. The diarrhea may occa-

sionally be severe; with as many as 20–30 watery stools per day (Morris and Black 1985). Almost all of the cases of non-01 *V. cholerae* infections in the U.S. have been associated with eating raw oysters.

Considering the relative frequency of isolates from seafood, the incidence of illness is very low. There is evidence that victims often have an underlying liver disease, which might be a host factor for the disease. Also, in most cases the disease may not be severe enough to warrant medical attention and therefore, the incidence may be unreported. However, it can be observed from Table 1 that non 01 *V. cholerae* accounted for a large percentage of the cases associated with the naturally occurring vibrios.

Non-01 *V. cholerae* strains are widely distributed in the environment of the United States, Asia and Europe. They occur most frequently in bays and estuaries with salinity in the area of 0.4–1.7% (Colwell and Kaper 1978); but have also been found in rivers and brackish inland lakes of salinity levels as low as 0.01%. Their presence in oysters and water samples does show a seasonal variation with the highest numbers being isolated June–August (Madden et al. 1982). Non-01 *V. cholerae* are free living organisms and are part of the autochatonous flora.

Vibrio parahaemolyticus

V. parahaemolyticus was first associated with food poisoning in 1950 in Osaka, Japan (Fujino et al. 1974). Since its discovery, *V. parahaemolyticus* is implicated in greater than 1,000 outbreaks per year in Japan and accounts for 45–70% of that country's bacterial food poisonings. Food poisoning in Japan is usually related to the consumption of raw seafood during the warm months. Typical symptoms include diarrhea (sometimes bloody), abdominal cramps, nausea, vomiting, headaches, fever and chills (Fujino et al. 1974). The infective dose for humans is between 10^5 and 10^7 viable cells; however, a decrease in stomach acidity may decrease infective dose. The time for onset of symptoms is usually 9–25 hours and the duration of the illness is usually 2.5–3 days. No deaths have been reported in the United States, but a death rate of 0.04% is reported for Japan. In Japan, raw seafood is the usual vehicle for the organism, but in the U.S. most of the foods implicated in *V. parahaemolyticus* outbreaks are cooked seafoods that have been recontaminated; although raw oysters and raw crabs have been implicated in some outbreaks (Barker 1974, Blake 1980, Spite et al. 1978). CDC data indicates that it is the agent most responsible for illness associated with molluscan shellfish. The NETSU data base indicates that it ranks sixth as a leading cause of illness. In any case, it is a significant cause of illness in shellfish.

V. parahaemolyticus is widely distributed in nature and has been isolated from coastal waters worldwide. Its presence has been documented in virtually all the marine coastal environs of the United States from the coast of Maine, south to the Gulf of Mexico, all along the west coast and from the coastal waters of Hawaii (Fujino 1974, Blake 1980), It is not considered to be a microorganism of the open sea because of its sensitivity to cool temperatures and high hydrostatic pressure (Kaneko and Colwell 1978, Colwell 1984, Schwarz and Colwell 1974). Its presence in estuarine environments and in the seafood harvested from these environments usually shows a seasonal variation, being present in the highest numbers during the summer months (Kenako and Colwell 1978, Hackney et al. 1980). Thompson and Vanderzant (1976) did not observe a positive correlation between numbers and season in the waters of the Gulf of Mexico off the Texas coast. However, Paille et al. (1987) observed seasonal variation in numbers of *V. parahaemolyticus* in oysters and waters of Louisiana.

While *V. parahaemolyticus* is a common contaminant of seafood, often present in high numbers, almost none of the isolates from seafood are capable of causing gastroenteritis in man (Fujino et al. 1974, Blake 1980, Hackney 1981). The test most widely used to differentiate between virulent and avirulent strains is the Kanagawa reaction, which tests a strain's ability to produce a heat stable hemolysin in an agar medium containing 7% NaCl, mannitol and fresh human or rabbit red blood cells. The heat stable hemolysin is the main virulence determinant for *V. parahaemolyticus*. Isolates from the marine environment and seafood are predominantly Kanagawa negative. Thompson and Vanderzant (1976) reported only 0.18% of the isolates from water, shellfish and sediments of the Gulf of Mexico were Kanagawa positive. In Japan 99% of the sea and fish isolates are Kanagawa negative (Sakazaki 1979). Food poisoning victims usually only excrete Kanagawa positive isolates. Studies have demonstrated that isolates do not change in the intestines and that Kanagawa positive types are probably part of marine *V. parahaemolyticus* populations, but present in low numbers.

Vibrio vulnificus

V. vulnificus has been called the new "terror of the deep" and is one of the most invasive species ever described (Oliver 1985). It has been identified as a halophilic "lactose-positive" marine vibrio. Foodborne infection may result after consuming contaminated, raw or undercooked seafood, particularly oysters and clams, with illness usually starting 16–48 hours after ingestion. The organism penetrates the intestinal tract and produces a primary septicemia. The illness usually begins with malaise, followed by chills, fever, and prostration. Vomiting and diarrhea are uncommon, but sometimes occur shortly after chills and fever. Hypotension (systolic blood pressure $\geq$80 mmHg) is present in approximately 33% of the cases (Blake et al. 1979). The fulminating infection progresses rapidly and may cause death in 40–60% of the patients (Oliver 1985). Primary septicemia by *V. vulnificus* is NOT OBSERVED in normal healthy people and is ONLY associated with certain risk factors including: liver disease, gastric disease, malignancy, hemochromatosis and chronic renal insufficiency (Oliver 1985, Blake et al. 1979). Healthy individuals can develop a gastroenteritis from this bacterium. The most common vehicle for the organism is raw oysters.

V. vulnificus is wide spread in the environment and has been isolated from estuarine waters of most coastal states. Infection via the intestinal tract is most often associated with the consumption of raw oysters, but it is sometimes difficult to isolate from the mollusks. Oliver (1981) demonstrated that antimicrobial factors in oysters could be lethal to *V. vulnificus* when the oysters were homogenized for analysis. Kelly and Dinuzzo (1985) demonstrated that the presence of *V. vulnificus* in oysters was probably due to filtration of the bacterium from sea water rather than active multiplication in oysters.

The presence of *V. vulnificus* in water and shellfish is seasonal being most prevalent when the water temperature is high (>20°C). Low salinity (0.5–1.6%) also favors the presence of *V. vulnificus* in seawater (Kelly 1982). Some strains of *V. vulnificus* show bioluminescence and these strains may also be pathogenic (Oliver 1986). Environmental isolates are phenotypically indistinguish-

able from clinical isolates and produce virulence factors identical to clinical isolates (Tison and Kelly 1986).

It is interesting that *V. vulnificus* is not listed in the CDC data base. This is because this data base only lists outbreaks and not individual cases. Since this organism only affects comprised individuals no outbreaks have been reported, just individual cases. This organism is causing concern. For example, in Louisiana, warning labels are now required on sacks of oysters. Also, in California, oyster from the Gulf coast must have a warning label. The warning suggests that individuals who have a compromised immune system or have other risk factors described above, should not eat raw oysters.

V. mimicus

V. mimicus is biochemically similar to *V. cholerae*, with the exception that the strains are sucrose negative. In earlier publications, they were listed as *V. cholerae* of the Hieberg group 5; however, DNA homology studies demonstrated that may of the sucrose negative strains were a separate species and in 1981 the name *mimicus* was proposed because of their similarity to *V. cholerae* (Shandera et al. 1983, Colwell 1984). Both toxigenic and non-toxigenic strains have been isolated, however, the food poisoning cases have been mostly from the non-toxigenic strains. Symptoms of the illness have included diarrhea in most cases, but approximately 67% of the cases had nausea, vomiting and abdominal cramps. Diarrhea may be bloody and will last 1 to 6 days.

Raw oysters and boiled crawfish (crayfish) have been implicated as vehicles for the organism. *V. mimicus* is widely distributed in nature and can be found in fresh as well as brackish waters. It does show seasonal variation, being present in highest numbers in the warmer months (Bockemuhl et al. 1986, Colwell 1984).

V. hollisae

V. hollisae (formerly EF 13) has been implicated in approximately 36 cases of food poisoning. Symptoms have included diarrhea and in approximately half the cases vomiting and fever. Seafood was implicated as the vehicle for *V. hollisae*, including raw oysters, clams and shrimp (Morris et al. 1982).

The ecology of *V. hollisae* is not well understood because it grows poorly or fails to grow in TCBS, the medium most used in isolation of members of the genus *Vibrio*.

V. furnissi and *Vibrio fluvialis*

V. furnissi was previously classified as biovar II of *V. fluvialis*. *V. furnissi* has been implicated in food borne illness (Brenner et al. 1983). It produces gas from glucose, which is an unusual characteristic among *Vibrio* species. Symptoms of illness include diarrhea, abdominal cramps, and sometimes nausea and vomiting. Most of the cases listed by NETSU are probably *V. furnissi*.

Pleisomonas shigelloides

P. shigelloides (formerly *Aeromonas shigelloides*) has been implicated in human gastroenteritis for 40 years (Miller and Koberger 1985). *P. shigelloides* is widespread in nature, being mostly associated with fresh surface water, but may also be found in seawater. It shows a seasonal variation in its isolation similar to that of marine vibrios; being more often isolated during the warmer months (Miller and Koburger 1985).

Foods implicated as vehicles for *P. shigelloides* include cuttle fish salad, salt mackerel, raw oysters and undercooked oysters. In the U.S. raw oysters are probably the most implicated food.

According to the NETSU data base, *P. shigelloides* has only been implicated in 18 cases during the 15 year period from 1973–1987. This accounted for less than 0.5% of the cases of illness associated with molluscan shellfish.

Miller and Koburger (1985) reviewed infections by *P. shigelloides* and reported by the percent of people experiencing symptoms which included diarrhea (94%), abdominal pain (74%), nausea (74%), chills (49%), fever (37%), headache (34%), and vomiting (33%). The onset of symptoms usually occurred 24–50 hours after ingestion of the food. The illness was self limiting and usually lasted 24–48 hours.

Most strains of *P. shigelloides* have a minimum growth temperature of 8°C, but at least one strain has been reported to grow at 0°C. They seem to survive well in shellstock oysters held at refrigeration temperatures. The organism is sensitive to pH of <4 and salt concentrate of $>5\%$ (Miller and Koburger 1986). In addition, being a member of the family Vibrioneae, it should be killed by relatively mild cooking temperatures.

Aeromonas

Aeromonas hydrophilia is listed as a cause of diarrheal illness by the NETSU data base. However, there is some question as to whether it is truly a pathogen.

Other agents that have caused illness from consuming molluscan shellfish that are of natural origin include *Clostridium perfringens* and *Bacillus cereus*. There is some question as to whether these organisms caused illness from consuming raw shellfish or were contaminants of cooked products that were temperature abused. The data bases do not make this clear. *B. cereus* was most likely associated with cooked products or products that were stored for a long period of time. On the other hand, it is probable that *C. perfringens* was in some outbreaks associated with raw products.

Clostridium perfringens

Clostridium perfringens has been associated with human disease, mostly gas gangrene, for over 90 years. However, it was not until the 1940's that it was first associated with food poisoning. *C. perfringens* food poisoning is associated with proteinaceous food products. The bacterium has exacting growth requirements, requiring thirteen amino acids and six vitamins. Foods of animal origin are more likely to provide these needed growth requirements. Meat and poultry products account for most of the reported illness with seafood products only accounting for approximately 2% of the reported outbreaks (Banwart 1989).

Most of the outbreaks of *C. perfringens* food poisoning have been associated with food service establishments. Cooking of foods contaminated with *C. perfringens* will kill vegetative cells of the organism, but the spores will survive. Cooking tends to lower the oxidation/reduction potential of foods and heat shocks the spores into activation, creating ideal conditions for growth of the organisms. Time-temperature abuse of the cooked food allows the organism to grow to high numbers. *C. perfringens* grows very quickly, with a generation time of as low as 8.5 minutes reported in some foods (Willardsen et al. 1979). Growth can occur at temperatures as high as 50–52.3°C (Shoemaker and Pierson 1976). Thus, if warming trays in food service establishments are not kept at proper temperatures, growth can occur. Time-temperature abuse of cooked products is usually a critical factor in most food poi-

sonings of *C. perfringens* origin. The number of organisms normally found in foods is usually low compared to the high number required to induce illness. The critical number needed to induce illness has been estimated at between $10^6 - 5 \times 10^8$ (Labbe, 1988 and Hatheway et al. 1980).

The source of the *C. perfringens* can be from soil, dust, water, spices, or the food itself. Type A is the strain mostly associated with food poisoning. It is considered to be part of the microflora of soil. Virtually all soils examined have contained type A *C. perfringens* at levels between log 3–4 per gram (Labbe, 1989). Also, it is associated with the intestinal contents of most animals being present at levels of log 3–5. This level is usually observed in infants after 6 months of age (Labbe 1988). This bacterium has also been isolated from the intestinal contents and surface of fish but at considerably lower levels. In addition, it is often isolated at low levels from shellfish and has been suggested as an indicator of fecal pollution.

The presence of *C. perfringens* in shellfish has been documented worldwide. Burow (1974) reported that 56% of mussel samples were positive for the organism. Inal et al. (1974) also reported the isolation of *C. perfringens* from mussels in Turkey. Ayres (1975) reported the organisms isolation from a number of shellfish including the European flat oyster, mussels, and hard clams. Fruin (1978) reported that most of the *C. perfringens* isolated from foods including clams were type A. Saito (1990) reported high incidence of *C. perfringens* in oysters in Japan. Furthermore 12% of the isolates from oysters were positive for enterotoxin production. This compared to six percent of the isolates from food handlers, 2% of isolates from dogs, and 10% of water isolates being positive for enterotoxin production.

Tia Son and Fleet (1980) observed that oysters (Crassostrea *commercialis*) were commonly contaminated with low levels of *C. perfringens* and *Bacillus cereus*. These organisms could be removed by depuration or relaying to clean water. Their depuration rates were similar to that of enteric bacteria such as *Escherichia coli*. They further observe that in artificially contaminated oysters that *C. perfringens* rapidly died off during storage, whereas counts of *B. cereus* remained stable to refrigeration.

Examination of CDC (Chapter 8 of this report) data shows that *C. perfringens* accounted for 5.4% of the outbreaks and 16.6% of the cases of illness associated with fish over the 15 year period from 1973–1987. Additionally, it was responsible for 4.3% and 3.1% of the outbreaks and cases associated with shellfish respectively during the same period. Since the illness associated with *C. perfringens* is usually mild, the number of cases are probably much higher.

The NETSU data base did not report any cases of shellfish born illness since 1894. Since the CDC data base includes crustaceans in its classification of shellfish, it is possible that the shellfish borne illness caused by *C. perfringens* reported by CDC may not have involved molluscan shellfish.

Analysis of the incidence of seafood borne illness caused by *C. perfringens*, indicates that *C. perfringens* is of little importance as a seafood borne pathogen. The number of outbreaks are low and most likely due to contamination and temperature abuse. It may be of greater importance as an indicator of pollution than as a pathogen. Madden et al. recommends that *C. perfringens* be the indicator of choice for depuration systems. These workers noted that *C. perfringens* was far more likely to be present in polluted shellfish than *Escherichia coli* because the spores survive well in the environment. Yet, they are depurated from shellfish at similar rates. By using *C. perfringens* as an indicator of depuration the public could be assured that the shellfish were indeed depurated. In addition, enumeration of the organism is easier.

The symptoms of *C. perfringens* food poisoning include severe abdominal cramps and a pronounced diarrhea. Nausea and vomiting are rare and headache and fever are usually absent. The on-set of symptoms is usually 8–12 hours after ingestion of the food and the illness usually does not persist for more than 24 hours. The illness is caused by sporulation of the vegetative cells in the intestine accompanied by production of an intracellular enterotoxin. The enterotoxin can be produced in food during sporulation but it has not been proven that illness has resulted from preformed toxin in foods (Labbe and Harmon, 1992).

Bacillus cereus

Bacillus cereus is a Gram-positive, facultatively aerobic spore-forming rod. The cells are large and the spores do not swell the sporangium. These and other characteristics including biochemical features are used to differentiate and confirm the presence *B. cereus* although these characteristics are shared with *B. cereus* var. mycoides, *B. thuringiensis* and *B. anthracis*. Differentiation of these organisms depends upon determination of motility (most *B. cereus* are motile), presence of toxin crystals (*B. thuringiensis*), hemolytic activity (*B. cereus* and others are beta hemolytic while *B. anthracis* is usually non-hemolytic) and rhizoid growth which is characteristic of *B. cereus* var. mycoides (Harmon et al. 1992).

Bacillus cereus food poisoning is the general description although, two types of illness are recognized which are caused by two distinct metabolites. The diarrheal type of illness is caused by a large molecular weight heat labile protein while the vomiting (emetic) type of illness is believed to be caused by a low molecular weight, heat-stable peptide.

The symptoms of *B. cereus* diarrheal type food poisoning mimic those of *Clostridium perfringens* food poisoning. The onset of watery diarrhea, abdominal cramps and pain occurs 6–15 h following consumption of contaminated food. Nausea may accompany diarrhea, but vomiting (emesis) rarely occurs. Symptoms persist for 24 h in most instances. The emetic type of food poisoning is characterized by nausea and vomiting within 0.5 to 6 h after consumption of contaminated foods. Occasionally, abdominal cramps and/or diarrhea may also occur. Duration of symptoms is generally less than 24 h. The symptoms of this type of food poisoning parallel those caused by *Staphylococcus aureus* food-borne intoxication.

The type most likely associated with shellfish is the diarrheal type. The emetic type has almost exclusively been associated with rice and starchy products. The presence of large numbers of *B. cereus* (greater than 10^6 organisms/g) in a food is indicative of active growth and proliferation of the organism and is consistent with a potential hazard to health. These high numbers could be reached during prolong storage out of water, or during transport to other states.

THE EFFECT OF HARVESTING, TRANSPORTATION AND STORAGE ON THE NUMBERS OF MICROORGANISMS IN SHELLFISH

This section will only be concerned with bacteria since enteric viruses do not multiply in shellfish.

Only a few studies have addressed the fate of pathogens and indicators during transportation and storage. In our laboratories we have examined the fate of indicators during transportation from Louisiana to Florida and Virginia. Non *E. coli* fecal coliforms

increased much faster than *E. coli* and often reach extremely high counts by the end of the trip. The oysters were harvested from waters meeting the fecal coliform standard of 14 or less per 100 mLs. The oysters were harvested and put into sacks on the boats. The first oysters were harvested before 6 AM and the boat arrived at the dock at approximately 4 PM. Approximately 425 sacks were loaded onto a refrigerated truck. The refrigeration was turned on after loading and the oysters were transported to Virginia over a period of 27 hours. Both *E. coli* and fecal coliform counts were <18/100 g for samples taken dockside. During the trip fecal coliform levels increased to levels of greater than 400 per 100 grams. *E. coli* levels remained very low. The fecal coliforms were identified to be *Klebsiella* species. In other studies, oysters were monitored in route from Louisiana to Apalachicola Bay, Florida. These studies were conducted in the months of July and August. In these studies the results were far more dramatic. The initial fecal coliform counts averaged 13,000 per 100 grams when the oysters reached the dock. *E. coli* only accounted for a small fraction of the fecal coliform count. In one trip the *E. coli* MPN was 50 per 100 g and in the other study the MPN was 20 per 100 grams. Four hundred sacks of oysters were loaded onto a truck and during the 15 hour trip the fecal coliform counts increased from 13,000/100 grams to 240,000/100 gm. The *E. coli* counts increased from 50 to 70/100 gms. In other studies from our laboratories, oyster samples were taken dockside as the harvesting boats landed and at the wholesale market during June and July. A total of 53 samples were taken dockside and 30 samples were taken at the wholesale level. Fecal coliform counts averaged 1112/100 grams dockside and 10,000/100 grams at the wholesale market. This data clearly shows that fecal coliforms increase in numbers during storage and transportation of shellfish harvest during the summer from the Gulf coast.

Cook and Ruple (1989), also examined the fate of fecal coliforms and *E. coli* during the trip from the harvest area to the plant. In general *E. coli* increased only during the time on the boat. Non *E. coli* fecal coliforms increase at all stages of transportation and during the summer months dominated the fecal coliform population. These studies clearly indicated that fecal coliforms are not adequate indicators of fecal contamination in shell stock oysters. Similar studies with soft shell clams have demonstrated that fecal coliforms are not good indicators of fecal contamination during the summer.

Cook and Ruple (1989) also studied the effect of transport on levels of vibrios in oysters. Many of the vibrios including *V. vulnificus* and *V. parahaemolyticus* increased by 3–4 orders of magnitude during time from harvest to the plant.

Marine vibrios do not depurate at the same rate as enteric bacteria and may be present far longer than indicators. This observation, coupled with the growth of vibrios demonstrated by Cook and Ruple may indicate that immunocompromised individuals should not assume that depurated shellfish are safe to consume. A significant reduction in bacterial counts is observed during depuration (not relaying); however, a certain bacteria of the normal microflora are resistant to depuration. These include vibrio species (Richards 1991). *V. parahaemolyticus* counts of naturally contaminated oysters were unchanged during depuration. Likewise, depuration does not significantly affect counts of *V. vulnificus* or *V. cholerae*. These pathogens could increase in numbers during storage and transportation.

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GENETIC ASPECTS OF INTRODUCTION AND TRANSFER OF MOLLUSCS

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ABSTRACT Attempts to predict the biological impact of an introduction have traditionally focused on the ecological dynamics of competition and predation, or the concomitant introduction of parasites or disease organisms. We focus here on a subject that has received less attention: the genetic effects of introductions on native populations. These may be broadly defined as direct or indirect changes in the genetic composition of an endemic population attributable to the arrival and establishment of a non-native population. Direct effects occur when the gene pool of the native population is open to the introgression of genes from the introduced population. Indirect effects occur when hybridization between the native and introduced populations is not possible, but alterations in gene frequencies result from ecological interactions with the introduced organism.

A transfer is defined here as the movement of individuals of a given species to another area within the current geographic range of that species. An introduction is defined as the importation of individuals of a given species into an area where it is not endemic. The nature and extent of genetic effects are determined primarily by the degree of reproductive isolation between the introduced and resident populations, the nature of the isolating mechanisms (pre- vs. postzygotic), and the relative sizes of the two populations.

We consider the introduction of the Pacific oyster *Crassostrea gigas* to mid-Atlantic waters and conclude that the genetic impacts of such an introduction are likely to be indirect only. The magnitude of such impacts will depend on ecological factors affecting the success of the introduction and cannot be accurately predicted at present.

KEY WORDS: introductions, hybridization, oyster, *Crassostrea*

Attempts to predict the biological impact of an introduction have traditionally focused on the ecological dynamics of competition and predation, or the concomitant introduction of parasites or disease organisms. We focus here on a subject that has received less attention, the genetic effects of introductions and transfers on native populations. These may be broadly defined as direct or indirect changes in the genetic composition of an endemic population attributable to the arrival and establishment of a non-native population. Direct effects occur when the gene pool of the native population is open to the introgression of genes from the introduced population. Indirect effects occur when hybridization between the native and introduced populations is not possible; alterations in gene frequencies result from ecological interactions with the introduced organism.

Three considerations are important for assessing the genetic impacts of introductions and transfers: time scale, the ameliorating role of natural selection, and the meaning of fitness. Immediate genetic effects—those evident in the first few generations following an introduction—may differ substantially from long-term effects. This is because natural selection continually acts to remove less adapted genotypes from a population. For example, the interbreeding of an introduced population with natives may at first lead to the production of poorly adapted hybrid progeny, thus lowering mean population fitness. Over time, however, natural selection will act to improve the mean fitness of the population, either by eliminating the alleles responsible for hybrid inferiority, or by favoring the development of reproductive isolation between the native and introduced populations. Finally, attributes that enhance biological fitness, the ability of an individual to survive and transmit its genes to the next generation, may not be desirable attributes from a human perspective. For example, genetic changes resulting in earlier reproduction or smaller adult size may increase fitness, to the chagrin of the human consumer.

In order to estimate the genetic effects of a particular introduction, we must consider two factors: 1) the strength of the barrier, if any, to gene flow between the native and introduced populations, and 2) the degree of genetic differentiation between them (Figure 1). We will consider three cases along this spectrum.

TRANSFERS

At one end of the spectrum are "transfers," which we define as admixtures of native and introduced populations belonging to the same biological species. Although the two populations may differ to some extent genetically, they readily interbreed. The genetic consequences of interbreeding will depend on the degree to which the introduced population differs from the native population.

If the species is characterized by the existence of locally adapted stocks or populations, the immediate result of introgression will be the disruption of coadapted gene complexes and a consequent reduction of fitness in the descendants of hybrid matings. Only when the number of animals introduced is large relative to the native population will this transient effect be noticeable. Following the transfer, natural selection will act to restore mean population fitness and form new coadapted gene complexes. After the winnowing action of natural selection, the native population may even reach a higher "adaptive peak" as favorable new genes contributed by the introduced population increase in frequency.

When the endemic population is small and locally adapted, as may commonly occur in terrestrial or island populations of organisms with restricted dispersal capacities, transfers may destroy the unique phenotype of the local population, even if overall population fitness is not compromised. The homogenizing effect of indiscriminate transfers is popularly labelled "genetic pollution," and results in the loss of interpopulation diversity and distinct local phenotypes. This concept is most appropriately applied to rare or

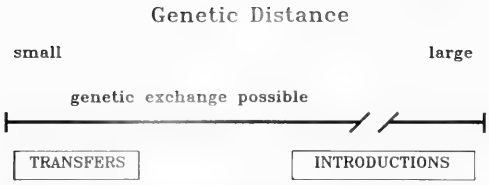

Figure 1. The continuum of genetic difference between populations in relation to reproductive isolation. Transfers involve freely interbreeding conspecific populations; introductions involve distinct species between which varying amounts of gene flow are possible.

endangered terrestrial vertebrates restricted to small isolated populations; it is not particularly apropos in the case of shellfish populations, which are typically very large and characterized by extensive gene flow between geographic regions.

In cases where the native population does not represent the pinnacle of adaptedness—for example, where rapid environmental change has outstripped the capacity of the population to respond genetically—the introgression of new genes may result in immediate benefits. Similarly, when the population possesses commercially undesirable characteristics, the influx of genes conferring a more desirable phenotype may be beneficial from the human perspective. Moav et al. (1978) demonstrated how the introduction of selected populations may be used for the genetic improvement of commercially exploited wild fish populations.

Perhaps the most important factor affecting the immediate genetic impact of a transfer is the size of the introduced population relative to the native population. In most cases, involving commercial shellfish, the transferred population is infinitesimal compared to the resident population, with the result that any immediate genetic impact, negative or positive, will be difficult to detect. However, if the transfer introduces beneficial genes to the native population, these will increase in frequency over time and a long-term positive effect may result from even a small transfer.

Transfers have been a regular practice in commercial shellfish populations for over a century (e.g., Ingersoll 1881, cited in Haven et al. 1978); indeed, Elton (1958) considered oyster culture to be "the greatest agency of all that spreads marine animals to new quarters of the world." Unfortunately, no clear evidence on the genetic impact of such transfers is available. Interpopulation crossing of *C. virginica* produced negligible or positive effects on larval survival (Newkirk 1978) or reduced larval survival (Mallet and Haley 1984). Juvenile growth (Mallet and Haley 1983) and survival (Mallet and Haley 1984) were higher in the progeny of interpopulation crosses than in the progeny of intrapopulation crosses. These limited results suggest that both immediate and long-term genetic effects of transfers will range from negligible to positive. However, as these authors noted, environment typically plays a larger role than genetics in overall performance, and genotype-environment interactions are common. The effect of a particular transfer is thus difficult to predict accurately without detailed information on the resident and introduced populations and their performance at the site of introduction.

INTRODUCTIONS: DIRECT GENETIC EFFECTS

An introduction is defined here as the importation of a species into an area where it is not endemic. The genetic effects of an introduction on an endemic species will be determined largely by the permeability of the barriers to interspecific hybridization. Although the classical biological species concept of Mayr (1963) defines species on the basis of reproductive isolation, there are many cases where good biological species produce hybrids, even under natural conditions. Contemporary species concepts (reviewed by Templeton 1989) more readily accommodate situations where reproductive isolation is less than absolute yet species nevertheless behave as distinct, cohesive evolutionary lineages.

When interspecific hybridization is possible, we must ask whether it is probable. This requires a careful consideration of the biology of the native and introduced species, and the nature of the mechanisms that effect reproductive isolation. Reproductive isolating mechanisms (RIMs) are conveniently categorized as pre- and postzygotic. Examples of the prezygotic RIMs range from behavioral differences that prevent interspecific mating (e.g., time of spawning) to gametic incompatibility. Postzygotic reproductive isolation occurs when hybrids are formed, but are less viable or sterile.

If the primary barrier to hybridization is prezygotic, direct genetic effects will occur when occasional breaches result in gene flow between the two species. The immediate results may range from detrimental to beneficial, while long-term effects—from the perspective of the organism, not the human consumer—may range from negligible to positive. As discussed above, the size of the introduced population and the extent of gene flow play key roles in determining the magnitude of short- and long-term genetic impacts.

If on the other hand the primary barrier to hybridization is postzygotic, then the mere presence of one species may impose a burden on the other. This occurs when the two species readily cross-fertilize, but the progeny show reduced viability or sterility, effectively resulting in gametic wastage. The possibility of wasted gametes becomes important when the introduction is massive, or if the introduced species is able to become established and attain high density. In this case, both species will lose gametes to the formation of interspecific hybrids. If the two species occupy the same niche and have no prezygotic RIMs, then the loss of gametes becomes critical, and one species may drive the other to extinction. This situation is analogous to the use of sterility induced by chromosomal rearrangements in insect population control (Foster et al. 1972). Which species wins the competition will depend on the population sizes and reproductive outputs of the two species. In practice, it is unlikely that two distinct species will occupy precisely the same niche; this, coupled with the widespread larval dispersal typical of shellfish, would likely lead to the stable coexistence of the two species in some areas, with other habitats supporting one or the other species only.

When interspecific hybridization does occur, the evolutionary dynamics of the hybrid and parental populations can be complex (e.g., references in Levin 1979). The fate of an introgressed gene depends not only on its fitness on the new genetic background, but also on the fitness of alleles at linked loci, and the rate of recombination between it and linked loci (Barton and Bengtsson 1986). Consequently, it is very difficult to predict the nature and extent of genetic changes in a recipient population due to the introgression of heterospecific genes.

INTRODUCTIONS: INDIRECT GENETIC EFFECTS

In the event the barrier to hybridization cannot be breached, the only genetic effects the introduced species may exert on the native species will be indirect, and will depend on the nature of interactions between the two species. Two different scenarios may be outlined: 1) The alien has only marginal success in becoming established. Its genetic effect on the native species is negligible. 2)

The alien becomes well-established, occupying a niche that overlaps partially with the native species. Ecological interaction in areas of sympatry will drive genetic changes in both species. The effects of such changes on the two gene pools will depend on the relative abundance and reproductive output of sympatric vs. allopatric populations, and on the amount of gene flow among populations of each species.

A Concrete Example: Crassostrea virginica *and* Crassostrea gigas

The continued decline of the American oyster (*C. virginica*) fishery in the mid-Atlantic region has raised the prospect of introducing the Pacific oyster (*C. gigas*) to areas which no longer support commercial harvests of the former (Mann 1979, Virginia Sea Grant 1990). At the same time, this notion is strongly opposed by those who fear dire biological impacts, in the form of introduced parasites or disease organisms, competitive exclusion or even "genetic pollution" of the American oyster. We leave the question of parasites, diseases and ecological impacts to others in this symposium, and address here the potential genetic effects of the introduction of *C. gigas* to the mid-Atlantic region.

The first issue to be resolved is whether any direct genetic effects are likely, i.e., what RIMs exist between the two species? Both eggs and sperm from one species are effective at stimulating spawning by the other species in the laboratory (Galtsoff and Smith 1932). Cross-fertilization also appears to occur readily in both directions (reviews in Menzel 1987, Gaffney and Allen in prep.). We have found no published data on the interspecific competitive abilities of sperm, but preliminary evidence indicates that the schedule of meiotic events is not altered in either species by heterospecific fertilization (Bernat and Gaffney unpubl., Scarpa, Allen and Gaffney unpubl.). Overall, it appears that prezygotic RIMs between the two species are very weak.

The question of postzygotic RIMs between the two species is problematic. The literature (see Menzel 1987 for review) is inadequate to settle this question, because hybridization experiments have rarely been followed by genetic verification (Gaffney and Allen 1991). Recent experimental data confirm the view that hybrids do not survive to metamorphosis (Allen and Gaffney 1991). Therefore it seems likely that introduced *C. gigas* would be capable of cross-fertilizing native oysters, and that the hybrids so formed would represent wasted gametes. In places where native oysters vastly outnumbered the introduced species, the loss of gametes would seriously hinder the spread of the latter. Any *C. gigas* zygotes formed during a mass spawning of the two species would probably be spread so thin after larval dispersal that they would be incapable of propagating a second generation by homospecific mating. In areas devoid of indigenous oysters, on the other hand, if ecological conditions were favorable and minimum critical densities were attained, an introduced species such as *C. gigas* might stand a good change of becoming established. Such areas could act as reservoirs from which larvae would be dispersed to sites where growth and survival were satisfactory, but reproduction effectively undermined by gametic wastage.

The Pacific oyster has been introduced repeatedly into eastern waters, including Maine (Dean 1979), Massachusetts (Galtsoff et al. 1950, Dean 1979, Hickey 1979), Long Island Sound (Dean 1979), the Chesapeake Bay (Cranston Morgan, pers. comm.) and several southern states (Galtsoff et al. 1950). Its failure to become established in these localities may be the result of the "gametic warfare" described above, rather than an inhospital environment, as it has been successfully introduced to a wide range of environmental regimes (Mann 1983).

Where the American oyster has been introduced to exotic waters, it has sometimes succeeded in establishing small but stable populations. Examples include Pearl Harbor, Hawaii (Brock 1960) and Boundary Bay, British Columbia (Elsey 1933, Quayle 1964). In Hawaii, there appears to have been no indigenous oyster adapted to the relatively limited estuarine habitat present there, and the establishment of *C. virginica* followed the planting of almost 40,000 oysters at the end of the nineteenth century (Brock 1960). This population persists today (John Ewart, pers. comm.). In British Columbia, the only indigenous oyster is *Ostrea lurida;* oysters of the genus *Ostrea* are generally incapable of cross-fertilizing *Crassostrea* species (Davis 1950, Menzel 1987). In any case, by the time *C. virginica* was introduced there, the native oyster population was severely depleted. Repeated introductions beginning at the turn of the century eventually resulted in the establishment of extensive American oyster beds in two small tributaries of Boundary Bay (Elsey 1933). It is possible that the introduction of the Pacific oyster at about the same time may have limited the subsequent spread of the American oyster on the west coast of North America, by either genetic (i.e., "gametic warfare") or ecological interactions. The apparent persistence of *C. virginica* populations as discrete entities coexisting with sympatric populations of *C. gigas* (Bourne 1979) is further evidence against the likelihood of successful hybridization in nature.

In conclusion, we believe on the basis of presently available data that the introduction of *C. gigas* to mid-Atlantic waters is unlikely to have any direct genetic effects on native oyster populations. Indirect genetic effects might occur if the Pacific oyster succeeded in becoming established; the magnitude of such effects could range from negligible to extensive, depending on the nature of ecological interactions between the species. Our current understanding of the ecology of bivalve introductions does not allow us to predict confidently the nature or extent of any such indirect genetic effects.

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AVOIDING THE TRANSMISSION OF DISEASE IN COMMERCIAL CULTURE OF MOLLUSCS, WITH SPECIAL REFERENCE TO *PERKINSUS MARINUS* (Dermo) AND *HAPLOSPORIDIUM NELSONI* (MSX)

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ABSTRACT Epizootic mortalities of oysters in the United States and Europe over the last several decades have stimulated a great deal of concern over the potential spread of disease-causing agents by introduction or transfer of molluscs in commerce. Whereas there is good evidence for the spread of some pathogens in this manner, especially those that are demonstrably contagious, evidence for others is purely circumstantial. When making decisions concerning shipments of stocks, shellfish regulators, managers, biologists, and industry members must critically evaluate such evidence, and add to it all other available information about the disease and its causative agent. Rational decision-making should consider biological information on life cycles and transmission of the pathogens, their distribution patterns in enzootic waters, environmental limits to their spread or survival, and a knowledge of the history of the animals to be shipped. In the United States, two major oyster pathogens, exhibiting distinctly different biological characteristics, are used to illustrate problems and to provide advice, concerning potential transfer of disease agents. *Perkinsus marinus*, cause of Dermo disease, is a highly contagious pathogen with a documented history of spread through movement of oysters. Until 1990, it had not become epizootic in northern estuaries (Delaware Bay and north) despite repeated large scale introductions from southern areas (Chesapeake Bay and south). Coincident with abnormally high winter temperatures from 1990 through 1992, *P. marinus* was reported as far north as Cape Cod, and caused an epizootic in Delaware Bay, underscoring the probable influence of temperature in control of this parasite. *Haplosporidium nelsoni*, cause of MSX disease, has not been demonstrated to be contagious and oysters can become parasitized in the absence of nearby infected oysters. Its spread has not been convincingly linked to transfers of oysters. Decision-makers are urged not to dwell solely on the "unknowns" in molluscan disease situations, but to make full use of what *is* known about the diseases, their causes and controls.

KEY WORDS: disease, introduction, mollusc, oyster, *Haplosporidium nelsoni*, *Perkinsus marinus*

INTRODUCTION

The documented, suspected, and potential transfers of disease-causing organisms in transplantations and introductions of commercially valuable molluscs have received considerable attention over the past two decades (Mann 1979, Rosenfield and Kern 1979, Andrews 1980, Elston et al. 1986). Since the middle of the twentieth century, concern over possible introduction of disease has been stimulated by epizootic mortalities associated with previously undescribed pathogens in several species of oysters on the east coast of the United States and in western Europe (Andrews 1980).

In response to these and other disease problems in marine species, the Working Group on Disease of the International Council for the Exploration of the Seas (ICES) established criteria for the introduction of exotic species, which are designed to limit the spread of disease (Sindermann and Lightner 1988). The guidelines specify that broodstock must be quarantined prior to and during spawning, and subsequently destroyed. First generation progeny can be transplanted to the natural environment if no diseases or parasites become evident in quarantine. When an introduced or transferred species is part of current commercial practice, ICES recommends periodic inspection of material (including microscopic examination) by the receiving country prior to mass transplantation. Each shipment must be inspected upon arrival and quarantined or disinfected whenever possible or appropriate. Importation must be immediately discontinued if inspection reveals any introducible pests or diseases.

In the United States, several conferences have considered the overall problems surrounding the introduction of exotic species and the movements of shellfish in commerce, and have attempted to standardize regulations of the various states affected. Austin Farley and Fredrick Kern of the U.S. National Marine Fisheries Service have proposed the establishment of shellfish management zones and embargo areas based on the known distribution of infectious diseases, parasites, predators, pests, and competitors (Proceedings of a Shellfish Relocation Conference, Marine Biological Laboratory, Woods Hole, MA. February 3–4, 1982). Movement of species between zones would require approval by a "controlling authority" and transfers between embargoed areas would, in addition, be permitted only after thorough assessment of the proposed transfer, including a review of the biology of the species and associated organisms, and compliance with the ICES recommendations, including inspection by a certified laboratory.

Despite efforts to establish uniform regulations for the transfer of native species, shellfish are commonly shipped between areas of the United States without concern for potential disease transfers—as they have been for centuries. In other instances, it may be impractical to follow ICES recommendations because of the expense and time required to provide the needed information. For example, movement of seed stocks from areas of high natural setting to other areas for growth and conditioning, or relays from condemned to clean water, are rarely accompanied by inspection for disease agents. Some states have no regulations and many that do are lax in enforcement. Some shellfish growers are unaware of the potential risks or willfully ignore the rules. Managers and regulators are often caught between the desire to foster shellfish industries that rely on transfer of animals and fear of allowing

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introduction of a pest or pathogen that could result in catastrophe. They almost always are forced to make decisions with too little information.

A decade ago, Matthiessen (1979) stated that "many decisions made by regulatory authorities relating to the importation of shellfish inevitably will be made on the basis of best guess rather than fact." This statement is true today. Some of the guess work is because we don't completely understand the biology of the parasites and their hosts, but some is because individuals making decisions (whether regulator or industry member) are not aware of what *is* known about them. In this situation, the scientist can be most helpful by evaluating available information as accurately as feasible, by presenting it as clearly as possible, and by taking pains to distinguish between fact and speculation (Bowden 1979, Mann 1979).

Evidence implicating shipments of molluscs in the spread of disease is convincing in some cases. For instance, the spread of *Bonamia ostrea,* a parasite of the flat oyster *Ostrea edulis* (Linnaeus, 1970) (Grizel et al. 1988), can be followed along a documented path tracing introductions of host and parasite from the east coast of the United States to the west coast and then to Europe (Elston et al. 1986, Farley et al. 1988). The linkage, however, is not in itself sufficient evidence. What fortifies this argument is the fact that *B. ostrea* can be transmitted directly from oyster to oyster (Poder et al. 1982).

Much of the evidence for transmission of disease along with movement of molluscs, however, is circumstantial. The outbreak of Malpeque Bay disease of oysters, *Crassostrea virginica* (Gmelin, 1791), in Prince Edward Island in 1914–15 was preceded by transplantation of oysters from New England, which first took place on a large scale just before the mortalities occurred (Needler and Logie 1947). Nevertheless, Fraser (1938) reported that direct inoculation of material from sick to healthy oysters failed to cause disease symptoms, and the disease was unknown in New England, although the oysters there may have been resistant.

Two diseases of oysters appeared in France shortly after the introduction of Pacific oyster, *Crassostrea gigas,* (Thunberg 1793) seed. Gill disease of the Portuguese oyster, *Crassostrea angulata* appeared in late 1966 in an area of southwestern France where *C. gigas* had been introduced at approximately the same time (Grizel and Héral 1991). The disease, caused by a virus, almost completely destroyed *C. angulata* culture in France. Aber disease, caused by the protozoan *Marteilia refringens* (Alderman 1979, Balouet 1979), appeared in Brittany in 1968, in an area where Pacific oysters were being held, and subsequently caused extensive losses of the flat oyster, *Ostrea edulis* (Andrews 1980). *Marteilia* sp. has been found occasionally in *C. gigas,* (Cahour 1979), but experimental transmission (between or within the two oyster species), has never been successful (Balouet et al. 1979, Figueras and Montes 1988).

Two important protozoan parasites of oysters have been responsible for catastrophic mortalities of *C. virginica* on the Gulf and East Coasts of the United States over the past forty to fifty years. The recognition of *Perkinsus marinus* (Mackin, Owen, Collier 1950) as the cause of Dermo disease in southern estuaries and *Haplosporidium nelsoni* (Haskin, Stauber, Mackin 1966) as the cause of MSX disease in the mid-Atlantic estuaries has spurred most of the concern over the spread of shellfish disease in the United States. Many of the greatest worries of industry members, state regulatory officials, and biologists in the United States center on the very real and immediate problems caused by these two pathogens. To illustrate some problems commonly faced by these individuals, I'd like to cite some specific concerns about potential spread and control of MSX and Dermo diseases. The questions are of immediate practical importance and they illustrate what we do and do not know concerning these diseases as they impact movement of the shellfish:

1. What is the evidence for introduction of *Perkinsus marinus* and *Haplosporidium nelsoni* by oyster transport?
2. Can the pathogens be transmitted in hatchery-produced larvae or small seed?
3. Can the pathogens be spread through overboard disposal of contaminated meats, shells, or other wastes by processors, dealers, restaurants, or consumers?
4. Can the pathogens be transmitted to and from other species?
5. Are there methods for treating small lots of oysters (broodstock, larvae, small seed) to eliminate pathogens?

EXAMPLES

1. What is the Evidence for Introduction of Perkinsus marinus and Haplosporidium nelsoni by Oyster Transport?

One of the well-documented, but unpublished, instances of transmission of a disease-causing organism affecting molluscs occurred in the early and mid 1950s in Delaware Bay. Because the supply of native seed was low during this period, many Delaware Bay planters bought "seed" oysters from private leases in the Hampton Roads area and other higher salinity regions of Chesapeake Bay where *P. marinus* was present and causing heavy losses (Andrews 1988). Infected oysters were brought by the shipload for planting in Delaware Bay (H. Haskin, Haskin Shellfish Research Laboratory, personal communication, 1989).

A survey conducted by Rutgers University in 1955 and 1956 found evidence that the disease had spread from the imported to native oysters (Christensen 1956). The highest prevalences of *P. marinus* were in the oysters brought from Virginia and in the native oysters growing close to them. Prevalences were negligible on the seed beds and on the eastern edge of the planting grounds. Prevalences and intensities of infection were low compared to those in fully epizootic areas of Virginia and the Gulf of Mexico (Andrews and Hewatt 1957, Mackin 1962) and there were no reports of heavy mortalities in Delaware Bay (Christensen 1956).

Although the proximity of imported oysters to infected native oysters was highly suggestive of transmission, lack of monitoring for the period before introduction precluded a clear assessment of the origin of *P. marinus* in native stocks. Within two years of this survey (spring 1957), however, the epizootic caused by *H. nelsoni* (MSX) had begun (Haskin et al. 1966) and all imports and exports into and out of Delaware Bay were embargoed. Intensive monitoring in 1958 and 1959 to determine the cause of the epizootic failed to show significant presence of *P. marinus* (unpublished records of this laboratory).

We interpreted these observations as evidence that *P. marinus* was introduced into Delaware Bay and sustained by importations of infected oysters from lower Chesapeake Bay, but was unable to maintain itself once that source was stopped (Ford and Haskin 1982). Andrews (1988) pointed out that *P. marinus* also disappeared from major planting areas in the lower Chesapeake after the MSX epizootic of 1959–1960 killed most of the oysters there. The

same occurrence in Delaware Bay between 1957 and 1959 undoubtedly contributed to the elimination of *P. marinus* in that estuary, but in contrast to Chesapeake Bay, *P. marinus* never reappeared to cause problems in Delaware Bay, even after intensive plantings of native seed resumed in the late 1960s and 1970s (Haskin and Ford 1983). Rather, low temperature was considered to be the controlling factor in the failure of *P. marinus* to persist in Delaware Bay or to become epizootic north of Chesapeake Bay (Christensen 1956, Andrews and Hewatt 1957).

In the summer of 1990, *P. marinus* was found in oysters at a number of sites in Delaware Bay (Ford, unpublished) where it caused localized epizootics. In 1991, the disease intensified causing severe mortalities over much of the New Jersey portion of the Bay. Coincidentally, temperatures in the Delaware Bay area during 1990 and 1991 were among the highest on record (U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Climatological Data for New Jersey). The 1990–91 epizootic was not linked with large-scale transplants of infected oysters and, in fact, an apparent focus of infection appeared on the New Jersey seed beds where oysters would never have been introduced. At the same time, infected oysters were found at several locations on the Atlantic coast of New Jersey, including Raritan and Manasquan Bays (W. J. Canzonier, Maurice River Oyster Culture Foundation, personal communication) where oyster industries have not existed for many years. Our current interpretation of these data is that pre-existing non-lethal infections in a few native oysters, or introductions by transient ships or overboard disposal (see below), were stimulated to proliferate and spread by unusually warm temperatures. It is significant that the previous incursion of *P. marinus* into Delaware Bay in the 1950s, which occurred during a more typical temperature regime, never caused epizootic mortalities and disappeared after importation of infected seed was stopped. We expect that a return to more normal temperatures will attenuate the cycle of parasite proliferation, host death (releasing infective forms), and reinfection of new hosts, but its effective disappearance (not causing mortalities or being detectable through routine sampling) will probably require unusually low temperatures. It is not yet clear, however, whether the critical controlling temperatures occur in the winter or summer, or both (Ford and Tripp 1992).

It is much more difficult to evaluate evidence of possible introduction of MSX disease because the complete life cycle and means of transmission of its etiologic agent, *Haplosporidium nelsoni*, are not known. Nevertheless, there is information available of use to those making decisions about possible introduction of this disease into non endemic areas.

After the first outbreaks of MSX disease in Delaware and Chesapeake Bays in the late 1950s, considerable effort was put into elucidating the life cycle of *H. nelsoni* and in trying to transmit the parasite experimentally. None of these experiments resulted in transmission, but most of them have involved the plasmodial stage of the parasite (Canzonier 1968, 1974). Few have used the spore stage (Andrews 1979), which is most likely involved in transmission, but which has been reported only rarely in oysters. Most researchers have concluded that another host may be involved in the life cycle (Farley 1965, Andrews 1968, Ford and Haskin 1982, Haskin and Andrews 1988). Recently, Barber et al. (1991) have found that sporulation may occur regularly in spat (oysters under a year of age) if infections reach the advanced stage. Andrews (1979) also reported heavy spore production in a single group of spat in Virginia in 1976. These observations have led us to consider the possibility that direct transmission from oyster to oyster may indeed occur, with the source of infective stages being very young oysters in which spores are produced.

For several years before the first MSX epizootic in 1975, Delaware Bay planters had been importing large quantities of seed oysters from the seaside bays on the eastern shore of Virginia (N. Jeffries Sr., personal communication), as well as from the lower Chesapeake. When the first oyster disease survey was initiated in that region in mid-1959, *H. nelsoni* was found, although a newly discovered, related species, *H. costale* (Woods and Andrews 1962) (cause of SSO disease), was more prevalent in these high salinity waters (Andrews et al. 1962, H. Haskin, personal communication). Andrews (1968) speculated that a new and virulent "race" of *H. nelsoni* may have developed by "interbreeding" of *H. costale* and *H. nelsoni* when the imported oysters were moved into the lower salinity waters of Delaware Bay. A simpler hypothesis also presupposes that *H. nelsoni* was enzootic to the seaside of Virginia, but was masked by the better adapted (to high salinity) *H. costale*. If spores of *H. nelsoni* were present in the huge numbers of young, rapidly growing oysters moved into the Delaware Bay, then they, rather than a hybrid strain, might have initiated the epizootic once *H. nelsoni* was in a more favorable salinity.

Outbreaks of MSX disease in at least two areas on Cape Cod have followed importation of seed oysters from areas where *H. nelsoni* was present (Krantz et al. 1972, Haskin and Andrews 1988), but it is equally significant that other outbreaks have occurred in the absence of any known importations. Notable among these was the initial epizootic in Chesapeake Bay in 1959, which occurred in the midst of "native [James River] transplants no different from beds in surrounding areas [which did not experience mortalities]" (Andrews 1968). Seed oysters were not moved into this area from seaside bays (J. D. Andrews, Virginia Institute of Marine Science, personal communication). Mortalities caused in 1983–85 by MSX in Oyster Bay, Long Island, a location totally controlled by one company, were not associated with imports (D. Relyea, F. M. Flower and Son Oyster Co., personal communication to H. Haskin) nor were outbreaks in North Carolina in 1988 (M. Marshall, North Carolina Division of Marine Fisheries, personal communication, 1989). Also relevant is that grounds on the Delaware side of Delaware Bay, heavily planted with Chincoteague Bay seed between 1953 and 1957, did not experience losses due to MSX disease until the spring of 1958, a full year after epizootic mortalities had begun on the New Jersey side (N. Jeffries, Sr., personal communication, 1989).

The link between movement of infected oysters and outbreaks of MSX disease is thus quite tenuous compared to that for *P. marinus*. In addition to its introduction into Delaware Bay in the 1950s, the latter has been spread around Chesapeake Bay by transplants of infected seed (E. Burreson, personal communication in (Andrews 1988)). In contrast, there are as many examples of *H. nelsoni* appearing in areas with no known history of introductions or transfers as there are cases with connections, although many undocumented transfers of oysters are undoubtedly made.

2. Can the Pathogens be Transmitted in Hatchery-produced Larvae or Small Seed?

The ICES measures designed to reduce risk of disease introduction involve the quarantine of broodstock. A recent report sug-

gested that a parasite of bay scallops, *Argopecten irradians*, which was identified as *P. karlssoni* and was reported to occur in scallop eggs, might undergo vertical transmission (McGladdery et al. 1991). *Perkinsus marinus* has never been reported to occur in oyster eggs, although it does survive intracellularly in hemocytes. Greater concern exists that larvae could become infected by the adults during spawning in a hatchery. Although there is no absolute reason that larvae could not become infected by *P. marinus* in this manner, it has never been reported and there are a number of biological reasons why it is unlikely. The presumed site of infection by *P. marinus* is the digestive tract (Mackin 1951) and the spawning stock would normally be removed from contact with the embryos long before the latter developed into veligers (18–24 hr) with the capacity to feed. If viable infective particles were discharged during spawning and survived in sufficient numbers until the larvae were capable of ingesting them, the larvae theoretically could become infected. There is, however, no reason to believe that lightly infected oysters would discharge *P. marinus* cells during spawning, and heavily infected individuals will not spawn because they do not produce gametes (Mackin 1962). Thus, the chances of larval contamination, although possible, are extremely slight. Hatchery operators could minimize the possibility by thoroughly cleaning the shells of parent stock, including placing the oysters in dilute (0.3%) hypochlorite solution for 15–20 minutes to kill epibionts that might harbor *P. marinus* cells and removing parent oysters from spawning containers as soon as they have spawned. As a further safeguard, broodstock could be screened for systemic *P. marinus* by non-destructive blood diagnosis (A. Farley, Oxford Cooperative Laboratory, personal communication 1989; Gauthier and Fisher 1990) before selecting spawners.

Because of the life cycle and transmission considerations already discussed, there is no danger that larvae could acquire *H. nelsoni* from infected broodstock. The parasite has never been observed in eggs and, as a matter of fact, is typically extracellular. Spat, which might be carrying spores capable of producing infective stages, are hardly likely to be chosen as broodstock, and oyster-to-oyster infection does not occur from plasmodia. As with *P. marinus*, cleaning of shells (to remove potential alternate or intermediate hosts) and screening for the presence of systemic *H. nelsoni*, would be added safety measures.

Juvenile oysters (spat) can become infected with either pathogen, but because they "pump" much smaller volumes of water than do adult oysters, their chances of encountering either of these water-borne parasites is considerably reduced. If the juveniles are maintained in an on-shore nursery where water flow is restricted compared to the field, their chances of becoming infected would be further reduced. The potential for seed being infected is thus a combination of their size, the length of time they have been "exposed," and the concentration of infective particles in the water surrounding them. We cannot presently measure the abundance of either pathogen in water samples, but inferences as to relative abundance can be made based on the history of infections in the immediate area.

3. Can the Pathogens be Spread through Overboard Disposal of Contaminated Meats, Shells, or Other Wastes by Processors, Dealers, Restaurants, or Consumers?

There is no conclusive evidence of which I am aware that any molluscan disease-causing organism has been transmitted through shucking wastes or shell transplants; however, Andrews (1980) cites a case involving the presumed introduction of a sacculinid parasite (*Loxothylacus panopaei*) into Chesapeake Bay. This parasite, which devastated two species of mud crab in the mid 1960s, may have been introduced in shipments of oysters from the Gulf of Mexico brought "to Virginia for shucking at waterside plants where shells and wastes were discarded near native oyster beds."

We do know that *P. marinus* can be very easily transmitted in a laboratory simply by water splashing from a tank holding infected animals (W. J. Canzonier, personal communication, 1989) and that any stage is infective (Andrews 1988). We also know that oysters with high levels of *P. marinus* appear glassy and emaciated, and might well be discarded (overboard) by shuckers, as would infected gapers (dead oysters). On the other hand, injection experiments with measured numbers of *P. marinus* cells indicate that a threshold inoculum is required to initiate infection and cause mortality (Mackin 1962). In the laboratory, a relatively small number of infective cells may initiate an epizootic because of the limited volume of water and the high density of oysters involved making the chance that each infective particle will come into contact with an oyster very high. If wastes are disposed of in an areas with restricted circulation where oysters are present nearby (within several hundred yards) in relatively large numbers, the chance of transmission is high. That possibility would be reduced if infective stages from wastes were diluted before they contact a host, either because of flushing patterns or distances of oysters from the disposal site. Andrews (1988) found that isolation of oysters by as little as 15 m substantially delayed the transmission of *P. marinus*, although transmission over longer distances is possible.

Between 1986 and 1989, when local oysters were scarce, several shucking plants bordering the Maurice River, a New Jersey tributary of Delaware Bay, processed oysters from the Gulf of Mexico and the Chesapeake Bay, where *P. marinus* was enzootic. During the initial stages of the 1990 epizootic in Delaware Bay, very high prevalences of *P. marinus* were found in oysters growing in the river adjacent to the shucking houses. We do not think that *P. marinus* was necessarily re-introduced into Delaware Bay by this means because of other apparent infection foci in the Bay and along the New Jersey Atlantic coast (see above), but the intensity of the early outbreak near the shucking houses suggests that a combination of waste disposal and suitable temperature may have stimulated a localized epizootic in the river.

The proximity of processing plants to oyster populations and the characteristics of the water into which they are discharging wastes should be considered in assessing the potential for transmission of *P. marinus* in this manner, but because of the extremely contagious nature of this disease, processors should be encouraged not to dispose of fresh shucking wastes overboard in non-enzootic areas if oyster populations exist nearby. Additionally, appropriate means for treating *P. marinus*-contaminated wastes should be investigated (Goggin et al. 1990).

Transmission of *P. marinus* via the movement of shells from shucked infected oysters is less likely, but probably not impossible. Andrews and Hewatt (1957) reported survival of *P. marinus* (i.e., it could be cultured in fluid thioglycollate) after infected tissues had been frozen or dried, although the authors did not attempt transmission with material that had been subjected to freezing or drying. Also to be considered is the possibility that carriers such as the parasitic snail *Boonea impressa* (White et al. 1987), crabs, oyster drills, polychaetes, etc. (Table 1) might survive for extended periods in the interior of shell piles, particularly during cool weather, and infect oysters when reintroduced into the

TABLE 1.

Organisms in which *Perkinsus marinus*, or *Perkinsus*-like cells culturable in fluid thioglycollate, have been identified.

Transmission to Oysters Demonstrated	Cells Found in/on Scavengers	Perkinsus-Like Cells in Bivalves
Boonea impressa[W]	*Opsanus tau*[H]	*Mercenaria mercenaria*[A]
Gobiosoma bosci[H]	*Chasmodes bosquianus*[H]	*Macoma balthica*[A]
Ostrea lurida[(U)R]	*Urosalpinx cinerea*[H,C]	*M. phenax*[A]
	Neopanope texana[H]	*M. tenta*[A]
	Rhithropanopeus harrisii[H]	*Tagelus plebeius*[A]
	Nereid worms[C]	*Mya arenaria*[A]
		Mulinia lateralis[A]
		Anomia simplex[A]
		Anadara transversa[A]
		Laevicardium mortoni[A]
		Ensis minor[A]
		Lyonsia hyalina[A]
		Ostrea frons[R]
		O. equestris[R]
		Crepidula fornicata[R]
		Argopecten irradians[R,M]

[A] (Andrews 1955)
[C] (Christensen 1956)
[H] (Hoese 1963)
[M] (McGladdery et al. 1991)
[R] (Ray 1954)
[(U)R] (Ray 1954)
[W] (White et al. 1987)

water. It is unlikely that more than a few organism would survive for long in this environment and, further, Andrews (1988) considers that scavengers do not carry sufficient infective stages of *P. marinus* to make "major contribution to the high dosage necessary to produce infections."

The concerns discussed above apply also to overboard disposal of infected oysters, or their remains, by restaurants, seafood markets, or consumers. Such disposal is practically impossible to prevent except by education, but is likely to introduce only a small amount of infective material.

Transmission of *H. nelsoni* by this means is far less likely than for *P. marinus*. As mentioned already, *H. nelsoni* has proved impossible to transmit in the laboratory, whereas special care must be taken to prevent contamination by *P. marinus*. We are confident that plasmodial stages of *H. nelsoni*, even when injected or transplanted into recipient oysters, cannot initiate infections (Canzonier 1968, 1974; Ford unpublished). Thus overboard disposal of whole animals or tissues infected with only this stage (which is by far the most common form in oysters) could not be a source of infective stages for oysters. The fact that most processors, distributors, and consumers would not be dealing with spat minimizes the potential for distributing spore stages from young oysters; on the other hand, shuckers would not open spat on shells of market-sized oysters and they might be discarded overboard.

4. Can the Pathogens be Transmitted to and from Other Species

Perkinsus-like organisms (i.e., those that culture in fluid thioglycollate) have been found in many North American species other than oysters (Table 1). Some of these species, like the gastropod *Boonea impressa*, carry *P. marinus* that can infect oysters (Hoese 1963, White et al. 1987). Most, however, appear to carry related, but not identical, organisms. Ray (1954) and Andrews (1955) reported finding them in many species, but always in very low abundance. Attempts at cross-species transmission between oysters and *Mercenaria mercenaria* (Linnaeus, 1758) *Macoma balthica* (Linnaeus, 1758), and *Mya arenaria* (Linnaeus, 1758), by direct inoculation or feeding, failed in nearly all cases (Ray 1954, Andrews and Hewatt 1957). The same techniques easily transmit the parasite between oysters. Apparent invasion of *M. mercenaria* tissues did occur at the site of injection, but no parasites spread from there. Several *M. arenaria* did become infected when injected with material from infected oysters (Ray 1954). Presently available evidence indicates that the chances are remote of transmitting the oyster parasite by moving other commercially important bivalves, such as clams, in which thioglycollate-culturable organisms have been found.

Although *H. nelsoni* has never been found in any species other than the eastern oyster, members of the family Haplosporidiidae parasitize a variety of marine invertebrates. Until the complete life cycle of *H. nelsoni* is known, the possibility that the pathogen exists in, and is spread by, another host must be considered very real.

5. Are There Methods for Treating Small Lots of Oysters (Broodstock, Larvae, Small Seed) to Eliminate Pathogens?

Both *P. marinus* and *H. nelsoni* are found primarily in the higher salinity portions of estuaries, where salinities are between 15 and 30 parts per thousand (ppt). At temperatures of 20°C or more it has been shown that *H. nelsoni* can be eliminated from infected oysters if they are submerged for two weeks at salinities below 10 ppt (Ford 1985). The use of low-salinity immersion to clear *H. nelsoni* infections from broodstock or seed would appear to be a very inexpensive and practical means for reducing the risk of transmitting this parasite through aquacultural practices. Additional research is needed, however, to pinpoint the exact time-temperature-salinity requirements needed to assure complete elimination of the parasite.

P. marinus cannot be cleared under similar conditions as it is much more tolerant of low salinity than is *H. nelsoni* (Andrews and Ray 1988). There are currently no anti-protozoal agents known to be effective and practical in ridding oysters of *P. marinus*. Ray (1966) demonstrated that exposure of infected oysters to cycloheximide reduced disease levels, but when the "treatment" was stopped, even after 164 days, the parasite recovered and again started causing deaths.

SUMMARY AND RECOMMENDATIONS

Catastrophic losses caused by oyster pathogens over the last several decades have justifiably frightened persons concerned with shellfish transfers. In attempting to prevent the spread of disease, most individuals, particularly regulators, are extremely conservative. While caution is appropriate, over-reactions, sometimes approaching paranoia, can result if those responsible are ignorant of, or are reluctant to emphasize, biological knowledge in their decision-making.

It is not sufficient to conclude that a disease agent has been introduced through transfer of the host species simply because it has been newly discovered in a particular location (or something resembling a known pathogen has been found in tissue sections or culture media). Even when mortalities associated with a parasite

occur suddenly after transfer of host species, there may be alternate explanations. For instance, review of the vast numbers of species moved about the world in ballast water or on the bottom of ships (Carleton, this volume?) suggests that some potential introductions may be well out of the immediate control of shellfish regulators! A good example is the recent finding of *P. marinus* in native oysters in Raritan Bay (see above). Because of water pollution, an oyster industry has not existed for more than half a century (H. Haskin, personal communication, 1989) and it is difficult to believe that oysters would have been imported into this area by commercial shellfish growers, but there is heavy boat traffic through the area.

Alternatively, a parasite may have existed in limited numbers, and gone undetected, in areas where environmental or culture conditions prevented its development to epizootic proportions. If those conditions change, even temporarily, the parasite may multiply to a critical threshold that results in an epizootic. Further, parasites, like their hosts, experience unexplained long-term natural cycles in abundance. A host species may be harvested for the first time when it is at peak abundance, at which time the abundance cycle of a major pest or parasite is at an ebb. Later, when the pest or parasite becomes abundant enough to detect (usually when it causes mortalities), it may be considered "new."

In addition to a critical evaluation of these kinds of observations, rational decision making will take into account all available information on the diseases and their etiological agents. These include:

1. What is known of the life cycle and method of transmission of disease agents? For instance, it would be unwise to introduce animals from areas known enzootic for contagious pathogens such as *Perkinsus marinus* or *Bonamia ostrea*. There is somewhat less cause for concern in the case of agents that are not contagious (i.e., host species do not require proximity to infected individuals of the same species to become infected) such as *Haplosporidium nelsoni* and *Marteilia refringens*. In cases where direct transmission has not been demonstrated, much greater attention should be paid to possible introductions of other hosts in shipments of wild seed. Our recent findings concerning spores of *H. nelsoni* in oyster spat do, however, dictate caution in transferring young oysters (which are precisely the ones most likely to be shipped) from regions where MSX disease is enzootic.
2. What information is available about the distribution of the disease agents in known enzootic water? For instance, *H. nelsoni* is distributed fairly evenly over wide areas and can move miles up estuary during a drought (without concurrent transplant of oysters). It thus makes little sense to ban movement of oysters within an estuary, or even between subunits of the same general water system, to prevent the spread of *H. nelsoni* in an area where it already exists. The presumed infective stage of this parasite is a spore, which may last for years outside the host and be transported great distances in the water or in vectors. In contrast, *P. marinus* may take several years to move naturally from one location in an estuary to another. For instance, certain regions of Delaware Bay remain free of the disease. If the disease persists, experience from other areas indicates that it will eventually spread to all oyster-growing areas of the lower estuary, but moving infected oysters would only hasten this process and might introduce it to areas that would remain disease free until the return of more normal temperatures, which should inhibit its further spread.
3. What is known about environmental constraints, especially salinity and temperature? Is it likely that the pathogen could survive and/or cause damage in the new environment? *Perkinsus marinus* was introduced in tremendous quantities into Delaware Bay over several years, yet failed to cause serious problems at the time and effectively disappeared after importation of diseased oysters ceased. Historically, southern oysters, presumably carrying the same pathogen, were repeatedly shipped to New England without introducing detectable levels of *P. marinus* (Andrews 1988). It is probable that low temperature prevented the development and spread of the parasite in these areas, but some low-level parasitism may have persisted over many years and provided a source of infective material that caused the recent outbreaks in New Jersey when environmental temperatures became favorable for the parasite. A similar origin can be argued for the *P. marinus* recently found in several Cape Cod estuaries (E. J. Lewis, Oxford Cooperative Laboratory, personal communication, 1991) where it was previously undetected and where there are stringent prohibitions against introductions of southern oysters.
4. What is known about the history of the animals to be moved and the area from which they originate? Histological examination is often a prerequisite to such shipments, and is reasonable, but it should be clearly understood that **it is impossible to "certify" them as being "disease or pathogen free."** A "negative" rating simply means that in that particular sample of animals collected at a certain place and time, and in the subsample of tissues examined, no recognizable pathogens were found by the diagnostic method(s) used. Subpatent infections are common in certain seasons and in resistant or tolerant animals. Thus, it is critical that the histological examination be accompanied by a background profile of the animals to be shipped.

Clearly, indiscriminate shipment of molluscs, particularly large quantities of wild stocks, is unwise if not downright foolish. Long distance shipment of commercial species is currently more likely to be to or from a hatchery than from the wild, so that ICES guidelines can be followed to a much greater extent than previously. Further, the costs of hatchery produced seed and the relatively large investment in the shellfish as they are grown under intensive culture makes the aquaculturist much more wary of possible disease problems than were earlier planters who had vast reserves of plentiful and cheap natural seed.

Even after taking into account all possible known factors, we will still be faced with unanticipated or unknown elements that could confound our best judgement. Yet, we should avoid making decisions based solely on what might conceivably happen if our worst fears come true. Rather, we should decide using the best available information, while assessing potential risks and benefits. Above all, we should dwell less on the "unknowns," and make more rational and complete use of what we do know.

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CONTENTS

PREDATION BY THE RED ROCK CRAB *CANCER PRODUCTUS:* THE ROLE OF CRAB DENSITY, TIDE LEVEL, AND PREY SIZE ON PREDATION RATE. Bart C. Baldwin,* Sylvia Behrens Yamada, and **Heidi Metcalf.** Department of Zoology, Oregon State Univ., Corvallis, OR 97330.

The ecological role of predatory crabs on Pacific Northwest shores has not been well documented. We identified the red rock crab, *Cancer productus,* to be the most devastating crab predator on intertidal organisms. In previous experiments we attributed predation rates on tethered snails and mussels of over 80% per day to this species.

The object of the present study was to determine the impact *Cancer productus* has in structuring intertidal communities around San Juan Island, WA. This was accomplished by setting out a model hard prey organism that is common to the intertidal; the snail *Littorina sitkana.* The predation rate on two size classes of tethered snails at 2 tidal levels on 4 beaches was monitored for three days. Relative crab densities were determined for the 4 beaches by setting out crab rings during high tide at the 0 m tidal level with standardized bait.

Average predation rates on the 4 beaches ranged from 7 to 77% per day. These rankings were consistent for 3 days and were directly correlated with *Cancer productus* abundance ($r^2 = 94$; $p < 0.001$). Beaches with high crab abundance experienced high predation rates at both tidal heights, while beaches with low crab abundance experienced lower predation rates at the higher shore level. *Cancer productus* also showed a size preference for larger snails. These results explain why one only find a few very small prey organisms on beaches with high *Cancer productus* densities.

GEODUCKS, PREDATORS, AND VOLUNTEERS. J. H. Beattie, Washington Department of Fisheries, Point Whitney Laboratory, 1000 Point Whitney Road, Brinnon WA 98320, USA.

The geoduck enhancement project began in 1982. By 1987 the program had developed sand substrate nurseries. From 1987 to 1990, the hatchery/nursery produced 18 million seed.

The present area of concentration is field survival of planted geoduck seed. From the 18 million broadcast planted seed, survival after 2 to 3 years was less than 1%, only about a tenth of that expected. Field observations and laboratory experiments suggest that these losses are due to predation within the first year after planting. Predators include crabs, starfish and flatfish. Work in 1991 used volunteer labor for intertidal plants on four State Park beaches. Predator protection devices were made of PVC tubing and plastic screening. Resulting survival ranged from 20 percent to nearly 70 percent and averaged about 40 percent among the beaches tested. Present laboratory and intertidal and subtidal field experiments on predator protection devices include evaluation of tube composition, size, planting density and screening the tops of the tubes.

DOES STARFISH REMOVAL INCREASE MUSSEL PRODUCTIVITY? Sylvia Behrens Yamada,* Bruce A. Menge, Bart C. Baldwin, and **Heidi Metcalf.** 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 wave-exposed shores in the Pacific Northwest. *Mytilus californianus* form extensive beds, up to 1 meter deep, in the mid- but not the low intertidal zone. We explored the possibility of opening up the low intertidal zone to mussel production by 1) monitoring the growth of caged mussels in low and mid-intertidal cages and by 2) monitoring the survival of transplanted mussels in low intertidal starfish removal and control plots on the central Oregon coast.

The growth rate of mussels was greater in low than in mid-intertidal cages; and greater in wave exposed than in protected cages. 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.

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.

EFFECT OF STARVATION ON BLOOD-GLUCOSE AND TISSUE-GLYCOGEN LEVELS IN THE NORTHERN ABALONE *HALIOTIS KAMTSCHATKANA*. Thomas H. Carefoot,* P.-Y Qian, B. E. Taylor, T. G. West, and **J. Osborne.** Department of Zoology, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada.

As with any industry, product quality and marketability are important concerns in aquaculture. It is vital to know how various culture practices and feeding regimes influence meat quality in a cultured animal. The overall effect of starvation on glycogen reserves in *Haliotis kamtschatkana* has been investigated. 50 adult abalone were held without food for 27 days while an equivalent group was fed *ad libidum* on a natural kelp diet. Blood-glucose and tissue-glycogen levels were monitored throughout the experimental period. Starvation induced a marked decline in blood-glucose and glycogen reserves. After just 6 days of starvation blood-glucose was reduced by 50%, and glycogen stores in the digestive gland and muscles were reduced by 100% and 60% respectively. Glycogen reserves in the gonad, however, were not significantly decreased by 27 days of starvation. The influence of reduced tissue-glycogen and blood-glucose on palatability for human consumers is also reported.

GENETIC IMPROVEMENT OF DIPLOID AND TRIPLOID PACIFIC OYSTERS. Ken Cooper,* Taylor United, Inc., Shelton, WA and **Dennis Hedgecock,** U. C. Davis, Bodega Marine Lab, Bodega Bay, CA.

Production of farmed Pacific oysters along the west coast of the United States is based on hatchery seed. While hatchery technology provides a stable source of seed, it creates both potential pitfalls and opportunities for genetic improvement. We review research relevant to two fundamental methods by which oysters might be improved, selection, in which brood stock is chosen on the basis of performance and inbreeding followed by crossbreeding, in which crosses among inbred lines yield progeny showing hybrid vigor. We also review the use of triploids by the oyster industry and discuss how inbreeding and crossbreeding might be used to improve quality and performance.

Results from the WRAC project suggest that variation in growth to market size is heritable, yet, in our experiments, genetic differences were often obscured by environmental factors. Thus, methods developed in this project for creating pedigreed brood stock and estimating components of variation in growth are not yet adequate for a selection program. We are presently tripling the sizes of experimental crosses to evaluate 24 sires simultaneously.

The WRAC experiments found significant variation in growth resulting from particular sire-by-dam combinations. This observation is consistent with other studies suggesting that growth in bivalves shows hybrid vigor. Thus, a proposed continuation of the WRAC project and a recently awarded grant for the USDA Competitive Grant Program will undertake a systematic inbreeding program for the Pacific oyster, utilize crosses among inbred lines to determine whether inbreeding and crossbreeding is a more efficient method for genetic improvement, and determine the genetic and physiological mechanisms of hybrid vigor. The proposed WRAC project will make triploids by two-way and three-way crosses among inbred lines and test their growth on commercial oyster grounds. Crossbreeding is the only means for improving the performance of sterile triploid oysters.

CHARACTERIZATION OF KUMAMOTO-LIKE PACIFIC OYSTERS FROM TASMANIA, AUSTRALIA WITH STARCH-GEL ELECTROPHORESIS. Robert H. Deupree Jr.,* Sandra L. Downing, William K. Hershberger, and **Kenneth K. Chew.** University of Washington School of Fisheries WH-10, Seattle WA 98195 USA.

The Kumamoto variety of the Pacific oyster (*Crassostrea gigas*) is distinctive because of its deeply cupped left valve. Decades of inbreeding, over-fishing, and pollution have combined to drastically reduce the availability of this desirable subspecies to the oyster industry. A population of oysters that resemble the Kumamoto exists in Tasmania, Australia.

The Tasmanian oysters were originally introduced from Japan during the 1940s and 1950s. They have deeply cupped left valves similar to Kumamoto oysters, but apparently grow at a much greater rate. Characterization of the Tasmanian stock with starch-gel electrophoresis was initiated as a first step in establishing their genealogy.

Gene frequencies at known polymorphic loci were measured for Washington State and Tasmanian oysters and correlated with published electrophoretic data on Japanese Kumamoto oysters. This information will be useful in initiating effective conservation efforts and differentiating environmental from genetic influences on shell growth and shape.

THE EFFECT OF BURROWING SHRIMP AND THE PESTICIDE CARBARYL ON THE BENTHIC COMMUNITY IN WILLAPA BAY, WASHINGTON. Brett R. Dumbauld,* and **Dennis Tufts,** Washington State Dept. of Fisheries, P.O. Box 190, Ocean Park, Washington, 98640; **David A. Armstrong,** School of Fisheries, University of Washington, Seattle, Washington 98195; **Martin H. Posey,** Dept. of Biological Sciences, University of North Carolina, Wilmington, North Carolina 28403.

Experiments on the efficacy of the pesticide carbaryl, used to control the mud shrimp *Upogebia pugettensis* and ghost shrimp *Neotrypaea californiensis* on oyster culture grounds in Washington state coastal estuaries, were initiated in 1989. Benthic core samples were taken to monitor the effects of the pesticide and the shrimp themselves on the abundance of other macro-infauna. Burrowing shrimp are known to exert a strong influence on benthic community structure via bioturbation from their burrowing activities. Previous studies have also examined the immediate effect of carbaryl on the benthos, however we wished to compare both the direct effect of carbaryl and the slightly longer term effect of reduced shrimp density on communities dominated by each species of shrimp. Results showed similar short term reductions in the dominant species of crustaceans and polychaetes in each community, but differences in both initial diversity (mud shrimp highest) and recovery rate (mud shrimp slowest) for the two communities.

SHELLFISH PROTECTION DISTRICTS IN WASHINGTON STATE. Stuart D. Glasoe, Puget Sound Water Quality Authority, P.O. Box 40900, Olympia, Washington 98504-0900.

Shellfish are a valued but threatened resource in Washington state. Today, over 40 percent of the commercial shellfish growing areas in Puget Sound are restricted by pollution. These restrictions have more than doubled over the past decade due largely to non-point pollution from such sources as failing on-site systems, farm animal wastes, stormwater runoff, and boater wastes.

Washington counties were originally given authority in 1985 to create shellfish protection districts to help protect water quality and shellfish resources. The legislation was never used though, due in part to the lack of flexibility and authority needed by counties to carry out effective programs.

Realizing the need for immediate and aggressive action, the state amended the statute (Chapter 90.72 RCW) in 1992 to give counties greater latitude in creating the districts and designing and

funding the accompanying water quality programs. The statute now also *requires* counties to establish districts and programs in response to growing area downgrades caused by nonpoint source pollution.

In addition to the attention being given to shellfish protection districts, the legislation has triggered broader discussions about other authorities available to counties for establishing and financing comprehensive programs dedicated to clean water and shellfish protection. While some counties are considering using shellfish protection districts as the basis for their countywide water quality programs, others are looking at using the districts to complement other authorities, such as stormwater utilities, or to enhance particular programs in priority watersheds.

A NEWLY DEVELOPING COMMERCIAL FISHERY FOR THE SUBTIDAL CLAM, *COMPSOMYAX SUBDIAPHANA*, IN BRITISH COMUMBIA. R. M. Harbo, Department of Fisheries and Oceans, 3225 Stephenson Point Road, Nanaimo, B.C., Canada, V9T 1K3; **N. Bourne,*** Pacific Biological Station, Nanaimo, B.C., Canada, V9R 5K6.

The deep water clam, *Compsomyax subdiaphana,* belongs to the family Veneridae and is widely distributed along the Pacific coast of North America in soft muddy substrate in subtidal depths of 2-550 m. Populations of this species were known to occur in British Columbia as a result of deep water clam surveys and extensive dragging for scallops. However, industry expressed little interest in attempting to harvest this resource.

In 1991 a small fishery began for this species in Trincomali Channel in the Gulf Islands region of the Strait of Georgia in British Columbia and it continued in 1992. Originally harvesting was done with a home made dredge but has continued with a Fall River rocker dredge. Catches have ranged from 27–50 kg per 20 minute tow, the average is about 40 kg. Annual landings to date have not been large, about 1.4 tonnes.

Monitoring of catches has been carried out to obtain biological information. There has been a good range of clam sizes in catches, mean size has ranged from 25 to 47 mm shell length. There have been good numbers of clams under 30 mm shell length indicating regular recruitment in recent years. Information on age, growth, length-height-width and weight relationships, time of spawning and associated fauna are being collected.

The fishery will probably never be large because of limited stocks and its future will depend on the extent of the resource, markets and the economics of harvesting.

RESULTS OF SURVEYS TO ASSESS INTERTIDAL CLAM RESOURCES IN NORTHERN BRITISH COLUMBIA. G. D. Heritage* and **N. Bourne.** Pacific Biological Station, Nanaimo, B.C., Canada, V9R 5K6.

Except for razor clams, *Siliqua patula,* harvest of intertidal clams has not occurred in the north coast district of British Columbia, from the northern tip of Vancouver Island to the Alaska border, in the past twenty years because of distance from markets and chronic low levels of PSP in butter clams, *Saxidomus giganteus*. Recently industry has expressed interest in harvesting intertidal clam resources in this area because of strong markets. In 1990 and 1991 surveys were undertaken to assess intertidal clam resources in the north coast district and to obtain further information on the northward dispersal of manila clams, *Tapes philippinarum*.

Littleneck clams, *Protothaca staminea,* were the most common intertidal clam species found during the surveys. Extensive populations occurred on many beaches and large numbers of small clams indicated good recruitment in recent years. Butter clams were abundant on most beaches in the lower third of the intertidal zone. Good recruitment has occurred in recent years as seen by the number of small clams on many beaches.

Results of the surveys showed that manila clams have not spread farther north than observed in surveys in 1980 and 1981. They have not spread north of about latitude 54 N. Manila clams were abundant on many beaches in the Bella Bella area. There was a preponderance of large size clams on many beaches but there was evidence of good recruitment in most areas. Of interest was the fact that manila clams did not occur in abundance on beaches south of the Bella Bella area.

The possibility of developing a commercial fishery in the northern area is discussed.

BALLAST WATER AND SEDIMENTS AS MECHANISMS FOR UNWANTED SPECIES INTRODUCTIONS INTO WASHINGTON STATE. Janet M. Kelly.* Institute for Marine Studies, University of Washington, Seattle, Washington 98195.

Ballast water and sediments from bulk cargo carriers have been implicated in the transfer of a diverse assortment of non-native species to near-shore marine environments worldwide. Dinoflagellate cysts present in discharged ballast sediments are believed to be responsible for the recent introduction of PSP-causing algal blooms and disruption of the shellfish culture industry in Tasmania.

Examination of ballast water and sediments from Japanese woodchip carriers arriving at the Ports of Tacoma and Port Angeles revealed the presence of living mollusc larvae, crustacea, macroalgae and numerous species of diatoms and dinoflagellates. With up to 20,000 metric tons of water and several cubic yards of sediment present in each cargo hold, the threat of introduction of harmful algae, pathogens, predators and resource competitors is valid. However, interviews with ship's officers indicated that at least some practice ballasting and deballasting procedures that decrease the risk of introductions, such as offshore ballast loading, open-ocean exchange of ballast water and open-ocean discharge of ballast sediments. Recent regulatory efforts to prevent introductions via ballast water and sediments will be discussed.

EFFECT OF DIETARY PROTEIN CONTENT ON GROWTH OF JUVENILE *MYTILUS EDULIS TROSSULUS*. **Daniel A. Kreeger,*** and **Christopher J. Langdon.** Hatfield Marine Science Center, Oregon State University, Newport, Oregon 97365.

Juvenile mussels, *Mytilus edulis trossulus,* were fed for three weeks on different rations of *Isochrysis galbana* (clone T-ISO) of either high protein content (HP; 43% w/w) or low protein content (LP; 28% w/w). The asymptote of the curve of mussel growth rate (shell height, wet weight and ash-free dry tissue weight [AFDTW]) against ration occurred at a ration of about 27.5% body weight (bw; ash-free dry algal weight/AFDTW of mussels) d^{-1} for both LP and HP diets. However, maximum growth of mussels fed on LP algae was lower than that of mussels fed on HP algae, indicating that growth of mussels fed on satiation-level rations of LP algae was limited by qualitative nutrient imbalances rather than by deficiencies of nutrients or energy per se.

In order to determine if the protein content of satiation-level LP rations was limiting mussel growth, LP rations of 27.5% bw d^{-1} were supplemented with 8, 16, or 24% bw d^{-1} protein microcapsules (PM). Mussel growth rates increased with increasing supplements of protein capsules and equalled those of mussels fed on HP algae at the highest PM supplementation level. Mussel growth was not increased by increasing rations of LP algae, indicating that growth improvement of mussels fed LP algae supplemented with PM was due to increased dietary protein content rather than to increased ration size. Mussels require dietary C/N ratios of less than or equal to 12 (approximately 40% protein w/w) for maximum growth, when fed satiation-level rations.

SHELLFISH WATER QUALITY WORLDWIDE. **Dorothy L. Leonard,** National Marine Fisheries Service, Southeast Fisheries Science Center, P.O. Box 12607, Charleston, SC 29412-0607.

Between 1985 and 1990, the United States suffered a six percent loss in waters approved for harvest. In the Chesapeake Bay, oyster production declined from 32 million pounds in 1959 to less than one million in 1991. Some countries can no longer harvest for direct consumption; the product is purified or heat processed. This paper reviews standards used by shellfish producing countries, particularly changes projected as a result of the European Community Shellfish Directive scheduled to be implemented January 1, 1993. A brief overview of water quality addresses many of the countries producing molluscan shellfish. World production and trade is summarized. Production and export of molluscan shellfish has shifted from developed and industrialized nations to those experiencing rapid economic growth. For example, from 1982 to 1988, Korea took the lead in oyster production from the US, landing 30 percent while the US dropped from 32 to 16.5 percent. During the same period China took the lead in clam and mussel landings. A decrease in available resource has forced countries such as the United States to import. This snapshot of world molluscan shellfish production is one of declining water quality, intensification of shellfish farming activities and dependence on processing to provide a safe product.

OVERVIEW OF WASHINGTON STATE DEPARTMENT OF HEALTH SHELLFISH AREA RESTORATION PROJECTS. **Donald J. Melvin,*** and **Jack Lilja,** Washington Department of Health, Office of Shellfish Programs, Bldg. 4, Airdustrial Park, P.O. Box 47824, Olympia, WA 98504-7824.

The Washington State Department of Health has been engaged in shellfish area restoration activities for a period of five years. Restoration projects focus on pollution source identification and correction. Emphasis is placed on involvement and coordination with other state and local/tribal governments.

Source identification strategies, correction techniques and project results are discussed for projects at: Dosewallips State Park, Hood Canal; Bay Center, Willapa Bay; North Bay, Puget Sound; and Burley Lagoon, Puget Sound.

A THREE-YEAR STUDY OF PARASITES AND DISEASES OF OVERWINTERING DREDGED BLUE CRABS, *CALLINECTES SAPIDUS*, IN UPPER CHESAPEAKE BAY. **Gretchen A. Messick,** NOAA National Marine Fisheries Service, Cooperative Oxford Laboratory, Oxford, Maryland 21654.

Blue crabs, *Callinectes sapidus,* are the target of the largest and most valuable fishery in the Chesapeake Bay. It has long been noted that catches fluctuate from year to year due to various factors such as winds, currents, fishing pressure, and disease. Recent reports indicate that landings are not increasing proportionately with increased fishing efforts. To explore the possibility that mortalities caused by disease might be a factor in this dilemma, a three-year study was made to determine the extent of disease in overwintering crab populations from Maryland portions of Chesapeake Bay. Many crabs exhibited hemocytic inflammation and nodule formation, indicative of stress and defense against foreign materials. Parasites identified included viruses, bacteria, microsporidans, hyperparasitized trematode metacercariae, nemerteans, and gregarines. In addition, crabs were found infected with a blood ciliate and a rickettsia-like organism not previously reported in blue crabs.

OYSTER CULTURE IN BON SECOUR BAY, ALABAMA. **Christopher L. Nelson,** Bon Secour Fisheries, Inc., Bon Secour, Alabama 36511; **Richard K. Wallace,** Auburn University Marine Extension and Research Center, 4170 Commanders Drive, Mobile, Alabama 36615.

Oyster culture along the Gulf of Mexico coast has not progressed much beyond providing substrate for spat and transplanting seed 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 ± 0.5 SE (n = 100) to 42.8 mm ± 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 ± 1.6 SE, n = 33), than those from the middle (mean = 48.3 mm ± 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 reached 60 percent of harvestable length (75 m) with negligible mortality in the 201 d of nursery and bay culture.

Oysters completed growout from nursery to marketable size in 15 months. The current phase of the project is to determine economic feasibility.

CONTROL OF SHRIMP INFESTATION ON OYSTER BEDS. John Pitts, Aquatic Farm Program, Wash. State Dept. of Agriculture.

Local, state and federal agency representatives met with crab fishers, oyster farmers and other citizens, as the Burrowing Shrimp Control Committee for 18 months in 1991–92. The goal was to develop a strategy to reduce the use of carbaryl for the control of shrimp, while maintaining oyster farmers' ability to raise their crop in an economically viable manner. Integrated Pest management Plans used in terrestrial agriculture were the basis for the development of a plan to deal with burrowing shrimp infestation. Washington State University (WSU) specialists participated with the committee in developing criteria and selecting alternatives to the existing control or growing methods. WSU extension agents developed economic data to help guide the process for selection of these alternatives.

The committee selected alternative culture methods, biological predator control, mechanical control and alternative application of carbaryl to control the shrimp infestation. The committee recognized the need for continued pest control with carbaryl and recommended its expanded use from the current 400 acres annually, to 800 acres annually in order to control shrimp infestation and loss of oyster beds and resultant oyster production.

Grants were developed and two areas of exploration have been funded to date: 1. A benthic/epibenthic study to explore the effects of carbaryl on various key species on shrimp beds after carbaryl application, 2. Washington and Oregon State University engineering studies to explore alternative culture and alternative shrimp control methods.

Impediments to the process have been "perception" problems based on objections by some crab fishermen who feel that serious impacts occur with the use of carbaryl treatment. To date, all biological evidence indicates that while acute impacts do occur, there is no long term negative environmental impact, and in fact, many believe that the existence of oyster culture results in increased biodiversity in the estuaries. Additional problems relate to the modification of Integrated Pest Management formats. State agencies will not allow shrimp control on private oyster beds on an "as needed" basis, even though treatment is not considered a serious biological impact. Farmers have been asked to justify increased treatment through "Economic Injury Thresholds." Limitations on total acreage, treatment season, carbaryl concentration and the ability to treat seeded beds are inconsistent with standard integrated pest management plans and have resulted in constraints in the process.

MECHANISMS CONTROLLING THE SPREAD OF PARALYTIC SHELLFISH POISONING (PSP) IN PUGET SOUND. Jack Rensel. School of Fisheries, University of Washington, Seattle, Washington 98195.

The geographic distribution and intensity of PSP has increased in Puget Sound since the mid 1970's. Formerly a rarity in central Puget Sound, now all but parts of southern Puget Sound (SPS) and central and southern Hood Canal (CHC and SCH) are affected by shellfish toxicity due to PSP from the toxic dinoflagellate *Alexandrium catenellum*. The initial spread of PSP has been traced to major physical events, but lack of PSP in most of SPS and all of CHC and SHC has been a mystery. I present monitoring data and preliminary experiments that suggest supply and vertical distribution of nitrogen in the water column prevents *A. catenellum* growth during the summer in the unaffected areas.

After the spring diatom bloom, surface *and* subsurface (10 m) waters of CHC are annually depleted of nitrogen until fall, while deeper waters (30 m) are nutrient rich. Filtered water from the surface and subsurface depths of this area did not support growth of *A. catenellum,* and unexpectedly, neither did the nutrient-rich deep water. These factors, along with slow physical transport in CHC, apparently form a barrier to the passage of *A. catenellum* to the more nutrient-rich SHC.

SPS waters (except Carr Inlet and Nisqually Reach) are seasonally depleted of surface and subsurface nitrogen, similar to CHC. Subsurface nutrient concentration greater than 15 μM and water temperature above 13° in Carr Inlet were strongly correlated with the presence of PSP toxin in mussels. Increased nutrification from rapid urbanization and non-point sources could lead to annual PSP problems in areas presently unaffected by PSP, unless mitigative measures are taken.

DEVELOPMENT OF THE COMMERCIAL AQUACULTURE OF THE SUMINOE OYSTER (*CRASSOSTREA RIVULARIS*). Anja M. Robinson and **C. J. Langdon.** Department of Fisheries and Wildlife, Oregon State University, Hatfield Marine Science Center, Newport, Oregon 97365.

Crassostrea rivularis was introduced to the West Coast of the U.S.A. from Japan. It does not reproduce in our cool waters and it becomes sexually mature in late summer in Yaquina Bay, Oregon. Sexual maturation can be accelerated by holding adult oysters at elevated water temperatures (20°C). Conditioned broodstock oysters can be spawned by temperature shock. Larvae were raised to setting and metamorphosis by culturing them at 25°C in 20 ppt seawater, fed on a mixed diet of flagellate *Pseudoisochrysis paradoxa* and the diatom *Chaetoceros calcitrans*.

Using the techniques developed at the Hatfield Marine Science Center, commercial hatcheries have been able to raise larvae through setting and metamorphosis. Growth of planted spat varies at different locations.

Cultchless spat were obtained either by setting eyed larvae on shell chips or by exposing competent larvae to 2×10^{-4} M epinephrine solution. Cultchless spat were held in upwellers until large enough to plant at growout sites.

THE EFFECTS OF WATER TEMPERATURE ON THE SEXUAL DEVELOPMENT OF ADULT OLYMPIA OYSTERS, *OSTREA LURIDA*. Jose M. Santos,* Sandra L. Downing, and **Kenneth K. Chew.** University of Washington, School of Fisheries WH-10, Seattle, WA 98195.

Stocks of the native Washington State oyster, *Ostrea lurida* (Carpenter), are now so low that hatchery efforts may be needed. Thus a study was initiated to examine broodstock conditioning, gametogenesis, and spawning in the hatchery under four temperature regimes (12, 15, 18, and 21°C). Three hundred Olympia oysters were placed for conditioning under each regime for 6 to 9 weeks depending on temperature. Oysters were sampled on day 0, day 14, and then once or twice a week until the end of the experiment. At each sampling period, 36 individuals held at each temperature were taken: 18 for histologically determining the gonadal index and the other 18 for establishing the condition index. The numbers of brooding oysters, brooded larvae, and released larvae were also recorded.

Results showed that the higher the temperature regime, the faster a gametogenic peak was reached: the 12°C group peaked at week 8, the 15°C group peaked at weeks 3 and 5, the 18°C group peaked at 2.5 and 5.5 weeks, and the 21°C group peaked at 2 and 3.5 weeks. Although the number of brooding oysters was higher in the 18°C group (43) than the 21 group (33), the 21 group released more larvae such that the total number of larvae produced by both (around 15 M) was not significantly different. The condition indices for the 18 and 21°C groups decreased rapidly over the first 3 weeks while the 12°C group remained close to the initial sample's level over 9 weeks. These and other results will be discussed as they apply to hatchery production of this species.

A REVIEW OF THE EFFECTS OF ALGAL BLOOMS ON SHELLFISH AND AQUACULTURE. Sandra E. Shumway, Maine Department of Marine Resources, and Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine 04575 USA.

Toxic algal blooms occur worldwide and in some areas they are a common and seasonal occurrence. Historically, attention has been focused on blooms of toxic dinoflagellates (e.g. *Protogonyaulax tamarensis*). Attention has been now turned to other genera including, *Dinophysis, Aureococcus, Gymnodinium* and most recently, *Nitzschia*. Responses of shellfish to the individual algal species vary between both algal and bivalve species and are specific to the algal/bivalve interaction under consideration. These blooms often present problems with respect to optimal utilization of the shellfish resources and the magnitude of economic losses can be catastrophic. Nevertheless, successful culture facilities and commercial harvests persist in areas prone to toxic algal blooms.

Occurrence of toxic algal blooms and the means by which harvesters, managers, and industry cope with the problems associated with them are reviewed and recommendations for the most efficient and successful utilization of resources in the face of environmental instability are presented.

ABALONE NUTRITION: OPTIMUM PROTEIN LEVELS IN ARTIFICIAL DIETS FOR *HALIOTIS KAMTSCHATKANA*. Barbara Taylor. Department of Zoology, University of British Columbia, Vancouver, Canada, V6T 2Z4.

Protein is the most expensive ingredient in rations for cultured abalone. To avoid costly waste and enhance production, diets should be formulated such that protein requirements are met but not exceeded. This demands identification of the optimum level of dietary protein, a level at which protein utilization (protein retention relative to intake) and growth efficiency (weight gain relative to food intake) are both maximized. To this end, adult abalone were fed diets containing 0, 10, 20, and 30% casein for 8 weeks to determine optimum dietary protein level. Protein utilization was greatest on a 10%-casein diet, though not significantly greater than that on a 20% one. However, only 20%- and 30%-casein diets yielded a positive growth efficiency. Therefore, when casein is used as a protein source, the optimum dietary protein level for this species of abalone is estimated to be 30%.

HIGHER PARALYTIC SHELLFISH POISONING TOXICITY OBSERVED WITH LOWER pH (pH < 1) SAMPLE EXTRACTION. Debra A. Wadford,* and **R. E. Danielson.** California Department of Health Services (CADHS), Sanitation and Radiation Laboratory (SRL), Berkeley, California 94704; **Gregg W. Langlois,** CADHS-Environmental Management Branch, Berkeley, CA 94704.

When an aliquot of shellfish meat (100 g) is prepared according to the standard AOAC mouse bioassay for paralytic shellfish poisoning (PSP) determination, a pH value between 2–4 is specified as the appropriate solution pH to extract PSP. PSP is toxic under acidic conditions, as in the human stomach (pH $<$ 2), but its toxicity is neutralized under alkaline conditions. We compared the results of the standard AOAC method with the results using an extraction pH $<$ 1 on mussel and oyster homogenates. We found that PSP toxicity is greatly increased with an extraction pH $<$ 1 relative to the results obtained via the standard AOAC method of extraction. We believe that extremely acidic conditions may allow for the conversion of toxins to more toxic species of PSP (e.g., conversion of 11-hydroxysaxitoxin (11-(OH)SXT) to the diol, 11-(OH)STXOH). The implications of this finding are two-fold: first, that strict adherence to the AOAC method and quality control of biotoxin sample extraction is necessary in order to minimize variability in an already variable method; second, that a more accurate method of PSP toxin determination, one that would indicate the exact toxin species present, may serve as a better indicator of shellfish toxicity than the current AOAC mouse bioassay method.

TECHNIQUES FOR THE ENHANCED RECOVERY OF *BACTEROIDES VULGATUS* AS AN INDICATOR OF FECAL CONTAMINATION IN SHELLFISH. **Debra A. Wadford,*** and **Beverly A. Dixon,** Dept. of Biological Sciences, California State University, Hayward, CA 94545; **Mike E. Cox,** Anaerobe Systems, San Jose, CA.

Coliforms are the current indicator organisms of fecal contamination in shellfish. Recently there has been a desire to investigate other microorganisms that would more definitively indicate the source of fecal contamination. *Bacteroides vulgatus*, a member of the anaerobic *Bacteroides fragilis* group, is present in higher concentrations in the human intestine, and outnumbers fecal coliforms a thousandfold. Well-established clinical techniques for the isolation and identification of anaerobic bacteria were applied to environmental samples, namely oysters and mussels, to identify *B. vulgatus*. Isolation and identification of *B. vulgatus* was facilitated by the organisms's resistance to kanamycin, vancomycin, colistin, and bile. *B. vulgatus* has the potential to be an indicator organism of recent fecal contamination because it is more aerotolerant than other anaerobes.

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ADDENDUM ABSTRACTS

EXPERIMENTAL STUDIES ON LARVAL HABITAT CHOICE IN THE MACTRID BIVALVE *MULINIA LATERALIS*. **Judith P. Grassle.** Institute of Marine and Coastal Sciences, Rutgers University, P.O. Box 231, New Brunswick, NJ 08903; **P.V.R. Snelgrove,** Biology Department and **C.A. Butman,** Applied Ocean Physics and Engineering Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543. USA.

Still-water and flume flow experiments on habitat choice in larvae of the opportunistic coot clam, *Mulinia lateralis,* show that larvae always prefer organically rich mud over sediment lacking in organic matter in flow. The same significant preference is often but not always demonstrable in still water. An examination of larval competency and near-bottom behavior over the period of ~5 days in which larvae pass from precompetent, to competent, then to spontaneously-metamorphosing stages, suggests that a choice is more readily made by late pediveligers when they are moved by the flow between different sediment patches. These results will be compared with what is known about settlement behavior in certain other bivalve larvae.

SUSPENDED CULTURE GROWOUT STRATEGIES EXPLOITING THE LIFE-HISTORY CHARACTERISTICS OF VARIOUS POPULATIONS OF SEA SCALLOPS, *PLACOPECTEN MAGELLANICUS*. **Michael J. Dadswell*** and **Jay Parsons.** Dept. of Biology Acadia University, Wolfville, Nova Scotia BOP 1XO Canada.

Populations of sea scallops on the Atlantic coast of Canada exhibit both annual and biannual spawning cycles depending on their geographical location. Populations with annual cycles normally spawn in late summer (Aug-Sept.) and spat collected can be grown to commercial size in 33-36 months (90mm S_h, 15 g meat). Populations with biannual spawning cycles usually spawn both in late spring (June) and fall (Oct.). Spat collected from the early set of these populations can be grown to commercial size in 24-26 months. Use of spring-spawning populations for collection of naturally occurring spat or for hatchery production could significantly reduce the required growout period for this species. Strategies exploiting both stock types would yield the aquaculturist with a steady supply of market-size animals.

SEASONAL AND SIZE-RELATED SWIMMING BEHAVIOR IN THE SEA SCALLOP, *PLACOPECTEN MAGELLANICUS*. **G. Jay Parsons*** and **Michael J. Dadswell.** Department of Zoology, University of Guelph, Guelph, Ontario, Canada N1G 2W1 and Department of Biology, Acadia University, Wolfville, Nova Scotia, Canada B0P 1X0.

The swimming behavior of the sea scallop, *Placopecten magellanicus,* was studied on about a bimonthly basis over a four year period in Passamaquoddy Bay, N.B. in order to test the hypotheses that scallops showed a seasonal pattern of swimming activity in relation to environmental variables and that the size-related swimming pattern agreed with theoretical predictions of swimming efficiency. Underwater observations and video photography, using SCUBA, were made on the swimming response of scallops when approached by a diver. Scallops showed a seasonal pattern of swimming activity. Few scallops swam in the winter months, when temperature was <4°C. Swimming activity increased in summer and was related to temperature. Swimming rates (claps s-1 and velocity) were also positively correlated with temperature. Peak activity occurred in autumn and coincided with the annual temperature maxima. Peak activity was also coincident with and subsequent to spawning. Scallops from 40 to 80 mm shell height were more active compared to other sizes which agreed with theoretical predictions based on the hydrodynamics of this species. Scallop aquaculturists considering a bottom growout strategy will have to account for the impact of movement and swimming on their operation.

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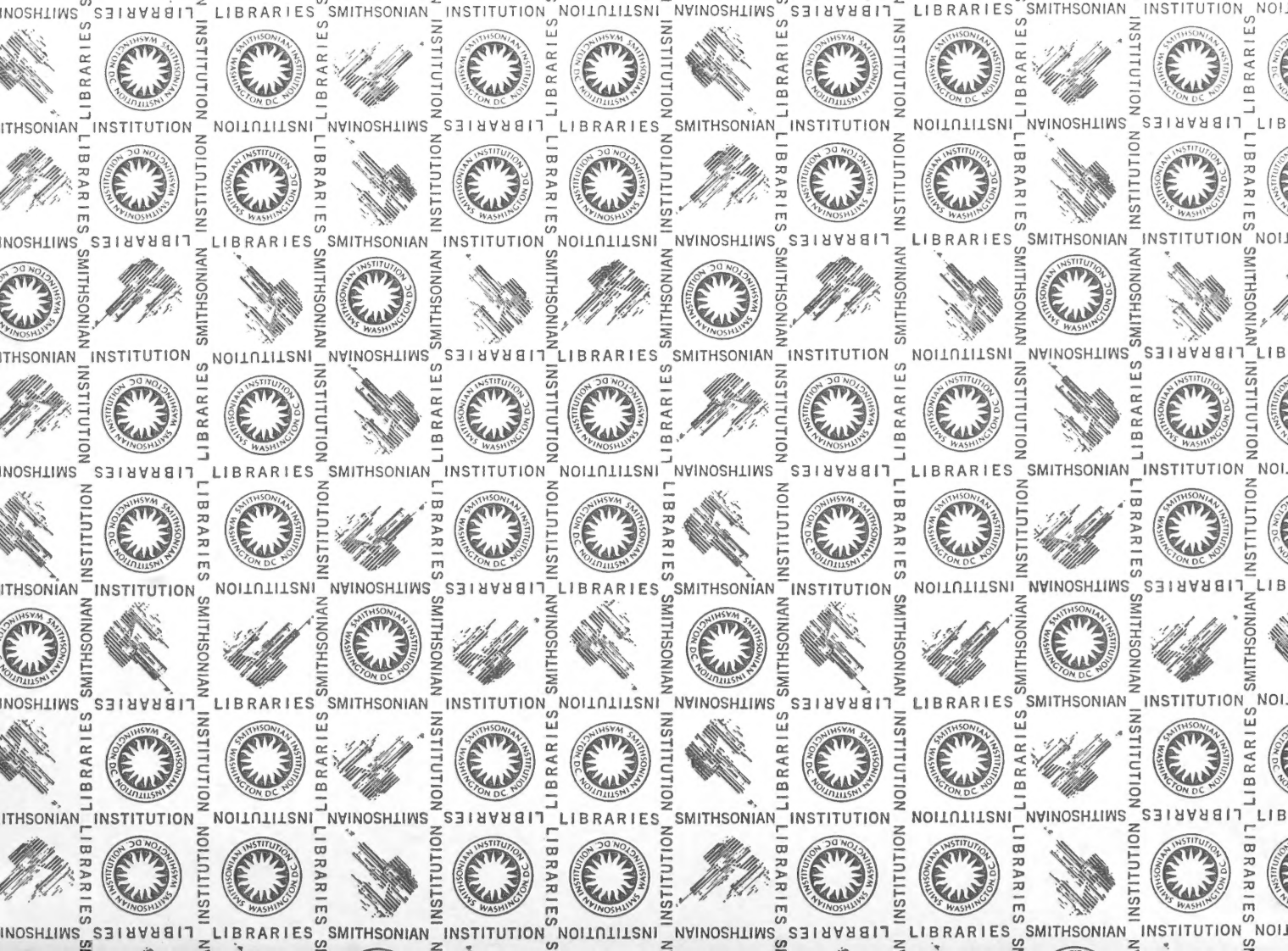
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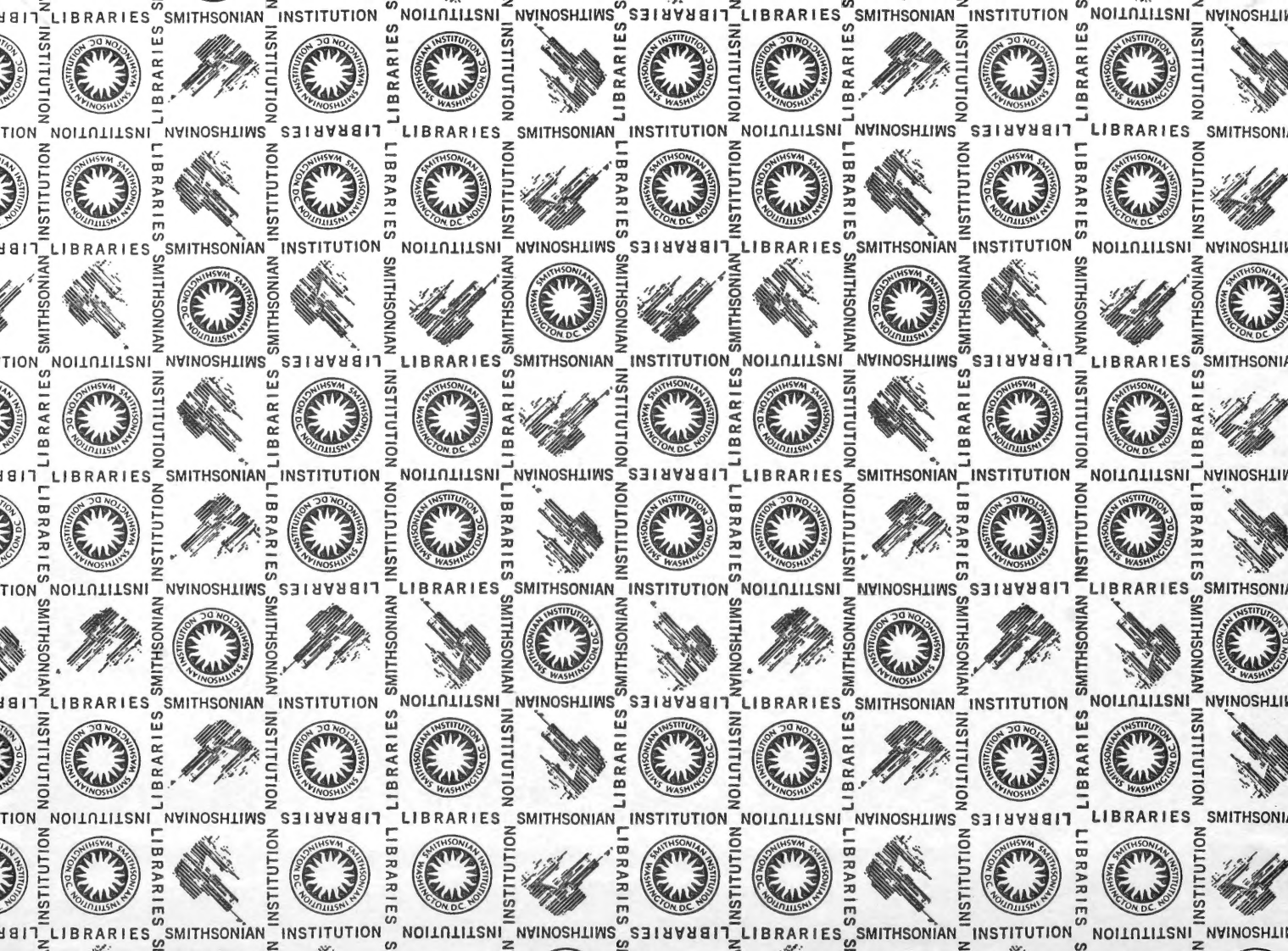
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