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DANIEL BRANCH QUAYLE
(1913–1993)

Daniel (Dan) Branch Quayle, a recognized world authority in the field of molluscan biology and bivalve culture, passed away October 19, 1993, at his home in Nanaimo, British Columbia. He was 80 years old.

Dan was born in the U.K. in 1913 but the family emigrated to Canada when he was three years old and settled in Ladysmith, British Columbia, where his father worked in the local coal mines. Dan received his elementary and secondary education in Ladysmith. He undoubtedly developed his love for the marine environment at an early age as he played around the shores of Ladysmith Harbour. One note in his C.V. includes the notation, "1929—coal miner (stimulus to Academe)."

In 1929 Dan attended the Provincial Normal School in Victoria, British Columbia, graduating as a teacher in 1930. He taught for three years, 1930–34, in the Michel-Natal area in southeastern British Columbia. He continued to maintain a love of young people and teaching for the rest of his life.

His love of the sea brought him back to the coast and in 1934 he returned to Vancouver where he enrolled in the University of British Columbia receiving his B.A. degree in Zoology in 1937. During this period he worked as a summer student under the direction of Dr. C. R. Elsey and began his life-long interest and association with oysters. In 1936 Dan assisted Dr. Elsey with studies of Pacific oyster, *Crassostrea gigas*, breeding in Ladysmith Harbour. A large general breeding occurred in Ladysmith Harbour that year and in 1937, Dr. Elsey and Dan took juveniles from this set and planted them in isolated inlets along the west coast of Vancouver Island. Offspring from these plantings still remain in these inlets. Dan undertook his M.A. degree in Zoology under Dr. MacLean-Fraser at U.B.C., receiving his degree in 1938.

In 1938 he joined the staff of the Fisheries Research Board of Canada at the Pacific Biological Station in Nanaimo. This was the beginning of his life-long association with this Station that spanned a period of 55 years. He assumed responsibility for the molluscan research program and began an investigation of molluscan shellfish resources in the Province. Although his work focused on molluscan resources that could support commercial fisheries he began studies of the invertebrate fauna and ecology of marine invertebrates that would continue for the rest of his life.

In 1941 he joined the Royal Canadian Air Force and became a navigator in an elite pathfinder squadron. He was shot down over Germany and was a prisoner of war for 1.5 years.

At the end of the Second World War in 1945 he returned for a brief period to the Pacific Biological Station, assuming responsibility for the molluscan shellfish research program. He left shortly after to undertake his PhD degree at the University of Glasgow under the direction of Sir C. M. Yonge, receiving his degree in 1948.

After receiving his PhD degree, Dan returned to British Columbia but went to work for the British Columbia Provincial Department

of Fisheries as Director of Biological Services. He built a small laboratory at Ladysmith and commenced extensive studies on oysters and oyster culture in British Columbia. During this period he worked closely with industry, pioneering culture methods to improve oyster production. He continued to maintain a close association with people in the industry, not only in British Columbia but elsewhere, for the rest of his life.

From 1956–1957, Dan was Director of Fisheries for the Province of British Columbia.

In 1957 Dan left the Provincial government and British Columbia and went to the United States where he worked from 1957–1958 as a technical advisor to the Coast Oyster Company in South Bend, Washington and then as a Technical Advisor to a Chesapeake Bay oyster consortium in Virginia.

In 1958 he returned to the Pacific Biological Station and continued his lifetime study of the ecology of marine invertebrates of British Columbia until his retirement in March 1973. During this period he undertook extensive research on marine invertebrate populations along the coast and became recognized as a world authority in the field. He maintained a keen interest in a wide range of marine invertebrate subjects and published numerous scientific and semi scientific papers. Dan's close association with the industry continued and during this period he published his book, "Pacific Oyster Culture in British Columbia", which became a standard text for oyster culture, particularly for the west coast of North America. Much of the success of the British Columbia oyster industry is due to the efforts of Dan Quayle.

After his retirement in 1973, Dan became a consultant for Canadian Overseas Aid organizations; Canadian International Development Agency (CIDA), International Development Research Centre (IDRC), and Canadian Executive Services Overseas (CESO). This work took him to developing countries throughout the world where he assisted in the development of shellfish culture industries. Results of this work resulted in various publications which served as texts for bivalve culture in developing countries. He also taught courses on oyster culture at Dalhousie University in Halifax, Nova Scotia.

When not involved in overseas consulting, Dan continued as an active volunteer research worker at the Pacific Biological Station from the time of his retirement until shortly before his death. When he was in Nanaimo he was at the Station almost daily. He continued with studies of marine invertebrates and in particular on molluscs and bivalve culture and published widely. He undertook a major revision of his "Pacific Oyster Culture in British Columbia" publication which appeared in 1988. Shortly before his death he completed a major work on Wood Borers in British Columbia. He was an inspiration to the staff of the Pacific Biological Station and was highly respected for his never ending quest for knowledge. Dan was a kind and patient person and always had time to talk with younger staff members and give them the benefit of his long years of experience.

Three marine invertebrates were named in honour of Dan. The shrimp, Quayle's spinytail, *Systellaspis braueri*, a hermit crab, *Pagarus quaylei* and a bivalve, *Lyonsiella quaylei*.

In 1989 the University of Victoria awarded him an honourary LLD degree in recognition of his work in the field of marine invertebrates and molluscan culture in British Columbia and throughout the world.

Dan was always interested in athletics. During his University days he was a member of the U.B.C. soccer team and a member of the University of Glasgow basketball team. He was a keen golfer and a regular member of a Saturday morning golf group from the Pacific Biological Station.

Dan was a long time member of the National Shellfisheries Association. He served on the Editorial Board for the Proceedings and the Journal for many years and was active in the west coast section of the Association. He was made an Honorary Member of the Association in 1973 and awarded the Wallace Award in 1992.

Dan was a wonderful field and ship-board companion. Those of us who were privileged to travel with him will remember the close companionship and long philosophical discussions we had on a wide variety of biological and other topics. They were stimulating to all of us who accompanied Dan on trips and have done much to assist and direct us in our work.

Dan is survived by his wife Ann, his daughter Moura and by a host of friends whose lives have been enriched by their association with him.

Neil Bourne
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CANADA V9R5K6

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THE PEA CRAB, *PINNOTHERES PISUM* (LINNAEUS, 1767), AND ITS ASSOCIATION WITH THE COMMON MUSSEL, *MYTILUS EDULIS* (LINNAEUS, 1758), IN THE SOLENT (UK)

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ABSTRACT Pea crabs (*Pinnotheres pisum* (Linnaeus)) from 5366 mussels (*Mytilus edulis* (Linnaeus)) were collected from two sites in the Solent, southern England, between 1972 and 1974 in order to determine the relationships between pea crab sex, size and occupancy, mussel size, time of year and position on beach. Larger mussels were more likely to be occupied by larger, female crabs, but pea crabs will occupy smaller mussels if fewer hosts are available. Mussel occupancy tends to increase from high to low water where food is more readily available. Pea crabs do not occupy mussels at random; there were more male/female pairs and fewer single males and pairs of females than expected. Possible mechanisms that explain these results are discussed. Berried females were found between April and October with a peak of 70% in berry in June and July. Larger females came into berry earlier, and may have been a year older, than smaller females. The association with mussels is best described as amensalism for male pea crabs and parasitism for females.

KEY WORDS: amensalism, host-parasite interaction, logistic modelling, pea crab-mussel association

INTRODUCTION

Pinnotheres pisum (Linnaeus) is a brachyuran crab found in the mantle cavity of bivalves, including the mussel *Mytilus edulis* (Linnaeus). Pea crabs have been recorded in the literature for over four hundred years (e.g. Rondeletius 1554) (Fig. 1), and from many different habitats such as the internal cavities of ascidians and oysters and the tubes of tubicolous polychaetes (Silas and Algarswami 1965, Warner 1977, Ingle 1980). Whilst resident within a mussel, pea crabs benefit from the relationship by feeding on strings of mucus collected from the mussel's gills (Orton 1920; pers. obs. CMCH).

The distribution and frequency of pea crabs in mussels has previously been studied in the English Channel by Houghton (1963) and Seed (1969) with very limited sampling, and by Huard and Demeusy (1968). In this study we selected two sites in the Solent where pea crabs occurred, one with a high, the other with a low density of mussels. Samples of mussels were collected with a view to exploring the factors influencing occupancy of mussels by pea crabs including size of mussel, time of the year and position on the beach.

MATERIALS AND METHODS

Sampling Sites

The results from a ten month pilot survey at Lee-on-Solent led to the choice of the site at Elmore. At this site (Grid reference SZ 566 997, Fig. 2) an intertidal raised mussel bed, referred to as 'the spit,' projected SW from the shore. The spit was the only intertidal mussel bed in the vicinity and arose in an area where the easterly and westerly currents in this region of the Solent converged. The mussel bed was on a substrate of shingle and sand overlying predominantly soft grey mud on the NW side and peat on the SE side. It was 60 m long, with a maximal width of 14 m and maximal height above the mud and peat of 0.2 m. At LWN, only the area of maximal height was exposed, but at LWS the entire mussel bed was uncovered. The vertical distance between LWN and LWS was about one metre. The time of exposure varied with the strength and direction of the prevailing wind. The tidal range was from 2.5 to 4.5 metres.

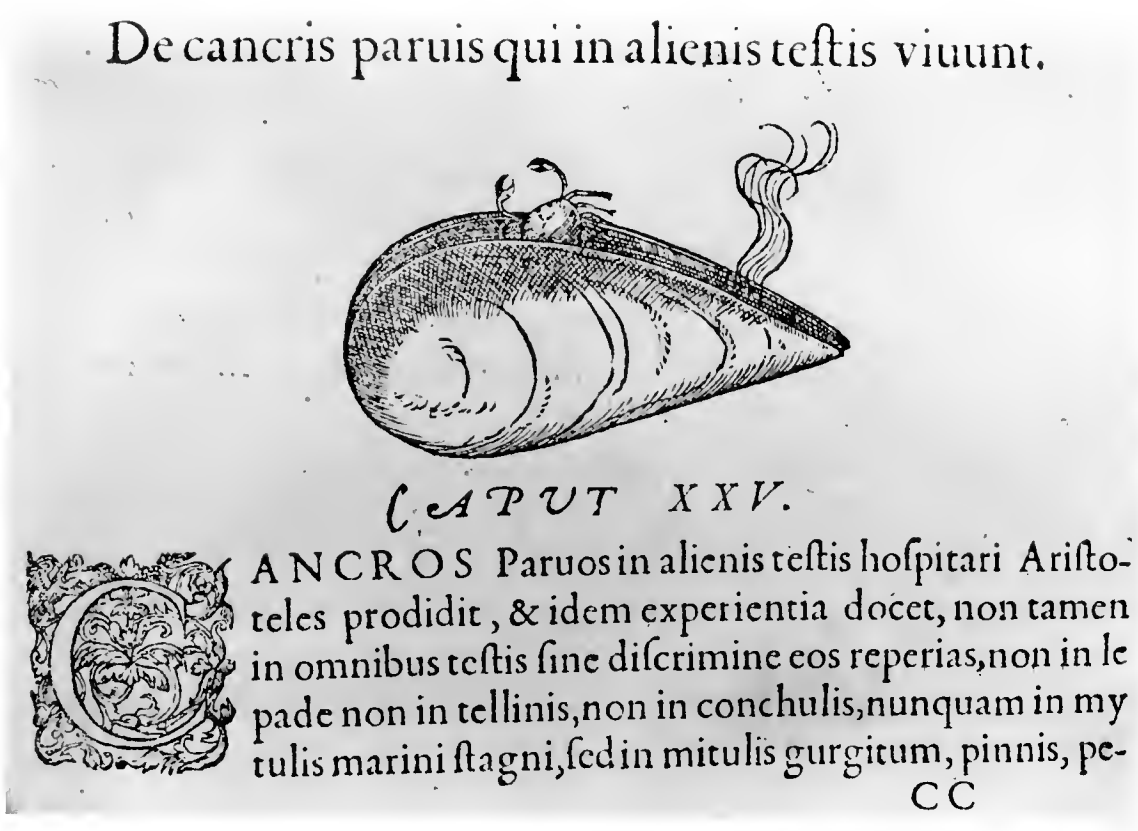
Mussels were collected from the surface of the bed at monthly intervals from August 1972 to July 1973 with additional collections in September 1973 and March 1974. At each sampling time twenty-five mussels were collected from twelve stations at 5 metre intervals on a transect from HWN to LWN. All collections were made at low water spring tides.

The site at Calshot (Grid reference SU 487 018, Fig. 2) was on the south-east side of a very narrow spit. The area studied extended 60 m seawards from HWN to midway between HWN and LWN. The site was more level than that at Elmore and the mussels were sparsely distributed. Samples were collected from twelve stations every second month from August 1973 to April 1974.

Treatment of Samples

Mussels were transported in polythene bags, kept cool in a refrigerator and examined the following day. If the mussels were placed in water prior to examination, many of the male crabs left the mussels, as was also reported by Huard and Demeusy (1968). All mussels were opened and any visible crabs removed. Mussel length and crab carapace width were measured with vernier callipers to the nearest 0.5 mm. The shell length ranges of the mussels collected from the two sites were similar, being 23 to 80 mm at Elmore and 23 to 75 mm at Calshot, although the mussels collected at Elmore were on average larger than those at Calshot (49.1 mm compared with 45.8 mm).

The sex of each crab was established by inspecting the width of the abdomen. Females in berry were also noted. Occasionally a mussel was found to be dead and filled with mud; these mussels have not been included in the subsequent analysis.


De cancris paruis qui in alienis teſtis viuunt.

CAPVT XXV.

CANCROS Paruos in alienis teſtis hoſpitari Ariſto-
teles prodidit, & idem experientia docet, non tamen
in omnibus teſtis ſine diſcrimine eos reperias, non in le
pade non in tellinis, non in conchulis, nunquam in my
tulis marini ſtagni, ſed in mitulis gurgitum, pinnis, pe-
CC

Figure 1. Copy of illustration of a pea crab from Rondeletius (1554, chapter XXV, p. 569).

Statistical Analysis

Within our analysis, basic statistical summaries were generated, and exploratory data analysis undertaken, using the Minitab statistical package (Ryan et al. 1985).

As part of a preliminary analysis the Poisson distribution was fitted to the frequency distributions of the number of crabs occupying mussels in order to investigate the hypothesis of random occupation of mussels by pea crabs. Integral parts of the Poisson model are: (i) the mean number of crabs occupying mussels is constant, and (ii) crabs occupy mussels independently of one another. Given differences in the observed frequencies for males and females and between the two sites, a separate Poisson distribution was fitted to the frequency distribution for each sex/site combination. The goodness of fit of the Poisson model was established using standard chi-squared procedures.

The GLIM statistical package (Aitken et al. 1989) was used to carry out logistic regression analysis in order to investigate the relationship between the probability of a mussel being occupied and various physical and environmental variables. Specifically, the fitted models were linear binomial response models with logistic link functions. Model fitting within GLIM is carried out via a maximum likelihood method. Subsequent, analysis of variance (ANOVA) based, cross-classification analyses to investigate the relationship between the carapace width of occupying pea crabs and environmental variables were also carried out using this piece of software. For both forms of analysis a step-down approach based on the criterion of deviance reduction was used to obtain parsimonious descriptions of the data structure.

RESULTS

Mussel Occupancy by Pea Crabs

2481 pea crabs (953 at Calshot and 1528 at Elmore) were found in the 5366 mussels collected at the two sites (1402 at Calshot and 3964 at Elmore). At Calshot, 45% of the crabs were males, while at Elmore only 28% were males. Table 1 provides a more detailed breakdown of mussel occupancy by pea crabs.

Investigating the Hypothesis of Random Occupation

The relevant data used in fitting the Poisson model of random occupation are given in Table 2.

Chi-squared goodness-of-fit statistics, calculated after combining categories for which the expected values were less than 5, were very highly significant ($p \ll 0.001$) for all but the males at Elmore. In the three significant cases the major disparities between the observed and expected frequencies arose because the Poisson model underestimated the frequencies of single occupancy, and overestimated the frequencies of multiple occupation, by crabs of the same sex. These disparities from the Poisson model could have been due either to crabs not locating themselves independently of one another or variations in the mean number of crabs occupying a mussel, or both. These possibilities were explored further, yielding the remaining results of this section.

Rejection of Independence

Chi-squared contingency table analysis rejected the hypothesis that males and females locate themselves in mussels independently

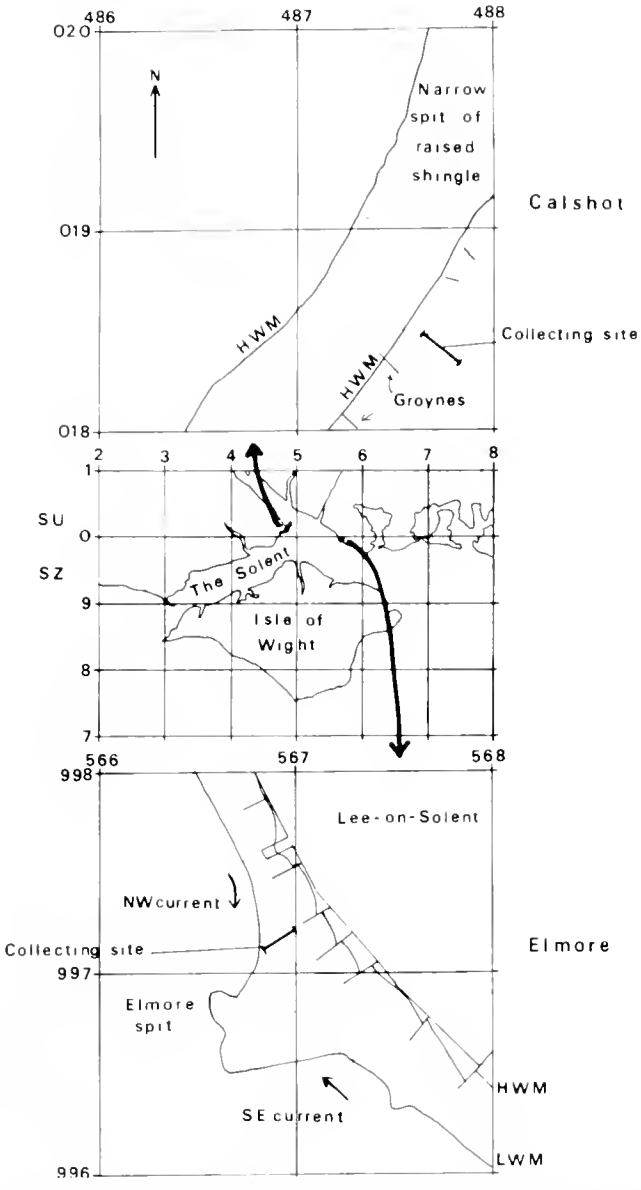


Figure 2. The collection sites in the Solent, southern England (centre) with detail of Calshot (above) and Elmore (below). Ordnance Survey National grid lines are shown giving 10 km squares (centre) and 0.1 km squares (above and below).

of one another ($p < 0.001$ at both sites). The relevant data is that given in Table 1. The predominant significant effect at Calshot resulted from the fact that no females were found to occupy mussels together with two or more males. A secondary significant effect was that more male/female pairings and fewer single males were found than would have been the case under the independence hypothesis. This latter effect was also found to be significant at Elmore.

Variables Influencing Occupancy

A much higher proportion of the sampled mussels were occupied by pea crabs at Calshot than at Elmore (54% compared with 34%). One-way ANOVA for mean mussel length with four categories of occupation gave highly significant results for both sites ($p \leqslant 0.001$ in both cases). Table 3 gives the sample mean and a 95% confidence interval for the population mean for each of the four categories of occupation at the two sites.

Allowing for multiple testing using the Fisher-Bonferroni method (Kotz et al. 1982) with an overall significance level of 5%, at both sites the mean length of mussels occupied by single males was not significantly different from that for unoccupied mussels, while the mean for mussels occupied by single females was significantly different; the mean for mussels occupied by more than one pea crab was significantly different from that for single females.

Logistic regression analysis was used to describe the relationship between the probability that a mussel would be occupied and the time at which sampling took place, the position on the beach and the shell length of the host mussel. The three occupancy categories of mussels occupied by single male pea crabs, single females and male/female pairings were considered individually at each of the two sites. At both sites, occupancy by single females and male/female pairs was found to increase with the size of the host mussel. Also, at Elmore, where the sampling stations covered the complete tidal range of the beach, the proportion of mussels occupied by crabs was higher near low water than near high water for all three categories of occupation.

TABLE 1.

Numbers of mussels at each site occupied by the indicated number of male and female pea crabs.

	Number of Female Crabs		
Number of Male Crabs	**0**	**1**	**2**
Calshot			
0	642	353	2
1	216	161	0
2	26	0	0
3	2	0	0
Elmore			
0	2601	954	0
1	245	147	0
2	16	1	0

For example, at Calshot 642 mussels contained no pea crabs and 161 mussels were occupied by male/female pairs.

TABLE 2.

Numbers of mussels occupied by specified number of pea crabs of the given sex (disregarding the number of occupying crabs of the opposite sex).

Number of Occupying Crabs of Specified Sex	Site	Calshot		Elmore	
	Sex	Male	Female	Male	Female
0		997	886	3555	2862
1		377	514	392	1102
2		26	2	17	0
3		2	0	0	0

For example, at Elmore 392 mussels contained just one male (and unspecified numbers of females).

TABLE 3.

Mussel length (mm) for four categories of occupation.

	Calshot		Elmore	
	Mean	95% CI	Mean	95% CI
Unoccupied mussels	44.61	(44.1,45.1)	47.70	(47.4,48.0)
Mussels occupied by a single male	44.45	(43.5,45.4)	48.80	(47.9,49.7)
Mussels occupied by a single female	47.15	(46.5,47.8)	51.93	(51.5,52.3)
Mussels with multiple occupation	49.15	(48.0,50.3)	54.50	(53.4,55.6)

Relationship between Pea Crab Size and Environmental Variables

Table 4 shows that female crabs were on average nearly twice as large as males, and that the average size of each sex was similar at the two sites.

For each of four categories of pea crab occupation ANOVA was carried out for pea crab carapace width cross-classified according to three environmental variables. The four categories were single males, single females, males in male/female pairs and females in male/female pairs. The three environmental variables considered were sampling time, position on beach and shell length of the host mussel. From this analysis it was found that, across the two sites, the size of occupying females increased with the size of the host mussel, paired males were on average almost 1 mm larger than single males and paired females were on average 0.6 mm larger than single females.

Berried Females

In all, 203 female crabs were found to be berried at Elmore (181 single females, 21 in male/female pairs and 1 in a 2 male/1 female triple). Berried females were found only in the months April to October. Table 5 gives the proportion of berried females and the total number of females recorded for these seven months. It shows the proportion of berried females rising sharply from April and peaking at around 70% in June and July.

Very few berried crabs were found in the smallest mussels collected (with lengths in the range 22 to 42 mm), or in the quarter of the beach closest to high tide. Generally, the proportion of mussels occupied by berried females increased with the size of mussel and from high to low tide.

TABLE 4.

Carapace width (mm) of male and female pea crabs at Calshot and Elmore.

	Mean	SD	Range
Calshot			
Males	4.48	1.43	1.0–9.0
Females	7.82	2.09	2.0–13.0
Elmore			
Males	4.38	1.26	1.0–7.0
Females	8.13	1.79	1.5–13.0

The mean carapace width for the berried females was 8.80 mm whilst that for the unberried females was 7.98 mm. The difference between the two was very highly significant ($p \ll 0.001$) with a 95% confidence interval for the difference in population means being (0.60,1.05) mm. Overall, there was no significant difference between the mean shell length of mussels occupied by berried females compared to that for unberried females ($p = 0.11$).

ANOVA for the carapace width of berried females, cross-classified according to position on beach, sampling time (May to September) and shell length of the host mussel, showed that mussel size was the most important of these variables followed by the month in which sampling took place and then sampling position. Specifically, the mean carapace width of berried females increased with mussel size, was 0.75 mm larger in May than for the other four months and was 1.2 mm larger near low water than that near high water.

DISCUSSION

Occupancy

The marked difference in occupancy of mussels by pea crabs at the two sites (54% at Calshot, 34% at Elmore) was most likely due to the much greater density of the mussels at Elmore: if similar numbers of megalopae arrive at the two sites they are likely to enter a higher proportion and less optimal size of mussels at Calshot than at Elmore. This is supported by the data in Table 3 which show that for any given class of pea crab the mussels they occupy are smaller at Calshot than at Elmore.

Our various findings lead us to conclude that the Poisson model for random occupation is untenable. The results from the tests of randomness and independence, together with an inspection of the frequencies in Tables 1 and 2, indicate that for female pea crabs there exist mechanisms which inhibit co-occupation by two or more females and, at Calshot (where the average size of the sampled mussels was smaller than at Elmore), co-occupation by females with two or more males. Given the observed frequencies for the males, it is unlikely that similar mechanisms exist which have any strong effect on multiple occupancy by males. However, across the two sites there is evidence that mechanisms exist which promote occupation by male/female pairings and militate against single male occupation. These mechanisms could involve the behaviour of either full-grown crabs of one or both sexes, or megalopae and newly metamorphosed crabs which have just settled on the mussel bed.

The well camouflaged and tough carapace of mature male pea crabs, together with their swimming ability, would appear to enable them to survive outside a host for some time. Mature males in aquaria have been observed to leave mussels, live freely for over a month, and re-enter new mussels (pers. obs. CMCH). Mature males have also been collected from plankton hauls by Baan et al. (1972). Female crabs have a transparent, soft carapace and swim inefficiently (Hartnoll 1972; pers. obs. CMCH). Mature free-living females have not been found in nature. This suggests that female crabs normally remain inside bivalves.

Young crabs of both sexes settling on the mussel bed will occupy empty mussels. If they encounter an already occupied mussel their response would appear to depend on the number and sexes of the existing occupants. Immature single female crabs will enter mussels occupied by single males and cohabit, and *vice versa*. However, if the mussel is already occupied by a female (perhaps paired with a male) and another female encounters the

TABLE 5.

Proportion of berried females and total number of females recorded at Elmore

	Month						
	April 73	**May 73**	**June 73**	**July 73**	**Aug. 72**	**Sept. 72**	**Oct. 72**
Proportion of berried females	0.01	0.50	0.72	0.71	0.39	0.11	0.01
Total number of females recorded	80	70	83	70	116	104	93

mussel, then some form of interaction would appear to operate which results in one of the females (at the very least) abandoning the mussel. Similarly, if a male crab encounters a mussel occupied by a male/female pair then, as a result of some interaction, at least one of the crabs will abandon the mussel. The nature of these interactions is unknown to us but possibilities include: the prospective resident experiencing a lack of available space; aggressive behaviour resulting in occupation by the victor(s); the release by a paired female of a pheromone that deters other crabs from entering the host mussel. Two or more male crabs appear able to coexist, although presumably either, or both, of the first two possible sources of interaction mentioned above might apply when living space is found to be overly constrained. It is also possible that males move between mussels after occupation times of random length but they remain in a mussel longer if they are sharing it with a female. Carefully designed laboratory trials are required to explore these various possibilities.

From the results concerning the variables influencing occupancy and crab size it was found that the mussels occupied by single females were larger on average than those occupied by single males, and those occupied by more than one pea crab were larger still. Also, occupancy by single females and male/female pairs increased with the size of the host mussel, and the size of occupying female crabs increased with the size of the host mussel. These findings are no doubt all related to the lack of space available within smaller mussels to accommodate the larger size of the females and the combined size of multiply-occupying crabs. Houghton (1963) and Seed (1969) found a positive correlation between crab size and mussel size, but they did not distinguish between male and female crabs. Since it is likely to be the larger crabs that are sexually mature, this relationship may explain why we also found that paired males were larger on average than single males, and paired females were larger on average than single females.

At Elmore, where the sampling stations covered the complete tidal range of the beach, the proportion of mussels occupied by crabs was higher near low water than it was at high water for all three categories of occupancy (single male, single female and male/female pairs). This could be because mussels can only feed when covered by water, the pea crabs feed on the food collected by the mussels, and there is therefore more food available for crabs in the mussels found closer to low water. The lack of similar findings for Calshot could have been due to patchiness in crab occupancy within the more restricted sampling region at this site and/or the more level profile of the beach.

The higher proportion of male crabs found at Calshot than at Elmore was probably due to the fact that males tend to occupy smaller mussels than females, and more small mussels were sampled at Calshot than at Elmore (Table 3). However, these data together with those of Table 1 also indicate that, whilst being subject to the constraints of the interaction mechanisms between crabs, pea crabs will occupy smaller mussels if the number of available hosts is limited.

Reproduction

Crabs have been observed mating inside hosts in February (pers. obs. CMCH). Mating may occur at other times, but, because berried crabs were only found from April through to October (with a peak in their numbers in June and July), fertilization is only likely to occur from February to May or June. Christensen and McDermott (1958) report that for *Pinnotheres ostreum* mating occurs in open water. Mature male *P. pisum* have been recorded swimming in plankton during June and July (Huard and Demeusy 1968) and September (Baan et al. 1972), but no free-living mature females have been found in nature, and this, together with the numbers of male/female pairs in our mussels and the observation of mating recorded above, indicate that for *P. pisum* mating probably occurs only inside hosts. One might therefore expect more male/female pairs to be found from February through to June. However, the seasonal occurrence of male/female pairs at Elmore does not support this expectation: they were found with more or less equal frequency throughout the year. One possible explanation for this is that the crabs face the same problem as dioecious parasites, namely, of finding a host that contains a potential mate out of a great many that do not. Selection will then favour any individuals which find a mate if they remain together, perhaps for several months. Our data also show that berried females are on average larger than unberried females, presumably reflecting the sexual maturity of larger females. Moreover, the larger females came into berry before the smaller ones, indicating that the former are likely to be the more mature females from the previous year's spawning.

Nature of Association

In the literature there is no consistency when defining the nature of the association between the pea crab and its habitat (or host). It is variously described as symbiotic (Anderson 1974), parasitic (Calman 1911), commensal (Huard and Demeusy 1968), or inquiline (Grassé 1960). A further problem is the lack of consensus as to what is meant by these terms. Following Odum (1971), mutualism and symbiosis are associations where both partners benefit; commensalism is where one partner benefits and the other neither benefits nor suffers harm; amensalism is where one animal benefits from the association whereas the other suffers, but the relationship is facultative for the amensal; parasitism is also a relationship where one animal benefits at the expense of the other, but it is obligatory for the parasite which usually lives in or on the other animal.

For *P. ostreum*, McDermott (1962) found that the oysters containing pea crabs often had damaged gills and palps. He attributed this damage to the crabs, which would indicate a parasitic asso-

ciation. For *P. maculatus*, mussels with pea crabs had lower filtration rates and lower oxygen consumptions than mussels without crabs (Bierbaum and Shumway 1988). In nutrient poor environments mussels with crabs had slower growth rates than mussels without crabs, but there was no such difference in nutrient rich environments (Bierbaum and Ferson 1986). *P. maculatus* collects food from the mussels's gills but does not appear to destroy the living tissues. For *P. pisum*, the observations of Orton (1920) and CMCH show that it too feeds on the food collected on the gill filaments of the mussel and does not eat the mussel tissue itself. Since the mussel loses some of the food it has filtered it must therefore ingest less than it would have done in the absence of the crab. Given that no free-living mature females have been found in nature, their association with a host would thus appear to be akin to true parasitism. However, for mature males the association is best described as amensalism as they are able to survive outside hosts.

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SHELL WEAKENING IN MARINE MYTILIDS ATTRIBUTABLE TO BLUE-GREEN ALGA *MASTIGOCOLEUS* Sp. (*NOSTOCHOPSIDACEAE*)

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ABSTRACT *Mastigocoleus* sp. was found infesting shells of *Mytilus galloprovincialis*. SEM figures depict *Mastigocoleus* sp. burrows in the shell, and micrographs of decalcified shell show the algal filaments. Fast growing subtidal mussels were least infested; slow growing high intertidal mussels suffered heavier infestation. Prevalence varied from 94% at the high intertidal site to 1% at the subtidal site. Infestation prevalence and intensity increased with shell length. Aspects of infestation intensity were examined by studying severity (depth of pits in the shell) and extent (infestation patch coverage on the shell) of burrowing. Holes, caused by fracture of the shell, were most abundant over the adductor muscle scar area, but infestation focus lay anterior to this. Heavily infested mussel shells had lower penetration strengths than those of the same length with lesser infestations. Mean penetration strength of infested samples was reduced by between 36% to 43%.

KEY WORDS: Burrowing cyanophyte, *Mytilus galloprovincialis*, *Perna perna*, *Choromytilus meridionalis*, *Aulacomya ater*, *Mastigocoleus*.

INTRODUCTION

Shells of living *Mytilus galloprovincialis*—the introduced Mediterranean black mussel—on the Western Cape coast of South Africa have recently been found with grey corroded patches caused by burrows of the filamentous cyanophyte *Mastigocoleus* sp. These infested patches are often weak, and in extreme cases may fracture to produce large holes in the shell (Fig. 1).

Endolithic (rock burrowing) micro-organisms are important bio-erosive agents of animal hard-structures and limestone (Peyer 1945). For reviews see and Kobluk and Risk (1977) and Kinne (1983). Noteworthy work on the identification of marine endolithic algae from their burrowing patterns has been done by Golubic (1969) who in collaboration (Golubic et al. 1970) also pioneered study techniques and has proposed a new scheme of terminology for endolithic organisms (Golubic et al. 1981). Although infestation of mollusc shells by endolithic micro-organisms is common, serious structural weakening of mollusc shells by *Mastigocoleus* sp. has not been reported. Furthermore, direct damage resulting from algal penetration of bivalve shells is usually negligible (Kinne 1983). And although Raghukmar et al. (1991) reported that bivalves, including the mytilid *Perna viridis*, suffered shell weakening when infested with boring algae including *Mastigocoleus* sp., they did not, however, report any extreme effects such as the production of fracture holes in the shell. This is consonant with their findings that the burrows did not penetrate deeply into the shell. Nevertheless, burrows by this alga up to two millimeters deep have been reported in intertidal calcareous rocks (Anand 1937).

Mastigocoleus sp. and other cyanophyte protophytes are widespread in marine habitats (Fogg 1973) and endolithic algae occur in all types of carbonate substrata. According to Humm and Wicks (1980) this genus contains only the species *Mastigocoleus testarum* Lagerheim, and only this species is mentioned in the literature. *M. testarum* is a common species found mostly in temperate areas, but Lawson and John (1982) consider its distribution to be worldwide. The closest previous reports to South Africa are from Sierra Leone (John and Lawson 1977, Aleem 1980).

Endolithic cyanophytes share this niche with chlorophytes (Harris et al. 1986), rhodophytes (Nolan 1991), fungi—in lichens (Griffiths, pers. comm.), and some sponges, particularly of the genus *Cliona* (Kinne 1983). Nolan (1991) reports an infestation by a *conchocelis* phase, probably of the red alga *Bangia* sp. or *Porphyra* sp., in shells of the limpet *Nacella concinna* from the South Orkney Islands. This infestation affects the density of the shell and furthermore induces other grazers to abrade and sometimes perforate the shell in an attempt to harvest the alga.

Harris et al. (1986), in South Africa, detail the interaction between the sandy-beach whelk *Bullia digitalis* (Dillwyn) (Gastropoda, Nassariidae) and the endolithic chlorophyte *Eugomontia sacculata*. Also in South Africa, lichens of the genus *Pyrenocollema* sp. have been observed (Griffiths, pers. comm.) infesting a number of molluscs, including limpets and mytilids. Although *Pyrenocollema* sp. appears to pose a threat to the physical integrity of shells, it has not been noted on any of the mussels examined here.

In this study we examine *Mastigocoleus* sp. infestations in *Mytilus galloprovincialis* for three reasons: it seems to be the most heavily infested of the mussel species on these coasts; it is an important maricultural component of the industry centered in Saldanha Bay on the west coast of South Africa and, not least, *M. galloprovincialis* has proved to be a vigorous invader. In places it has become a dominant intertidal species. Thus, the ecological and economic significance of this mussel render it particularly interesting.

MATERIALS AND METHODS

Collections of mussels were taken from four sites at Saldanha Bay (33°02′S, 18°00′E) which is about 120 km north of Cape Town, South Africa. These sites comprise one high intertidal of mixed rock and sand at Saldanha Beach with light to moderate wave action (collected on 19/7/90), and three at Sea Farm—an aquaculture enterprise located in a dam next to the Saldanha–Sishen iron ore jetty (collected on 18/7/90). The dam, at which the tidal range is about one meter, covers several hectares. Wave action is nil to very light. Flow in and out of the dam is conducted by a 1.5 meter diameter pipe to the bay. Wave action at the high intertidal site on the outer (sea) side of the dam wall is moderate.

The differing tidal levels at collection sites provided a range of

Figure 1. ***Mytilus galloprovincialis*** **showing shell damage and fracture holes caused by** ***Mastigocoleus*** **sp., scale bar 30 mm.**

different mussel growth rates varying from very fast in the subtidal, to slow in the high intertidal (Van Erkom Schurink and Griffiths, in press).

Other sites examined for infested mussels were: Simonstown (34°10′S, 18°25′E), this is the only site not on the west coast, instead it lies on the western shore of False bay about 30 km south of Cape Town. Blouberg (33°48′S, 18°27′E) is about 20 km north of Cape Town and Stompneusbaai (32°45′S, 17°55′E) is just north of Saldanha. Duiwegat (31°30′S, 18°05′E) is about 270 km north of Cape Town, and Port Nolloth (29°16′S, 16°52′E) lies another 330 km further north from Duiwegat.

Shell fragments were prepared for SEM examination by fixation in 3% glutaraldehyde, then dehydrated in an alcohol series and critical point dried. Shell pieces were then fractured to expose a fresh face and mounted on stubs before they were coated with gold-palladium. Decalcified shell specimens were obtained by immersion in 10% acetic acid overnight. Algal filaments could then be teased apart for examination.

With the exception of mussels from the culture ropes, a representative 0.1 m^2 quadrat sample of mussels from the Saldanha collection sites was taken which included all mussels down to the rock surface; each mussel was measured. Cultured mussels were taken randomly from ropes brought in for harvesting. Shell length is as defined in Seed (1968): the greatest measurement along the anterior-posterior axis.

To determine the location of the infestation patch on the shell, we divided each valve was into areas A, B and C (Fig. 6A) corresponding to the areas designated 3, 2 and 1 on the shells of *Mytilus edulis* by Laihonen and Furman (1986). Extent of the infestation patch was graded out of 5 on each of these divisions: clear areas being graded 0 and covered areas 5. The overall extent of the infestation patch on each mussel was determined as the sum of values, each out of 5, for divisions A, B, and C (maximum 15).

Location of the infestation patch on the valve surface was plotted by hand onto the diagram of a shell as an infestation patch perimeter line for each individual. Contours reflecting 10%, 50% and 80% frequency of infestation were obtained by evaluating groups of ten superimposed infestation patch perimeter line plots from the same site. Individual perimeter lines were then counted from the outer margin of the valve to a point at its center. Counting was aided by drawing a series of lines radiating from the nominated center of the shell (Fig. 6B). The 10% contour is the outermost 1st line, it signifies that the area between the 10% and 20% lines is encroached upon in 10% of cases. The other contour lines have corresponding meaning. In samples of fewer than 10, the distance between the first and last line was split *pro rata* and percentage contour lines constructed. These composite contours were then combined with others similarly constructed until the sample size for each collection site was achieved. Probability contours for holes broken in the shell were similarly derived.

Shell penetration strength in Newtons was obtained by use of a spike penetrometer over the adductor muscle area. The figures given are for comparison of infested with uninfested shells. Absolute values of force per unit of shell area are not available as a

conical spike was used. Two mussel samples, one lightly and one heavily infested, were taken from each collection site and their strengths compared using Student's one-tailed *t*-test. Equal numbers of infested and uninfested mussels were used, and each was matched to an equal sized counterpart, thus ensuring an identical size distribution and mean size of population. This is to eliminate any bias that change in shell strength with size might introduce. In the case of the sample from the outer side of the Sea Farm dam wall, it was not possible to match all mussels with equal sized counterparts, here mussels of size within one millimeter were paired.

In this work an infestation of *Mastigocoleus* sp. is defined as any presence of the alga as indicated by grey roughening of the shell in areas where the periostracum is absent. Pilot studies employing decalcification methods to free algae from the shell matrix showed that this characteristic in the mussel samples studied here was invariably associated with the presence of *Mastigocoleus* sp. filaments in the shell. The identification *Mastigocoleus* sp. was confirmed by H. Stegenga (pers. comm.): it is a cyanophyte of order Stigonematales and family Nostochopsidaceae. See descriptions in Humm and Wicks (1980), Lawson and John (1982) and Fogg et al. (1973). Because the genus has only one previously recorded species, *M. testarum*, it is likely that this study also deals with this species. Identification to specific level was not confirmed and in consequence the subject of this study is referred to as *Mastigocoleus* sp.

RESULTS

Figures 2 and 3 depict the exposed *Mastigocoleus* sp. filaments in a decalcified piece of infested mussel shell. Scanning electron microscopy (Figs. 4 and 5) revealed that the filamentous thallus of this endolithic blue-green alga had honeycombed the shell with burrows about 8 μm in diameter. In severe infestations they may occur in such density as to honeycomb the shell matrix and cause it to disintegrate. The burrows are invisible to the naked eye and light microscopy of the shell did not disclose them.

Mastigocoleus sp. also affects the brown mussel, *Perna perna*, and the black mussel, *Choromytilus meridionalis*, but with less severity (pers. obs.). *Aulacomya ater*, the ribbed mussel, was

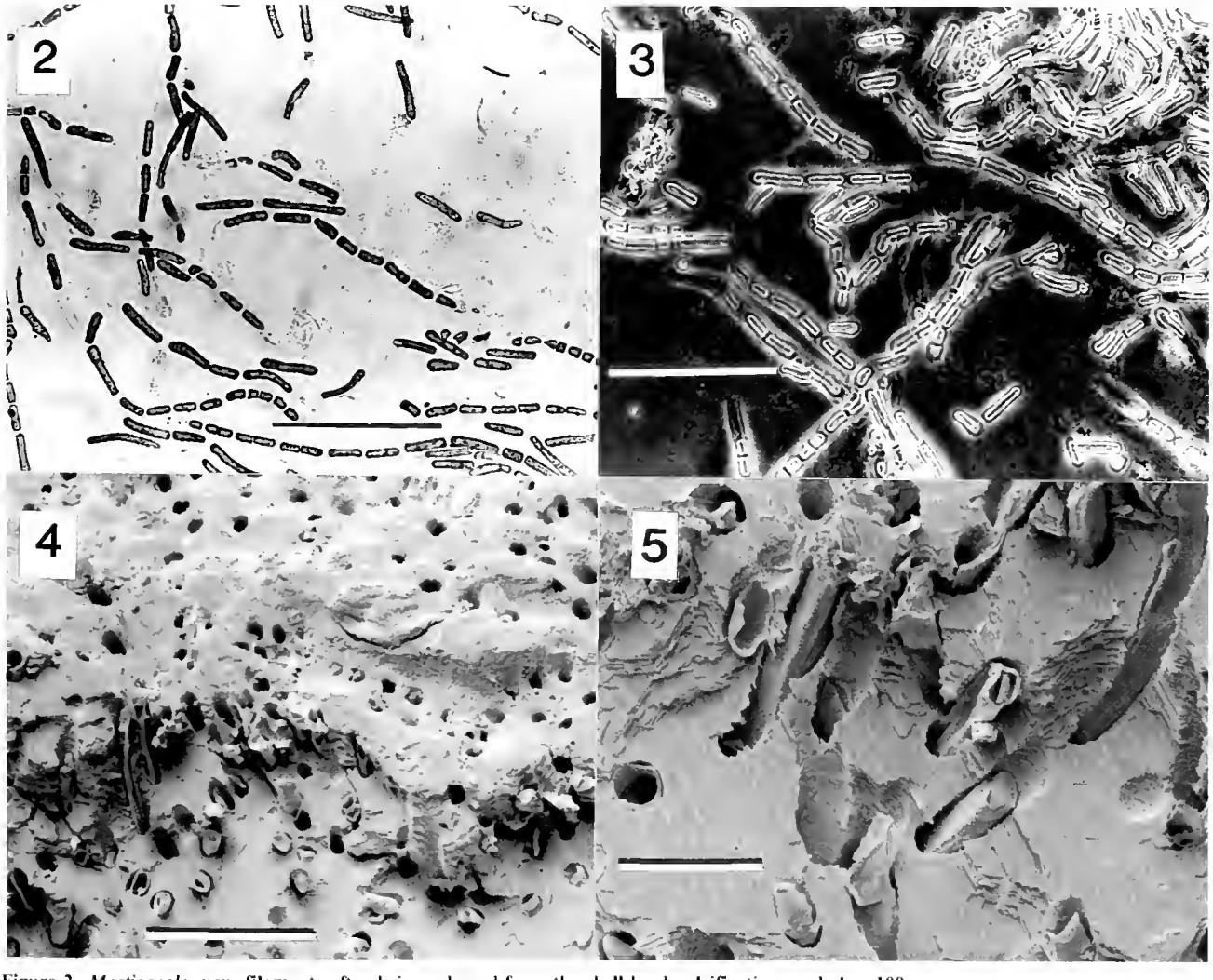

Figure 2. *Mastigocoleus* sp. filaments after being released from the shell by decalcification, scale bar 100 μm.
Figure 3. *Mastigocoleus* sp. filaments (phase contrast) after being released from the shell by decalcification, scale bar 100 μm.
Figure 4. *Mastigocoleus* sp. infested mussel shell, SEM scale bar 50 μm.
Figure 5. Details of *Mastigocoleus* sp. tunnels in a mussel shell, SEM scale bar 20 μm.

largely uninfested except at Port Nolloth where severe shell weakening was common (pers. obs.).

Mean length, population density and prevalence in *M. galloprovincialis* are summarized in Table 1. Subtidal mussels attain a much greater maximum size than those from intertidal populations; in fact, very few intertidal mussels exceed 70 mm, which is a common size for cultured mussels.

Prevalence, the proportion of mussels in a population showing signs of infestation, was ascertained (Table 1) from the data on extent and severity. When these were both nil the mussel was deemed uninfested.

Intensity, the number of infesting individuals per host, could not be measured directly, but aspects of it were ascertained under the headings of extent, severity and shell penetration strength. Extent, the area of infestation patch coverage on the shell (Fig. 9 A, B and C), the extent of shell fracture holes (Fig. 9D), and the most common areas of both are given on Figure 10. The mean extent in each size group for each of the three shell areas at the different collection sites is given in Figure 7 A, B and C. Infestation extent at each site increased with increasing mussel length. Figure 8 shows a typical plot. Extent shows a trend that increases with increasing shell length (Figs. 7, 8 and 11).

Shells were graded for severity—the amount of damage caused by the infestation—from 0 (free) to 5 (holed). The amount of damage apparent was always ascertained on the most heavily affected valve, as any perforation will critically reduce survival. Regression equations are given below; their significance in all cases is $P = 0.001$.

At the outer side of the Sea Farm dam wall (high water) 50% of variation in severity is accounted for by shell length: $Y = 0.10979X - 2.30436$, $R^2 = 0.50$ (df = 141). At Saldanha (intertidal) 64.1% of variation in severity is accounted for by shell length: $Y = 0.133461X - 0.75587$, $R^2 = 0.64$ (df = 142). At the inner side of the Sea Farm dam wall (low intertidal) 38.2% of variation in severity is accounted for by shell length: $Y = 0.047953X - 0.97872$, $R^2 = 0.38$ (df = 137). Thus, severity increases with shell length (Fig. 11). Regression analyses show that components of this relationship are highly significant. The smallest mussels are often undamaged, whereas larger ones tend to suffer deep pits, or even have holes broken in their shells.

Mussels whose shells are severely infested with *Mastigocoleus* sp. have weaker shells than those of the same length with lesser infestations. The mean penetration strength of infested samples is reduced by between 36% to 43%. Furthermore, *t*-tests comparing penetration strengths of shells with light and heavy infestations show that they are significantly different.

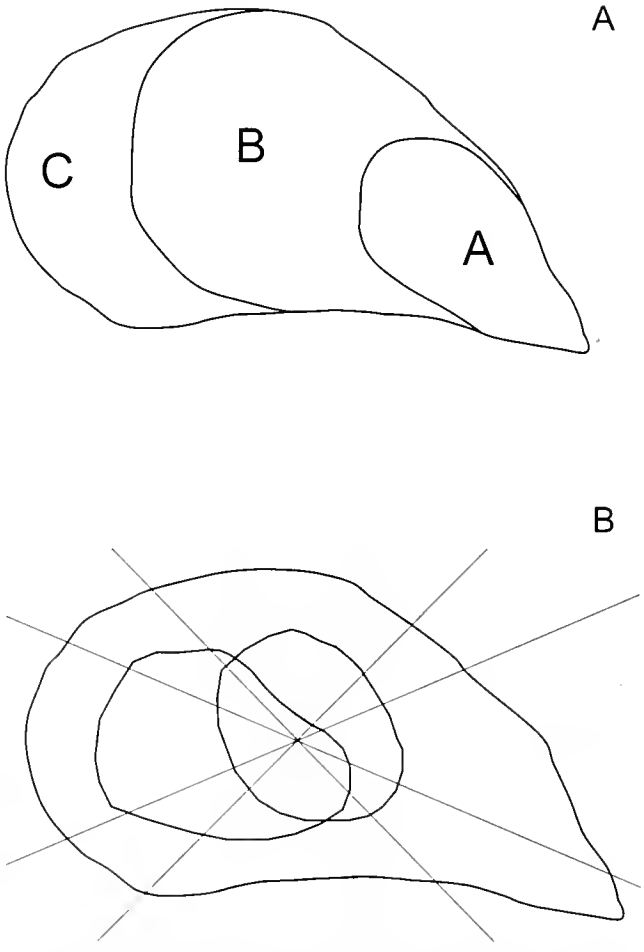

Figure 6. A: valve areas nominated A, B and C. B: the radiating lines show the contour method for determining infestation patch size and location of *Mastigocoleus* sp. on the shell.

The Saldanha Beach mussels had the highest prevalence (Table 1) and severity (Fig. 11), and were the only sample to contain mussels with holes broken in their shells. Saldanha mussels also had the most marked strength differences: samples of infested and uninfested mussel shells differed in mean penetration strength ($P = 0.005$; one-tailed *t*-test). Shell strength was 76.9 N and 134.3 N for heavy and light infestations respectively. The sample size was eight of each class of mussel and the mean sample shell lengths in both classes was 34.51 mm with a Standard Deviation in both samples of 2.601 mm. The critical region $t_s \geq 2.977$; the test statistic = 3.1389 and the degrees of freedom = 14.

At the outer side of the Sea Farm dam wall, samples of infested and uninfested mussel shells differed in mean penetration strength ($P = 0.025$; one-tailed *t*-test). Shell strength was 97.2 N and 152.6 N for heavy and light infestations, respectively. The sample size was nine of each class of mussel. The mean sample shell lengths were 46.89 mm (S.D. = 3.923 mm) for heavily infested mussels and 46.87 mm (S.D. = 3.911 mm) for those with light or no infestation; these figures were very close so no size dependent differences may be expected. The critical region $t_s \geq 2.12$; the test statistic = 2.46 and the degrees of freedom = 16.

TABLE 1.

Population density, mean shell length, and prevalence of infestation by *Mastigocoleus* sp. in *Mytilus galloprovincialis* from different collection sites.

Site	Mean Shell Length (mm)	Population Density per m^2	Prevalence	*n* for Prevalence
Outer causeway	18	15240	51.4%	142
Saldanha	19	7120	94.4%	144
Inner causeway	45	1380	66.2%	139
Culture ropes	53	—	0.96%	104

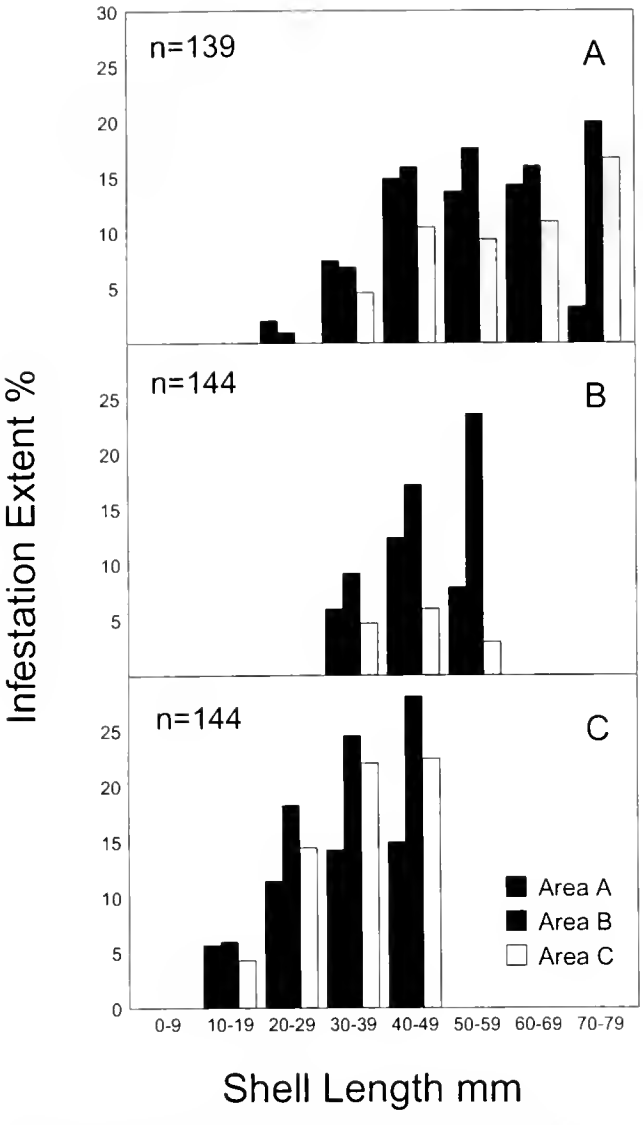

Figure 7. A: Extent of *Mastigocoleus* sp. infestation on each of the three shell areas (collection from the inner side of the Sea Farm dam wall). B: Extent of *Mastigocoleus* sp. infestation on each of the three shell areas (collection from the outer side of the Sea Farm dam wall). C: Extent of *Mastigocoleus* sp. infestation on each of the three shell areas (collection from Saldanha Beach).

At the inner side of Sea Farm dam wall, samples of infested and uninfested mussel shells differed in mean penetration strength ($P = 0.005$; one-tailed t-test). Shell strength was 63.4 N and 99.6 N for heavy and light infestations, respectively. The sample size was sixteen of each class of mussel. The mean sample shell lengths were 52.86 mm for each class with a Standard Deviation of 6.39 mm in both cases. The critical region $t_s \geq 2.75$; the test statistic = 3.1389 and the degrees of freedom = 30.

A study of infestation distribution on the shell valves (Figs. 7, A, B and C) shows that it is predominantly the mid-area on the outer surface of each shell valve that is most commonly infested by the alga and that the extent of infestation tends to rise with increasing shell length. Figures 9 and 10 indicate specific areas of infestation and the location of holes. Note that the pattern of infestation is similar, irrespective of the sample sites, and also that

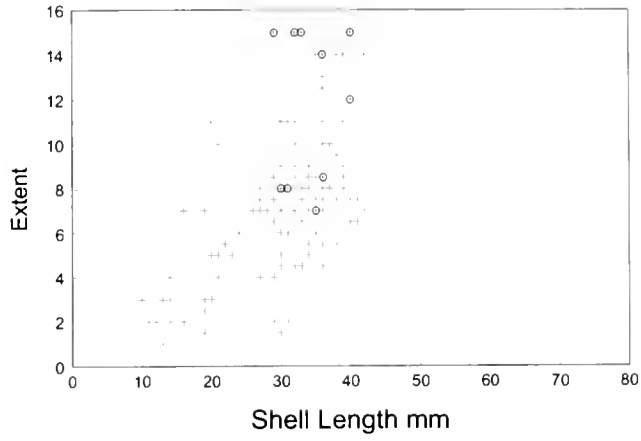

Figure 8. Extent of shell coverage by *Mastigocoleus* sp. versus shell length of *Mytilus galloprovincialis* from Saldanha beach. Holed individuals are circled.

the 80% infestation area does not correlate with the oldest part of the mussel.

Only one mussel out of 104 from the culture ropes at Sea Farm was infested with *Mastigocoleus* sp. This infestation had an extent and severity rating of less than 1, and it occurred in the typical area on the valve. In contrast, the Saldanha Beach sample contained shells with holes broken in them. Holes were most common over the adductor muscle insertion points, which fall outside of the 80% infestation probability contour. Figures 8 and 11 show that shells with holes in them tend to be larger than most.

DISCUSSION

Within populations the prevalence of *Mastigocoleus* sp. infestations rises with increasing shell length (Figs. 8 and 11; any score above 0 signifies an infestation). These figures also show that the smallest mussels are commonly disease free. If one assumes a correlation between shell length and age in any collection, then the larger mussels are older: the duration of opportunity for infestation increases with age and therefore size (Kinne 1983).

When we compare the relationship of mean shell length and prevalence among the populations (Table 1), the converse of

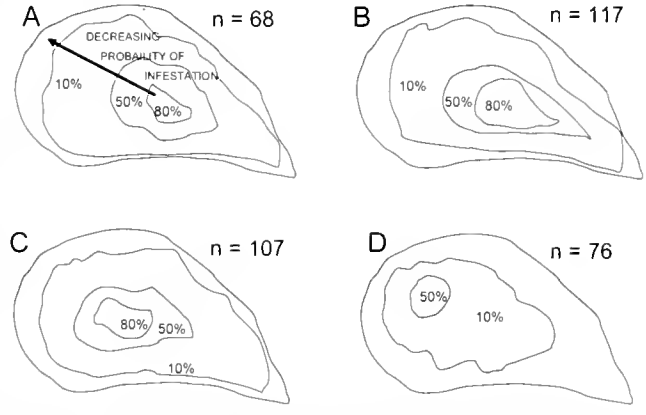

Figure 9. Areas and locations of *Mastigocoleus* sp. infestations at four sites. A: Low intertidal—inner causeway; B: High intertidal—outside causeway (Sea Farm); C: High intertidal—Saldanha Beach; D: Area and location of holes in shells of *Mytilus galloprovincialis* collected from Saldanha Beach.

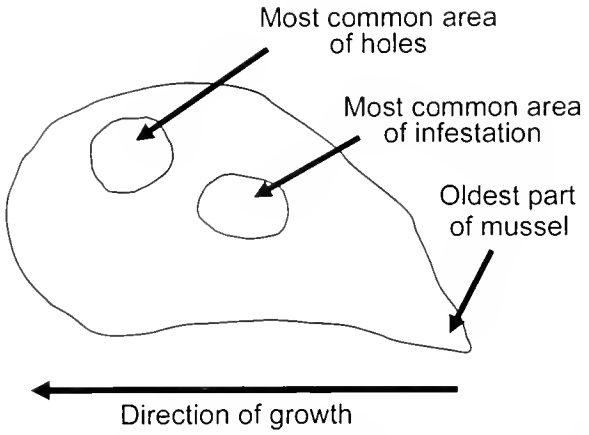

Figure 10. Summary diagram indicating the most common area of *Mastigocoleus* sp. infestation on the shell, and the most common area where holes occur.

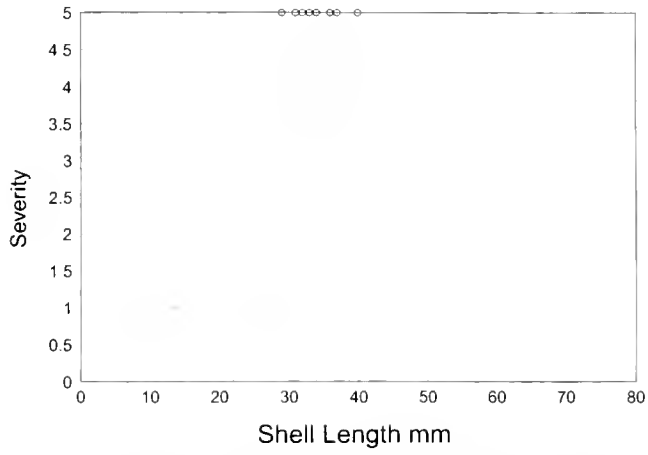

Figure 11. Severity of shell damage caused by *Mastigocoleus* sp. versus the shell length of *Mytilus galloprovincialis* in a sample from Saldanha Beach. Individuals with fracture holes in their shells are circled.

above is suggested: populations with larger mean shell lengths suffer lower prevalences. The explanation appears to rest with differential growth rates and shell abrasion brought about by tidal conditions. Faster growing mussels may outrun the growth of the alga resulting in a lesser degree of infestation severity and extent on the shell. The sample with the smallest mean shell length—that from the outer side of the Sea Farm dam wall—was subject to the highest wave action and was also high on the intertidal. The sample with the largest mean size was from the most favorable conditions: subtidal culture ropes with no wave action.

Faster growth of the subtidal mussels is attributable to the unbroken feeding time provided by permanent immersion (Van Erkom Schurink and Griffiths, in press). Moreover, such favorable conditions, lacking either emersion or mechanical threat by wave action, allow the mussels to put more effort into growth even at the expense of shell thickness (Raubenheimer and Cook 1991).

Mastigocoleus sp. is mainly restricted to shell areas where the periostracum has worn away. This is also supported by Raghukmar et al. (1991) who found the same for the mytilid *Perna viridis*. It seems that an intact periostracum layer may be an important factor in preventing infestation. Indeed, according to Bottjer and Carter (1980) this conchiolin layer, in living bivalves, offers some protection from epibionts.

Subtidal cultured mussels had relatively thin shells, but they still had an intact and shiny periostracum. And coincidentally, they had a very low prevalence of *Mastigocoleus* sp. Intertidal mussels, at their points of maximum shell width, often had patches where the periostracum was worn through. This is coincidentally the area of most frequent *Mastigocoleus* sp. infestation (Figs. 7, 9 and 10). This damage may be caused by wave action in combination with water borne sediments and exacerbated by high mussel population densities. Closely packed mussels will suffer more abrasion of the periostracum at the point of maximum width because this area is more likely to rub against that of the neighboring mussel when mussels are moved on their byssus by wave action.

Though the most common area of infestation on the shell is not the oldest part (Fig. 10), absence of heavier infestation in the older regions of valves may be explained by these parts usually being wedged in between neighboring shells and buried under sand where there may be insufficient light for algal growth.

Holes caused by fracture of the shell, as shown in Figure 1, are indicative of extreme shell weakening. The Saldanha Beach sample contained some holed specimens and they were usually larger mussels (Figs. 8 and 11). It is curious that these fracture holes often lie outside of the most common area of infestation and instead are frequent at the point of adductor muscle insertion (Fig. 10). The shell here is thinner because there is no nacreous layer beneath the point of muscle insertion. In consequence, the shell here is more highly stressed and any weakening coupled with the concentrated force applied by the adductor muscle may be critical. Other parts of the shell may be more severely infested, and thus be weaker, but they are not so subject to mechanical forces.

This work demonstrates that burrows of *Mastigocoleus* sp. can drastically weaken the shells of mussels. Indeed, some of the more heavily infested mussels may be crushed between finger and thumb (pers. obs.). This weakening is likely to render them more vulnerable to predation and mechanical effects of wave action. As yet there is no explanation for the outbreak or for its unusual destructiveness, but it is clear that the intensity of *Mastigocoleus* sp. infestations have worsened in the past five years (pers. obs.) and that further work is needed to gauge its impact.

ACKNOWLEDGMENTS

We thank students of the Third year 1990 Zoology field camp for their assistance, and Associate Professor Griffiths and Professor Brown for their support. The work was funded by a grant from the University of Cape Town. Thanks also to Candy Lang of the Department of Materials Engineering, UCT, for instruction on the use of the penetrometer.

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SPATIAL AND TEMPORAL VARIATION IN ADDUCTOR MUSCLE RNA/DNA RATIO IN SEA SCALLOPS (*PLACOPECTEN MAGELLANICUS*) IN THE BAY OF FUNDY, CANADA

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ABSTRACT The ratios of RNA to DNA (RNA/DNA) were determined from the adductor muscle of the sea scallop *Placopecten magellanicus* (Gmelin 1791) collected from the commercial fishing beds off Digby, Nova Scotia, Canada. The study was designed to examine the effects of and interactions between scallop size and position within the bed and interannual variability in adductor muscle RNA/DNA. The smallest size class of scallops had significantly higher RNA/DNA values than the two larger size-classes. A consistent fine-scale spatial patchiness in RNA/DNA was observed. In the Digby area this pattern showed an interaction with size, that is some "patches" produced higher ratios in particular size-classes. Large-scale spatial variability was also observed, although there was no significant interaction with sampling year. These data indicate that it may be possible to use RNA/DNA values as an indicator of nutritional stress in wild populations of *Placopecten*.

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

INTRODUCTION

The sea scallop, *Placopecten magellanicus* (Gmelin 1791), has sustained a lucrative fishery in the Bay of Fundy, Canada for more than 70 years. Recently this resource suffered from high mortality during the summer months (Kenchington and Lundy 1991, Robinson et al. 1992) with a significant impact on the fishery. The cause of this mortality has not been determined, however starvation and/or disease may have been major factors, at least in one population (Kenchington and Lundy 1991). In response to this catastrophe a method for assessing the health status of the scallops was desired as a means of monitoring the condition of the stock.

Measurement of the ratio of ribonucleic acid (RNA) to deoxyribonucleic acid (DNA) in somatic tissue has been suggested for detecting a response to environmental change before growth rate changes become severe (Haines 1973). The ratio of RNA to DNA (RNA/DNA) is a self-calibrating measure of cellular metabolic activity which has also been used to assess nutritional condition. Apart from polyploid individuals, the amount of DNA per nucleus is constant for all somatic cells within a given species. Conversely, RNA content appears to respond rapidly to changes in food availability and temperature (Wright and Hetzel 1985) and is directly related to protein synthesis (Devi et al. 1963, Munro and Fleck 1966).

RNA/DNA ratios may also be useful in detecting stress due to disease. Squibb et al. (1968) found that virus infections in birds resulted in significantly lower amounts of ribonucleic acids. Changes in the levels of RNA and protein occurred at the onset of infection and were negatively correlated with the progression of the disease.

The RNA/DNA ratios have been determined for a number of organisms under various conditions. In fish larval studies, they are used as a growth and condition index (e.g. Buckley 1979, 1980, 1984, Buckley and Bulow 1987, Clemmesen 1987a, 1988, 1989, 1990, Robinson and Ware 1988, McGurk and Kusser 1992). However, in larval Crustacea, interpretation of the RNA/DNA ratio is complicated by the molt cycle (Sulkin et al. 1975, Juinio et al. 1992). In both fish and crustacean larvae, the sensitivity of this ratio to environmental change appears to be on the order of 2 to 4 days (e.g. Sulkin et al. 1975, Buckley 1981), rendering it useful for experimental situations.

There is a paucity of information available on this index in mollusks. Martinez et al. (1992) compared RNA/DNA ratios between hatchery and open-ocean-reared Chilean northern scallops, *Argopecten purpuratus* Lamarck (1819), at two life-stages in order to assess optimum rearing conditions for the aquaculture of this species. The gonadal RNA/DNA ratio of the ocean-reared adult broodstock decreased after 12 days of transfer to the poorer growth conditions of the hatchery. Thus it appears that in sexually mature animals the sensitivity of the gonadal RNA/DNA ratios to environmental change may be protracted. The time scale of this response renders the adult life history stage useful for identifying nutritional stress in the field, that is, the ratio does not appear to fluctuate widely in response to feeding activity and so changes in the ratio would appear to reflect substantive changes in nutritional condition as seen in the hatchery example detailed above. Gonadal RNA/DNA has also been used to evaluate seasonal variability in synthetic activity and sexual state in the scallop *Pecten maximus* L. (Robbins et al. 1990).

In the American oyster, the RNA/DNA ratios of both mantle (Wright and Hetzel 1985) and adductor muscle (Pease 1976) tissues have been evaluated. Of these two somatic tissues, the mantle tissue showed higher RNA/DNA values reflecting the rapid production of shell proteins observed in growing oysters (Wright and Hetzel 1985). The RNA/DNA ratio in this tissue decreased within 48 hours of starvation. The adductor muscle RNA/DNA ratio in natural oyster populations shows a marked seasonal cycle, with higher values reported in the fall (Pease 1976). Pease (1976) also observed that there was no relationship between the observed muscle RNA/DNA ratio and oyster year-class, a feature important in assessing wild populations.

The current study was designed to examine spatial and temporal changes in the RNA/DNA ratios of natural populations of adult sea scallops on the commercial fishing grounds in the Bay of Fundy in order to assess the use RNA/DNA values as an indicator of nutritional stress under field conditions.

MATERIALS AND METHODS

A survey of the extensive scallop bed off Digby, Nova Scotia was made in June 1990 to examine the effects of, and interactions between, scallop size and position within the bed on RNA/DNA ratios. Position included two aspects: "area" defined by position along the shoreline, and "distance" defined by distance away from the shoreline toward sea (Fig. 1). Results of the analysis of the 1990 data showed that the factor "distance" was non-significant in all of the tests. This survey was repeated in June of 1991 in order to test for year-to-year effects and more specifically for area-year interactions. In 1991 the sampling design was modified by removing distance from shore as a factor, and reducing the number of areas to four (due to an inability to obtain enough animals in each size-class in the Centreville area). Inter-annual variation was further assessed in June in one area, Digby Gut, by sampling six random tows for each of three years (1990–92).

At each sampling location (Fig. 1) an eight-minute tow over the bottom was made using Digby buckets, the traditional scallop fishing gear of this commercial fishery. The length of an average tow is approximately 1 km (pers. obs). Bottom temperature was recorded for each tow using a digital sub-surface thermometer. Depth was also recorded. Scallops caught in the buckets were brought on board and sorted from the mix of rubble and other species. Three scallops were randomly selected for RNA/DNA analysis from within each of three, five millimeter increment size-classes based on shell height. These size-classes, 80–95 mm, 96–110 mm, and 111–125 mm represent mature individuals of commercial size. The corresponding ages for these size-classes in this area, based on the counts of annual rings formed on the shell (Bourne 1964), are approximately three, four and five years, respectively.

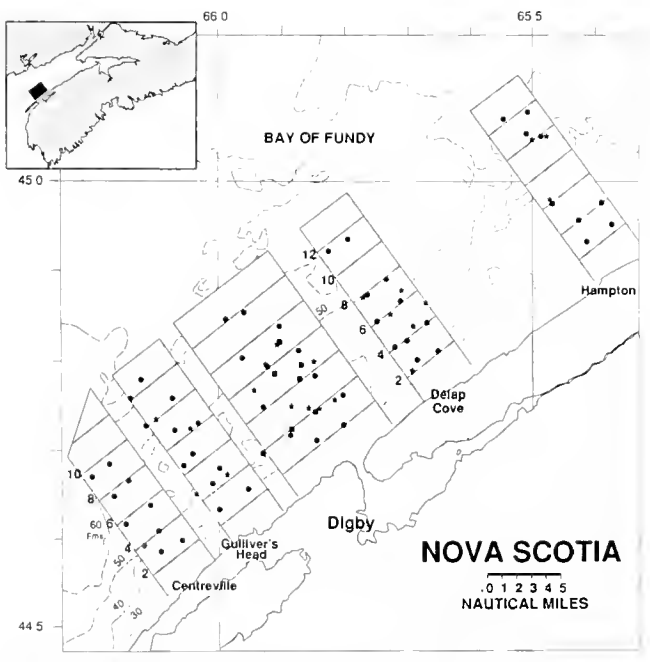

Figure 1. Map showing Digby, Nova Scotia, Canada with experimental areas (Hampton, Delap Cove, Digby Gut, Gulliver's Head and Centreville) and distances (2–12 miles) depicted. The location of individual tows are represented by solid circles in 1990, solid stars in 1991, and solid squares in 1992.

RNA/DNA was determined for the adductor muscle (as opposed to the mantle or gonad) with the hope of mitigating small-scale variability due to local feeding conditions. The seasonal component expected to be present in this tissue (Pease 1976) was standardized somewhat through sampling data. In scallops, the mantle tissue contains the eyes and tentacles which must be removed prior to RNA/DNA analysis as they contain differing amounts of nucleic acids. Some scallop mantles are also pigmented which could potentially interfere with fluorescence. For these reasons, the mantle tissue was not evaluated beyond preliminary testing. The adductor muscle and top shell were dissected live from each animal. The tissue was wrapped in foil and immediately placed in liquid nitrogen and held frozen there until the samples could be transferred to a −85°C freezer for storage. Tissue samples and shells were cross-coded and the shells were retained to verify size-classes. The sex and the shell height (mm) of the animals were recorded.

RNA/DNA Analysis

RNA/DNA were measured fluorometrically as described by Karsten and Wollenberger (1972, 1977) with two modifications. A 1 mm transverse section was made midway through the adductor muscle. The tissue was homogenized in 7 ml of an ice-cold heparin solution (3.75 μg/ml) and centrifuged at 2000 rpm for 5 min at 5°C. Centrifugation, a modification to the Karsten and Wollenberger protocol (1972, 1977), greatly increased the reproducibility of the results (e.g. Clemmesen 1987b), and typically produced three phases. The lower phase was largely cellular debris and when tissue was homogenized as above, negligible amounts of nucleic acid were detected in the precipitate. However, if the tissue were homogenized in phosphate buffered saline solution (PBS) as described in Karsten and Wollenberger (1972, 1977), significant amounts and differing proportions of RNA and DNA were precipitated through centrifugation. Of the three phases, the upper phase was largely foam and did not contain nucleic acid. The middle, clear phase was used in the RNA/DNA analyses and the pellet was discarded.

Total nucleic acid concentration and total DNA concentration were determined in replicate for each sample as in Robinson and Ware (1988). The effect of storage at −85°C for up to 2 years on samples of adductor muscle and tissue homogenate on RNA/DNA ratio was tested and found to be non-significant, however all samples were analyzed within 6 months of collection. Ethidium bromide-nucleic acid fluorescence was assessed using a Turner Model 112 fluorometer. A 320–390 nm filter was used for excitation and a >570 nm filter was used for emission. Calf thymus DNA and yeast RNA were used for calibration of standard curves. All chemicals used in the procedures were analytical grade.

Data Analysis

RNA/DNA ratios were natural log-transformed to correct for skewness. The data were tested for normality using the Kolmogorov-Smirnov test, and for homogeneity of variances using Bartlett's test. The transformed ratio was regressed against depth, temperature, shell height and sex in univariate analyses. Because a significant amount of the variation in the data set was explained by the depth variable, subsequent analyses were performed on both the ln-transformed ratio data and on the residuals extracted from the regression of this variable against depth. RNA/DNA ratio means were analyzed by univariate nested ANOVA to test for

area, distance from shore, size, year and interaction effects. Tow was nested within the appropriate factors (i.e. distance, area or year). Post-hoc comparisons of the group means were performed for each significant ANOVA ($\alpha = 0.05$) where more than two sample means were included in the analysis. Contrasts were selected across the sample means in order to determine which subgroups differed (Rodger 1974, 1975).

RESULTS

The mean and standard deviation of the RNA/DNA ratios for each size-class within each area for each year are given in Table 1. All data were normally distributed after transformation and the variances were not significantly heterogeneous.

The regression of the ln-transformed RNA/DNA ratio against depth was significant at $\alpha = 0.05$, however less than one percent of the variation in the data was explained by the regression model ($r^2 = 0.007$). Residuals from this regression were extracted. The regression of the ratio against temperature was non-significant. Temperature ranged from 4.5 to 8.6°C. In 1990 the mean temperature and standard deviation was 7.6°C ± 0.6°. In 1991 the mean temperature and standard deviation was 7.4°C ± 0.2°. In 1992 water temperatures were colder with a mean temperature and standard deviation of 5.0°C ± 0.2°. Temperature was correlated with depth. There was no significant correlation between ln-transformed RNA/DNA and sex, however, there was a significant negative correlation between the ratio and shell height.

There were no significant interactions between any of the factors in any of the analyses. The data were thus analyzed to test only the main effects and nested terms using both the ln-transformed ratio variable and the residual of a regression of this variable against depth. In 1990 five areas, six distances and three size-classes were sampled. ANOVA of this three-factor design is presented in Table 2. Area was a significant factor, however post-hoc analysis showed that all of the areas had indistinguishable RNA/DNA ratio means except for Delap Cove which had a smaller mean than the other four areas. Size was also significant, and the smaller three year-old scallops in the 80–95 mm size class had larger ratios than the two larger size-classes (Table 2). Tow means were significantly different indicating a fine-scale patchiness in the RNA/DNA ratio. The power of the test for both area and size factors was high, however, the power of the ANOVA to detect an effect of distance was low (0.41). This factor was non-significant. Re-analysis of a subset of the data (four areas and five distances) confirmed the non-significance of the distance factor with a high power value. Analysis of the depth-independent residuals increased the value of F in most cases (distance decreased) but did not alter the significance of any of the factors or terms.

In 1991 the survey was restricted to four areas. The Centreville data collected in 1990 was excluded from the analysis and the 1990 and 1991 data combined were analyzed to test for the effect of year, area and size on the ratio (Table 3). Year and size factors were significant and the powers of the tests were high. The values of the ratio were higher in 1991 than in 1990, and again the smaller size class had significantly higher values than the two larger size-classes which were non-significantly different from each other. Analysis of the depth-independent residuals produced a significant effect of area ($F = 3.15$, $P = 0.05$) as well as of year, size, and tow. In this analysis the Digby Gut and Hampton areas had means indistinguishable from each other and lower than the means for Gulliver's Head and Delap Cove which were also non-significantly different from each other.

Analysis of inter-annual variation in the Digby Gut area over three years (Table 4) showed that both year and size had significant effects on RNA/DNA. The ratio was higher in 1991 than in the other two years and in this analysis both of the two smallest size-classes had higher ratios than the larger animals. Most interestingly there was a significant effect of size by tow within year, suggesting that within the fine scale patchiness observed above, some patches are better for some sizes in this area. This interaction was non-significant in all of the other analyses. ANOVA of the depth-independent residuals showed the same significance of factors and interaction terms as did the unadjusted data.

TABLE I.

Summary statistics of RNA/DNA ratio values in adductor muscles of scallops collected in the Bay of Fundy from 1990–1992.

Area	Year	Size Class	Mean RNA/DNA	Standard Deviation	n
Centreville	1990	80–95 mm	0.599	0.138	30
		96–110 mm	0.613	0.182	29
		111–125 mm	0.611	0.315	30
Gulliver's Head	1990	80–95 mm	0.652	0.144	36
		96–110 mm	0.621	0.151	35
		111–125 mm	0.635	0.229	35
	1991	80–95 mm	0.809	0.187	12
		96–110 mm	0.841	0.179	12
		111–125 mm	0.640	0.115	12
Digby Gut	1990	80–95 mm	0.652	0.218	35
		96–110 mm	0.561	0.191	35
		111–125 mm	0.510	0.141	35
	1991	80–95 mm	1.019	0.301	43
		96–110 mm	0.932	0.272	46
		111–125 mm	0.841	0.235	48
	1992	80–95 mm	0.612	0.232	32
		96–110 mm	0.558	0.193	29
		111–125 mm	0.533	0.230	36
Delap Cove	1990	80–95 mm	0.523	0.116	30
		96–110 mm	0.431	0.093	33
		111–125 mm	0.416	0.094	31
	1991	80–95 mm	0.862	0.138	14
		96–110 mm	0.834	0.265	17
		111–125 mm	0.779	0.158	18
Hampton	1990	80–95 mm	0.821	0.369	27
		96–110 mm	0.724	0.189	27
		111–125 mm	0.671	0.236	18
	1991	80–95 mm	0.788	0.190	9
		96–110 mm	0.632	0.079	9
		111–125 mm	0.655	0.228	8
Grand Mean:	0.684 ± 0.260, N = 961				
Range:	0.065–2.325				

DISCUSSION

The RNA/DNA values obtained from the adductor muscle of the sea scallop are consistent with values reported for the same tissue in the American oyster in Nova Scotia during June (Pease 1976). The present study has shown that smaller scallops (80–95 mm) had significantly higher ratios than the larger size-classes.

TABLE 2.

Results of analysis of variance of ln-transformed RNA/DNA ratios from scallops collected in 1990 testing the effects of size, area and distance from shore.

Factor	Factor Level	Error Term for Test	d.f.	F	Probability of F	Power
Area	Centreville Gulliver's Head Digby Delap Cove Hampton	l	4	7.85	0.000	0.993
Distance	2 miles from shore 4 miles from shore 6 miles from shore 8 miles from shore 10 miles from shore 12 miles from shore	l	5	t.36	0.268	0.410
Size	80–95 mm 96–110 mm 111–125 mm	w	2	9.25	0.000	0.976
Nested terms:						
Tow within distance × area (l)		w	30	3.29	0.000	1.000
Within cells (w)			301			
Post-hoc analysis of population means						
Factor: Area	Hampton = Gulliver's Head = Centreville = Digby > Delap Cove					
Size	80–95 mm > 96–110 mm = 111–125 mm					

Post-hoc analyses are presented for significant ($P \leq 0.05$) factors.

Scallop growth is known to decrease with age (c.f. Thompson and MacDonald 1991) and hence this result is consistent with laboratory studies of other species which have linked RNA/DNA ratio to age and growth rate (e.g. Haines 1973). The adductor muscle in particular may not sustain high metabolic activity once the scallops become more sedentary. The change in swimming behaviour is linked to the hydrodynamics of the shell and scallops generally become less active at shell heights of approximately 10 cm or 4 to 5 years of age (Dadswell and Weihs 1990).

Temperature was not correlated with adductor muscle RNA/DNA in this study, despite the fact that higher temperature conditions generally reflect higher somatic growth rates in *Placopecten* (MacDonald and Thompson 1985). Buckley (1982) also found that temperature did not directly affect RNA/DNA ratios in fish larvae.

Two spatial patterns were observed in relationship to RNA/DNA. A consistent fine-scale patchiness in RNA/DNA was recorded over this scallop bed. Different tows had significantly different RNA/DNA values. In the Digby area this pattern showed an interaction with size, that is some "patches" produced higher

TABLE 3.

Results of analysis of variance of ln-transformed RNA/DNA ratios from scallops collected in 1990 and 1991 testing the effects of size, area and year.

Factor	Factor Level	Error Term for Test	d.f.	F	Probability of F	Power
Year	1990 1991	l	1	22.21	0.000	0.993
Size	80–95 mm 96–110 mm 111–125 mm	w	2	8.39	0.000	0.962
Area	Gulliver's Head Digby Gut Delap Cove Hampton	l	3	2.71	0.080	0.547
Nested terms:						
Tow within area × year (l)		w	16	3.15	0.000	0.999
Within cells (w)			173			
Post-hoc analysis of population means						
Factor: Year	1991 > 1990					
Size	80–95 mm > 96–110 mm = 111–125 mm					

Post-hoc analyses are presented for significant ($P \leq 0.05$) factors.

TABLE 4.

Results of analysis of variance of ln-transformed RNA/DNA ratios from scallops collected in the Digby area from 1990 to 1992 testing the effects of size, year and their interaction.

Factor	Factor Level	Error Term For Test	d.f.	F	Probability of F	Power
Year	1990 1991 1992	1	2	13.28	0.001	0.990
Size	80–95 mm 96–110 mm 111–125 mm	2	2	3.80	0.034	0.646
Interaction terms:						
Year by size		2	4	0.34	0.848	0.117
Nested terms:						
Tow within year (1)		w	15	8.05	0.000	1.000
Size by tow within year (2)		w	30	1.98	0.003	0.997
Within cells (w)			195			
Post-hoc analysis of population means						
Factor: Year	1991 > 1992 = 1990					
Size	80–95 mm = 96–110 mm > 111–125 mm					

Post-hoc analyses are presented for significant ($P \leq 0.05$) factors.

ratios in particular size-classes. Large-scale spatial variability was also observed. In each of the two years studied "area" was a significant factor. However, there was no particular pattern evident in the data set. In the first year the beds were relatively homogeneous with only one area showing lower ratios than the other areas. In the second year the pattern changed and non adjacent pairs of areas were more similar to each other than adjacent pairs. These patterns are most likely linked to local variations in food availability.

In the study of interannual variation, year of sampling was found to be a significant factor: the ratios were higher in 1991 than in the other two years. A number of biological characteristics affecting the scallop have an annual cycle (e.g. growth, reproduction). In other bivalve species RNA/DNA ratios vary seasonally (e.g. Pease 1976) and in fish RNA/DNA ratios have been linked to the gonad maturation cycle (Bulow et al. 1981). In the sea scallop gonads begin to mature in the spring. It is possible that the observed inter-annual differences in RNA/DNA ratio reflect a shift in scallop maturation. In order to place these June observations into context with maturation processes, it will be necessary to determine the annual cycle in RNA/DNA for this stock. Subsequently, the level and associated duration of RNA/DNA which signals a severe stress in the population may be determined experimentally for critical phases of the annual cycle.

In conclusion, RNA/DNA ratios from the scallop adductor muscle show both spatial and temporal variation in wild populations. However, the extent of this variability is such that it can easily be incorporated into the error term of analysis of variance without the demand for extensive sampling regimes. Thus it will be possible to use RNA/DNA values as an indicator of nutritional stress in wild populations of *Placopecten*.

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COMPARISON OF SAMPLING TECHNIQUES, VIDEO AND DREDGE, IN ESTIMATING SEA SCALLOP (*PLACOPECTEN MAGELLANICUS*, GMELIN) POPULATIONS

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ABSTRACT The methods of video and dredge with lined and unlined buckets were compared in estimating shell height distribution and density in a population of sea scallops (*Placopecten magellanicus*) south of Îles-de-la-Madeleine from 1987 to 1989. For both the video and the lined bucket samples the dominant size mode increased over the years from about 30 mm to 78 mm, but the corresponding shell height distributions for the two techniques were statistically different. The shell height frequency distributions also show a marked difference between the lined and unlined dredge buckets, but only for scallops ≤85 mm. Densities obtained with video declined from 50 scallops per 100 m^2 in 1987 to 26 scallops per 100 m^2 in 1989. The scallop densities obtained from lined buckets were lower than video densities and declined from 23 to 34 scallops per 100 m^2 in 1987 to 3 scallops per 100 m^2 in 1989. The relative efficiency of the lined buckets compared to the video varied from 15 to 78% for scallops ≤85 mm. For scallops >85 mm the efficiency of the lined buckets was from 8 to 19%. The relative efficiency of unlined buckets compared to lined buckets increased from 3 to 29% over the three years, and the larger scallops were caught as, or more, efficiently in unlined buckets. Video surveys are the best method of estimating shell height frequency distributions and densities, but are time costly.

KEY WORDS: *Placopecten magellanicus*, scallop, video efficiency, dredge, shell height distribution, density

INTRODUCTION

Direct sampling methods currently used in marine resource assessment serve chiefly to estimate population abundance. Over the years scientists have attempted to correct for the bias associated with dredging by estimating the efficiency during sampling. Several studies have compared different types of dredge buckets (Serchuk and Smolowitz 1980, Worms and Lanteigne 1986, Robert and Lundy 1988, McLoughlin et al. 1991). Others compared abundance estimates from dredging with those from other techniques carried out in parallel, such as photography (Dickie 1955) and direct observation by diving (Baird and Gibson 1956, Caddy 1968, Thouzeau and Lehay 1988, Iribarne et al. 1991, McLoughlin et al. 1991). Finally, some have used video cameras to observe the dredge qualitatively during tows (Worms and Lanteigne 1986). This study was conducted to compare the quality and precision of population estimates of sea scallops (*Placopecten magellanicus*) obtained by video camera with those obtained by dredge over 3 years.

MATERIALS AND METHODS

The study site, an area of about 4.2 km^2, was situated in the Gulf of St. Lawrence, south of Îles-de-la-Madeleine (Fig. 1). The bottom was relatively flat and the substrate was fairly homogenous, composed of a mixture of sand and gravel. Depth varied from 24 to 28 m. The study was conducted during late July and early August each year from 1987 to 1989. Eight stations were sampled annually. The mean distance at each station was around 650 m. The first sampling was a video transect, followed by dredge tows the next day in the same track lines located by their Loran-C coordinates. The precision of the positioning system was 50 m. No commercial fishing took place on the site for the duration of the surveys.

A JVC trademark colour video camera was used and had the following features: step-energy decoding system (single tube); lens focal length of 8.5 to 51 mm; macro mechanism; F1.2; minimum illumination of 10 lux; resolution of 300 lines or more. The video camera was used in macro function and all other adjustments were kept constant (focus, diaphragm aperture). The video camera was enclosed in a water-proof case and mounted perpendicular to the bottom at the centre of a sled 2.4 m in length and with 2.2 m between the skates. The height of the camera was adjusted to obtain a field of view 70 cm wide when the two sled skates were on the bottom. A spot light consisted of a tungsten halogen lamp of 250 Watts. The boat speed was less than 2 km/h during which the transects were recorded on VHS videocassettes. Ten hours of video recordings were made over the three-year study.

During the viewing of the video recordings, two observers identified scallops as either live scallops or cluckers (empty with the two valves still attached). Shell height of observed scallops was measured using the morphometry software Bioquant system IV (R&M Biometrics, Inc.). Measurements were taken when scallops were immobile and sled skates were completely against the bottom, and thus the camera-object distance was exactly 74 cm. A maximum of 87% of the observed scallops could be measured. The distance of transects was estimated directly from the video recordings by systematically measuring the distance covered during the first 10 seconds of each minute of recording and recalculating for the total duration of the transect. This estimate was preferred to that calculated from the Loran-C coordinates because it was more precise, even though certain portions of the recordings were of poor quality and were unusable.

Two Digby dredges with 3 buckets each were used for bottom sampling. The bucket frame measured 76 cm in width and was edged with 6 cm wide teeth spaced at 6 cm. The rest of the bucket was constructed of steel rings of 26 mm diameter connected by steel washers. Some buckets had an inner lining of plastic netting (vexar) of 19 mm mesh (27 mm diagonal). In 1987 and 1988 the surveys used one dredge with two lined buckets separated by an unlined bucket and the other with 3 unlined buckets. In 1989 one dredge of 3 lined buckets and the other of 3 unlined buckets were used. The boat speed during tows was approximately 5 km/h. The length of the tow was determined from the Loran-C coordinates. In 1987 and 1988, all stations were dredged twice.

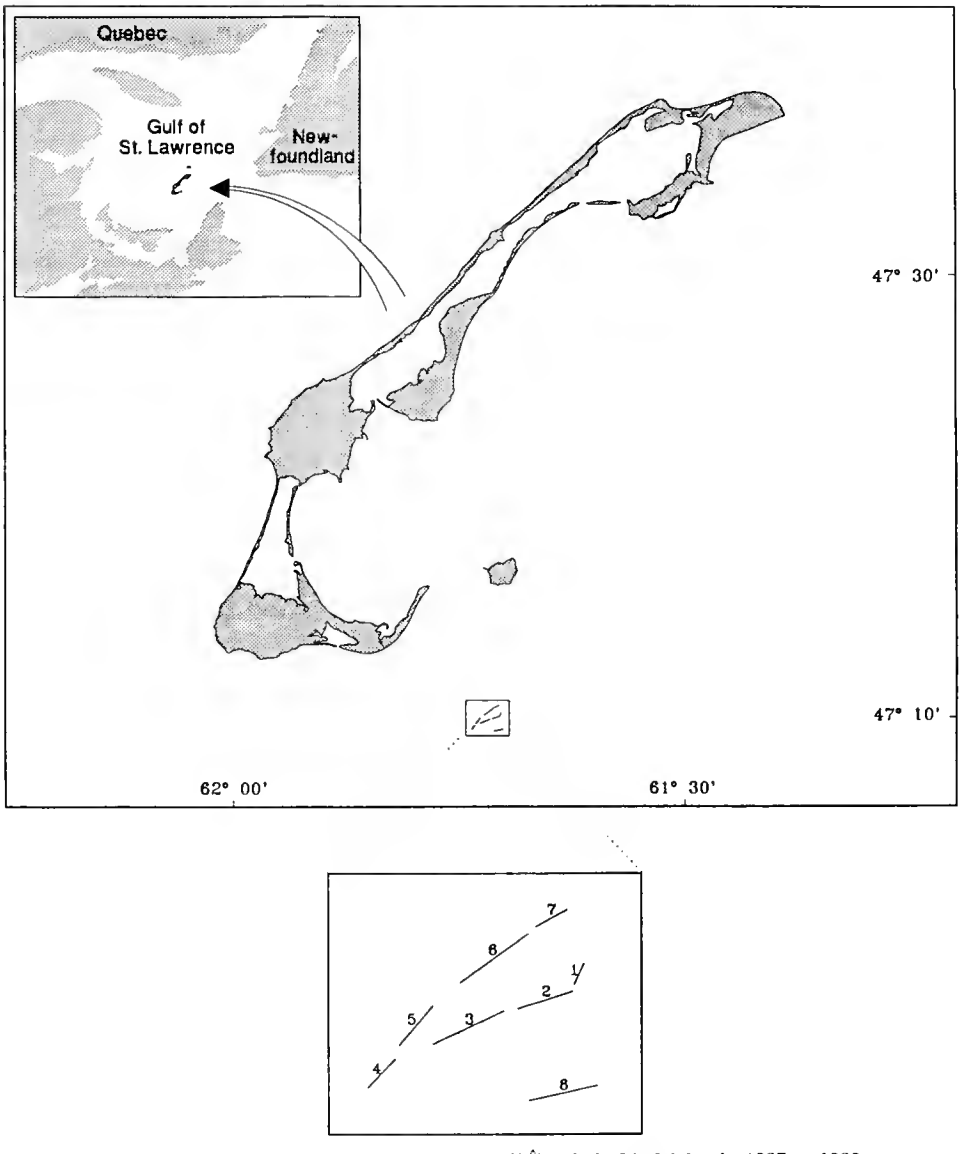

Figure 1. Positions of the sampling stations off Îles-de-la-Madeleine in 1987 to 1989.

When the dredge consisted of a combination of lined and unlined buckets, the samples were analyzed separately for each bucket. Otherwise, the bucket samples were grouped by dredge. All live scallops and cluckers were counted and measured, except in 1987 when all scallops were counted (N = 5051 scallops) but only 52% of them were measured. The Kolmogorov-Smirnov nonparametric test was used to compare shell height frequency distributions with $\alpha = 0.05$. Scallop densities were first standardized per 100 m^2 and then compared using Wilcoxon's nonparametric test for paired samples with $\alpha = 0.05$ (Scherrer, 1984).

RESULTS AND DISCUSSION

Mortality

The proportion of cluckers generally represented less than 2.5% of the whole catch, whichever sampling technique was used (Table 1). The number of cluckers collected by the dredge was usually less than that observed in the video recordings. This difference may be due to separation of the valves of some cluckers during the dredging; single valves were not counted in the samples.

Shell Height

The shell height distributions determined from video had generally one dominant mode each year. This mode was centred at 30 mm in 1987, 65 mm in 1988 and 78 mm in 1989, and corresponded with the annual growth of the 1985 cohort (Fig. 2). The shell height frequency distributions obtained from dredge samples differed noticeably with bucket type and year (Fig. 3). In the lined buckets, as with the video, there was one dominant mode, which was at 32 mm in 1987, 56 mm in 1988 and 78 mm in 1989. In the unlined buckets the distribution was polymodal with no dominant mode.

Few scallops less than 20 mm were caught, these scallops emanating from the spawning of the previous year. This absence was probably due to the difficulty in sampling these individuals using either technique. Individuals smaller than 20 mm were not retained by the lined buckets and the difficulty of observing small

TABLE 1.

Relative frequency (% of N) of scallop cluckers obtained by vidco and dredge sampling.

	1987		1988		1989	
Station	Video	Dredge*	Video	Dredge*	Video	Dredge*
1	2.9	0.0	2.6	0.0	0.0	0.0
2	1.3	0.0	6.1	1.8	0.0	0.0
3	1.0	0.0	4.2	0.0	1.4	0.0
4	4.7	0.0	2.2	2.4	2.1	0.0
5	0.0	0.0	2.3	4.2	2.4	0.0
6	0.9	0.1	0.9	0.2	1.5	0.0
7	2.1	0.0	1.8	0.0	0.0	0.0
8	0.0	0.0	7.9	1.6	0.0	0.0
All	1.4	0.0	2.1	1.2	1.5	0.0
N	1811	5061	1602	1333	949	480

* All buckets combined.

organisms in video recordings has previously been noted by Franklin et al. (1980). Furthermore, shell height distributions in subsequent years indicated a very low abundance of the 1986 and 1987 cohorts.

The shell height distributions from the video recordings and from the lined and unlined buckets differed significantly ($P = 0.0001$). However, the video modes appeared to be similar to those obtained with the lined buckets. In 1988, though, the curves are shifted slightly, i.e. the dominant mode for the video was at 65 mm and for the lined buckets was at 56 mm. The video size distribution was probably incomplete in that year since 20% of the observed scallops in 1988 were not measurable, compared to 6% in 1987 and 9% in 1989. This may be a result of decreased visibility in the water near the bottom in the 1988 recordings due to abundant particles and the presence of a fine sediment deposit on the organisms. This would especially affect the identification of small scallops and the precision of measurements, and result in a distortion of the size frequency and possibly an underestimate of the density.

A detailed analysis of the dredge results from 1987 and 1988 indicates that for a given tow there was no significant difference in the shell height distribution between the lined buckets on the same dredge ($P > 0.081$) or between unlined buckets mounted on two different dredges ($P > 0.683$). For stations that were dredged twice, there was no significant difference between the replicates for each of the bucket types ($P > 0.203$).

The shell height distributions of scallops differed significantly between the lined and unlined buckets ($P = 0.0001$). On the other hand, an *a posteriori* analysis of the distributions indicated no difference for shell heights greater than 85 mm ($P > 0.165$). Overall, the dredge results indicated that the size selectivity varied with bucket type, and under identical sampling conditions the shell height frequency distributions were similar.

Abundance Index and Relative Dredge Efficiency

The scallop density estimated by video was 50 per 100 m^2 in 1987, 43 per 100 m^2 in 1988, and 26 per 100 m^2 in 1989 (Table 2). For the lined buckets the density ranged from 23 to 34 scallops per 100 m^2 in 1987, 5 to 8 scallops per 100 m^2 in 1988, and approximately 3 scallops per 100 m^2 in 1989. For the unlined buckets the densities were much lower, on the order of 1 scallop per 100 m^2 for each of the three years studied because of the low retention of smaller scallops (Table 3). The video densities, in all cases, were significantly higher than those obtained from dredging ($P < 0.005$).

Analysis of the dredge densities shows that there was no significant difference between the lined buckets on the same dredge ($P > 0.100$). However, the comparison of the two dredges (unlined buckets) showed a significant difference for the second tow ($P < 0.004$). This difference may be explained by the presence of two lined buckets on one of the two dredges and the turbulence they generated. Worms and Lanteigne (1986) mentioned that lined buckets fill rapidly and create water currents that push organisms out of the path of the bucket. Thus the unlined bucket in the middle would also be affected by these currents.

In other cases, the comparison of replicate tows showed significant differences ($P < 0.001$), which may be explained by heterogeneity of the scallop density on a small scale (Langton and Robinson 1990, Brand 1991) and imprecision of the positioning system. Moreover, since the replicate tow was often in the opposite direction to the first tow, there is a possible indirect effect of current direction, since current can, according to Winter and Hamilton (1985) influence scallop movements.

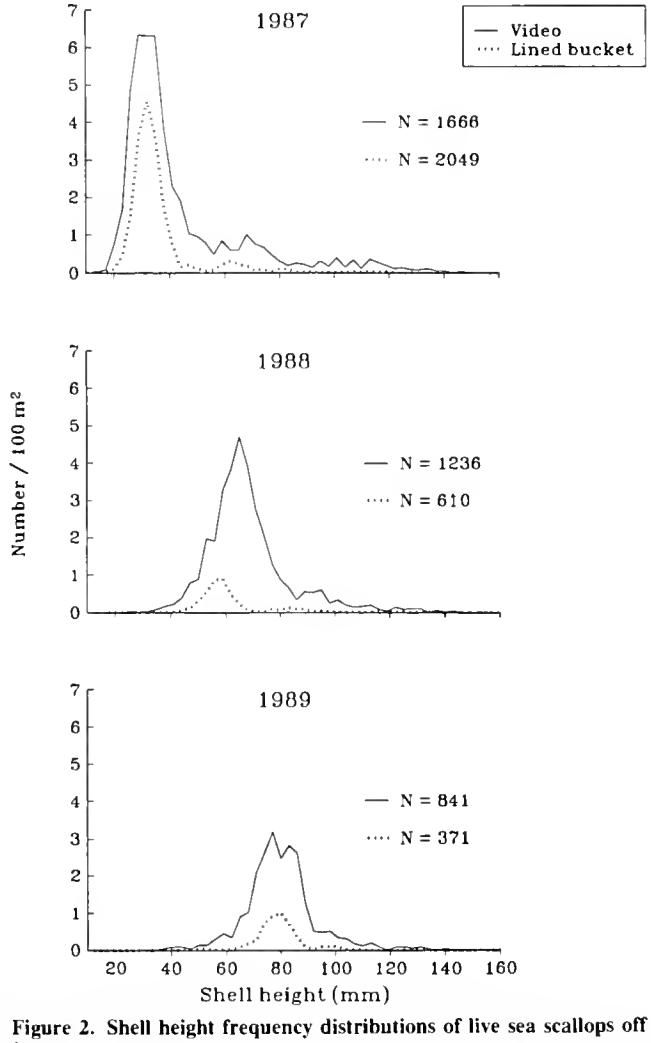

Figure 2. Shell height frequency distributions of live sea scallops off Îles-de-la-Madeleine determined by the two sampling techniques, video and dredge with lined buckets.

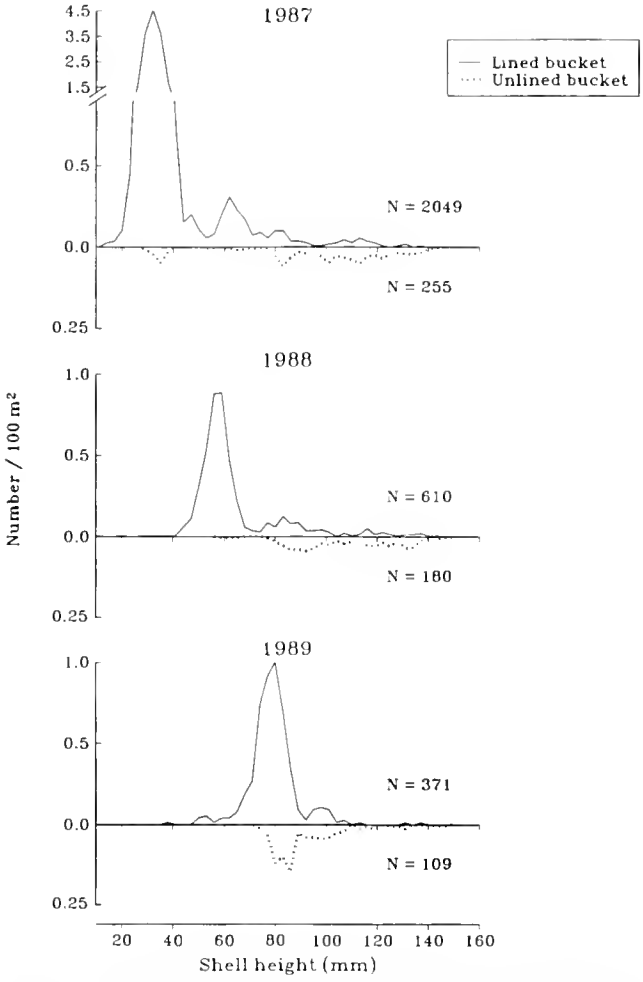

Figure 3. Shell height frequency distributions of live sea scallops off Îles-de-la-Madeleine sampled by dredge with lined and unlined buckets.

The video results were used to estimate the relative efficiency of the lined buckets, and it varied with the shell height of the scallops. In 1987 the dominant mode for shell height of the scallops was 32 mm, and the efficiency of the lined buckets was high, varying from 47 to 69% (Table 2). In 1988 and 1989 the dominant mode scallop shell height was 56 and 78 mm, respectively, and the efficiency dropped to between 12 and 19%. Yet, the relative efficiency of the dredge for scallops larger than 85 mm varied little from year to year, representing from 8 to 19% of the video counts.

The relative efficiency of the unlined buckets compared to the lined buckets, all shell heights included, varied from 3 to 29% (Table 3). Efficiencies for 1988 and 1989 were higher and were associated with larger shell heights. Analysis of the results indicated that the unlined buckets were as, if not more, efficient than the lined buckets in sampling scallops larger than 85 mm. The relative bucket efficiency for these scallops varied from 98 to 120%. These results agree with other published reports, that unlined buckets are more efficient for capturing large scallops (≥75 mm) than lined buckets (Serchuk and Smolowitz 1980, Worms and Lanteigne 1986).

The difference each year in the efficiencies of lined buckets compared to video may be explained by the mobility of scallops. The video results in 1987 indicated that more than 27% of the scallops observed were moving. The percentage decreased to 5% and 0% in 1988 and 1989, respectively. Several authors have remarked that the sea scallop is a very mobile species (Caddy 1968, Posgay 1981, Dadswell and Weihs 1990, Thouzeau et al. 1991). Dadswell and Weihs (1990) stated that the swimming ability of sea scallops is directly related to its shell height; scallops of 40 to 80 mm are very good swimmers, but outside this size range, their swimming ability decreases because of less power in small scallops and different hydrodynamic characteristics for the large individuals. In addition, Worms and Lanteigne (1986) noted that only scallops smaller than 50 mm are affected by an approaching dredge and swim, larger scallops not reacting to the dredge. Caddy (1968), as well, mentioned that scallop dredges elicit a flight response in scallops below 100 mm shell height.

The probability of scallops escaping being sampled was thus a function of shell height as well as the sampling technique used, since the dredge swept a track three times wider and at a towing speed two or three times faster than the video camera. In 1987, when the dominant mode in scallop shell height in the lined buckets was 32 mm, these small scallops may have more easily avoided the video camera than the dredge. Thus scallop density may have been underestimated by video, which would explain the relatively

TABLE 2.

Mean density (number/100 m²) of live sea scallops per sampling technique, and the relative efficiency (%) of the lined buckets with respect to the video estimates.

	Video			Lined Bucket			
Year	Density ≤85 mm	Density >85 mm	Total Density*	Tow	Density ≤85 mm (efficiency)	Density >85 mm (efficiency)	Total density (efficiency)
1987	43.12	3.46	49.82	1st	22.88* (53%)	0.47 (14%)	23.35* (47%)
				2nd	33.74* (78%)	0.64 (19%)	34.38* (69%)
1988	30.16	3.79	42.59	1st	7.49 (25%)	0.56 (15%)	8.04 (19%)
				2nd	4.43 (15%)	0.53 (14%)	4.98 (12%)
1989	17.93	6.02	26.29	1st	2.83 (16%)	0.49 (8%)	3.31 (13%)

* Includes sampled scallops that were not measured.

TABLE 3.

Mean density (number/100 m^2) of live sea scallops per bucket type, and the relative efficiency (%) of the unlined buckets with respect to the lined buckets.

		Lined bucket			Unlined Bucket		
Year	Tow	Density ≤85 mm	Density >85 mm	Total Density	Density ≤85 mm (efficiency)	Density >85 mm (efficiency)	Total Density (efficiency)
1987	1st	22.88*	0.47	23.35*	0.19	0.46	0.65
					(1%)	(98%)	(3%)
	2nd	33.74*	0.64	34.38*	0.39	0.63	1.02
					(1%)	(98%)	(3%)
1988	1st	7.49	0.56	8.04	0.08	0.56	0.64
					(1%)	(100%)	(8%)
	2nd	4.43	0.53	4.98	0.15	0.63	0.78
					(3%)	(119%)	(16%)
1989	1st	2.83	0.49	3.31	0.38	0.59	0.97
					(13%)	(120%)	(29%)

* Includes sampled scallops that were not measured.

high efficiency calculated for the lined buckets in 1987. In the case of scallops larger than 60 mm the results from the video technique are most likely close to reality, bearing in mind that this technique seemed to induce very little movement in larger scallops.

The dredge population estimates are probably only valid for the substrate we sampled, which was sand and gravel. In 1987 one dredge was monitored using a camera mounted directly on the towing cable. Observations have shown that the dredge is clearly more efficient on flat, sandy bottoms than on rocky bottom. On rocky bottoms, where the number of scallops is often higher, the buckets do not sample the substrate efficiently for much of the time. Caddy (1968), Robert and Lundy (1988) and McLoughlin et al. (1991) also noted that the type of substrate could affect dredge efficiency. Dredge efficiency is also related to other factors such as boat speed, bucket type, and environmental factors that might affect scallop mobility (water temperature, current direction and speed) (Orensanz et al. 1991).

Video surveying, although not perfect, is the best method for determining the shell height frequency distribution of sea scallops and especially for estimating the density. However, despite its greater precision, the video technique also has its limitations. It requires: 1) high precision in the instrument calibrations; 2) good atmospheric and environmental conditions; 3) about 4 additional hours of work per 650 m transect to analyze the video recordings. The use of the video technique in stock assessments of a population occupying a vast territory and requiring a large number of stations is unreasonable. The work involved in examining the video recordings is disproportionate to the requirements of routine stock assessments of exploited scallops, from which advice is requested promptly. The use of the scallop dredge with lined buckets is an acceptable method of sampling to determine the shell height frequency distribution of scallops when gear size selectivity is known, keeping in mind that it appears to strongly underestimate the population density.

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REPRODUCTIVE CYCLES OF THE SEA SCALLOP, *PLACOPECTEN MAGELLANICUS* (GMELIN), AND THE ICELAND SCALLOP, *CHLAMYS ISLANDICA* (O. F. MÜLLER), IN ÎLES-DE-LA-MADELEINE, CANADA

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ABSTRACT The reproductive cycles of the sea scallop and the Iceland scallop were studied over an 18 month period in Îles-de-la-Madeleine by periodically checking the gonadosomatic index and by microscopic observations of the gonads. Female sea scallops collected from fishing areas were ripe around the end of June while males were ripe in mid-August. Spawning occurred mainly at the beginning of September. Sea scallops kept in a lagoon had a reproductive cycle similar to those from the fishing areas. Furthermore, the gonadosomatic indices were comparable or sometimes even higher in the lagoon than in fishing areas. The reproductive cycle of the Iceland scallop is characterized by the permanent presence of all types of sex cells in males, including spermatozoa, by a protracted spawning period, and by a notable lyse of oocytes (up to 55%) in ripe females.

KEY WORDS: *Placopecten magellanicus*, *Chlamys islandica*, scallop, reproductive cycle, gonadosomatic index, Gulf of St. Lawrence

INTRODUCTION

The sea scallop (*Placopecten magellanicus*) and the Iceland scallop (*Chlamys islandica*) are exploited in the Gulf of St. Lawrence. The reproductive cycle of the sea scallop is well-documented for eastern Canada except for the northern Gulf (Naidu 1970, Beninger 1987, Davidson and Worms 1989). Few authors have studied the Iceland scallop, and the available data concerns only Norwegian and Icelandic populations (Sundet and Lee 1984, Thorarinsdóttir 1993).

The reproductive cycle of scallops is strongly influenced by environmental conditions such as food abundance and temperature (MacDonald and Thompson 1985, Langton et al. 1987, MacDonald and Thompson 1988, Barber and Blake 1991) as well as by the species' own genetic characteristics (Barber and Blake 1991). Sea scallop spawning is usually annual (MacKenzie et al. 1978, Robinson et al. 1981, Parsons et al. 1992) but occasionally it is semi-annual under particular environmental conditions (Naidu 1970, Beninger 1987) or, more pronouncedly, in the southern part of its distribution (Dupaul et al. 1989, Kirkley and Dupaul 1991, Schmitzer et al. 1991).

The objectives of this study were to: 1) document the reproductive cycles of the sea scallop and the Iceland scallop off Îles-de-la-Madeleine, and 2) verify the potential of the Havre-aux-Maisons Lagoon, an unnatural habitat, as a reproductive area for scallops to optimize spat collection.

MATERIALS AND METHODS

Sampling was carried out in parallel on a scallop fishing ground and on a culturing site in the Havre-aux-Maisons Lagoon in Îles-de-la-Madeleine (Fig. 1). The fishing areas were located in the Gulf of St. Lawrence, approximately 15 km south of the archipelago at a depth of 35 to 40 m. Sea scallops dominate in this region but Iceland scallops are also present. Both species were sampled by dredging on fishing areas periodically from April 1990 to September 1991 (Table 1), although there was no sampling between January and March because of ice. Gonads were sampled for histological section until July 19, 1992 for Iceland scallops and until August 12, 1991 for sea scallops.

Prior to the present study scallops were absent from the Havre-aux-Maisons Lagoon. This area is distinguished from fishing areas by different environmental conditions. The summer temperature was higher in the Havre-aux-Maisons Lagoon; temperatures were not comparable until September (Fig. 2). In 1991 and 1992, the maximum bottom temperature was 21°C in the lagoon and 16°C in the fishing areas. In addition, muddy and sandy bottoms, such as are found in the lagoon, are considered unfavourable for settlement of scallop larvae.

Sea scallops from the fishing areas were transferred to culture cages and placed at a depth of 2 to 5 m in the lagoon. Two transfers took place. The first, in May 1990, was to compare the gonad development of sea scallops in the lagoon with those from fishing areas. These scallops were sampled from June to October 1990 and from May to August 1991 (Table 1). A second transfer was carried out in October 1990 in order to observe the gonad development during the winter. It was assumed that during this period the temperature and gonad development in the lagoon would be similar to that in the fishing areas. These scallops were sampled from November 1990 to August 1991 (Table 1).

The mean shell height of the sea scallops was 107 ± 11 mm for those sampled on the fishing areas and 103 ± 9 mm for those transferred to the lagoon. The mean shell height of Iceland scallops sampled on the fishing areas was 80 ± 6 mm.

Samples of each species were analyzed for the gonadosomatic index and microscopic examination was made of the gonads. Twenty individuals of each species, 10 per sex, were collected from each site at each sampling date. The gonadosomatic index (GSI_i) was calculated for each individual as the percent ratio be-

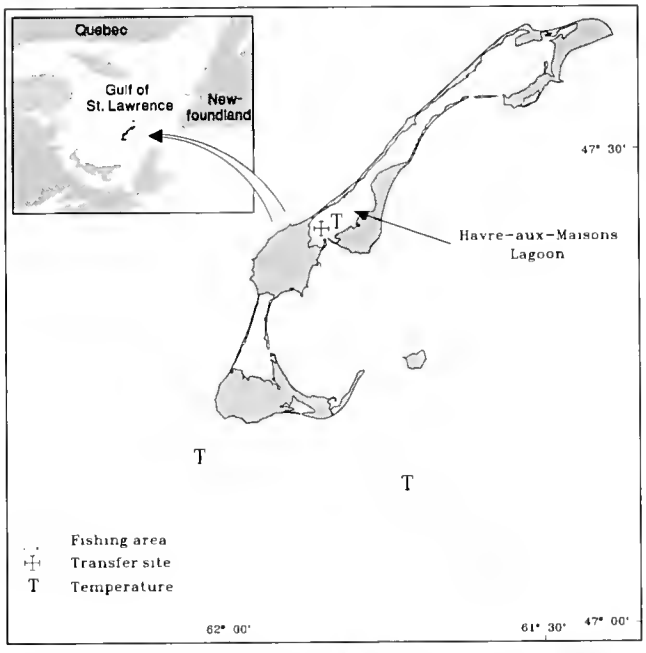


Figure 1. Locations of the Îles-de-la-Madeleine sampling sites (Gulf of St. Lawrence, Canada), the fishing areas and the Havre-aux-Maisons Lagoon transfer site.

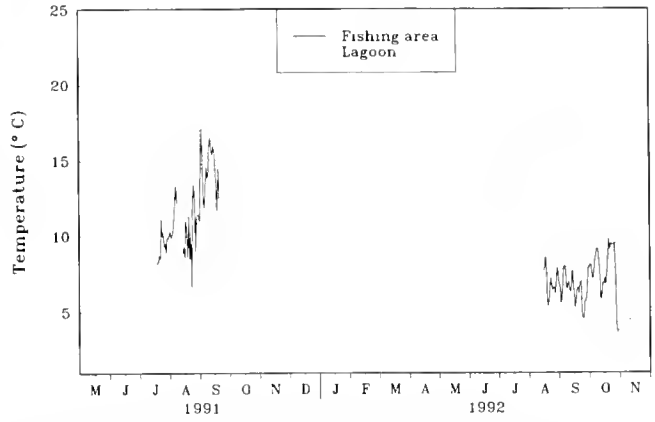


Figure 2. Bottom temperature of fishing areas at 35 m depth and in the Havre-aux-Maisons Lagoon in 1991 and 1992.

tween the wet weight of gonad and that of remaining soft parts. GSI should be taken as the mean GSI_t per species, per sex, per sampling date, and per site.

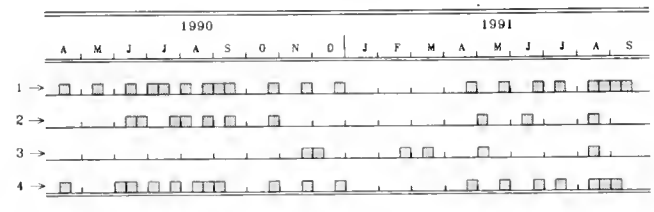
Histological sections of gonads were used to determine the developmental stage of the gonad. A 2 to 3 mm transversal slice through the central portion of the gonad was made and preserved in Bouin's fixative. After 48 hours the tissues were rinsed and kept in 70% ethanol. Following paraffin embedding, 8 μm sections were mounted on slides and stained with haematoxylin-eosine. A magnification of 100× was used for all microscopic observations. The developmental stage of the sea scallop and Iceland scallop gonads was determined according to the classification established by Davidson and Worms (1989). The following gives a general description of the stages:

Stage I—Differentiated

The follicles are well-defined and occupy approximately 44% of the gonad. The lumen of the follicle may occupy up to 63% of the follicle. The interfollicular space is highly visible.

TABLE 1.

Sampling periods, grey areas, for sea scallops and Iceland scallops from the two sampling sites (1 = Fishing areas sea scallops; 2 = Lagoon sea scallops transferred in May 1990; 3 = Lagoon sea scallops transferred in October 1990; 4 = Fishing areas Iceland scallops).



♂: Only a few layers of cells (germ cells and spermatogonia) along the follicle walls.

♀: Presence of germ cells, oogonia and primary oocytes (I) with diameters varying between 10 and 24 μm. The oocytes I appear to be incorporated in the follicle wall.

Stage II—Developing

The general organization is similar to stage I except the follicular lumen occupies only 45% of the follicle.

♂: Along with germ cells and spermatogonia are several layers of spermatocytes I and II.

♀: Oocytes I are now 24 to 39 μm and protrude into the follicular lumen.

Stage III—Filling

The follicles occupy approximately 85% of the gonad. The follicular lumen is much reduced or has disappeared. The interfollicular space is still present but limited.

♂: Follicles are full of spermatocytes I and II and spermatids. At the beginning of this stage spermatozoa can be present in the centre of the follicles.

♀: Presence of stalked oocytes I still attached to the wall by a thin thread and free oocytes of 40 to 60 μm diameter.

Stage IV—Ripe

The follicles are completely full of ripe gametes, and the follicular walls are touching with no interfollicular space.

♂: Spermatozoa occupy almost 100% of the follicles and some are oriented radially with the flagella toward the centre of the follicle.

♀: Follicles are full of free oocytes of up to 70 μm diameter. They have polygonal shapes, being pressed against each other.

Stage V_1—Spawning

Ripe gametes are being released through the gonoducts. The follicles are in various degrees of emptiness. The follicular lumen and interfollicular spaces become enlarged. Presence of phagocytic cells.

Stage V_2—Spent

Most follicles are empty, although certain follicles may have failed to spawn. Interfollicular spaces become very noticeable. Phagocytic cells may be very abundant. Residual gametes may or may not be present, but with no sign of gametogenesis.

Stage VI—Latent

At this stage it is impossible to distinguish between the sexes. No gametogenesis activity.

Gametogenesis was also characterized by: 1) measuring the diameters of 50 oocytes with clearly visible nuclei; and 2) estimating the surface area of the spermatozoa in 10 follicles. These observations were made on a single specimen for each species and sex per site and sampling date. Mean oocyte diameter was calculated for each development stage per species, per site, and per year. Measurements were made using an image analyzing system, BIOQUANT IV. The *t*-test was used to compare the GSIs and the oocyte diameters ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Hermaphroditism

As a general rule, sea scallops and Iceland scallops are both gonochorics (Naidu 1970, Sundet and Lee 1984, Parsons et al. 1992). However, some cases of hermaphroditism (0.6%) were identified in Îles-de-la-Madeleine sea scallops during microscopic examinations of the gonads. In all of these cases, male and female gametes were present in the same follicle, usually with a dominance of male gametes. The developmental stage was not determined in any of these individuals. Naidu (1970) observed 1.3% of hermaphroditism in sea scallops off the west coast of Newfoundland.

Sea Scallops—Fishing Areas

The general description of the sea scallop gonadal development from fishing areas off Îles-de-la-Madeleine is similar to that defined by Davidson and Worms (1989) for the same species in the southwestern Gulf of St. Lawrence. However, during the present study the Îles-de-la-Madeleine sea scallops displayed no latent period (stage VI) between spawning and the beginning of gametogenesis. Davidson et al. (1993) noted that the latent stage is sometimes absent in sea scallops of the southern Gulf of St. Lawrence.

The male and female gonads developed in parallel over time and the pattern was similar for the two years studied (Fig. 3). The reproductive cycle began around the end of September. During the fall and winter the male follicles filled with developing sex cells (stages I and II). The first spermatozoa appeared around mid-May (beginning of stage III). In mid-August the majority of males were ripe (stage IV) and the spermatozoa occupied 80 to 100% of the follicle space. In 1990 spawning began between September 7 and 13. On September 7 all males collected were at stage IV; on September 13, 80% of the males were spawning (stage V_1) and 10% had already spawned (stage V_2). At the end of October all males were at stage I.

Females remained at stage I from October to December and the mean oocyte diameter was 22 μm (Table 2). In April and May the gonads were at stages II and III. The gonads were at stage IV at the end of June and the follicles were full of ripe oocytes until the end

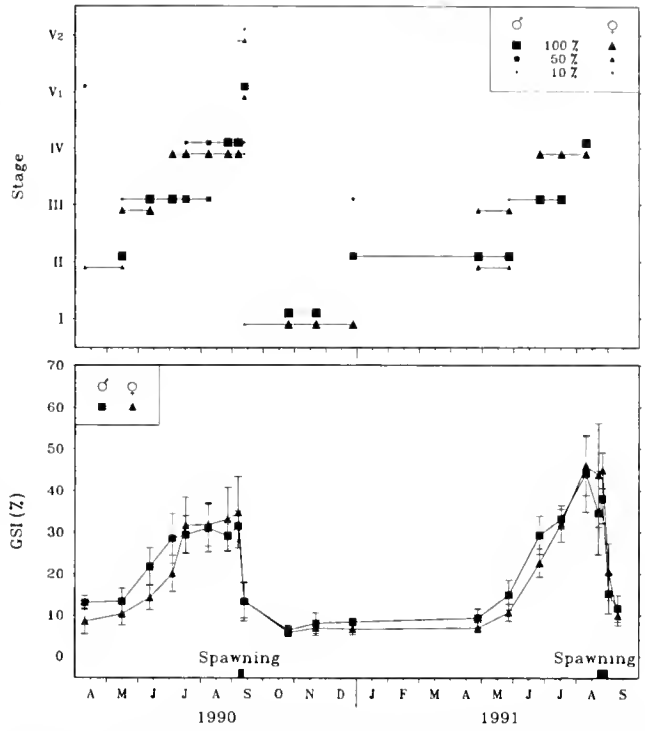


Figure 3. Gonad developmental stages (% of specimens) and mean gonadosomatic index (± standard deviation) of sea scallops, *Placopecten magellanicus*, in fishing areas off Îles-de-la-Madeleine.

of August. A ripe oocyte is defined as a free, non-stalked, oocyte (Dorange and Le Pennec 1989). During this stage up to 20% of the oocytes were observed in lysis within the follicles; a mean oocyte diameter of 74 μm was measured. Ninety percent of the females were ripe and 10% had already spawned (stage V_2) by September 7, 1990. On September 13, 40% of the specimens were spawning (stage V_1) and 50% were at stages V_2 or I. By October all of the females had spawned and were at stage I. The oocytes that were still stalked during spawning remained attached to the follicular walls and were reabsorbed during the winter.

The male and female gonadosomatic indices (GSIs) followed the same pattern. In the spring of 1990 GSIs increased gradually then stabilized at 29 to 35% around mid-July. On September 13 GSIs dropped drastically, indicating synchronous spawning. GSIs were at their lowest from October 1990 to April 1991 and increased slightly from 6 to 9%. GSIs started to increase more rapidly in May. In August 1991 GSIs were significantly higher than during the same period in 1990 ($P < 0.001$), attaining 33 to 46%. In 1991 spawning again took place in the beginning of September.

Reproduction in the sea scallop off Îles-de-la-Madeleine is an annual cycle and a synchronous spawning period occurs mainly in the beginning of September, although some males appeared to be spawning in April 1990. Naidu (1970) observed a similar phenomenon on the west coast of Newfoundland for the same species. Beninger (1987) also noted that 33% of the male sea scallops analyzed in December and January from the Bay of Fundy, New Brunswick were ripe even though spawning here occurs mainly in September.

Sea Scallop—Lagoon

One month after scallops were transferred to the lagoon in the spring of 1990, the gonads were at stage III (Fig. 4). The mean

TABLE 2.

Mean oocyte diameter per developmental stage for each species, site and sampling year.

Species	Site	Year	Mean Oocyte Diameter (μm) ± std			
			I	II	III	IV
Sea scallop	Fishing areas	1990	22.2 ± 6.3	40.7 ± 7.5	48.6 ± 10.0	74.3 ± 11.0
		1991		48.9 ± 6.8	44.7 ± 11.5	80.1 ± 11.7
	Lagoon May	1990	18.4 ± 2.9		54.7 ± 17.5	66.3 ± 15.2
		1991			51.1 ± 16.6	72.8 ± 17.6
	Lagoon October	1990	23.9 ± 4.2	27.5 ± 7.3		
		1991		33.5 ± 8.7	47.4 ± 13.5	73.5 ± 16.0
Iceland scallop	Fishing areas	1990	24.1 ± 6.7	36.6 ± 8.3	54.9 ± 11.8	73.4 ± 14.6
		1991		51.6 ± 15.3	47.1 ± 22.1	72.6 ± 12.6

oocyte diameter was 55 μm (Table 2). The females were ripe by June and the males around mid-July. During this period the mean oocyte diameter in ripe females was 66 μm. On September 11, 1990, 90% of the males and 60% of the females were spawning or spent. In the following sampling on September 19 only 20% of the males and 10% of the females had not yet spawned (stage IV). In October all the specimens were at stages V_1, I or II. GSIs followed the same pattern as the development stages. The maximum values occurred in mid-July and were 46 to 54% for both years studied. From July to August 1990 variations in the GSI were notable.

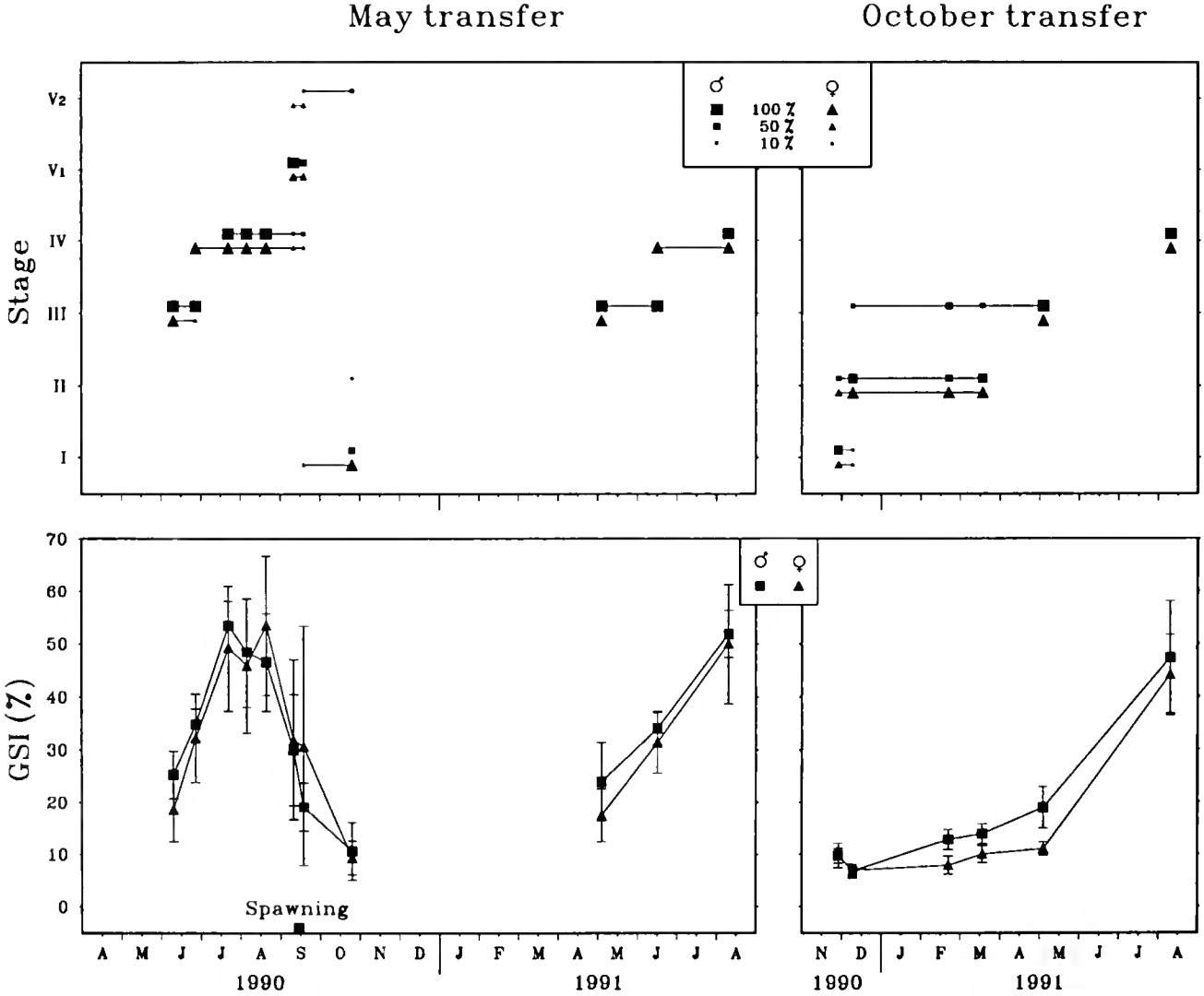


Figure 4. Gonad developmental stages (% of specimens) and mean gonadosomatic index (± standard deviation) of sea scallops, *Placopecten magellanicus*, transferred to the Havre-aux-Maisons Lagoon in May and October 1990.

However, in the microscopic examinations no difference between individuals was noted and there was no sign of spawning. In 1991 GSIs decreased at the beginning of September as in the previous year.

The scallops transferred to the lagoon in October 1990 were at stages I or II at the end of November (Fig. 4). From December to March development slowed, although a slight increase in GSIs was observed, especially in the males. Development increased again in the spring. At the beginning of May 1991, the GSI of female scallops transferred in spring 1990 to the lagoon was significantly higher than for those transferred in October ($P = 0.004$). The males, however, showed no significant difference ($P = 0.051$). In any case, GSIs in August 1991 were similar for the two transfers.

In general, the reproductive cycles of sea scallops in the lagoon and of those in fishing areas are comparable, but some differences are observed in the gonad development. The oocyte diameter in ripe females, although variable between the years at a given site ($P < 0.001$), was always significantly smaller in the lagoon than in the fishing areas ($P < 0.001$) (Table 2). As well, GSIs of lagoon transferred scallops were significantly higher than for those collected in fishing areas in the summer of 1990 and the spring of 1991 ($P < 0.006$). These variations in oocyte diameter and GSI are probably related to environmental conditions, as Barber et al. (1988) and Skreslet (1973) have found for GSIs.

The logarithmic regressions between the weight of soft parts (excluding the gonad) and shell height or the weight of gonad and shell height show no significant differences in the slopes between the two sampling sites ($P > 0.11$). On the other hand, for mature individuals of a given size, the weight of soft parts was less in 1990 and the gonad weight was greater in the lagoon then in fishing areas ($P < 0.005$). These differences, observed only in 1990, are explained by a reduction in somatic growth due to the transfer occurring during their growth period in combination with an important increase in gonad growth in the lagoon, where environmental conditions are particularly favourable. Sundet and Vahl (1981) and MacDonald and Thompson (1985) have shown that the adult scallop's somatic growth is relatively constant and that variations in available energy affect mostly gonad growth.

Iceland Scallop—Fishing Areas

The gonad developmental stages in Iceland scallops off Îles-de-la-Madeleine were similar to those described by Davidson and Worms (1989) for sea scallops. However, in males the first stages of development, I and II, were absent (Fig. 5). Outside of the spawning period, the male gonads were usually at stage III. In July, when the gonads were ripe, spermatozoa occupied only 70 to 80% of the follicles, the remaining volume being taken up by unripe sex cells. This is the final phase of stage III or the beginning of stage IV according to Davidson and Worms (1989). The spawning period was long; on July 24, 1990, 20% of the males had spawned and the rest were at stage IV. The proportion of mature males decreased progressively as males at other stages (V_1, V_2, and III) became more numerous. All males finally reached stage III at the end of October. In 1991 there were males spawning as early as mid-July. After spawning, unripe sex cells were conserved in the follicles and continued to develop. At the very beginning of the reproductive cycle the gonads were small but the follicles were full of these unripe sex cells. The first spermatozoa thus appeared early in the fall.

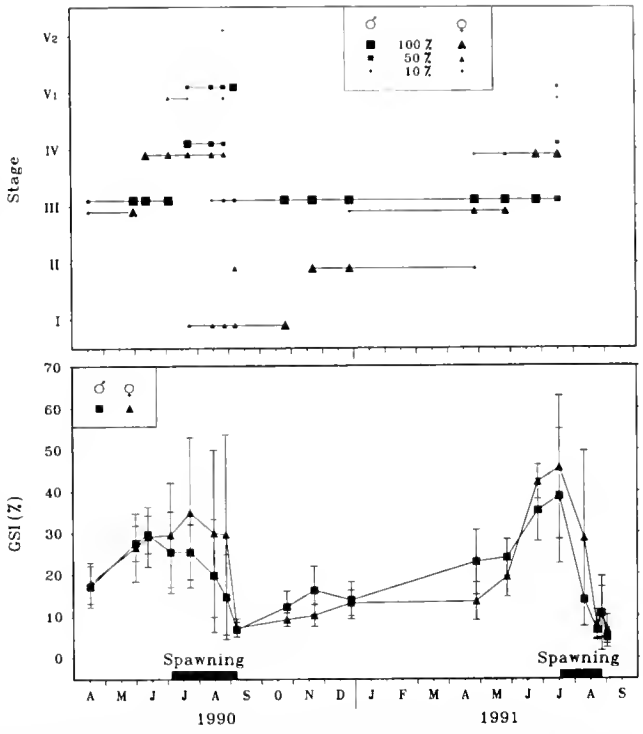


Figure 5. Gonad developmental stages (% of specimens) and mean gonadosomatic index (± standard deviation) of Iceland scallops, *Chlamys islandica*, in fishing areas off Îles-de-la-Madeleine.

All females were at stages I and II at the beginning of September. The mean oocyte diameter increased from 24 to 37 µm between September and December (Table 2). The gonads were at stage III in the spring, but approximately 20% of the oocytes were in lysis. In mid-June the females were ripe. At this point oocyte lysis was quite noticeable, involving up to 55% of the oocytes. In spite of this, the mean oocyte diameter is large, around 73 µm. On July 5, 1990, 20% of the females were spawning. From July to mid-September 1990 the females were at stages IV, V_1, V_2, I, or II in different proportions. All the females were at stage I at the end of October. On July 19, 1991 females were observed spawning. Thus it appears that there is a lack of synchronism among individuals since the spawning period is spread over a few months. As with the males, a large number of unripe oocytes remained attached to the follicular walls after spawning that apparently continued to develop.

GSIs of Iceland scallops reached 28 to 35% by June 13, 1990 and remained at this level until the end of July in males and the end of August in females. In July and August, great variability in GSIs, especially in females, is due to a lack of spawning synchrony. In September 1990 GSIs were at their lowest values, around 7%. Male and female GSIs started to increase again by October. At the end of June 1991 GSIs reached 35 to 40%, which is significantly higher than those for the summer of 1990 ($P < 0.001$), as was previously noted for sea scallops in the fishing areas. In 1991 a decrease in GSIs began in mid-July.

The reproductive cycle of Iceland scallops off Îles-de-la-Madeleine is comparable to that of other populations of the same species. Sundet and Lee (1984) and Thorarinsdóttir (1993) studied the gonad development in a population of Iceland scallops located north of Norway and west of Iceland and also observed all types of sex cells, including spermatozoa, at the very beginning of the

reproductive cycle and the synchronous spawning in July. Îles-de-la-Madeleine Iceland scallops differ from those northern populations in two aspects: 1) its lengthy spawning period, probably to ensure better survival of the larvae when conditions are not favourable, as indicated by Langton et al. (1987) and Parsons et al. (1992); and 2) the high percentage of oocyte lysis, which is mainly associated with a lack of energy available for reproduction (Lubet et al. 1987, Barber et al. 1988, Dorange and Le Pennec 1989).

The results obtained during the study were not due to exceptional environmental conditions. In particular temperature data of the surface layer (Gregory et al. 1993) and of the 15 to 20 m depth layer (unpublished data) for southwestern Îles-de-la-Madeleine, in 1990 and 1991, show no important temperature anomalies compared to the means obtained for the period from 1915 to 1987 (Petrie 1990). Thus our results probably represent the reproductive cycle of Iceland scallops in this area and indicate that the conditions are not optimal for the species reproduction. It must be pointed out that the fishing areas off the Îles-de-la-Madeleine are located at the southern limit of the geographical distribution of the Iceland scallop.

In general, the results obtained from Îles-de-la-Madeleine indicate that: 1) reproduction in the sea scallop is an annual cycle; 2) sea scallops introduced into the Havre-aux-Maisons Lagoon can survive and reproduce; and 3) conditions are suboptimal for the reproduction of Iceland scallops. A better knowledge of the characteristics of the two scallop species would hasten the initiation of the scallop culture industry of Îles-de-la-Madeleine by allowing the optimization of spat collection of sea scallops, the targeted species.

ACKNOWLEDGMENTS

We wish to thank Nathalie Moisan, Carole Cyr and Jacques Richard for their cooperation in this study. We also thank Roberta Miller for the English translation, and Marcel Fréchette for his valuable comments. The work is involved with REPERE, a research program on scallop culture and restocking.

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GAMETOGENIC AND SPAWNING PATTERNS OF MANILA CLAMS *TAPES PHILIPPINARUM* (BIVALVIA: VENEROIDA) IN TWO LAGOONS OF THE RIVER PO DELTA, ITALY

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ABSTRACT The reproductive cycle of the manila clam (Japanese littleneck clam), *Tapes philippinarum* (Adams and Reeve), was studied in the Po River Delta of Italy. Different stages of the reproductive cycle were described: active, ripe, spawning, resorption and inactive. Specimens collected from two areas, Sacca Degli Scardovari and Sacca Del Canarin, were compared for morphological characteristics of the gonads. The two lagoons are characterized by different environmental and hydrographic factors. Onset and development of the maturing stages was not identical in the two lagoons suggesting a correlation between reproductive events and different trophic conditions.

KEY WORDS: Bivalves, reproductive cycle, *Tapes philippinarum*, gametogenesis, spawning

INTRODUCTION

Tapes (*Ruditapes*) *philippinarum* (Adams and Reeve, 1850), (Bivalvia: Veneroida) is an Indo-Pacific bivalve that was introduced to the northern Adriatic Sea for commercial purposes (Breber 1985). Suitable habitat exists for the species in the lagoons of Venice and in the bays of the Po River Delta and extensive populations of high density, extending from intertidal to subtidal zone, now occur there. Manila clams have replaced the indigenous species *Tapes decussatus* (Jeffreys nec Linnaeus, 1873) in many areas of the Delta. The success of the introduction of *T. philippinarum* is probably due to the fact that manila clams are a larger species and have a faster growth rate than the native species (Mattei and Pellizzato, 1990).

Knowledge of the reproductive cycle of commercially important species is important in aquaculture. The purpose of this study was to determine the annual reproductive cycle of *T. philippinarum*. This study it was undertaken in two lagoons of the River Po Delta, Sacca Degli Scardovari and Sacca Del Canarin, and is the first phase of a study of the population dynamics to determine correct management procedures for exploitation of the stock.

MATERIALS AND METHODS

Description of the Experimental Areas

Two areas were chosen for this study, Sacca Degli Scardovari (Scardovari) and Sacca Del Canarin (Canarin) located in the Po River Delta (Fig. 1). The first lagoon, Scardovari, is a large inlet (about 32 km^2), located between the Tolle and Gnocca branches of the Po River Delta. It has a southern opening to the sea between the mouths of the branches of the river. Depth varies from 1.5 to 4 m. Distinct gradients in environmental parameters exist from the mouth to the head at the northern end which is highly productive (Ceccherelli et al. 1985). Canarin has an area of about 6 km^2 and is located between the Pila and Tolle branches of the Po River Delta. It has a continuously changing environment that has been modified by both human and natural causes (Ceccherelli et al. 1985). Because of freshwater inflow, the indigenous zooplankton and zoobenthos communities are impoverished in both number of species and total numbers. Mean water depth is less (maximum depth about 1 m) in Canarin than in Scardovari. Water temperatures and salinities as recorded in spring and summer at both lagoons are shown in Fig. 2. In these lagoons tidal amplitude is about 50–70 cm although Scardovari may occasionally reach 1 m.

Sampling Procedures

Individual *T. philippinarum* were sampled, from the subtidal zone, at monthly intervals from January 1991 to March 1992. At least 50 individuals (25 settled in a central zone of Canarin and 25 from a Scardovari zone near the opening to the sea) were collected at each sampling to assess the stages of gonadal maturity. Size of individuals ranged from 40 to 50 mm shell length. The gonads were fixed in 2.5% glutaraldehyde in cacodylate (sodium dimethylarsinate) buffer, postfixed in 1% osmium tetroxide, embedded in Epon-Araldite, sectioned, stained and examined with both optical and transmission electron microscopes. Semi-thin sections were stained with Azure II-methylene blue and basic fuchsin. Other pieces of gonad were fixed in Bouin's solution, embedded in paraffin, sectioned and observed under an optical microscope after being stained with Azan-Mallory.

RESULTS

Males

Histological observations made during the first stages of spermatogenesis showed the follicles contained spermatogonia and proliferating spermatocytes (Fig. 3a and b). At maturity their lumen was full of spermatozoa typically clustered in rosettes, round-shaped structures in which sperm were arranged with their acrosome in a centripetal position (Fig. 3c and d). The spermatozoon was of the primitive type with a rather elongated acrosomial vesicle and axial rod (Fig. 3e). Its morphology was similar to that described for *T. decussatus* (Pochon-Masson and Gharagozlou 1970). After spawning and resorption (Table 1, Stage V) in which the gonad had follicles with residual spermatozoa undergoing phagocytosis (Fig. 3f), the inactive winter period began.

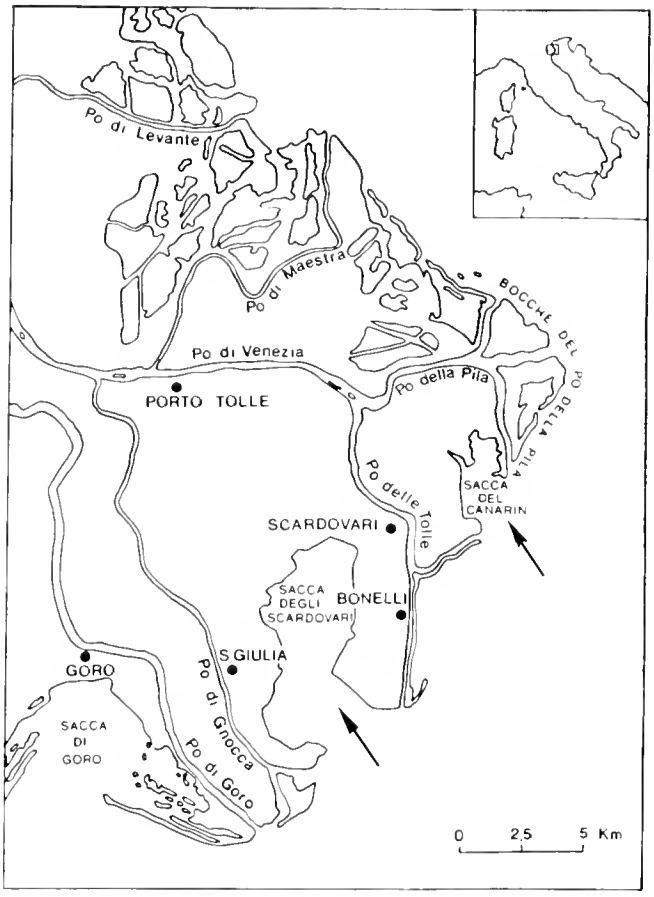

Figure 1. Map of the northern Adriatic Sea area showing the location of the Po River Delta and the two lagoons, Sacca Degli Scardovari and Sacca Del Canarin.

Females

Small clusters of previtellogenic oocytes were attached to the connective follicle wall in female follicles at the onset of gametogenesis. When their volume increased, they initially showed a pyriform shape (Fig. 4a and b). At the end of vitellogenesis, following increases in cytoplasm, they became more roundish and protruded towards the lumen (Fig. 4c and d). During summer months, particularly in samples from Canarin, irregular shaped oocytes suggesting an initial degeneration process were observed (Fig. 4c). After spawning and resorption by phagocytes of oocytes and egg residues, the follicles disaggregated and entered the resting stage as was observed in the male gonad (Fig. 4f).

Somatic cells characterized by a uniform cytoplasm and roundish shape with rather small nuclei were observed among the germ cells during the first stages of gametogenesis. These cells, called "vesicular cells" (Figs. 3a and 4a) were found in both males and females and could be intragonadic reserve tissue (Medhioub and Lubet 1988). They were observed with basic fuchsin staining. Later during gametogenesis the cytoplasm in these cells was markedly reduced because of the many developing gametes and when the gonad was fully developed it was more difficult to detect them with the same staining method.

Overall morphological and cytological data obtained from gonads of different individuals collected over the year allowed us according to (Shaw 1988) to arbitrarily divide the reproductive cycle into five stages as shown in Table 1.

Comparative Gametogenetic and Spawning Patterns

The percent frequency of the different stages of the gonadal cycle found in manila clams in the two lagoons at different times of the year is shown in Figures 5 and 6 for males and females respectively.

During winter (January–March 1991), individuals in both lagoons always had gonads that were reduced or in the inactive stage (Stage 1) and hence it was difficult to determine the sex of the animals. It was impossible to sex 20% of individuals from Scardovari and 30% from Canarin at this time.

By the end of March morphological differences began to appear in gonads of individuals from the two lagoons. These differences became more evident in April. Follicles of these bivalves, at Scardovari, were much smaller and had proliferating gonia in the first stage of gametogenesis (Stages Ib, IIa). In both sexes numerous "vesicular cells" with abundant cytoplasm were present to-

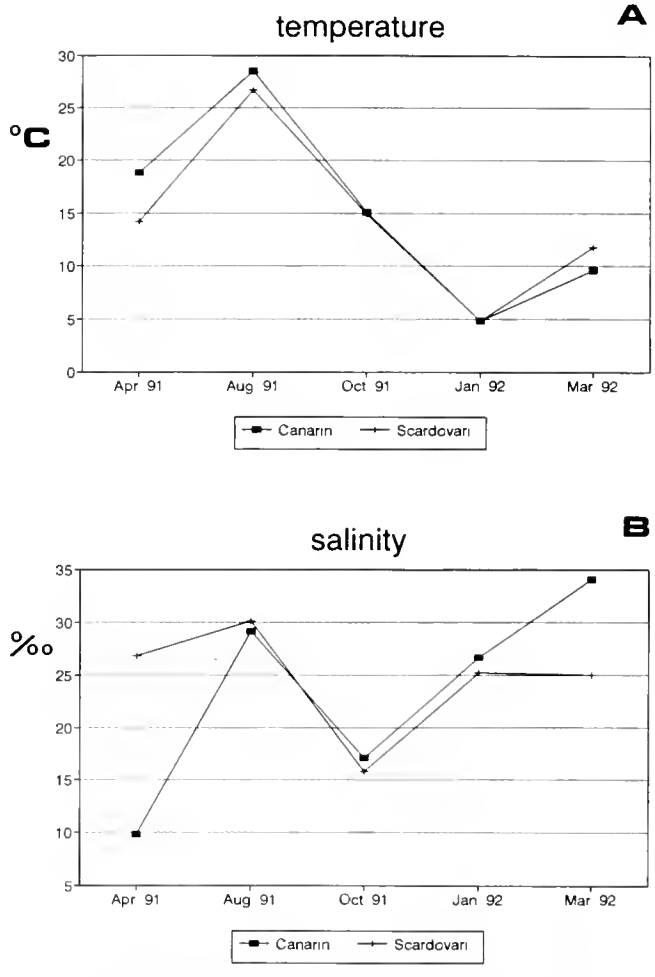

Figure 2. Water temperatures and salinities at both Canarin and Scardovari Lagoons. A: Temperature (°C); B: Salinity (ppt).

gether with germ cells. At Canarin however, gametogenesis appeared to be more advanced during the same month. Primary and secondary spermatocytes were present in males and actively proliferating oocytes in females (Stages IIa, IIb, IIc). Vesicular cells showed progressively reduced cytoplasm.

In May most of the gonads of manila clams from Canarin were in a more advanced stage of development. In both sexes, 66% of the gonads were fully ripe (Stage III) while 44% of females and 22.7% of males had almost empty gonads. Only 11.1% of the males there were only empty follicles and phagocytes. These morphological features suggest that a spawning had occurred in Canarin.

In May at Scardovari, 70% of males and 15% of females still showed active gametogenesis and only in July 90% of animals were fully ripe (Stage III).

During the summer months at Scardovari gonads of *T. philippinarum* became mature twice and therefore two spawnings occurred; once in June–July (in July 75% of male and 57% of females follicles were empty) and again in September–October (in October 100% of male and 55% of females follicles were empty). In this lagoon gonads of manila clams undergo all the developmental stages, including spawning twice a year.

It should be stressed that at Scardovari when gonads of both sexes became ripe during the first period (June–July) they began to spawn so that after about 15 days (in July) 75% of males and more than half of the females had completely discharged their gametes. However, in the second period (August–September) the percentage of ripe clams gradually increased. During August and the first week of September, all individuals examined had mature gonads (80% of males and 90% of females in Stage III) but only 30% of the population showed evidence of spawning. About half of the females in this August–September period had gonads with either irregular or cytolytic oocytes.

During the same summer season (July–September 1991) the gonadal cycle of manila clams at Sacca Del Canarin was different from that observed at Sacca Degli Scardovari. The spawning observed in July at Scardovari was not observed at Canarin. Furthermore at Canarin lagoon after the May spawning the gonads started a new gametogenic cycle at the end of which, in July, about 90% of gonads of both sexes were ripe and follicles were always packed with gametes. The gonads stayed in this ripe phase during the summer months between June and September. The oocytes in gonads of all mature females sampled in July–September were extremely irregular or in a more or less advanced degeneration (50%, 60% and 30% of clams examined in July, August and September respectively). In the remaining population (10%) the gonads had probably begun the spawning stage and this continued until August and involved a gradually smaller number of follicles. Morphological observations showed that in July, as was observed at Scardovari, at Canarin there was a slight increase in the number of empty gonads together with resorption of residual gametes (37% of males and 17% of females). A spawning peak was observed in clams at Canarin in October, slightly later than the one in Scardovari. The period when the gonads were emptied was longer which caused a delay in completion of the resorption stage. In December many gonads still had fully active phagocytes. From November onwards the resorption period occurred in both lagoons and the gonads had reduced volumes and showed a progressive loss of follicular organization. Clams from both populations entered the inactive period (Stage I) which continued until February (1992).

Morphological observations during the first months of 1992 supported the findings that gametogenesis in *T. philippinarum* populations in Canarin lagoon began earlier than at Scardovari as was observed in the previous year. The first gonial proliferations and beginnings of follicle increase (Stages IIb and IIc) occurred in clams at the end of February in Canarin but in March at Scardovari.

DISCUSSION

Reproductive Patterns

Morphological observations undertaken over a period of a year permitted us to determine the reproductive cycle and gonadal developmental stages.

Criteria that exist for separating the reproductive process in stages is often subjective since they depend on both the bivalves species examined (whose reproductive cycle is more or less continuous all over the year according to the geographical location) and the kind of histological and ultrastructural analysis conducted on gonads. Sometimes this different criteria for describing the gonadal stages make comparisons difficult. For example Holland and Chew (1974) divide the cycle of *Venerupis japonica* in five phases similar to those described by Ponurovsky and Yakovlev (1992) in *T. philippinarum* but these two species have only one gametogenic cycle each year.

According to Shaw (1988) we divided the reproductive cycle in manila clams, *T. philippinarum*, in the Po River Delta in five main phases namely active, ripe, spawning, resorption and inactive.

After a brief inactive period in winter (December–February) the gonads entered the active period at the beginning of spring (March–April). Gametogenesis culmina with the ripe stage which was reached in June followed by the main spawning period. During summer a second ripe stage was reached after gametogenesis and this lasted until the beginning of autumn when another spawning occurred. The gonad then entered the resorption stage prior to the winter inactive period.

No other studies have been reported about the reproductive pattern of *T. philippinarum* in other areas of the Adriatic Sea. However, similar studies have been carried out on other bivalves in the same area, e.g. *Rudicardium tubercolatum, Chamela gallina, Venus verrucosa* (Marano et al. 1980), *Crassostrea angulata* (Renzoni 1974). The reproductive cycle of *Scapharca inaequivalvis,* (Corni and Cattani 1990, Campioni and Sbrenna 1992), another Indo-Pacific bivalve now present in the Adriatic Sea, is similar to the one reported here for the manila clam settled in Scardovari.

A comparison can be made between the reproductive cycle of *T. philippinarum* in the Po River Delta and other areas of the world. In British Columbia, Canada (Quayle and Bourne 1972), in Hood Canal, Washington, U.S.A. (Holland and Chew 1974), northern Japan (Yoshida 1935), in Vostok Bay, northwestern part of the Sea of Japan (Ponurovsky and Yakovlev 1992), *T. philippinarum* spawns continuously during the summer and there was one gametogenic cycle each year. However, in southern Japan two spawning peaks were observed (Tanaka 1954) which is similar to the situation in Cadix Bay, Spain. In the latter location, just as in

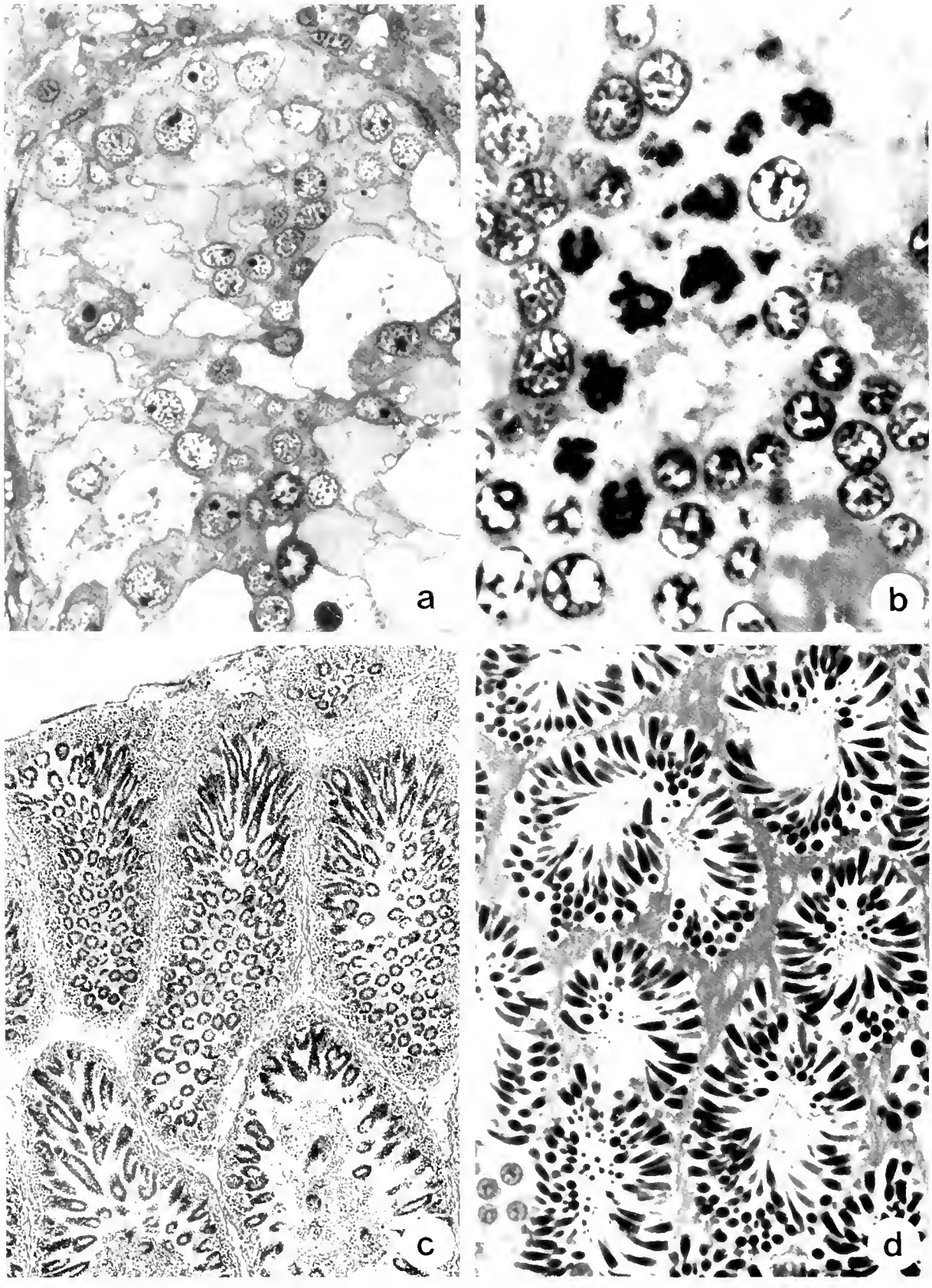

Figure 3. Gametogenic stages in the development of the male gonad of *Tapes philippinarum*. a: Male follicle at the beginning of gametogenesis. Among the large vesicular cells (arrow) the germ cells are visibile (1090×). b: Details of a follicle containing spermatogonia and proliferating spermatocytes (1600×). c: Mature gonad. Many follicles are filled with mature spermatozoa (120×). d: Details of the central area of a follicle. Spermatozoa are clustered in a typical rosette shape (1270×). e: TEM photograph of a spermatozoon. The gamete is characterized by an elongated acrosome (14400×). f: Follicle in the resorption stage. The follicles are almost empty because of spawning and resorption has begun.

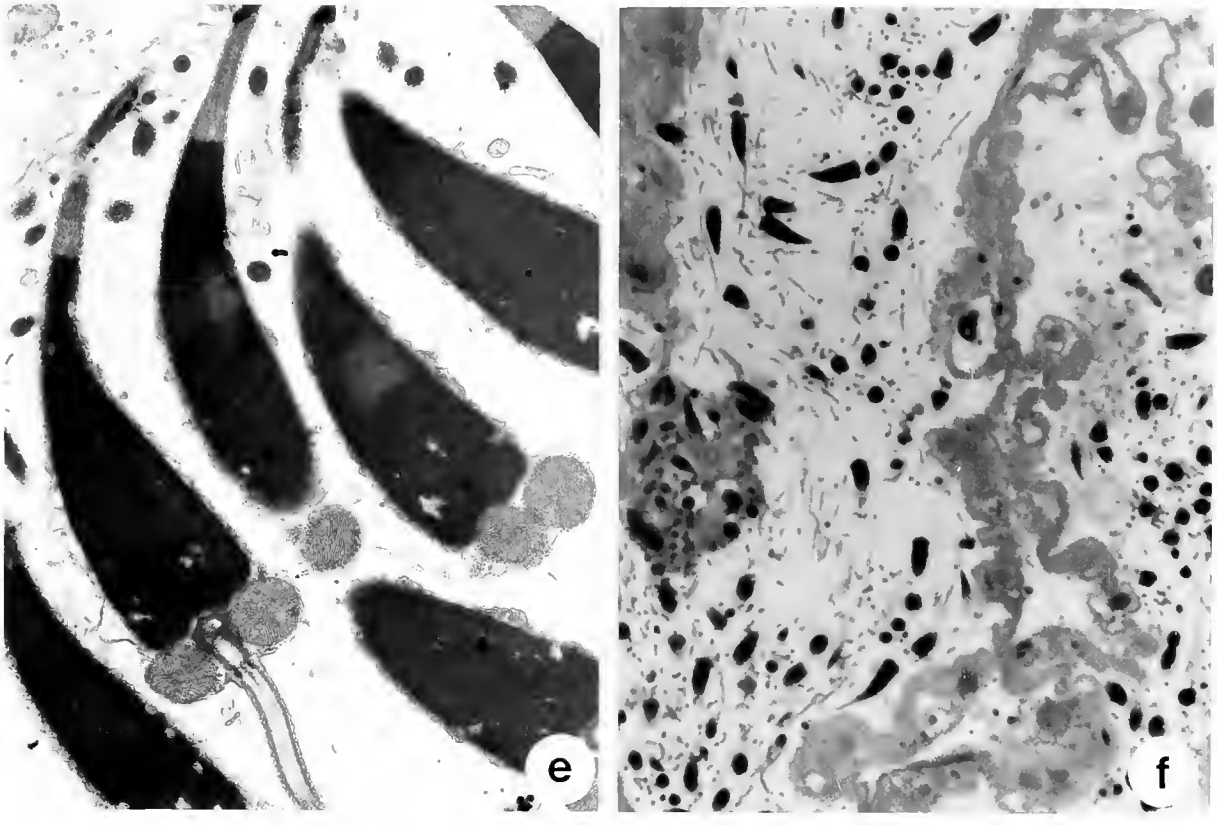

Figure 3. Continued.

the Adriatic Sea, repeated spawnings of varying intensity were observed, the first was rather weak (spring), the second was strong (summer) and the third was of variable intensity (autumn) (Sarasquete et al. 1990). Finally it is interesting to note that the reproductive cycle we observed for *T. philippinarum* did not overlap that of *T.* (*Venerupis*) *decussata* which occurs in the nearby Venice Lagoon. The latter species requires a temperature of 28°C for spawning which is only attained in some places for a short period of time (Breber 1980). This temperature threshold is a "restricting factor" and permits only one short and localized spawning (late August) for this species. The absence of such a restricting factor for *T. philippinarum* and the fact that the species spawns at a lower optimal temperature (20–24°C) has probably contributed to the dispersal and success of this species in the Adriatic region.

TABLE 1.

Stages in the annual reproductive cycle of *Tapes philippinarum*

Inactive
Stage I. The gonad has an extremely reduced volume with few follicles.
Active
Stage IIa. The number of follicles increases within the interstitial tissue.
Stage IIb. The follicles increase in number and size within the interstitial tissue.
Stage IIc. In males, small groups of spermatozoa appear within the follicles. In females, oocytes begin vitellogenic activity.
Ripe
Stage III. The gonads are full of gametes
Spawning
Stage IVa. Partial spawning. Some follicles appear empty.
Stage IVb. Spawning is almost completed in all follicles.
Resorption
Stage V. Within the empty gonad resorption begins on the few gametes that remain.

Comparison Between the Two Lagoons

A second goal of this project was to compare the reproductive cycle of *T. philippinarum* in the two lagoons, Sacca Degli Scardovari and Sacca Del Canarin. The morphological study of the reproductive stages showed some differences between the two areas; e.g. the early resumption of male gametogenesis at Canarin and the rapid regeneration of the active and mature stages during summer in Scardovari in contrast to the almost continuous ripe period at Canarin. Differences were also seen in spawning characteristics. At Scardovari manila clams spawned in the first week of July while at the nearby Sacca del Cararin the low percentage of empty gonads recorded from June to September and the simultaneous presence of fully mature individuals during the same time shows that *T. philippinarum* underwent a continuous spawning, albeit of low intensity, during this period. Similar differences in the length and number of spawning in population from different

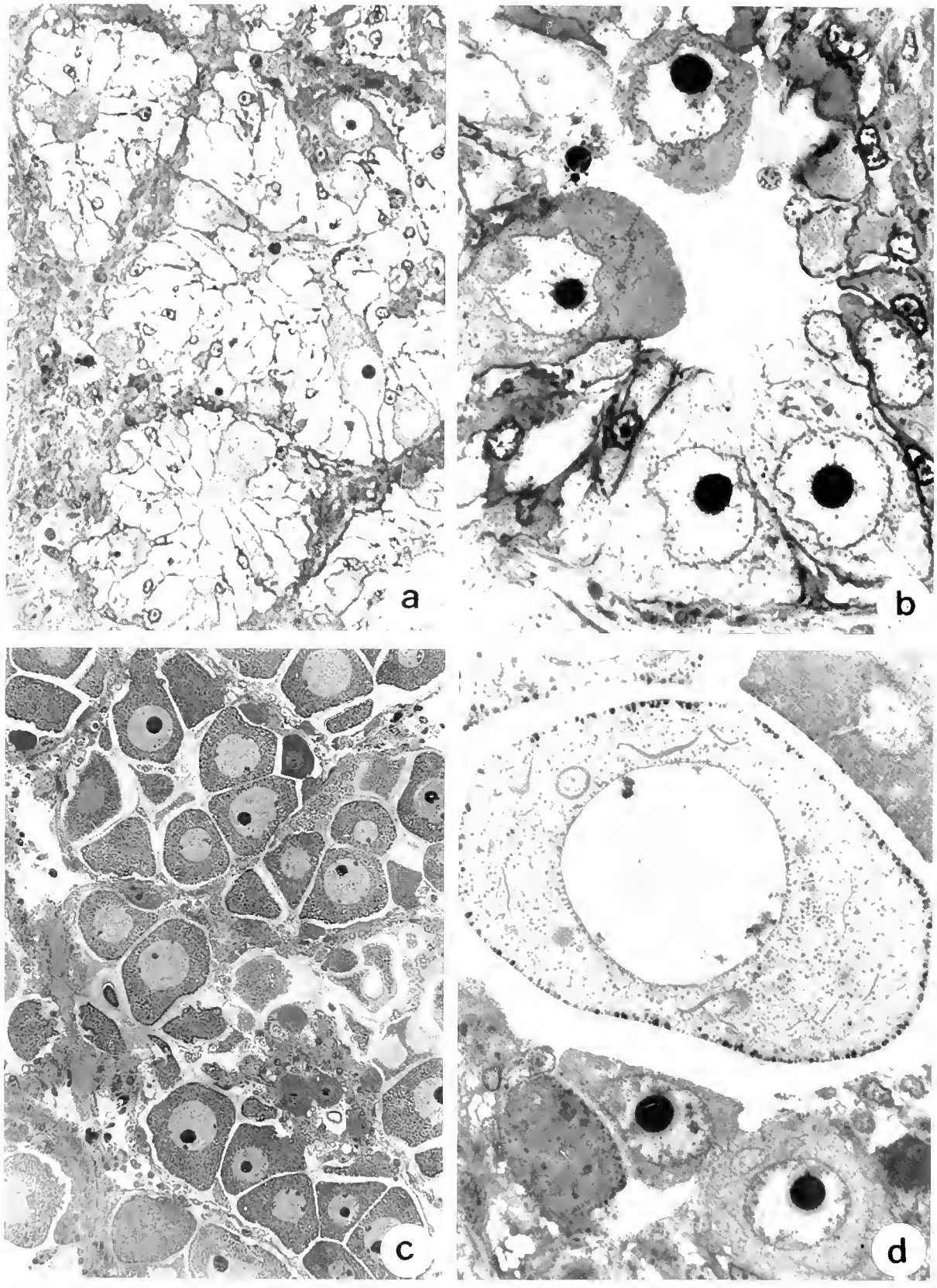

Figure 4. Gametogenic stages in the development of the female gonad of *Tapes philippinarum*. a: Female gonad at the beginning of gametogenesis. Previtellogenic oocytes are visible among vesicular cells in many follicles (250×). b: Details of a follicle wall in which previtellogenic oocytes with RER aggregates in their cytoplasm are still visible (1160×). c: Mature gonad. Follicles contain many vitellogenic oocytes (208×). d: Details of a vitellogenic oocyte. Yolk granules are visible in the ooplasm (1060×). e: Oocytes with a shrunken appearance that suggest the beginning of the degenerative process (940×). f: Gonad in the resorption stage. The resorption process, involving residual gametes, is beginning in the empty follicles (410×).

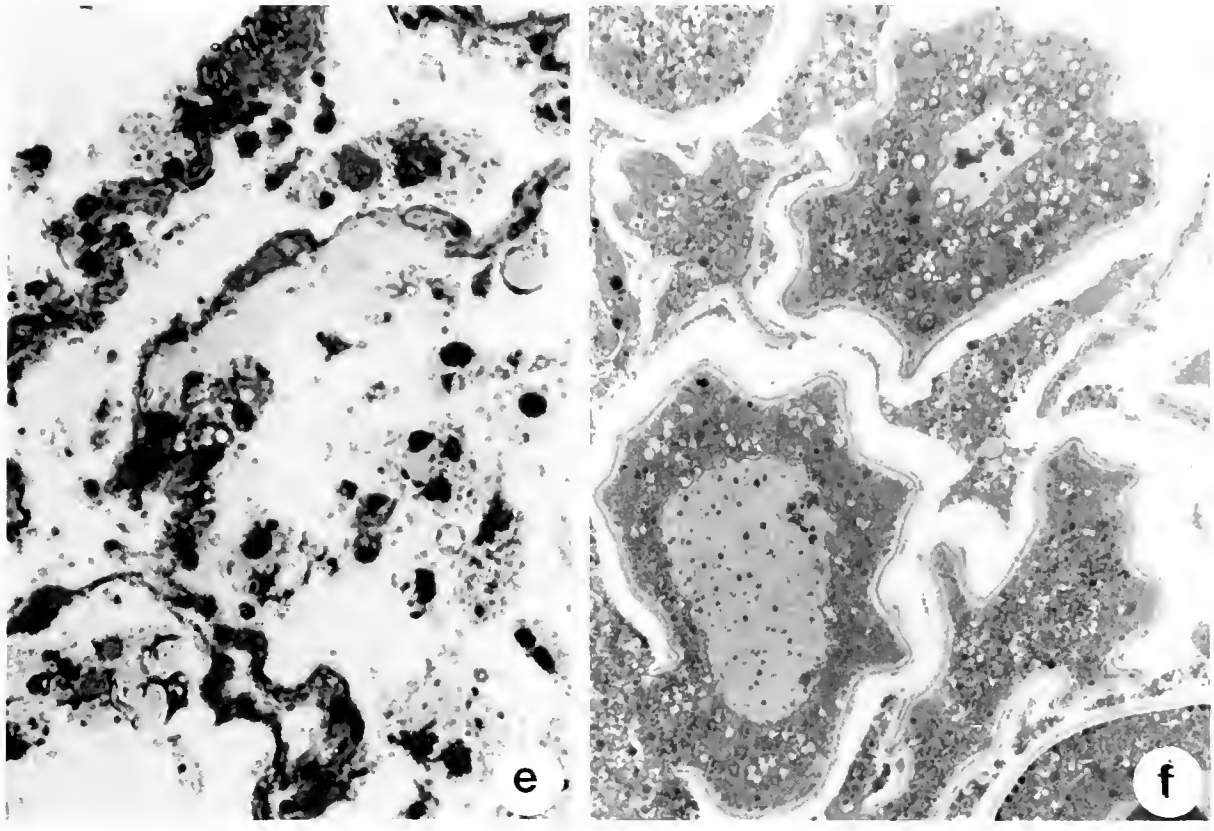

Figure 4. Continued.

areas of the same producing region are not found (Holland and Chew 1974, Ponurovsky and Yakovlev 1992).

The reason for the difference in reproductive patterns of *T. philippinarum* in the two lagoons is not clearly understood. Loosanoff and Davis (1963) stated that resumption of gametogenesis was due to the presence of a threshold temperature value (10°C). Other authors such as Sarasquete et al. (1990) stated that the resumption of the maturation process in *Tapes* was due mostly to the temperature regime during the period preceeding the winter inactive stage. In the two Po Delta lagoons, temperature differences were recorded during the first months of the year but in October–December, prior to the winter inactive stage, no differences were observed in temperature (Fig. 2). Our results support the conclusions of Mann (1977) who showed the direct role of temperature for the resumption of the maturation process in manila clams. He stated that in temperate climates a temperature between 20–22°C was optimal for the beginning and continuation of gamete development. Actually such a temperature is reached earlier in Sacca Del Canarin than in Sacca Degli Scardovari because of the shallow water depth at Canarin so that gametogenesis began there as early as March.

The difference in maturation may also be due to differences in trophic conditions between the two lagoons. Information on *Tapes* metabolism (Beninger and Lucas 1984) and comparison of data for lipid, glycogen and protein composition in the two populations (Ambrogi, pers. comm.) shows that metabolic reserves are higher in manila clams in Sacca del Canarin from late winter to early spring. Resumption of gonadal maturation and the life cycle of manila clams in this lagoon could be favoured by better synchrony between food availability and energy requirements. These optimal conditions may only allow maturation of the gonads but also permit spawning in May although it is of low intensity. As found by MacDonald and Thompson (1988) local variability in gonadal development can be ascribed to local variations in environmental factors, among which food availability is of major importance.

It is difficult to state what environmental factors trigger spawning. Information on the effects of temperature, salinity, tidal cycles and food availability on initiation of spawning are meager (Mann 1979, Dohmen 1985, Lubet et al. 1987). During summer, differences in temperature and salinity at the two lagoons were not sufficiently different to cause a difference in spawning behavior. For example, the almost perfect overlapping of temperatures in the two lagoons (Fig. 2) suggests that temperature may not be the main triggering factor for spawning in agreement to what observed by Chipperfield (1953). According to this author, timing and duration of the spawning and also the number of spawning each year is depending on species and is interrelated with different environmental factors.

The trophic component may affect gonadal development in these lagoons (Ceccherelli et al. 1987). In Sacca Degli Scardovari the highest yearly average density and biomass of zooplankton occurred in a spring peak followed by a protracted decline during the summer. In Sacca Del Canarin trophic conditions became worse during summer eutrophication which may be due to slower water renewal in this lagoon.

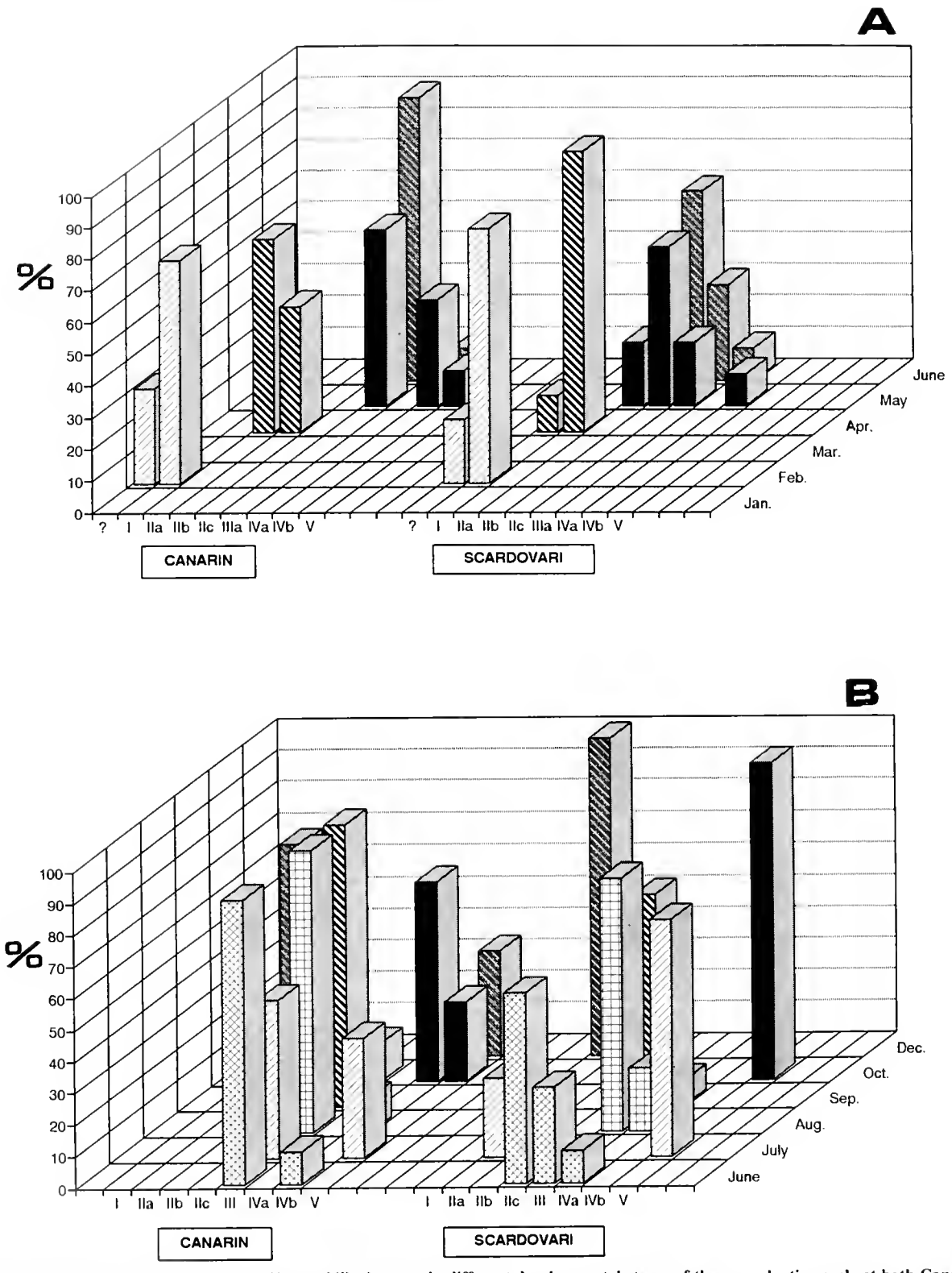

Figure 5. Percent of male manila clams, *Tapes philippinarum*, in different developmental stages of the reproductive cycle at both Canarin and Scardovari Lagoons. A: January–June 1991; B: July–December 1991.

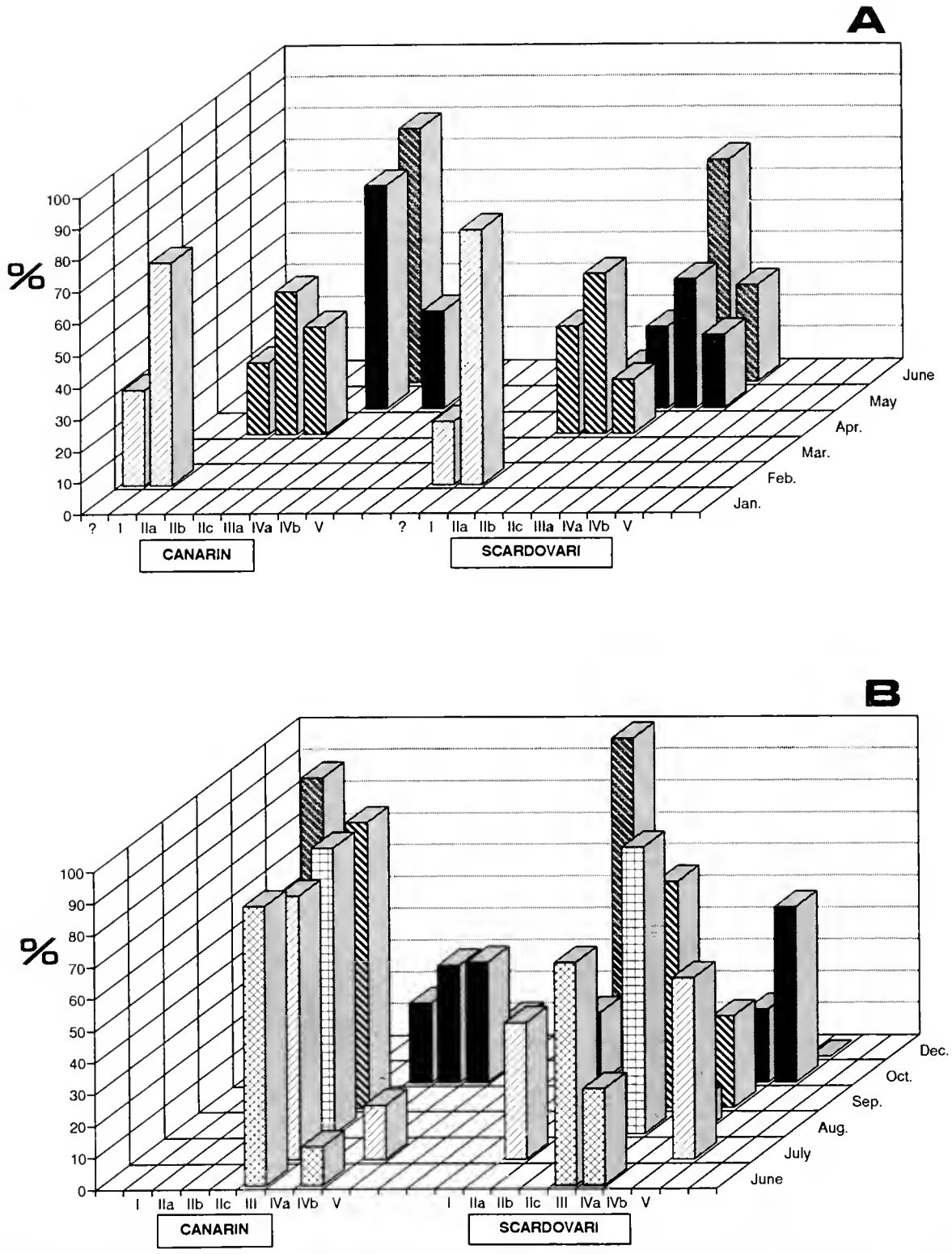

Figure 6. Percent of female manila clams, *Tapes philippinarum*, in different developmental stages of the reproductive cycle at both Canarin and Scardovari Lagoons. A: January–June 1991; B: July–December 1991.

Our observations show that although *T. philippinarum* is an exotic species it has adapted well to the Po River Delta environment. These lagoons favour completion of the reproductive cycle in spring and become less favourable for spawning in summer because of eutrophication.

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EFFECT OF ALGAL RATION ON FEEDING AND GROWTH OF JUVENILE MANILA CLAM *TAPES PHILIPPINARUM* (ADAMS AND REEVE)

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ABSTRACT Juvenile *Tapes philippinarum* (Adams and Reeve) were reared for three weeks on different rations of *Chaetoceros neogracile* which were either centrifuged and stored or fed together with the culture medium. Algal rations were adjusted daily in order to feed constant weight-specific daily rations. Growth of *T. philippinarum* fed the concentrate of *C. neogracile* was maximal at a daily ration of 1% (algal dry weight per clam live weight). *T. philippinarum* fed single or mixed diets of *C. neogracile* and *Isochrysis* sp. (clone T-Iso) directly from the algal cultures, maximized growth at a ration of 1.3% day^{-1}. Higher growth rates and gross growth efficiencies were obtained with *C. neogracile* fed together with the culture medium. A set of grazing experiments showed that the incipient limiting concentration was about 26 *C. neogracile* cells µl^{-1}. Measurements of cell concentration during the growth tests demonstrated a discontinuous feeding activity in the treatments receiving the optimal ration for maximum growth.

KEY WORDS: algal ration, feeding, growth, bivalve, Manila clam, *Tapes philippinarum*

INTRODUCTION

Earlier work examining the effect of various culture conditions on the growth of bivalve juveniles revealed that the amount of food available per spat was far more important than any other factor explored (Walne and Spencer 1974). Various methods have been used to describe food ration for bivalves. To maintain the link of ration with the concentration at which the food is presented, some authors refer to the cell density upon batch feeding a constant amount of food, independent of the size of the seed (Langton and McKay 1976, Hollett and Dabinett 1989). This method complicates comparisons between experiments which differ in density and size of the seed. For this reason, food rations are preferentially expressed as daily weight-specific rations, such as number of cells (Pruder et al. 1976) or percent dry weight (% DW, Urban et al. 1983) of algae per live weight (WW) of bivalves.

Empirical studies of the relationship between ration size and growth of bivalves weighing less than 1 g are very scarce, although the early juvenile stages are the largest consumers of intensively cultured micro-algae in most commercial hatchery operations (Manzi and Castagna 1989, Helm 1990). Ease of handling has prompted most researchers to use larger juveniles for nutritional studies and, moreover, empirical work has been mainly restricted to oysters. Several equations have been described relating ration size of *Crassostrea virginica* to oyster weight (Pruder et al. 1976, 1977, Epifanio and Ewart 1977, Epifanio 1979). However, these formulas are derived from measurements of maximum filtration rates for oysters mainly in the size range of 10–100 g and predict unreasonably high weight-specific rations for oysters weighing less than 10 mg. By contrast, Urban et al. (1983) concluded that the ration for maximum growth of the American oyster in the size range of 11 to 64 mg was probably greater than that predicted by the equations of Pruder and co-workers.

The optimal ration depends upon the species and culture conditions of algae making up the diet. Enright et al. (1986a) evaluated the nutritional value of 16 phytoplankton species fed individually to *Ostrea edulis* (5–25 mg initial live weight) at rations ranging from 0.1 to 6.0% of the oyster live weight and found that optimal algal rations for growth differed according to the algal species. In the same way, Epifanio and Ewart (1977) demonstrated that maximum daily rations removed from suspension by *C. virginica* (15 g live weight) varied from 0.4% for *Thalassiosira pseudonana* and *Carteria chuii* to 1.5% for *Isochrysis galbana*. Growth of *O. edulis* juveniles fed nutrient-limited cultures of *Chaetoceros gracilis* was saturated at a ration of ±2.5% DW WW^{-1} day^{-1}, whereas that of oysters fed algae grown in a complete medium showed a maximum between 2.5 and 4.9% (Enright et al. 1986b). Because optimal rations for maximum bivalve growth will vary according to the culture conditions, they must be determined through empirical growth studies which integrate culture conditions with the physiological, as well as nutritional requirements of the bivalves for maximum growth (Urban et al. 1983). The experimental data reported by Urban and Pruder (1992) showed a linear growth response in *Mercenaria mercenaria* juveniles (initial WW 25 mg) to increased ration from 0 to 1%. The latter authors did not provide the actual daily rations, which should have been lower because rations were adjusted only on a weekly basis. The optimal daily ration for growth of *M. mercenaria* seed (in the size range of 0.4 to 7 mg live weight) fed a mixture of *C. gracilis* and *Isochrysis* sp. (clone T-Iso) was found to be 1.5 to 2% DW WW^{-1} (Coutteau et al. 1994).

In the present study, the quantitative food requirements for growth of small juvenile *Tapes philippinarum* were determined in small-scale experiments. Grazing tests were performed to determine the critical concentrations for filter-feeding in juvenile Manila clams. Growth data were compiled from seven independent experiments. Growth and feeding rate of clams fed *C. neogracile* either after centrifugation and storage or together with the culture medium was compared. Finally, preliminary tests were run to compare two algal species' optimal algal rations for growth.

MATERIALS AND METHODS

Origin and Acclimatization of the Animals

Juvenile Manila clams *T. philippinarum* (Adams and Reeve) were obtained from commercial hatcheries (see Table 1). The spat were transported in a refrigerated styrofoam box from the hatchery to the lab. Upon arrival they were acclimated gradually to the experimental temperature (temperature increase rate <0.25°C

TABLE 1.

Overview of the size and origin of the juvenile *T. philippinarum* used for the various experiments.

Experiment	Initial Mean Unit Weight (mg)	Origin*	Arrival Date
1	5.13	SSW	25/11/89
2	5.65	SSW	26/01/90
3	5.63	GSF	12/03/90
4	1.31	GSF	18/04/90
5	1.35	TM	10/09/90
6	1.32	TM	12/10/90
7	4.91	TM	30/11/90

* SSW: Seasalter Shellfish Whitstable Ltd., UK; GSF: Guernsey Sea Farms Ltd., UK; Tinamenor S.A., Spain.

h^{-1}) and fed *Chaetoceros neogracile* (Vanland. 1968) *ad libitum* for three to seven days prior to the start of the experiment. This species has been referred to previously, and incorrectly, by many authors as either *C. gracile* or *C. gracilis* (VanLandingham 1968).

Culture Systems and Conditions

The experiments were performed in a recirculating system consisting of a 350 μm mesh silo which was submerged partially in a five liter aquarium. An air water lift maintained a flow of about 300 ml min^{-1} through the silo, which was stocked at the start of the experiment with 0.5 g of spat. An additional aeration point in the aquarium minimized settling of the food. The systems were cleaned and the seawater was renewed three times each week. The seawater was filtered through a 1 μm bag and run through UV prior to use. Cultures were kept in a thermostatic bath at 21 ± 1°C.

Feeding

C. neogracile, which has proven to be among the best algal diets for *O. edulis* juveniles (Enright et al. 1986a) and is used extensively in several commercial bivalve hatchery and nursery operations (Coutteau and Sorgeloos 1992), was selected as the algal control diet. The seed was fed a weight-specific daily ration which was divided between two feedings per day. Rations were adjusted daily for growth of the spat to feed approximately constant weight-specific daily rations throughout the experiment (Urban et al. 1983). The daily ration, calculated as % dry weight (DW) of food per wet weight (WW) of clams, was thus approximated each day by adjusting the amount of food as a function of an assumed growth rate by means of the formula:

$$[\text{DW food day } n] = [\text{DW food day } 1] \times (1 + \text{DGR}/100)^{n-1}$$

where n = day of the week (1 to 7), DGR = daily growth rate (% day^{-1}) measured during the previous week or assumed to be 10% day^{-1} for the first week, DW food day 1 = initial WW × weight-specific daily ration (%DW WW^{-1} day^{-1}) × 1/100.

In experiment five, one treatment was fed "on demand" twice daily, i.e. the objective was to keep the *C. neogracile* concentration above 20 μl^{-1} throughout the experiment.

Calculation of the daily rations was based on dry weight analysis of the algal food species used. Algal dry weights were determined by filtering algae from various volumes of suspension of known concentration. Algae were retained on tared, glass-fiber filters (1 μm pore size) which were subsequently washed with a solution of ammonium formate (0.5 M) to remove salts. Filters were then dried at 100°C for 4 h to volatilize the ammonium formate, and weighed on an analytical balance (modified from Epifanio and Ewart 1977). The same procedure was followed with control filters on which an equal amount of seawater was filtered. Cellular dry weight was determined from regression analysis of DW retained on the filter *versus* number of algal cells filtered. Dry weights were 23.8 ± 3.8 and 14.1 ± 0.8 pg $cell^{-1}$ for *C. neogracile* and *Isochrysis* sp., clone T-Iso, respectively (mean and standard deviation from analysis of five cultures).

Algae were grown semi-continuously in 20 l carboys using Walne medium. Only algal cells in the logarithmic phase of growth were used in the feeding experiments. In the experiments one to five, algae were separated from the culture medium by centrifugation and stored prior to use (Winter and Langton 1976). The algal pellet was resuspended and diluted in filtered seawater to obtain a *Chaetoceros* concentrate of $20 \cdot 10^6$ cells ml^{-1} using a haemocytometer or Coulter counter, model Zf, and stored in total darkness at 4°C for maximal three days. In experiments five to seven, algal suspensions were drained from the cultures and counted immediately prior to each feeding.

Clearance and Intake Rate as a Function of Food Concentration

During two short term grazing experiments, clearance rates were recorded for *T. philippinarum* (20 mg mean WW) at various concentrations of *C. neogracile*. The animals were derived from a population which was fed *C. neogracile* and transferred to 5 l culture systems which were placed in a thermostatically-controlled water bath at 21 ± 1°C. In order to achieve 10 to 20% decreases of cell concentration over a 2 h period, stocking density was varied between 0.3 and 0.7 g live weight per 5 l system, depending on the food concentration tested. The seed was acclimated to the food concentration for 1 h prior to the experiment (Sprung and Rose 1988). Algal concentration was monitored with a Coulter counter (model Zf) during two consecutive periods of 2 h. A control experiment with no animals present was run in duplicate for all concentrations tested.

Weight-specific clearance rate (CR) was calculated using the equation (Coughlan 1969):

$$\text{CR} = \frac{V}{\text{WW}\ t}\left[\ln\frac{C_0}{C_t} - \ln\frac{C_0'}{C_t'}\right] \qquad [ml\ g^{-1}h^{-1}]$$

where WW = total clam live weight (g), V = volume of the food suspension (ml), C_0, C_0' = initial, and C_t, C_t' = final concentration of, respectively, the experimental and the control aquarium, and t = elapsed time (h).

The rate at which cells are removed from suspension, to be referred to as intake rate (ir) since pseudofaeces production was not quantified (Foster-Smith 1975), was computed as:

$$\text{ir} = C_m \times \text{CR} \qquad [\text{cells } g^{-1}\ h^{-1}]$$

where $C_m = (C_t + C_0)/2$ the mean concentration (cells ml^{-1}) encountered by the animals during the measurement of the clearance rate. Clearance and intake rates were graphically presented as a function of C_m.

Average Intake Rate During a Growth Test

During the experiments evaluating the effect of *C. neogracile* ration on growth, food concentration was measured either at short

time intervals of 2 to 6 h (experiment 1) or before and after each feeding (experiment 5). The observed decreases in cell concentration could be related to the total live weight present in the culture system by calculating an average weight-specific intake rate (ir) over the elapsed time interval (t) by means of the formula:

$$\text{ir} = \frac{V\,(C_0 - C_t)}{\text{WW}_n\,t} \qquad [\text{cells g}^{-1}\,\text{h}^{-1}]$$

where V = volume of the food suspension; C_0 and C_t = initial and final, i.e. after time t (h), concentration; WW_n = total clam live weight (g) present at the moment of the measurement, estimated from the daily growth rate (DGR) and the initial live weight (WW_1, day 1) from:

$$\text{WW}_n = \text{WW}_1\,(1 + \text{DGR}/100)^{n-1}$$

The intake rates were not corrected for algal growth during the experiment, since the latter was found to be fluctuating in time, but negligible compared to the amount of cells removed by the clams.

Growth Parameters Followed

Animals were selected initially from a single population of juveniles and divided randomly in groups of equal weight, which were distributed among the culture systems. Initial parameters (shell length and individual live weight) were measured on three subsamples.

At 7-day intervals, the seed was removed from the respective silos and the total live weight determined. Enough clams were removed to return the weight to the initial value, and individual live and dry weight were determined on the culled animals.

The total live weight per silo was determined by collecting the clams on a mesh, which was blotted dry on paper towel. To avoid differences in water content between samples due to air-drying, the total biomass was then immediately weighed and reduced to the initial weight prior to returning to the culture systems. The culled animals from each silo were weighed and counted for the determination of the individual live weight. The seed samples were transferred subsequently to Teflon pots, previously dried in an oven at 60°C for 4 h, and weighed. The pots were returned to the oven for 24 h at 60°C and then weighed to give the dry weight. Live weight (WW) of *T. philippinarum* showed a high correlation with dry weight (DW):

$$\text{DW} = 0.571\ \text{WW} + 0.431 \qquad (r^2 > 0.99)$$

A constant relationship between live and dry weight was also reported by Urban and Langdon (1984) for *C. virginica* and demonstrated that the drying procedure used for determining the live weight resulted in a constant water content.

Daily growth rate was calculated from the weekly increase of total wet weight per silo (DGR) using the equation:

$$\text{DGR} = \left(\sqrt[n]{\frac{\text{WW}_n}{\text{WW}_0}} - 1\right) \times 100 \qquad [\%\ \text{day}^{-1}]$$

where WW_0 and WW_n are the live weight, respectively, at the start and after n days. Shell length was measured on 30 clams per silo using a dissecting microscope equipped with a calibrated ocular. Live weight (WW, in the range 0.2–32 mg) increased with increasing shell length (L, in the range 2–5.5 mm) according to the equation:

$$\text{WW} = 0.463\ \text{L}^{2.477} \qquad (r^2 = 0.98)$$

Experimental Design

Seven culture tests were performed with *C. neogracile* fed either from a concentrated stock suspension (experiments 1–5) or directly from the algal culture (experiments 5–7). Experiments one and five were run specifically for the study of algal ration, with the latter test comparing feeding and growth on both types of *C. neogracile*. For the other experiments, which were part of a study evaluating algal substitution by a manipulated yeast diet (Coutteau 1992), only growth data from the algal control treatment were used in the present study. In addition, three preliminary experiments were performed to evaluate the effect of ration on growth of *T. philippinarum* fed single and mixed diets of *C. neogracile* and *Isochrysis* sp. (clone T-Iso), both fed directly from the algal cultures.

Data Treatment and Statistical Analysis

Daily growth rates were used to compare the effect of the diets on juvenile growth because this allowed comparisons between experiments that differed in initial live weights of the seed.

Statistical analysis of the growth data included analysis of variance and Tukey HSD multiple range tests. The homogeneity of the variances of means for each experiment was checked by Cochran's C-test and Hartley's test. Because of the limited number of replicates, normality was tested on the deviations $Y_{ij} - Y_i$, which were computed separately for each treatment and pooled per experiment, by means of the Kolmogorov-Smirnov test. Departures from the assumptions of analysis of variance could be rectified in most cases by logarithmic transformation of the data. Inherently heteroscedastic data (Cochran's C-test or Hartley's test, $P < 0.05$, even after transformation) were indicated in the tables of the results with "H.D." and were analyzed using an approximate test of equality of means assuming heterogeneity of variances (MCHETV) or, when only two means were to be tested, an approximate t-test (Sokal and Rohlf 1981).

RESULTS

Effect of Ration Size on Growth of T. philippinarum *Fed* C. neogracile

Clam growth increased with increasing daily ration up to a ration of 1% of live weight (Table 2). Further increase of the ration to 1.5% did not result in a significant difference in growth or final size of the clams. The feeding regime was adapted daily to the growth of the clams in the various treatments based on an assumed daily growth rate for each week of the experiment. The actual rations, computed from the feeding regime and the observed growth rates, deviated from the initial ration in the course of each week depending on the accuracy of the assumed growth rate (Fig. 1). To obtain a better estimate of the effective weight-specific ration fed to the clams, the arithmetic mean of the actual daily ration was determined for each week of the experiment. Daily growth rate showed a saturation response around an effective ration of 1%, though growth rate fluctuated between 6.7 and 9.8% day^{-1} according to the week of the test (Fig. 2). Growth of the starved clams declined from more than 2% day^{-1} during the first week to less than 0.5% day^{-1} during the rest of the experiment.

The fluctuation of algal concentration showed a similar pattern in all replicates of each treatment and is represented for one rep-

TABLE 2.

(Experiment 1) Daily growth rate (DGR), final live (WW) and dry (DW) weight, and shell length (L) of *T. philippinarum* fed various daily rations of *C. neogracile*. Data represent mean and standard deviation from four replicates. Like superscripts indicate means which do not differ significantly (ANOVA, Tukey HSD, $P \leq 0.05$, unless stated otherwise).

Treatment§	Week 1 DGR (% day^{-1})	Week 2 DGR (% day^{-1})	Week 3 DGR (% day^{-1})	Week 3 WW (mg ind^{-1})	Week 3 DW (mg ind^{-1})	Week 3 L (mm)
1. Unfed control	2.36 ± 0.30^{b}	0.31 ± 0.50	0.48 ± 0.36^{c}	6.38 ± 0.37^{c}	4.05 ± 0.21^{c}	2.84 ± 0.08^{c}
2. 0.1% Chg	2.91 ± 0.25^{b}	2.05 ± 0.17	1.33 ± 0.26^{c}	8.23 ± 0.28^{c}	5.16 ± 0.17^{c}	3.23 ± 0.15^{c}
3. 0.5% Chg	5.80 ± 0.21^{a}	6.52 ± 0.11	5.74 ± 0.37^{b}	17.99 ± 1.50^{b}	10.82 ± 0.84^{b}	4.37 ± 0.16^{b}
4. 1.0% Chg	6.71 ± 0.67^{a}	9.77 ± 0.09	8.67 ± 0.43^{a}	29.59 ± 3.26^{a}	17.14 ± 1.89^{a}	5.34 ± 0.17^{a}
5. 1.5% Chg	5.84 ± 0.68^{a}	7.85 ± 0.71	9.21 ± 0.53^{a}	25.65 ± 3.27^{a}	14.92 ± 1.89^{a}	5.07 ± 0.18^{a}
ANOVA, F_s	69.0	H.D.*	407.3	88.8	84.4	157.5

§ Chg = *Chaetoceros neogracile* concentrate, rations expressed as initial daily rations (DW WW^{-1} day^{-1}) initial seed: 5.13 ± 0.58 mg ind^{-1} (mean ± SD, n = 3).

* Heteroscedastic data, significantly different means separated by / (MCHETV, $P \leq 0.05$): 1/2,3,4,5; 2/3,4,5; 3/4.

licate system per treatment in Fig. 3. By distributing a weight-specific ration of 1% day^{-1} over two feedings, a peak concentration was attained twice daily of 30–40 *Chaetoceros* µl^{-1}, which was cleared below a level of 10 cells µl^{-1} in the subsequent 5–7 h period (Fig. 3C). The highest ration often resulted in an accumulation of the algae between the periodic renewals of the seawater up to concentrations of 40 (week 3) or even 90 algal cells µl^{-1} (week 2, Fig. 3D). In the cultures fed the lowest rations, the algae were cleared to levels below 1 to 2 *Chaetoceros* µl^{-1} (Fig. 3A and B).

The intake rate, calculated from the decrease of cell concentration over short time intervals during the second and third week of the experiment, was mainly related to the food level present in the culture at the moment of the measurement (Fig. 4). Fluctuation of cell concentration in the cultures fed 1% day^{-1} thus resulted in a variation of intake rate between 35 and 8.10^{6} cells g^{-1} h^{-1}, and even lower values may have been observed at the concentrations below 10 *Chaetoceros* µl^{-1}. The rate with which the clams removed the algae from suspension increased linearly up to a concentration of about 30 *Chaetoceros* µl^{-1}. Estimates of intake rate beyond this algal density were mainly derived from the cultures fed 1.5% DW WW^{-1} day^{-1} during the second week of the test and fluctuated strongly irrespective of food concentration. Possibly, the strongly depressed intake rates observed at the high food concentrations were artefacts due to the calculation of intake rates from relatively small decreases of cell concentration. Also, the impact of algal growth, which was not taken into account for the computation of the intake rate, may have been relatively more important at the high algal loads.

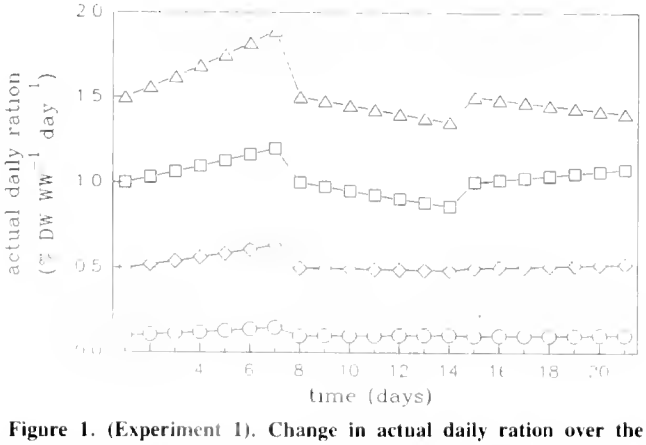

Figure 1. (Experiment 1). Change in actual daily ration over the course of each week of the experiment for *T. philippinarum* fed *C. neogracile* at an initial daily ration of either 1.5% (△), 1% (□), 0.5% (◇), or 0.1% (○).

Effect of C. neogracile *Concentration on Clearance and Intake Rate in* T. philippinarum

T. philippinarum maintained maximum clearance (CR_{max}) and intake (ir_{max}) rates at, respectively, low and high food concentrations (Fig. 5). The incipient limiting concentration, calculated from the ratio ir_{max}/CR_{max} (Sprung and Rose 1988), was similar in the two experiments and corresponded with the critical concentrations derived from the intersections of the fitted curves for the second experiment (Table 3). The deviating values obtained for the first experiment by the latter technique, especially for the

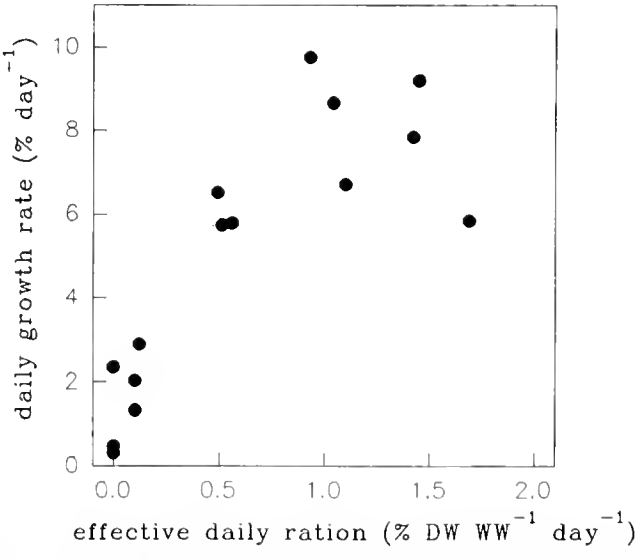

Figure 2. (Experiment 1). Relationship between the effective daily ration and the daily growth rate of *T. philippinarum* fed *C. neogracile*.

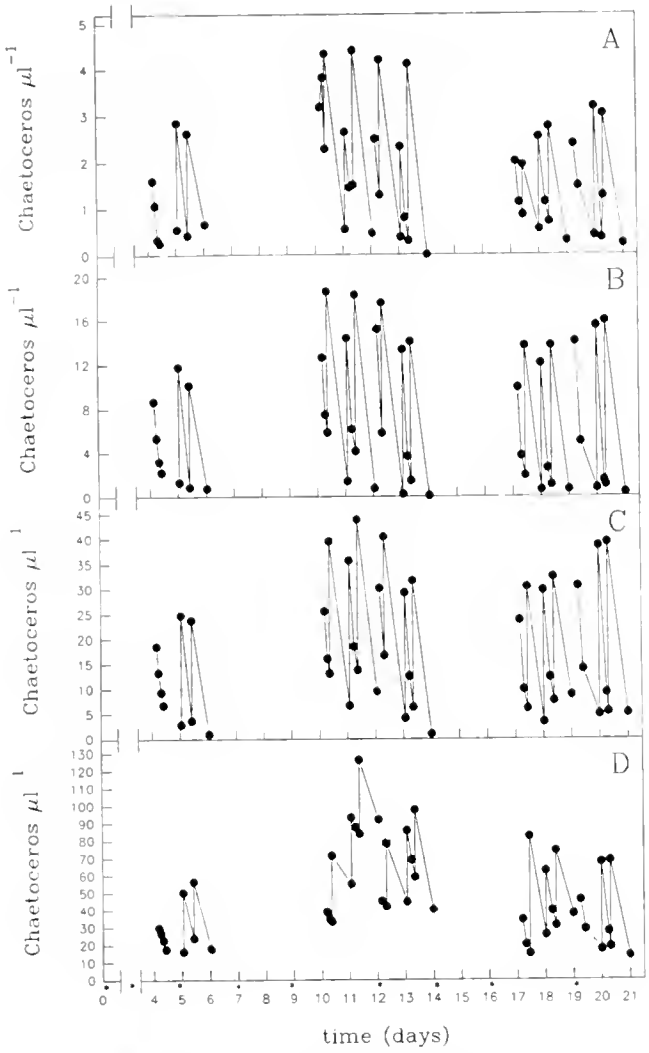

Figure 3. (Experiment 1). Fluctuation of food concentration in one replicate culture of *T. philippinarum* fed *C. neogracile* at a daily ration of either 0.1% (A), 0.5% (B), 1.0% (C), or 1.5% (D). Cell densities measured before and after feeding are connected with a vertical line. Data points are not connected when measurements were discontinued. The periodic renewal of the seawater is indicated on the time axis (*).

critical concentration of clearance rate, may have been due to the insufficient number of data points in the proximity of the incipient limiting concentration. Maximum weight-specific rates of filtration and feeding were about 30% higher during the second test than in the first experiment.

Comparison of Centrifuged Algae and Algal Cultures

Mean daily growth rate of clams obtained during the various weeks of seven experiments for clams fed *C. neogracile* at a daily ration of 1% is presented in Fig. 6. For the five experiments in which concentrated *C. neogracile* was fed, the average daily growth rate of the three weeks did not vary significantly between experiments (ANOVA, $F_{4,10} = 0.780$, $P = 0.56$). For experiments three and five, growth rates measured during the first week were highly deviating from those measured during the following weeks. Growth of clams fed the *C. neogracile* culture, averaged over all experiments, was significantly better than that of spat fed the algal

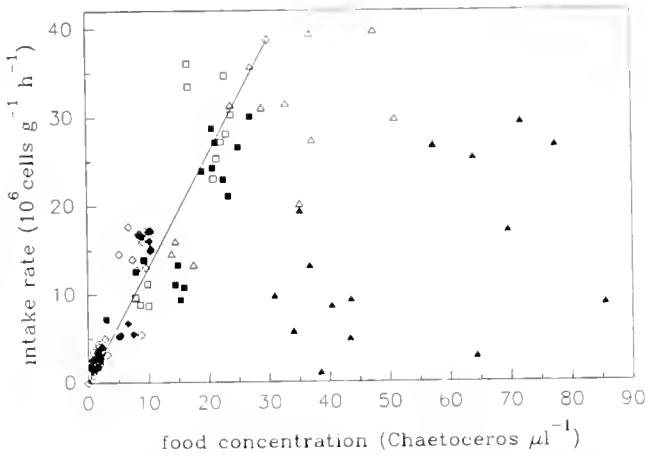

Figure 4. (Experiment 1). Intake rate (ir) as a function of food concentration (C) in *T. philippinarum*. Data are calculated from the decrease of food concentration measured over 2 to 6 h time intervals in the cultures fed various daily rations of *C. neogracile* (0.1%: ○, 0.5%: ◇, 1.0%: □, 1.5%: △) during the second and third week of the experiment (filled and unfilled symbols, respectively). Linear regression equation is given by: ir [10^6 cells g^{-1} h^{-1}] = 1.229 C [cells μl^{-1}] (r^2 = 0.81).

concentrate at the same daily ration (*t*-test: $P < 0.05$; $P < 0.001$ if the three aberrant growth rates for week one are excluded).

During the first week of the fifth test the seed exhibited a generally depressed growth, which was less pronounced for clams fed the algae derived directly from the culture. Clams fed the concentrated algae grew at a rate of about 30% of that observed during the subsequent weeks, whereas this was about 60% for seed fed *Chaetoceros* cultures (Table 4). The mean intake rates, calcu-

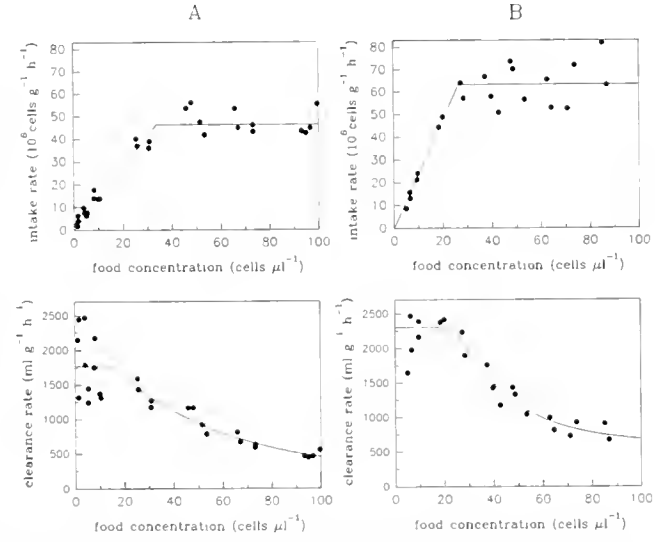

Figure 5. Clearance and intake rates as a function of food density in *T. philippinarum* (20 mg mean WW) fed *C. neogracile*. A and B represent the results of two independent experiments. Maximum clearance and intake rates are calculated from the mean of the data points below and above the critical concentrations, respectively. Curves were fitted to the data points for clearance and intake rate, respectively, above and below the incipient limiting level through, respectively, non-linear ($y = Ae^{-Bx} + Ce^{-Dx} + E$) and linear ($y = Ax$) regression. Derived parameters are presented in Table 3.

TABLE 3.

Maximal clearance (CR_{max}) and intake (ir_{max}) rate, and their critical concentrations for juvenile *T. philippinarum* (20 mg mean WW) fed *C. neogracile* in two independent experiments. Data are derived from Fig. 5.

	Experiment A	Experiment B
ir_{max} (10^6 cells g^{-1} h^{-1})	46.2	63.2
Minimum concentration for ir = ir_{max} (cells μl^{-1})	33.7	26.6
CR_{max} (ml g^{-1} h^{-1})	1771	2301
Minimum concentration for CR < CR_{max} (cells μl^{-1})	15.2	24.1
Incipient limiting concentration [ir_{max}/CR_{max}] (cells μl^{-1})	26.1	27.4

lated from the change in cell concentration between each feeding, were similar throughout the experiment and were not different between the treatments fed 1% DW of *C. neogracile* either as a concentrate or directly from the culture. As a result, gross growth efficiency was higher for clams fed the *C. neogracile* culture, in particular during the first week of the experiment (Table 5). It is of interest that the difference between the gross growth efficiency of clams fed either of the algal types is much reduced during the last two weeks. Possibly, the clams may have needed a longer acclimatization period to adapt to a diet of concentrated algae.

Feeding *Chaetoceros* cultures at daily rations of 1.5% or 1.73% (effective daily ration for treatment fed "on demand") resulted in significantly higher growth compared to the controls fed 1% during the last week of the test (Table 4). This suggests that the optimal ration for maximal growth may be higher for the *Chaetoceros* culture than for the concentrated algae. This was

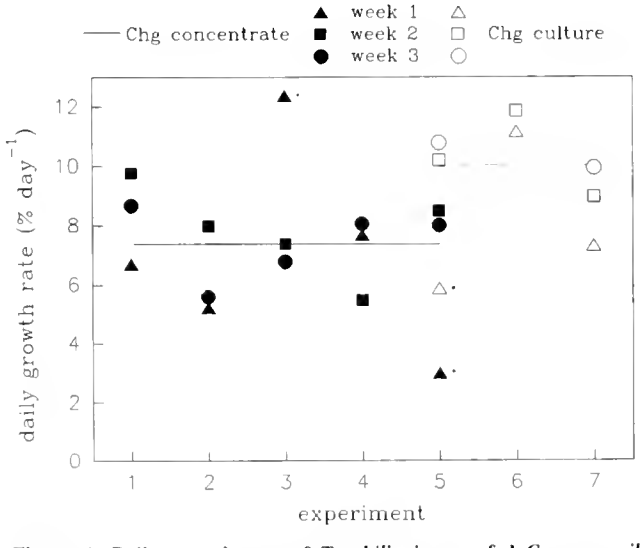

Figure 6. Daily growth rate of *T. philippinarum* fed *C. neogracile* either as a concentrate (filled symbols) or directly from the algal culture (hollow symbols) at a daily ration of 1% in seven independent experiments. Each data point represents the mean daily growth rate of three (four: Experiment 1) replicate cultures for each of the 1-week periods. Lines indicate mean daily growth rate obtained with the concentrate (7.4 ± 1.4% day $^{-1}$) and the culture (10.0 ± 1.5% day^{-1}) of *C. neogracile*, respectively (mean ± SD); aberrant observations from first week, indicated with *, excluded).

TABLE 4.

(Experiment 5) Daily growth rate (DGR) of *T. philippinarum* fed various daily rations of *C. neogracile* (Chg) either as a concentrate or directly from the algal culture. Data represent mean and standard deviation from three replicates. Like superscripts indicate means which do not differ significantly (ANOVA, Tukey HSD, $P \leq 0.05$).

Treatment	Week 1	Week 2	Week 3
1. Chg concentrate 1%	2.98 ± 0.20^b	8.50 ± 0.46^b	8.01 ± 0.37^c
2. Chg culture 1%	5.88 ± 0.45^a	10.20 ± 0.31^a	9.76 ± 0.57^b
3. Chg culture 1.5%	NA	10.41 ± 0.64^a	10.79 ± 0.20^a
4. Chg culture "on demand"	2.69 ± 0.13^b	10.55 ± 0.40^a	10.98 ± 0.19^a
ANOVA, F_s	108.3	12.5	41.1

corroborated by the measurements of cell concentration before and after feeding in one replicate of the algae-fed treatments. Feeding a daily ration of 1.5% maintained the food level above 5–10 *Chaetoceros* μl^{-1} throughout the experiment and resulted in a significantly larger amount of algae removed from suspension than in the treatments fed 1% (Table 5). However, the higher filtered ration was less efficiently converted into clam biomass and resulted only during the last week in a significant growth improvement (Table 4). The high food loads observed in the treatment fed "on demand", i.e. up to 90 *Chaetoceros* μl^{-1}, affected gross growth efficiency only to a small degree.

Clams fed the highest ration (1.73% day^{-1}, treatment fed "on demand") removed the largest weight-specific ration of 1.16% DW WW^{-1} day^{-1} (Table 5), which was equivalent to 67% of the offered ration. Feeding a daily ration of 1% resulted in a clearance of more than 80% of the food offered.

Single and Mixed Diets of C. neogracile *and* Isochrysis *sp. (Clone T-Iso)*

The preliminary series of experiments demonstrated a similar growth response to increasing ration size for *T. philippinarum* (live weight 1.7–6.9 mg) fed either *C. neogracile*, *Isochrysis* sp. (clone T-Iso), or a 50/50 mixture (on DW basis) of both species. Growth increased sharply up to a daily ration of 1%, reached a maximum value at 1.3%, and then decreased with a further increase of algal ration (Fig. 7). Because the experiments were performed with clams of different initial size and previous history, the data do not allow a comparison of the nutritional value of the three algal diets. However, it is interesting to note the consistency of the relationship between growth rate and ration for various algal diets.

DISCUSSION

The present series of experiments demonstrated that the optimal ration of *C. neogracile* for maximal growth of juvenile *T. philippinarum* in the size range of 1 to 40 mg live weight is situated between 1 and 1.5% DW WW^{-1} day^{-1}. Growth of clams showed a linear response to increasing rations up to 1% day^{-1}, was not significantly affected by rations ranging from 1 to 1.5%, and eventually decreased with a further increase of the ration. The set of preliminary tests indicated that growth attained a maximum for *T. philippinarum* fed either *C. neogracile*, *Isochrysis* sp. (clone T-Iso), or a mixture of both species, at a daily ration of 1.3%.

In clam cultures fed 1% *Chaetoceros* day^{-1}, the cell concen-

TABLE 5.

(Experiment 5) Mean intake rate and gross growth efficiency of *T. philippinarum* fed various rations of *C. neogracile*. Data represent mean and standard deviation for week one, and weeks two and three. Like superscripts indicate means which do not differ significantly (week 2 & 3: ANOVA, Tukey HSD test, $P \leq 0.05$).

Type of *Chaetoceros* → Feeding Regime → (% DW WW^{-1} day^{-1})	Concentrate 1%	Culture 1%	Culture 1.5%	Culture "On Demand" (1.73% effective DR)
Week 1				
Mean intake rate				
1. (10^6 cells g^{-1} day^{-1})				
mean ± SD (n = 7)	346 ± 131	346 ± 61		
min.–max. value	121–481	268–434		
CV (%)	38	18		
2. (% DW WW^{-1} day^{-1})§	0.82	0.82		
Daily growth rate (% day^{-1})	2.98	5.88		
GGE‡	3.6	7.2		
Weeks 2 and 3				
Mean intake rate				
1. (10^6 cells g^{-1} day^{-1})				
mean ± SD (n = 14)	301 ± 25[a]	342 ± 56[a]	457 ± 55[b]	486 ± 87[b]
min.–max. value	263–353	219–435	327–552	370–640
CV (%)	8	16	12	18
2. (% DW WW^{-1} day^{-1})§	0.72	0.81	1.09	1.16
Daily growth rate (% day^{-1})†	8.25	9.98	10.60	10.77
GGE‡	11.5	12.3	9.7	9.3

§ *C. neogracile:* 23.8 pg DW $cell^{-1}$.

† Average of the mean DGRs for the second and third week of each treatment (see Table 4).

‡ GGE = gross growth efficiency = live weight increase per dry weight of food cleared, estimated as DGR (%WW day^{-1})/mean intake rate (%DW day^{-1}).

tration was declining rapidly after feeding and was mostly below the incipient limiting level, i.e. about 25 *Chaetoceros* μl^{-1}. As a result, the clams exhibited fluctuating intake rates in the course of the experiment. The higher average food concentration occurring in the cultures that were provided daily rations exceeding 1%, allowed the clams to feed more continuously and maximize their daily food uptake, but also resulted in a lower efficiency of food utilization. This is in agreement with the observations for other bivalves showing that growth rate is maximized at higher rations than gross growth efficiency (Thompson and Bayne 1974, Goldstein and Roels 1980, Urban et al. 1983).

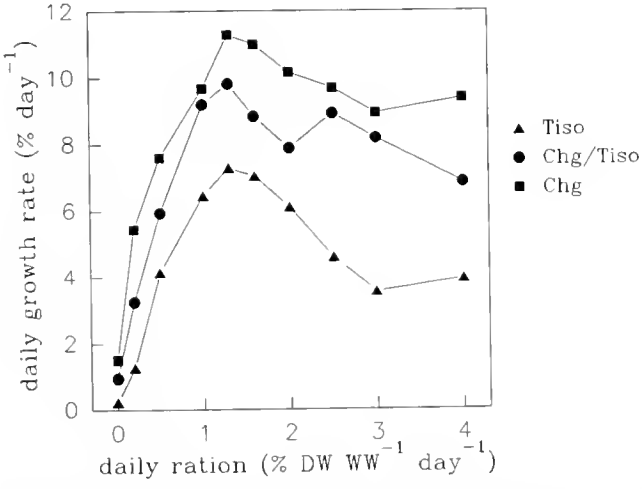

Figure 7. Daily growth rate as a function of daily ration for *T. philippinarum* fed *C. neogracile* (Chg), *Isochrysis* sp. clone T-Iso (Tiso), or a 50/50 (on dry weight basis) of both algal species (Chg/Tiso). Data sets were obtained from independent, one week experiments with one replicate culture per ration. Initial mean individual WW = 1.73 ± 0.03 mg (Chg), 6.94 ± 0.62 mg (Tiso), 3.92 ± 0.15 mg (Chg/Tiso).

It is interesting that clams fed the concentrated *C. neogracile* at a higher ration than 1% day^{-1} showed a tendency towards depressed growth, whereas the animals fed the same alga directly from its culture tended to further increase growth. Apparently, the optimal ration for maximal growth is shifted to lower values, but maximal growth obtained is lower for clams fed the concentrated algae. This is supported by the lower gross growth efficiency observed for the latter.

The periodic feeding activity of the clams fed *Chaetoceros* at 1% day^{-1} appeared to be mainly imposed by the discontinuous feeding regime which resulted twice daily in a depletion of the food. However, the latter feeding strategy yielded a higher gross growth efficiency and similar growth compared to that of clams fed 1.5% day^{-1} which filtered the food more continuously and at higher rates. From this it would appear appropriate to apply discontinuous feeding regimes in aquaculture systems in order to maximize the utilization efficiency of the algal food. In the same way, Epifanio and Ewart (1977) observed a discontinuous feeding activity for *C. virginica* in continuously replenished suspensions of algae and proposed to offer algae in pulses rather than maintaining constant food concentrations. This is further supported by the findings of Langton and McKay (1976) who reported better growth of *C. gigas* spat fed discontinuously than when feeding the same amount of food continuously.

The maximal daily amount of *C. neogracile* dry weight re-

moved from suspension averaged 1.16% of the clam's live weight. This is comparable with the weight-specific daily ration removed by *C. virginica* (15 g live weight) which ranged from 0.4% for *T. pseudonana* to 1.5% for *I. galbana* (Epifanio and Ewart 1977). Nevertheless, it appears difficult to explain why the optimal ration for juvenile Manila clams was as low as 1 to 1.5% DW WW^{-1} day^{-1} in the present laboratory experiments, whereas the standard regime in a commercial hatchery to feed a mixture of five algal species to seed of the same size and under comparable conditions of temperature and salinity consisted of 4% DW WW^{-1} day^{-1} (Albentosa et al. 1989). Also, the scarce literature data with regard to the effect of ration size on bivalve growth indicate higher values than those observed in the present study (Urban et al. 1983, Enright et al. 1986a,b). However, optimal ration differs according to the algal species (Enright et al. 1986a) and may be affected by the algal culture conditions (Enright et al. 1986b). Our data indicate that the optimal ration increases with increasing nutritional value of the algal diet, i.e. in ascending order: *Chaetoceros* concentrate, *Chaetoceros* culture, mixed algal diet used by Albentosa et al. (1989). Furthermore, various methodological factors may affect the value of the estimated daily ration, such as the accuracy of the algal dry weight analysis, the adaptation of the feeding regime to growth during the experiment, the natural food present in the seawater, and the settling and/or growth of the algae in the culture system.

Clam growth in the present experiments was acceptable when compared with the values of 7.6% day^{-1} and 14% day^{-1} reported for, respectively, *Mercenaria mercenaria* (initial live weight 25 mg) fed a mixture of *T. pseudonana* and *I. galbana* (Urban and Pruder 1992), and *O. edulis* (initial live weight 1.14 mg) fed *C. calcitrans* (Laing and Millican 1986). The superior growth obtained when feeding clams with algal culture showed that the nutritional value of *Chaetoceros* decreased due to centrifugation and storage for maximal three days. By contrast, Nell and O'Connor (1991) could not detect any deleterious effects on growth of larvae of the Sydney rock oyster *Saccostrea commercialis* when various species of diatoms were fed after concentration to a paste and storage for 7–14 days at 4°C. The aberrant values observed during the first week of experiments three and five, may have been due to the relatively short acclimatization period prior to the start of the experiment. In this way, a bad initial condition of the spat may have caused difficulties to adapt to the experimental food, resulting in reduced feeding rate and gross growth efficiency. Alternatively, animals that originate from a well-fed population may benefit from their food reserves and initially maintain higher growth rates. Laing and Millican (1986) thus found that greater lipid reserves in *O. edulis* spat were associated with higher growth rates of the seed when transferred to the sea. In this regard, it would be interesting to relate the initial biochemical composition of the seed to its performance in future culture tests.

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PLANKTONIC, METAMORPHIC, AND EARLY BENTHIC BEHAVIOR OF THE CHILEAN LOCO *CONCHOLEPAS CONCHOLEPAS* (MURICIDAE, GASTROPODA. MOLLUSCA)

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ABSTRACT Late stage veliger larvae of the loco *Concholepas concholepas* were collected in the surface plankton in the ocean off Coquimbo, Chile (30°S) and transported to the laboratory for a study of settlement, metamorphosis, and behavior under simulated natural conditions. Experiments were conducted on effects of seawater turbulence and presence of natural epibiota on settlement and metamorphosis. All loco veligers captured in the neuston appeared competent. They settled on natural substrata, resorbed the velum, and then spent hours or a few days grazing on microepiphyta, during which time the protoconch became darkly pigmented. After pigmentation was completed, juvenile locos began boring barnacles and depositing the adult shell (teleconch), growing to a mean height of over 6 mm in 30 d. A two-stage induction for metamorphosis is suggested, the first involving substances from microephiphyta and the second from barnacles. Grazing on films of microorganisms alone did not trigger formation of the teleconch. The loco has evolved several alternatives that safeguard its settlement in highly wave-stressed rocky shores. It may be stimulated to metamorphose by nonspecific inducers, resist stranding during tidal changes, overcome breakage of the protoconch, and attach firmly to the substratum in turbulent seawater. Scarcity of loco veliger larvae in coastal plankton argues against using present methods as a basis for mass culture of this species.

KEY WORDS: *Concholepas*, loco, muricid prosobranchs, teleplanic veliger larvae, juveniles, behavior, metamorphosis, experimental induction, culture

INTRODUCTION

Concholepas concholepas (Bruguière, 1789) known as the "loco" in Chile, is a muricid prosobranch gastropod (Kool 1989) of significant commercial value in the human diet (Castilla 1988). It has come under intensive study in recent years in order to protect it as an important renewable marine resource (Castilla 1988, Oliva and Castilla 1992).

Adult locos live in rocky subtidal habitats (Spight 1978), usually not exceeding 40 m in depth, where they actively consume barnacles, mussels and tunicates (Spight 1977, Castilla et al. 1979, Stotz et al. 1991a). On subtidal rocks they deposit egg capsules from which shelled veligers emerge one to two months after capsules are deposited (Castilla and Cancino 1976, Castilla et al. 1979, Gallardo 1979). Planktonic veligers grow from about 260 μm to 1.9 mm to height during a period of about 3 months in coastal waters (Castilla and Cancino 1976, Gallardo 1979, DiSalvo 1988). Recently settled locos, approximately 2 mm in height have been observed in subtidal inshore habitats to a depth of 18 m, usually associated with barnacles (Stotz et al. 1991a).

Little is known about the planktonic life of the loco except that late stage veliger larvae rise to the surface of the ocean at the termination of growth of the protoconch, which is marked by a conspicuous upturned lip on the leading edge of the shell aperture (DiSalvo 1988). Presence of the lip on the shell may signal readiness ("competence", Coon et al. 1990) for metamorphosis. Mass metamorphosis of these veligers has been observed on a natural substratum in the laboratory in flowing seawater (DiSalvo 1988).

Although the sea surface-seeking behavior of "lipped" loco veligers has made possible their capture at sea and the subsequent initial observation of their metamorphosis and early growth in the laboratory (DiSalvo 1988), there is little information on the biology of their settlement, metamorphosis, and initial growth because it is difficult to propagate them in the laboratory. Furthermore, field observations have been limited because of the difficulty of finding and observing newly settled wave-stressed locos in the rocky, coastal environment where they settle (Castilla et al. 1979, Gallardo 1979, Stotz et al. 1991b).

The present report, based on studies made during three seasons of field capture of veligers of *C. concholepas* between 1989 and 1992, provides the first detailed information of the premetamorphic, metamorphic, and postmetamorphic behavior of this species. The loco is probably representative of the broad group of long-lived planktotrophic muricid gastropod larvae that settle on rocky shores. Because *C. concholepas* is of such significant commercial value, another of the objectives of the present research was to develop a method for handling wild larvae and to determine the feasibility of their mass culture in managed systems, both for commercial production and for re-seeding of natural coastal banks.

C. concholepas is distributed along an impressively long stretch of coast, ranging from central Peru to the southern tip of Chile (Stuardo 1979). Its widely dispersed planktonic veliger stage (DiSalvo 1988) and interesting metamorphogenesis (for comparison, see Bonar 1978) are attracting increasing attention. Aspects of the biology of the species relating to the topic of the present publication are included in papers by Castilla and Cancino 1976, Spight 1977, Castilla et al. 1979, Gallardo 1979, Castilla 1988, DiSalvo 1988, Kool 1989, 1992, and Stotz et al. 1991a,b. Important references on dispersal, settlement, and metamorphosis of larvae of benthic invertebrates include Crisp 1974, Bonar 1978, Hadfield 1978, 1984, Fretter 1984, Scheltema 1986a,b, 1989, and Morse 1990.

MATERIALS AND METHODS

Collection of Lipped Veliger Larvae

Veligers were collected at the sea surface using a floating neuston net (DiSalvo 1988). One or more tows were made monthly

from October 1989 through July 1992 on north-south tracks 500 to 2000 m offshore of Coquimbo, Chile (30°S).

Natural Substrata

We used natural substrata (stones and shells) collected in wave-exposed subtidal environments where settlement of locos is known to occur. Surfaces of these substrata were encrusted with a variety of metazoans, primarily by juvenile barnacles (*Balanus laevis, Verruca laevigata, Austromegabalanus psittacus,* and *Notobalanus flosculus*), encrusting calcareous and fleshy red algae, encrusting brown and green algae, and films of microorganisms dominated by diatoms. Vagile epifauna on substrata were removed by thorough washing in seawater, and large invertebrates such as anemones and limpetlike molluscs were removed by hand. The volume of pieces of substrata employed varied from about 50 ml to 5000 ml displacement, depending on requirements of each phase of our study. For example, study of initial prey selection and feeding behavior by locos was done with small pieces of substrata easily placed under the stereoscopic microscope, whereas larger pieces were used to follow the growth of juvenile locos given an excess of prey. The fouling community on substrata included Polychaeta (e.g. *Polydora* spp., Serpulidae), Bryozoa (e.g. *Membranipora* sp., *Celleporella* sp.), *Semimytilus algosus,* and encrusting Foraminifera (Viviani and DiSalvo 1980).

Observations

Lipped veligers caught at sea were separated from other plankters within a few minutes of capture by taking advantage of the veligers' habit of settling out of the plankton and adhering to the bottom of plastic pails into which the net catch was poured. Veligers were held in fresh seawater until we arrived at the laboratory within one to 6 h after collection (see also, Taylor 1975). In the laboratory, veligers were examined microscopically to determine size, coloration, condition of protoconch, and presence of parasites. Pediveligers (swimming-crawling stage, Carriker 1990) undergoing metamorphosis were studied to determine the method of loss of the velum, activity of the foot, and other externally visible anatomical changes associated with metamorphosis. Individuals with damaged protoconchs were maintained in separate aquaria to note effects of the damage on survival and metamorphosis.

In addition, externally visible morphological changes and feeding behavior were examined in juvenile plantigrades (velum lost, locomotion limited to crawling on the foot, Carriker 1961, 1990—hereafter called "juveniles") that had settled on natural substrata. Pediveligers and juveniles were sacrificed to examine intestinal contents during the metamorphic period. This was accomplished by gently cracking and washing away fragments of the protoconch from the snail, then pressing the snail firmly between a microscope slide and a coverslip. This "squash preparation" made possible viewing of the radula and such internal anatomical structures as the esophagus, stomach and digestive glands.

Changes in strength of the protoconch in juveniles were measured using a depression slide with a 2 × 2 cm cover slip laid over the depression and taped to the slide by one edge to prevent slippage. The juvenile snail, blotted dry, was placed in the depression slide, and a 150 ml disposable plastic cup was centered over the cover glass. Water was then pipetted into the cup until the snail shell abruptly collapsed. Weight of the water plus that of the cup gave a relative measure of the resistence to breakage of the shell.

Once growth of the adult plantigrade shell (the teleconch) had begun, we observed juveniles to determine the onset of prey selection and methods of attack of prey, and other behavioral characteristics. Juveniles were routinely examined under the stereoscope microscope on small pieces of substrata in seawater. Observations and light micrographs were made at magnifications ranging from 8 to 45×. The shell of selected snails was photomicrographed with a JEOL Model JSM T-300 scanning electron microscope.

EXPERIMENTAL STUDIES

Settling behavior of pediveligers recently brought in from plankton hauls was observed in 10 ℓ glass aquaria to which were added two to three pieces of natural substratum. Aquaria contained daily changes of seawater at ambient temperature (18–20°C) and salinity (ca. 34‰), and were vigorously aerated by airstones. The pieces of substratum were each about 100 ml in volume and sparsely encrusted with fouling organisms. Five to 10 pediveligers were placed in each aquarium, the number of replicate aquaria depending on the success of the catch. Pediveligers easy to see were monitored by visual observation at irregular intervals to observe their position in the water column and settling behavior on or near substrata. All pediveligers were removed daily from aquaria and observed microscopically to ascertain whether loss of the velum had occurred and what other visible changes related to metamorphosis had taken place. Some pediveligers were examined in squash preparations to determine the extent to which they had rasped material from substrata.

Metamorphosis Relative to Water Motion and Food Organisms

Little is known about natural factors that induce loco pediveligers to alight and undergo metamorphosis. We began a quantitative evaluation of the effects on settling and velar resorption of water motion and presence of food organisms on natural substrata using groups of newly collected pediveligers.

As background for these experiments, we determined by direct microscopic observation the time in which pediveligers began spontaneously to resorb the velum in still seawater and absence of potential food organisms. Sixty-three freshly captured pediveligers were maintained in 25 μm-filtered seawater in plastic pails at a density of 2 snails per liter of seawater at 16–18°C, for a period of 11 days, with daily changes of seawater (see Table 1). Sea water was aerated very gently to provide necessary oxygen. Pediveligers were examined once daily for evidence of loss of the velum, extension of tentacles, and creeping on the foot, all characteristics indicative of metamorphosis to the juvenile plantigrade stage.

Quantitative testing of effects of water movement and presence of natural food organisms on onset of metamorphosis was carried out in the experimental systems illustrated in Figure 1. Each replicate consisted of a 35 ℓ plastic tank fitted with a wave generator that simulated a wave break every 10 to 20 sec. Each wave generator could receive a constant flow of seawater of 1 to 2 ℓ min^{-1} by means of an airlift tube (Fig. 1). Unfortunately, the relatively small number of lipped veligers caught in plankton hauls limited the scope for statistical analysis.

Experiment 1 (See Fig. 2)

System I lacked water movement; in II there was airlifted water flow plus simulated wave action; and in III there was water flow, wave action and potential food organisms. A number of pedive-

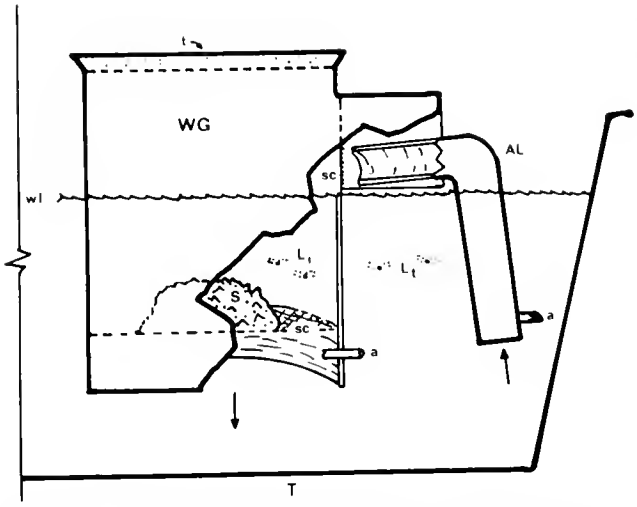

Figure 1. Wave generating chamber (WG) constructed of 110 mm diameter PVC with a 40 mm side arm, suspended in a 35 ℓ plastic tray (T), and fitted with a 15 mm diameter airlift tube (AL). Veliger larvae were placed inside (L_i) or exterior to the WG (L_t). Snails inside the chamber were prevented from escaping by 600 μm mesh screens (sc). Compressed air accumulated below the screen and burst through every 10–20 sec, producing a simulated wave break in the WG. wl, water line; t, acrylic top; a, compressed air inlets, S, natural substratum; arrows, direction of water flow.

ligers equal to that within the wave generator was placed in the water tank outside the wave generator as an internal control (Figs. 1, 2).

Experiment 2

All three systems had airlifted water flow but not wave action, and all pediveligers were placed inside the wave generators. System II contained a Petri dish fouled with a diverse film of microalage and bacteria placed inside the wave generating chamber; system III contained small pieces of natural substrata with barnacles and encrusting macroalgae in the chamber; and system I lacked pieces of substrata (Fig. 2).

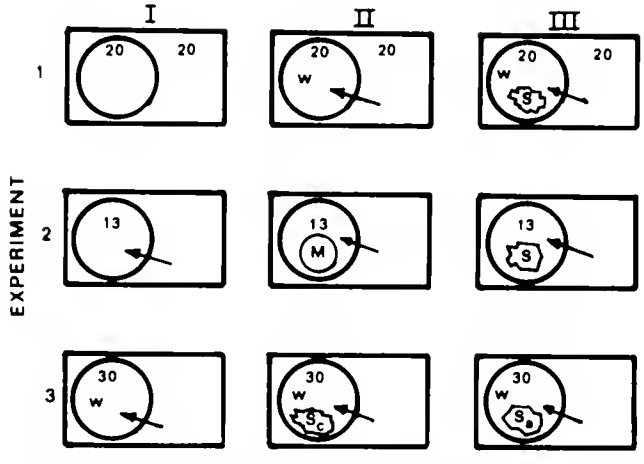

Figure 2. Diagram of experimental plan. Rectangle represent 35 ℓ seawater trays containing wave generators represented by large circles (not to scale). Numbers, number and location of veligers; arrows, airlifted water flow, 1–2 ℓ/min; W, simulated wave break every 10–20 sec; M, microorganism-coated petri dish; S, natural substratum encrusted with various epibiota; Sc, barnacle encrusted substratum; Sa, algal encrusted substratum.

Experiment 3

All systems had airlifted water flow and simulated wave action, and all pediveligers were held in the wave generators. System II contained a barnacle-encrusted substratum, and III an algal-encrusted substratum. System I lacked substrata (Fig. 2).

Experiments were carried out on different dates during the 1991–92 plankton survey. All pediveligers in each system in Experiments 1–3 were examined microscopically at the beginning and at the end of each experimental period to determine the number that had lost the velum and metamorphosed into the plantigrade stage.

Rate of Juvenile Growth

Snails that had lost the velum in Experiments 1–3 were removed daily and transferred to a simulated natural habitat for the study of shell growth and comparison of growth rate with that observed under laboratory conditions earlier by DiSalvo (1988).

The habitat was a 100 ℓ seawater tank (100 × 80 × 20 cm) containing 5 to 10 pieces of natural substrata, each 100 to 500 ml in displacement volume and dominated by the barnacle *Balanus laevis*. A current of seawater, 5–10 cm sec^{-1}, was provided by a battery of five airlift tubes similar to those illustrated in Figure 1. In addition, vigorous aeration was supplied with air stones. Seawater temperature was 18–20°C and salinity was about 34‰. Seawater was changed every 24 to 48 h. A 100 W incandescent daylight bulb was placed about 50 cm above the surface of the water and set at the local day/night cycle with an interval timer. Laboratory-cultured microalgae (*Isochrysis* sp., *Chaetoceros* sp.) were added *ad libitum* to help maintain the filter-feeding invertebrates populating the substrata. A total of 22 juveniles were studied in the system. Minimal and maximal rates of development observed were recorded for two of the juveniles (see Table 3).

RESULTS

Field Collections

During a three-year monitoring program lipped loco veligers were usually present at the sea surface off Coquimbo between August and March, in maximal densities between September and March (pers. comm.). However, they were always a rare component of the plankton. Only a few hundred were captured each year, the lipped veliger stage being the only one collected. Maximal catches per kilometer towed numbered 15 individuals on October 13, 1989, and 18 individuals on October 20, 1991. During the 66 field trips we made during which the net was towed 4–6 h per cruise, the total number of lipped veligers taken was 955, an overall mean of 1.6 veligers per kilometer towed! Veligers were patchily distributed; for example, none was collected on 25 cruises, and 66% of all captured was obtained on eight cruises.

Newly Caught Veligers

Veliger shells measured 1.6–1.9 mm in height (Fig. 3). The velum, tentacles, and a yellow lipid reserve near the apex of the shell were visible through the semi-transparent protoconch. Yellow ellipsoidal bodies about 300 μm in length were often seen inside the branchial chamber. In one plankton haul, 5 of 50 veligers contained from one to three of these bodies, which were

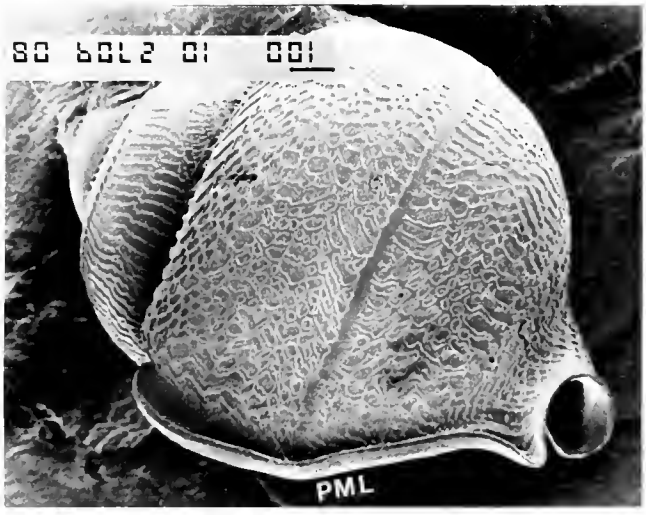

Figure 3. Protoconch of a lipped veliger of *Concholepas concholepas*. Height (spire tip to siphon tip) about 1.8 mm. PML, premetamorphic lip. Scanning electron micrograph.

regularly ejected by the snails within a few hours after snails were placed in laboratory aquaria.

Occasionally veligers were collected that had previously repaired cracks or perforations. In the laboratory, snails were capable of repairing these cracks or perforations, as well as the loss of shell margins suffered during collection. However, when broken shell was being regenerated, metamorphosis was delayed. One pediveliger, for example, which had accidently lost its premetamorphic shell lip, completely regenerated the lip in 48 h, and then underwent metamorphosis.

Some veligers were infected internally with *Chromobacterium* sp. that caused freeing of the shell from the body when snails were handled.

Pediveligers

In quiet water pediveligers tended to rest with the shell dorsum on the bottom of the aquarium, and velum and foot extended upwards. At intervals they rose into the water, sometimes attaching themselves to the underside of the air-water interface by the foot where they remained suspended for hours or a few days at a time. In one case, introduction into an aquarium of a small piece of natural substratum with epibiota caused 9 of 10 floating pediveligers to detach from the air-water interface and drop to the bottom of the aquarium within a 20 min period.

In water strongly agitated by aeration, pediveligers extended the propodium and foot, secreted a byssal thread, and were actively carried about the aquarium by the currents. They then settled on the glass bottom of the aquarium or on natural substrata and remained there for a time, before again circulating about the aquarium trailing the byssal thread from the extreme tip of the foot. They frequently settled on actively bubbling air stones, or explored the surface of natural substrata, or fell to the bottom remaining there in a resting position.

After pediveligers underwent velar loss on natural substrata, many left the substratum, crawling around the walls of the aquarium on the foot. Some also crawled above the waterline where they resisted some three hours of dessication apparently without harm.

Loss of the velum began with loss of preoral (locomotor) cilia. In some cases this loss was reversible, lost cilia being regenerated. Some snails, after loss of locomotor cilia, would rest on the bottom of the aquarium for days with the velum extended in a filter-feeding position. Loss of velar lobes could occur during a few hours by resorption and dropping off of fragments, or gradually over a few days by resorption alone. Ingestion of velar lobes was never observed. Other external changes visible during this phase of metamorphosis were loss of the byssus and byssal gland from the distal tip of the foot, and disappearance of the labial structures associated with the mouth (DiSalvo 1988).

Veligers captured at sea had well-formed radulae, approximately 1 mm in length, 50 μm wide, and bearing about 120 rows of teeth. Dissection of freshly captured veligers revealed large centric diatoms (e.g. *Thalassiosira* sp., *Stephanopyxis turris*) and dinoflagellates (e.g. *Dinophysis* sp.), and occasional remains of red and brown encrusting benthic algae in the stomach. A few premetamorphic veligers showed wear of the anterior rachidian teeth. This was seen under light microscopy and confirmed by SEM observations. That veligers actively rasped substrata in laboratory aquaria was confirmed by the presence of substratal diatoms in their alimentary canal.

Juveniles

After loss of the velum in the laboratory, plantigrade locos extended their tentacles and crawled over substrata where they sought shelter in surface irregularities and empty barnacle shells. An occasional juvenile deposited an initial narrow band (<300 μm) of teleoconch shelf to the right of the siphon, but further deposition of teleoconch did not occur at this early stage.

In a period as short as 12 h, or as long as 7 d, the protoconch of the juvenile became a chestnut brown color, resulting from deposition of pigmented material on its inner surface. For convenience of description, we estimated the percentage extent and intensity of pigmentation. Shell stress tests indicated a five-fold increase in strength of the protoconch as pigmentation reached a maximum intensity (Fig. 4). Dissection of juveniles during the pigmentation phase showed that esophageal and digestive structures were full of plant pigment, primarily golden-green in color, frustules of pennate diatoms, and occasional fragments of red and brown algae. A gastric protostyle was not seen. Some erosion of rachidian teeth was also observed (Fig. 5). Juveniles with unpigmented protoconchs rarely attacked newly set barnacles and were not observed to drill conical boreholes in prey.

Cannibalism occurred several times during our laboratory study (see also, Taylor 1975), one juvenile perforating the shell of another and consuming its flesh (Fig. 6). Darkly pigmented juveniles began feeding by attacking newly set barnacles through the scuto-tergal opening, or boring typical, muricid-like boreholes in barnacles about their own size. Boreholes were excavated on plates, or scuto-tergal sutures of *Balanus laevis*, *Austromegabalanus psittacus*, and *Verruca laevigata* (Figs. 7, 8). Teleoconch deposition began about 24 h after initiation of predation; this shell was white with occasional brown radial lines. Tissues of the head and foot, which were black in pediveligers and pigmented juveniles, changed to translucent white during the first 10 d of feeding on barnacles, and remained this color until the locos reached 10 mm or more in height. Their tissues then slowly changed to the grey pigmentation characteristic of adult *C. concholepas*.

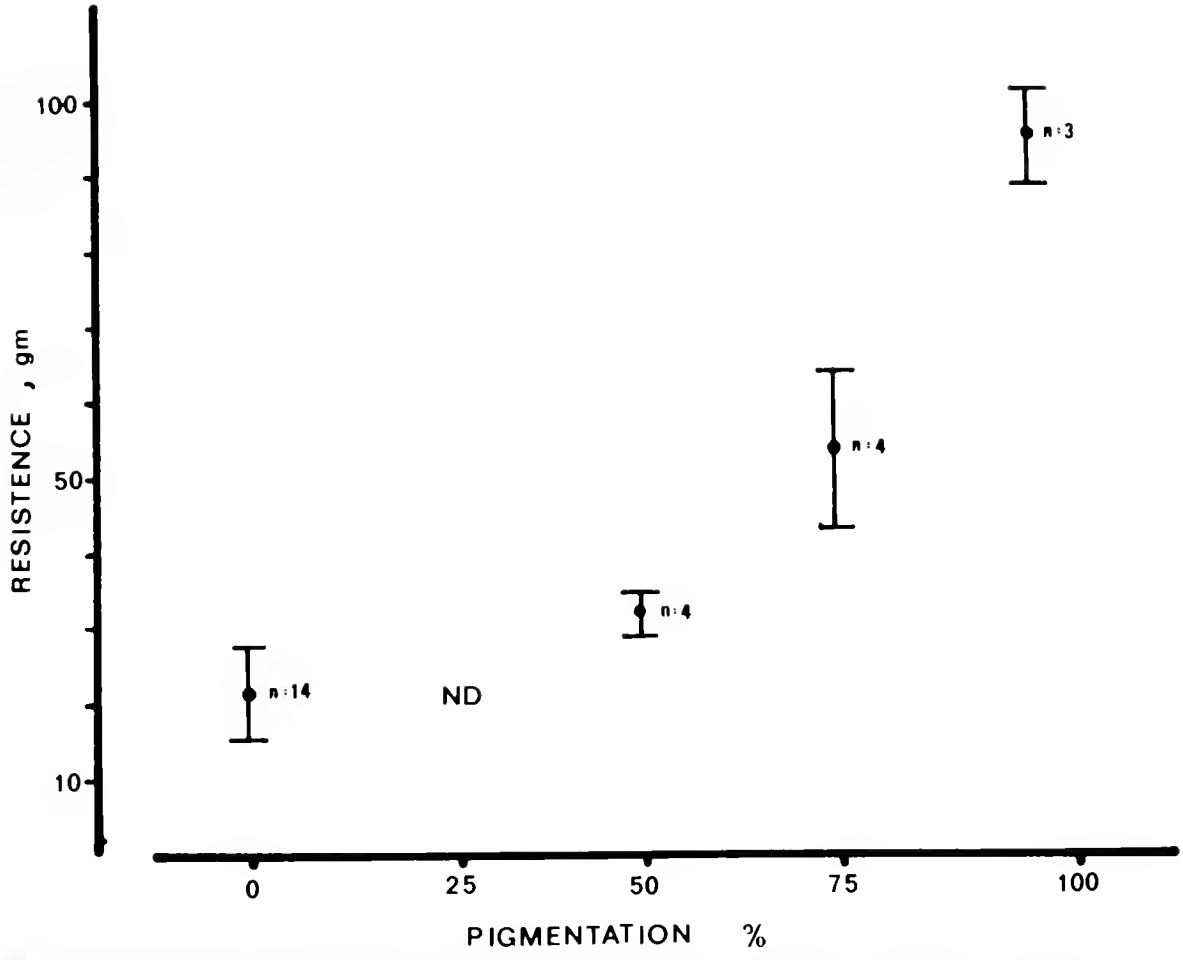

Figure 4. Relation between estimated percentage pigmentation of the protoconch of *Concholepas concholepas* after loss of the velum and resistance to breakage of the protoconch. Bars, ± one standard deviation.

Juvenile Growth Rate

A group of 8 darkly pigmented juvenile locos did not develop further when maintained for two weeks in aquaria fouled with films of microorganisms, primarily benthic diatoms. Dissection of some of these snails after the two weeks revealed that they had been feeding on diatoms. The remaining snails of this group, as well as numerous other pigmented juveniles, adapted well to barnacle-dominated substrata in aquaria, 22 snails growing to a mean height of 6.11 ± 0.84 mm in 30 days (Fig. 9).

Factors Influencing Metamorphosis

Some experiments indicated that pediveligers began to resorb the velum in the absence of natural substrata three to four days after capture. The rate of velar resorption in 63 pediveligers is tabulated in Table 1; 84% of the plantigrades survived in an 11 day period, over 50% of survivors resorbed the velum within 5 d of capture, and the remainder in little over one week. Further observations showed that a few pediveligers could resorb the velum after 18 days residence in laboratory aquaria in the absence of any nutritional sources, but no further development occurred in the absence of natural food.

Experimental Induction

The occurrence of initiation of metamorphosis (based on velar loss) in recently captured pediveligers in contrasting experimental systems is reported in Table 2. No locos began resorption of the velum within the first 24 h after capture unless they were exposed to simulated wave action (Experiment 1). The proportion of snails resorbing the velum was increased by the presence of natural food.

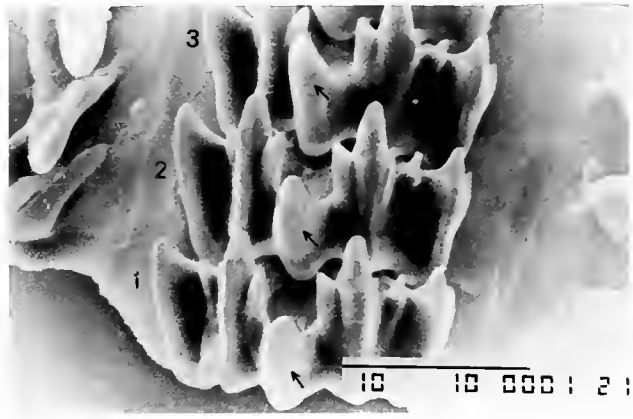

Figure 5. Erosion of anterior rachidian teeth of juvenile plantigrade *Concholepas concholepas* resulting from rasping on algae during the shell pigmentation period. Numbers indicate the order of each tooth on the radula from anterior to posterior. First and second teeth show major erosion; third tooth, minor erosion (arrows). Width of rachidian rows of teeth, 20 μm. SEM.

Figure 6. Circular borehole on the dorsal surface of the protoconch (P) of a *Concholepas concholepas* plantigrade that has been cannibalized by another juvenile loco. Approximately one week's growth of teleconch (T) had been deposited beyond the premetamorphic lip (PML) before predation occurred. Height of shell, 2 mm. SEM.

In the absence of wave action, the presence of natural food (barnacles) was sufficient to increase significantly $p \geq 0.01$ the percentage of velar resorption (Experiment 2). The presence of a microalgal film alone did not increase the percentage of velar resorption over that of the control.

Velar resorption over 60 h in the presence of wave motion (43%, Experiment 3) appeared well above the percentage expected in quiet water over an even longer period (8%, Table 1, 72 h). Presence of natural food (barnacles) increased the percentage resorption (Experiment 3) ($p = 0.05$), with no statistical difference in effect between substrata dominated by encrusting algae or by barnacles ($p \geq 0.01$).

Figure 7. Young plantigrade of *Concholepas concholepas* four days after initiation of teleconch deposition. Darkly pigmented protoconch, p; white teleconch, t; borehole excavated by this snail in a young unidentified barnacle. Height of snail shell, 2.3 mm. Light micrograph.

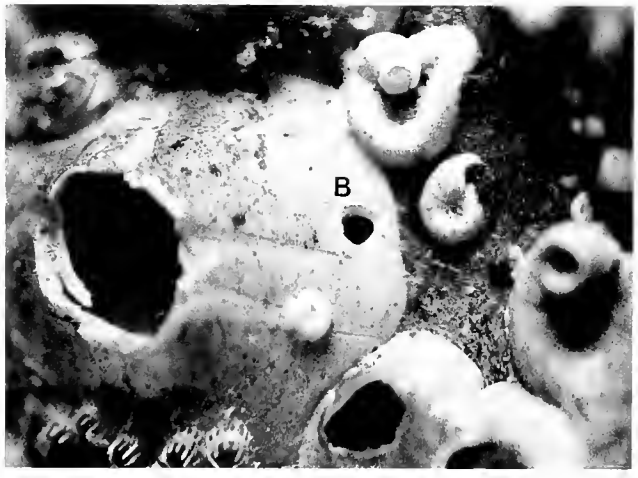

Figure 8. Borehole (B) excavated by a 5 mm plantigrade *Concholepas concholepas* in the shell of the barnacle *Balanus laevis* present on a piece of natural substratum. Internal diameter of borehole, 325 μm. Light micrograph.

Metamorphic Variation

Rate of development of metamorphosing pediveligers was not uniform (Table 3). Two different rates recorded here represent two pediveligers that settled on a barnacle-encrusted substratum and were closely observed for the first 10 days after metamorphosis.

DISCUSSION

The long planktonic developmental period of the loco, terminating in the lipped protoconch stage with large velar lobes, falls in the classification by Scheltema (1986a,b, 1989) of potentially long-lived teleplanic veliger larvae (Scheltema 1971), which reach a determinate size and then continue to feed on plankton until they metamorphose at settling sites (Hadfield 1978). A gastric protostyle may be present in veligers of *Concholepas concholepas* (as in *Thais haemastoma* veligers, D'Asaro 1966) but this has not been reported and calls for study. The early life cycle of *C. concholepas* is similar to that described for other muricids, such as for example, the serious oyster predator, *T. haemastoma* (D'Asaro 1966, Roller and Stickle 1988).

In our investigation a major problem was the serious difficulty of obtaining sufficient numbers of lipped loco veliger larvae for

Figure 9. Juvenile *Concholepas concholepas* cultured in the laboratory from lipped loco veligers collected in ocean plankton. Largest snail (right) measured 6.3 mm in height and was about 30 days beyond metamorphosis. Light micrograph.

TABLE 1.

Progress in velar resorption among 63 competent loco pediveligers in weakly aerated seawater in the absence of substrata; collected 13 October 1989.

	Days After Capture									
Velar condition	**0**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
No change	63	58	51	25	16	5	5	3	–	–
Completely resorbed*		5	12	34	43	47	52	53	53	53
Partially resorbed		0	0	0	0	7	0	1	4	4**
Died**		0	0	4	(4)	(4)	2 (6)	(6)	(6)	(6)

Numbers in table represent numbers of individuals; a total of 53 pediveligers metamorphosed, 4** partially metamorphosed, and 6 died. Cumulative total in parentheses.

* Cumulative numbers.

** Dying.

observation and experimentation. From the standpoint of the loco, however, the scarcity of loco veligers per unit surface volume of the ocean is probably beneficial, enhancing their survival by minimizing loss through predation, and thus increasing spatial and temporal dispersal (Scheltema 1971, Crisp 1974).

The complexity of factors dominating transport and settlement of long-lived teleplanic planktonic larvae was discussed by Scheltema (1986a,b, 1989). Presence of lipped loco veligers exclusively at the sea surface is the result of their upward vertical migration in the water column and their readiness as metamorphically competent veligers for shoreward transport in onshore circulation patterns. This has also been described for barnacle larvae by Shanks (1986), who hypothesized that barnacle cyprids inhabiting surface waters are transported onshore in slicks over tidally forced internal waves. That younger prelipped loco veligers are present in deeper water, was demonstrated by K. Knickmeyer (pers. comm.), who netted a few in midwater plankton hauls in the ocean off Coquimbo, Chile.

The "yellow bodies" often seen by us in loco veligers appeared to be an intermediate stage of an unidentified parasite. These, as well as infection of the kidney region of loco veligers by purple bacteria (pers. comm.), are two possible pathological conditions encountered by these plankters. The extent to which these parasites may be debilitating or lethal has not been reported.

Once near the shore, loco veligers (now swimming-crawling larvae, or pediveligers, Hadfield 1978, Carriker 1990) appear to be attracted to favorable substrata by dissolved substances released from potential food organisms (Scheltema 1961, Hadfield, 1978, 1984). Pediveligers alternately swim and crawl over the substratum, temporarily attaching by the byssal thread and the cupped propodium. When a favorable settling site is identified, perhaps by ingestion of suitable algal food scraped from the substratum by the radula, the loss of the velum occurs (Fretter 1984, DiSalvo 1988).

TABLE 2.

Effect of water movement and potential food organisms (natural substrata) on induction of metamorphosis (velar resorption) in pediveligers (PV) freshly collected in oceanic plankton samples (see Fig. 2).

Experimental Systems	Initial No. of PV	Location of PV	Stage after Expt. Period		
			PV	PL	% PL
Experiment 1, 24 h					
I. Control, no	20	Tank	20	0	0
water movement	20	WG	19	0	0
II. Wave	20	Tank	20	0	0
motion	20	WG	14	2	10
III. Wave motion,	20	Tank	15	5	25
barnacles	20	WG	10	7	35
Experiment 2, 48 h					
I. Control, water flow	13	WG	12	1	8
II. Water flow, microorganisms	13	WG	12	1	8
III. Water flow, barnacles	13	WG	6	7	54
Experiment 3, 60 h					
I. Control, wave motion	30	WG	16	13	43
II. Wave motion, barnacles	30	WG	9	20	67
III. Wave motion, algae	30	WG	10	19	63

PL, juvenile plantigrades; % PL, percent PV recovered as PL; WG, wave generator (see Fig. 1); h, duration of experiments.

TABLE 3.

Extreme variation in rate of development of two metamorphosing loco snails collected in oceanic plankton and held on natural substrata in vigorously aerated 100 ℓ aquarium.

Day	Snail A	Snail B
0	Velum lost within 2 h of collection, shell pigmentation began in 12 h.	Velum lost within 16 h of collection.
2	Protoconch darkly pigmented.	Explored substratum, no shell pigmentation.
4	Teleconch width 117 μm.	Shell pigmentation began, fed on 0.1 mm barnacles but no boreholes present.
5	Teleconch width 363 μm.	Teleconch width 167 μm.
6	Grazed on barnacle surfaces.	Protoconch darkly pigmented.
10	Fed on 0.1 mm barnacles, boreholes present, teleconch width 1400 μm.	Complete barnacle borehole on a barnacle scutum, teleconch width 984 μm.

Whereas, metamorphic competence, resulting in the metamorphosable stage, emerges with development of the pedal propodium (Hadfield 1978), in veligers of *C. concholepas*, as in most other prosobranch species, the propodium is developed well before competence (Fretter 1984, DiSalvo 1988).

The pediveliger searching period can involve significant risk to young *C. concholepas* because of turbulence on rocky substrata, which can cause damage to the protoconch. If this occurs, resulting, for example, in shell breakage, the pediveliger resumes planktonic existence for a time during which the broken protoconch is regenerated. Then settlement is attempted again. Significance of the pediveliger is indicated by the fact that the stage is represented in most modern molluscan classes (Carriker 1990).

After the velum is lost, *C. concholepas*, now a juvenile plantigrade, begins a period of feeding by rasping microepibiota on substrata. Concurrently, pigmentation of the protoconch occurs, making the shell increasingly resistent to breakage. When pigmentation is completed, the juvenile extends its proboscis and begins boring shelled animal prey (Carriker 1981, Graham 1985). Simultaneously, deposition of the post-larval teleconch begins. The conspicuous pigmentation of the protoconch could also signal tanning of the radula (Runham 1961), increasing its resistence to wear.

Information on rate of velar resorption resulting without overt environmental stimulation over a relatively long period (Table 1) might be valuable as a baseline for researchers examining the effects of biochemical metamorphic inducers on locos and possibly other muricids with similar early life histories (compare with abalone, Morse et al. 1979, 1980, Morse 1990; and *Phestilla sibogae*, Hadfield 1977, 1978, 1984).

Wave motion alone can induce a few loco pediveligers to metamorphose (Table 2, Exp. I, II). Pediveligers were active in highly aerated seawater with the propodium extended and byssal thread trailing; whereas, in quiet seawater they retracted the propodium, stopped secreting the byssus, and assumed a resting position (Exp. 1). This behavioral response was also described for the turrid gastropod *Oenopota levidensis* by Shimek (1986). Loco pediveligers possess a high rate of oxygen consumption in saturated seawater (ca. 0.5 μg O_2 per larva per hour, pers. comm.). The oxygen saturation of the coastal breaker zone along the coast alone may have an effect on induction of settlement by increasing basal metabolic rates. This occurred to a degree in our laboratory study (Table 2).

We observed that grazing by loco pediveligers on microalgal-bacterial surfaces triggered velar loss and deposition of initial teleconch shell in very few snails (Exp. 2, II); but that these anatomical changes were induced at a much higher rate on barnacle covered substrata. Extending the period of exposure of pediveligers to wave motion alone (Exp. 1 and 3) significantly increased the number metamorphosing. The loco pediveliger thus appears to be a species, not uncommon for a large variety of invertebrates, that is induced to metamorphose by several environmental signals, rather than by one finely tuned inductive cue. The latter occurs among species that are strictly prey specific (e.g. *Phestilla sibogae*, Hadfield 1984), or that do not feed during their planktonic existence and depend on chemical signals to effectively locate nutritionally favorable settling sites (e.g. *Haliotis rufescens*, Morse 1990, Morse 1991). The loco pediveliger, a typical swimmer-crawler (Carriker 1990), can repeatedly settle upon and then abandon potential settling sites before an acceptable location is found, delaying metamorphosis for days or even weeks without losing competence (Tables 1, 2). The same is true of pediveligers of *Crassostrea gigas* (Coon et al. 1990).

We found that some pigmented plantigrade locos could exist for many days in the laboratory without forming the teleconch. Many of these died even in the presence of natural substrata and barnacles without developing further; depletion of energy reserves or disease or lack of a specific inducer could have been responsible for the deaths. Juvenile locos predominantly prefer barnacles as initial prey, and feeding on them generally stimulates deposition of the adult shell.

Pigmented juveniles frequently used empty barnacle shells as refuges. They soon developed efficiency in perforating barnacles, extending the proboscis through the borehole to feed on the soft tissues within (Carriker 1981). As juveniles grew in size, their shell so closely mimicked barnacle valves that they could not be distinguished easily among their prey, a protective coloration that no doubt has enhanced survival of the young in natural habitats overrun by potential predators.

Our laboratory studies provided no evidence that barnacles are the primary source of inducers for metamorphosis of the loco pediveliger. In view of the fact that pediveligers and juveniles both grazed on encrusting algal films, yet did not develop past the pigmented stage on these films alone, but did so when barnacles were added to the diet, we suggest a two-stage metamorphic induction process, the first involving substances from diatoms or encrusting algae, the second from cirripeds. A similar two-stage metamorphosis was described for larvae for the family Architectonicidae by Robertson et al. (1970).

Potential for Culture

Several research groups in Chile have tried unsuccessfully to mass culture *C. concholepas* under controlled laboratory conditions (pers. comm.). In lieu of this, DiSalvo (1988) suggested capturing lipped loco veligers at sea and transporting them to the laboratory to undergo metamorphosis. This approach would avoid the long and difficult task of culture from the intracapsular stage. Growth rates of loco juveniles reared in our study did not differ significantly ($p = 0.1$) from that previously reported by DiSalvo (1988), indicating that "seed-size" locos about 10 mm in height could be grown within 60 days of their collection at sea under laboratory conditions. Negligible mortality occurred in the laboratory once locos began feeding on barnacles.

Seed locos produced by capture of lipped veligers at sea and culture of juveniles to seed size in captivity could be used to re-populate natural rocky areas depleted by overfishing pressure (Castilla 1988). Seed locos might also be grown to commercial size in captivity, provided an economical food source could be developed for them.

However, the primary limiting factor in the development of the culture of captive locos is the broadly and thinly dispersed distribution of lipped veligers in coastal waters. From the point of view of survival of juvenile locos, wide dispersal and sparse densities in native ocean waters could result in their non-gregarious recruiting habit, thus avoiding the cannibalism which we observed in the laboratory. Cannibalism is undoubtedly further redueed as juvenile locos individually take shelter in erypts and crevices.

Nonetheless, the mass culture of locos by the method suggested here and previously (DiSalvo 1988), is presently not a viable alternative, primarily because of the scarcity of lipped veligers in ocean waters and the lack of an economically available food supply for juveniles. The alternative for the present is conservation of the loco resource based on management and protection of its native rocky coastal habitat (Castilla and Cancino 1976, Castilla 1988).

CONCLUSIONS

Metamorphosis of *C. concholepas* is not the rapid, well defined event that has been described for some other molluscs. The change of behavior from a very long-lived, natatorially independent, planktonic veliger to a slowly crawling, shell-boring carnivore must have been a long drawn out evolutionary process. Even so, the event can now be interrupted for a few hours, days, or even weeks before a suitable settling site is identified and the teleconch begins to form. Settling on highly wave-washed rocky shores, rather than in quiet protected embayments, may provide some isolation from potential predators. The species has evolved a number of alternative choices that safeguards its life on rocky shores. For one, individuals can be stranded high on the intertidal zone and left to dessicate for three or more hours without becoming dehydrated or dying. They can also experience cracks and breaks in the protoconch, then repair them by returning to planktonic existence; repair of the protoconch can be made even after the velum has been resorbed. Furthermore, after loss of the velum during the period of grazing on encrusted algal films by the juvenile, the pigmented protoconch renders the shell harder. Finally, and probably the most significant, metamorphosis of pediveligers can be induced by more than one non-specific inducer.

One can only speculate on the morphological-physiological-behavioral "twists and turns" experienced by locos in their long phylogenetic odyssey through the ecological complexities of rocky coastal margins to reach the present anatomical sequence of early veliger, lipped veliger, pediveliger, juvenile plantigrade (Hansen 1978, Jablonski 1986). Whatever these may have been, they have contributed to the survival of a highly successful species.

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GROWTH AND BIOCHEMICAL PROFILE OF JUVENILE MUSSELS (*MYTILUS GALLOPROVINCIALIS* Lmk) FED ON DIFFERENT ALGAL DIETS

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ABSTRACT The food value of different microalgal diets to juvenile *Mytilus galloprovincialis* Lmk was assessed. Three different marine microalgae species (*Tetraselmis suecica*, *Dunaliella tertiolecta* and *Phaeodactylum tricornutum*) were fed singly and in various mixtures. Composition of the mixtures was based on the dry weight of the cells. Significantly higher growth rates in length and volume were obtained with diets containing *T. suecica*. Differences in food value between diets containing *T. suecica* and the remainder also were observed in terms of growth in dry and organic weights, gross growth efficiencies and condition indexes. All these parameters were correlated significantly with each other. The biochemical profile of the mussels also was modified by the diet. Mussels fed on diets containing *D. tertiolecta* showed increased levels of carbohydrates. Growth rates, gross growth efficiencies and condition indexes were correlated significantly with the protein and lipid deposited as body constituents, and lipid content (%DW) of the mussels. In the diets of higher growth, 44–47% of algal protein supplied was deposited as body protein, against 4.2–4.5% of lipids and 33–64% of carbohydrates.

KEY WORDS: mussel, *Mytilus galloprovincialis*, microalgal diets, growth, biochemical profile

INTRODUCTION

Extensive cultivation of mussels is a world-wide activity of growing economic importance. Mussel spat traditionally has been obtained from collector ropes on rafts, or natural banks on the coast (Mariño et al. 1983, Dardignac-Corbeil 1990). The first stages of culture (spat and young mussels) are critical in rearing bivalves, and improvements in the production require improvements in the quality of the spat.

The nutritional requirements of juvenile mussels are poorly understood (Hawkins and Bayne 1991). The nutritional value of different microalgal species as food for juvenile bivalves has been studied widely in clams and oysters, but only a few studies have been focussed on juvenile mussels (e.g. Walne 1970, Strömgren and Cary 1984).

Mussels are also used as test organisms or bioindicators in marine environmental research, and an important number of studies on their biology has been carried out. Convenient diets and optimum culture conditions improve such laboratory research. The optimization of the culture conditions in our department has produced an important increase in mussels survival and in the number of metaphases obtained for cytogenetic studies. In this way, differences in the physiological state of the mussels were reflected by the mitotic indices. Freshly collected mussels show variable, but rather low, mitotic indices. As the feeding time in the laboratory became longer, mitotic indices increase and deviations from the mean shorten (Martínez-Espósito et al. in press). Microalgal species used may also have an important influence on the results obtained from physiological experiments.

The algal diet must supply the nutrients required by juvenile bivalves, providing energy for metabolic demands and growth. The food value of a microalgal species depends on cell size, digestibility, toxicity and biochemical composition (Webb and Chu 1982). Mixed diets, with more than one algal species, have generally promoted better growth than monoalgal diets (Epifanio et al. 1976, Epifanio 1979, Strömgren and Cary 1984).

One useful way to evaluate differences in food value is estimate the relative growth efficiency of the spat fed with different diets. The importance of providing bivalve spat with a suitable algal diet is very clear: they will grow faster and more efficiently, reach higher quality and perform better when transferred to the natural environment (Laing and Millican 1986).

In the present work, the food value of different algal diets to juvenile *Mytilus galloprovincialis* Lmk, measured in terms of growth rate, biochemical composition of mussels and efficiency of food conversion, has been analysed.

MATERIALS AND METHODS

Microalgal Cultures

Three marine microalgal species were used as potential diets: *Dunaliella tertiolecta* Butcher (Chlorophyceae), *Tetraselmis suecica* (Kylin) Butch (Prasynophyceae) and *Phaeodactylum tricornutrum* var. *bicornutum* Bohlin (Bacillariophyceae). *D. tertiolecta* was obtained from the Culture Center of Algae and Protozoa, Cambridge, U.K., and *T. suecica* and *P. tricornutum* were obtained from Dr. Fábregas, University of Santiago. They were cultured in seawater filtered through a 0.45 μm filter, autoclaved at 120°C for 45 min, and enriched with commercial nutrients for marine microalgae (Algal-1; Nutrición Avanzada S. A., Santiago de Compostela, Spain) (Herrero et al. 1991). Salinity of seawater was 35‰. Unialgal cultures were carried out in 6 l PYREX vessels, and maintained in exponential growth. All cultures were incubated in a controlled environmental chamber at 18 ± 1°C and illuminated with 3 fluorescent lamps placed under the flasks and 3 above them (about 110 $\mu E\ m^{-2}\ s^{-1}$) on a 12:12 light:dark cycle.

Cellular density of the algal cultures was determined daily by cell counting with a Neubauer haemocytometer.

Culture of Mussels and Algal Diets

Juvenile mussels were collected from Ría de Arousa, Galicia, NW of Spain. On arriving at the laboratory, mussels between 6 and 13 mm were selected and randomly distributed in groups of 20 individuals. A group of mussels was used to measure length and volume; then the flesh was separated, pooled and stored at −20°C until biochemical analysis. This group represents the "wild mussels". The remaining groups were fed with *T. suecica* during an acclimation period of 7 days; after that mussels were pooled, and redistributed randomly in 20-spat groups. They were not fed during the 24 h before the feeding experiments. From these mussels, a sample was withdrawn for the initial biochemical composition.

Mussels were cultured in 1-liter polyethylene cylindric tanks filled with 0.45 μm-filtered ultraviolet light-treated seawater, in a culture room at 18 ± 1°C and 12:12 h light:dark. Tanks were aerated to maintain an adequate O_2 tension and to avoid food sedimentation. The pH of the seawater was 7.9. The tanks were emptied, cleaned and refilled with freshly filtered ultraviolet light-treated seawater twice each week. Each day, the seawater was filtered (5 μm) to remove faeces and pseudofaeces, pH was recorded and dead mussels were removed and measured.

Experiments were carried out for 60 days. The following algal diets, both mono-species and mixed-species, were assessed: *T. suecica* (Ts diet); *D. tertiolecta* (Dt); *P. tricornutum* (Pt); *T. suecica* + *D. tertiolecta* (TsDt); *T. suecica* + *P. tricornutum* (TsPt); and *D. tertiolecta* + *P. tricornutum* (DtPt). A control no fed was also established.

During the acclimation period, the food supplied was gradually increased and established in a dietary level of 1.20 mg algal DW per mussel per day. This food ration was supplied in several doses throughout the light period. The ration per doses was established as the ration at which pseudofaeces production was minimum. Before adding the algal cell suspensions, a volume of seawater equivalent to the volume of algal suspension was removed from experimental tanks to maintain a constant volume.

Equivalent cell densities were established, based on the dry weight of the algal cells of the different species used. For the mixed diets, rations were adjusted so that each microalga contributed an equal dry weight to the ration.

The food ration was increased up to 1.81 mg dry algae matter per spat per day throughout the experimental period.

Initial conditions for the different experimental diets are presented in Table 1.

Growth and Biochemical Analysis

The shell-length and volume were monitored at periodic intervals. Shell-length was measured by a calliper (±0.1 mm). Whole mussel volume was determined from weight of the displaced distilled-water volume, by a BOSCH balance (±0.1 mg).

At the end of the trials, flesh from the mussels fed on each diet was pooled and freeze-dried. Biochemical contents of the algae and mussels were measured using the phenol-sulphuric technique for carbohydrate (Dubois et al. 1956), and a charring method for lipid (Marsh and Weinstein 1966). Protein content of mussels was determined in a Tekator autoanalyser by the micro-KJELDAHL method (A.O.A.C. 1965). Protein of microalgae was measured by the dye-binding method (Bradford 1976), and RNA was determined as described by Kochert (1978). The ash weight was determined by ashing at 540°C (A.O.A.C., 1965), and the ash free dry weight (AFDW) was calculated by subtraction.

TABLE 1.

Initial length and volume of juvenile mussels (*M. galloprovincialis*) for experiments with different algal diets.

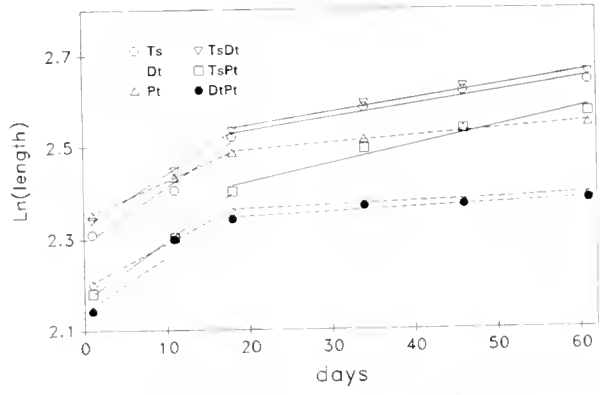
	Length (mm)		Volume (mm^3)	
Diet	**Mean**	**95% C.I.**	**Mean**	**95% C.I.**
Ts	10.07	9.78–10.38	93.06	84.55–102.42
Dt	9.03	8.12–10.03	70.00	50.00–97.00
Pt	10.52	9.83–11.27	116.32	96.23–140.62
TsDt	10.38	10.00–10.70	100.01	85.66–116.77
TsPt	8.86	8.16–9.61	70.96	57.30–87.87
DtPt	8.52	8.06–8.99	62.03	51.44–75.66

Diets: *T. suecica* (Ts), *D. tertiolecta* (Dt), *P. tricornutum* (Pt) and mixed diets (1:1 dry weight).

RESULTS

Growth in Shell-Length

Figure 1 represents the time course of shell-length (as the mean of natural logarithms of length) for each diet. Growth tended to fit curves with inflexion points on day 18 of culture. Because of this, the experimental time was divided in two periods: the first one, until day 18, and the second one after day 18. Straight lines were drawn using linear regression in each one of these periods; the regression coefficients were compared using the analysis of covariance described by Snedecor and Cochran (1989). No differences were observed among the slopes during the first experimental period ($P > 0.05$). Differences appeared in the second period.



	Days 1 - 18			Days 18 - 61		
	b_0	b_1 (x10^{-3})	r	b_0	b_1 (x10^{-3})	r
Ts	2.29±0.03	11.41±2.07	0.988	2.48±0.02	2.81±0.44	0.978
Dt	2.20±0.01	8.86±0.81	0.993	2.35±0.01	0.69±0.26	0.891
Pt	2.35±0.00	7.37±0.16	0.999	2.46±0.01	1.43±0.16	0.983
TsDt	2.33±0.01	10.69±0.71	0.999	2.49±0.01	2.86±0.26	0.990
TsPt	2.17±0.01	12.14±0.89	0.999	2.34±0.02	3.96±0.50	0.978
DtPt	2.14±0.03	11.41±2.11	0.978	2.33±0.01	0.86±0.20	0.937

Figure 1. Growth in length of juvenile mussels fed on different algal diets: *T. suecica* (Ts), *D. tertiolecta* (Dt), *P. tricornutum* (Pt), and mixed diets. Equations, for two growth periods, were adjusted by linear regression: $Y = b_0 + b_1X$; where Y is the length as Ln(length), and X is the time in days. The coefficients (mean ± SE) are shown below the figure.

Results of ANCOVA for the data of the second experimental period are presented in Table 2. Since significant differences were observed, comparisons of slopes were carried out between *T. suecica* (Ts) diet and each one of the remaining diets (analysis not shown). No differences were observed among the regression coefficients obtained in the diets Ts, TsDt and TsPt ($P > 0.05$), coefficient of Ts diet being different from slopes in Dt, Pt, and DtPt diets ($0.05 > P > 0.01$). The slope for Dt diet was not different from 0 ($P > 0.05$).

From these results, the diets were divided in two groups. Higher growth rates were observed with diets containing *T. suecica* (Ts, TsDt, and TsPt); TsPt being the diet which supported the highest growth throughout the experimental period. Lower growth rates were observed with the remaining diets.

A relative length growth rate (*Gr*, Table 3) was calculated, with *T. suecica* (Ts) diet as control, as:

$$Gr = (G \text{ diet}/G \text{ control}) \times 100$$

where G is the length growth rate, calculated using the equation:

$$G = \text{Ln}(L_t) - \text{Ln}(L_o)/t$$

being L_t the length at time t, L_o the initial length at day 1, and t the time in days.

Relative length growth rate obtained in the TsPt diet increased with respect to *T. suecica* diet throughout the experimental period, while replacing 50% *T. suecica* with *D. tertiolecta* (TsDt diet) resulted in a relative growth rate similar to Ts diet. In Pt diet, relative length growth rate remained stable, with values about 60%, after 18 days of culture. In Dt and DtPt diets, relative length growth rates declined continuously throughout the experimental period, because growth was almost zero from day 35.

A synergistic effect was observed in the mixed diets regarding their respective monoalgal diets (Table 3). *Gr* values for mixed diets were higher than the average of *Gr* values of monoalgal diets.

Length of unfed mussels did not vary throughout the experimental time.

TABLE 3.

Relative length growth rate (*Gr*) at days 11, 18, 34, 46, and 60, of mussels fed on different algal diets.

Diet	Gr_{11}	Gr_{18}	Gr_{34}	Gr_{46}	Gr_{60}
Unfed	29	16	15	12	8
Ts	100	100	100	100	100
Dt	97	69	61	56	52
Pt	82	64	59	59	59
TsDt	110	94	95	94	97
TsPt	126	107	114	115	120
DtPt	163	97	83	75	72

Growth in Volume

Figure 2 represents the time course of volume (as the mean of natural logarithms of volume) for each diet. The coefficients obtained by linear regression are presented below the graph. The slope for mussels fed on *D. tertiolecta* was not different from 0 ($P > 0.05$). Data were treated as data of length, and results of the comparison of slopes from day 18 are given in Table 4. Two groups of diets were again observed: growths observed for mussels fed on diets containing *T. suecica* were no different among them ($P > 0.05$), but faster than in the remaining diets: Dt and DtPt ($P < 0.01$) and Pt ($0.05 > P > 0.01$).

Volume growth rates (*Gv*) and relative volume growth rates (*Gvr*) were calculated as for length, but replacing L_t and L_o for V_t and V_o, expressed in mm^3. Relative volume growth rates are represented in Table 5. In Pt diet relative volume growth rate was stable (50–60%) throughout the experimental time, while relative volume growth rates of Dt and DtPt diets declined up to 40% lower values at the end of trials. After day 18, *P. tricornutum* supported faster growth than DtPt and Dt diets (Fig. 2), although not significantly different ($P > 0.05$).

Volume of unfed group did not change throughout the experimental period.

Length and volume growth rates for the whole experimental period ($t = 60$; Table 6) were closely correlated ($r = 0.95$, $P < 0.01$) (Table 7).

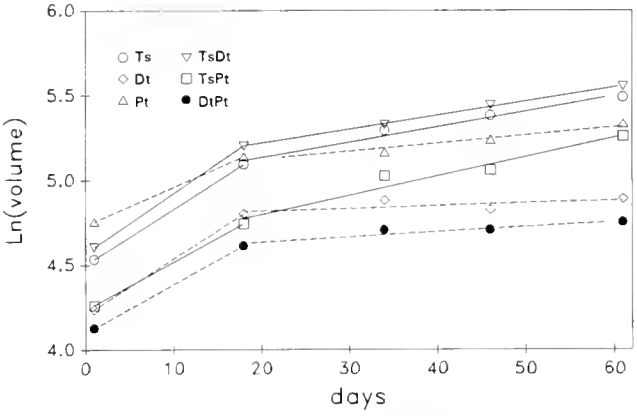
Growth in Weight

Growth rates in dry body weight (G_{DW}) and organic weight (G_{OW}) (Table 6) were calculated as $\text{Ln}(W_t/W_o)/t$, where W_t and

TABLE 2.

Comparison of regression lines obtained for the growth in shell-length of juvenile mussels fed on different algal diets. *x*: time (days); *y*: mean Ln(length).

	d.f.	Σx^2	Σxy	Σy^2	Regression Coefficient	Deviations from Regression		
						d.f.	SS	MS
Within								
Ts	3	996.75	2.792	0.00820	0.00280	2	0.00038	0.00019
Dt	3	996.75	0.688	0.00061	0.00069	2	0.00014	0.00007
Pt	3	1010.00	1.443	0.00211	0.00143	2	0.00005	0.00002
TsDt	3	1010.00	2.885	0.00838	0.00286	2	0.00014	0.00007
TsPt	3	998.75	3.954	0.01616	0.00396	2	0.00051	0.00025
DtPt	3	998.75	0.862	0.00082	0.00086	2	0.00008	0.00004
						12	0.00128	0.00011
Pooled, *W*	18	6011.00	12.624	0.03628	0.00210	17	0.00977	0.00057
		Difference between slopes:				5	0.00848	0.00170
Comparison of residual variances: (Test de Barlett)				F = 2.00		$P > 0.05$		
Comparison of slopes:				F = 15.85(5,12)		$P < 0.01$		



	Days 1 - 18		Days 18 - 61		
	b_0	b_1 (x10^{-3})	b_0	b_1 (x10^{-3})	r
Ts	4.500	33.01	4.94±0.04	9.11±0.99	0.988
Dt	4.207	33.17	4.79±0.05	1.48±1.24	0.644
Pt	4.734	22.52	5.03±0.04	4.62±0.90	0.967
TsDt	4.570	35.22	5.05±0.07	8.21±0.15	0.999
TsPt	4.234	28.42	4.56±0.08	11.32±1.17	0.974
DtPt	4.099	28.67	4.57±0.03	2.98±0.71	0.942

Figure 2. Growth in volume of juvenile mussels fed on different algal diets: *T. suecica* (Ts), *D. tertiolecta* (Dt), *P. tricornutum* (Pt), and mixed diets. Linear regressions were calculated for two growth periods, and the coefficients are shown below the Figure ($Y = b_0 + b_1X$; where Y is the Ln(volume) and X is the time in days).

W_o are final and initial dry weight (DW), or ash free dry weight (AFDW). Higher rates in weights were obtained in diets that promoved faster growth in length and volume: *T. suecica* + *P. tricornutum*, *T. suecica* and *T. suecica* + *D. tertiolecta* ($20.63 - 24.78 \times 10^{-3}$ d^{-1}).

G_{DW} and G_{OW} were closely correlated with length growth rate, G_{60} (r = 0.99 and 0.97, respectively; $P < 0.001$), and with volume growth rate, Gv_{60} (r = 0.93 and 0.89, respectively, $P < 0.001$) (Table 7).

Condition Index, Gross Growth Efficiency and Mortality

The selected condition index (Ci) relates dry flesh weight (mg) with total volume (mm^3):

$$\text{Ci} = \text{dry flesh weight/volume}$$

The traditionally used Ci in studies with bivalves relates flesh DW with internal volume. However, juvenile mussels present thin shells, and the calculated Ci was considered useful.

Ci values were between 90 and 100 in mussels fed with diets containing *T. suecica* (Table 6). In Pt and DtPt diets, Ci declined to similar values of "wild" mussels (69.13), and Ci was lower than "wild" mussels for those fed on *D. tertiolecta* (51.07). Ci fell to 25.80 in the unfed mussels. Condition index for freshly collecty mussels of equivalent volume to mussels fed on *Tetraselmis* diets was 59.88.

Gross Growth Efficiency (K_1) is the proportion of the organic weight of the algal cells cleared from suspension that is incorporated into organic growth of animals (Laing and Millican 1986).

$$K_1 = I/C$$

where I is the increase in total organic weight of the mussel in a certain time (in these trials, 60 days), and C is the organic matter of the algal cells cleared per mussel during the same period. Since cell deposition on culture vessels was minimal, an assumption was made that all supplied algal biomass was cleared by mussels. The organic matter contents of the microalgae used were 63.46 mg 10^6 cells^{-1} for *T. suecica*, 61.32 mg 10^6 cells^{-1} for *D. tertiolecta*, and 20.66 mg 10^6 cells^{-1} for *P. tricornutum*.

Higher K_1 values (Table 6) were observed in faster growth diets, those with *T. suecica* in their formulation: Ts, TsDt and TsPt. K_1 was correlated with G_{60} (r = 0.90, $P < 0.01$), Gv_{60} (r = 0.93, $P < 0.01$), G_{DW} (r = 0.94, $P < 0.01$), G_{OW} (r = 0.91, $P < 0.01$) and condition index (r = 0.98, $P < 0.001$) (Table 7).

Instantaneous mortality rate (Ricker 1973) was calculated using the equation:

$$Z = \text{Ln}(N_o - N_t)$$

where N_t is the number of living mussels at time t, and N_o is the initial number of mussels. The Z_{60} values (t = 60 days) are represented in Table 6. Unfed mussels maintained a high survival until day 40. From this moment, effect of starvation resulted in a Z_{60} of 0.43. Mortality rate was not correlated with any other parameter (Table 7), but Dt diets all had higher mortality rates.

Biochemical Profile and Food Conversion

Biochemical composition, as mg per mussel, and ratios between components, is shown in Table 8. The highest absolute contents of all components were obtained in mussels that grew faster (diets containing *T. suecica*).

Expressing data of Table 8 as % of dry weight, protein was the component more abundant, between 54.60–69.02% DW. Carbohydrates accounted for 9.04–25.14% DW, and diets containing *D. tertiolecta* showed the highest values (18.05–25.14%). Lipids were between 3.27 and 4.99% DW for cultured mussels, and 9.02 for "wild" mussels.

The lipid:protein ratio (Table 8) reached a maximal value in "wild" mussels (0.15); all the cultured mussels showed values between 0.059–0.083, being maximum in diets containing *T. suecica*. Carbohydrate:protein ratios were minimum in mussels fed on the diet of *Phaeodactylum*, and maximum in mussels fed on the three *Dunaliella* diets and in "wild" mussels. Lipid:carbohydrate ratio was minimum in *Dunaliella* diets (0.15–0.24) and increased up to values >0.30 in the remaining diets and in "wild" mussels.

Taking into account the gross biochemical composition of microalgal cells (Table 9), efficiencies of conversion of protein, carbohydrates and lipids from the diets into protein, carbohydrates and lipids deposited as body constituents were calculated. The components deposited as body constituents were obtained subtracting the initial values from final values. In the diets of higher food value (*T. suecica* diets) values between 44 and 47% of protein offered to mussels was deposited as body protein, compared with 33–64% of carbohydrates and 4.2–4.5% of lipids (Table 10).

Relationships Among Parameters

Correlation analysis among growth, physiological, and biochemical parameters was carried out using the statistical software SPSS/PC+ (*v.* 4.01). Parameters considered were growth rates in length (G_{60}), volume (Gv_{60}), dry body weight (G_{DW}) and ash free body weight or organic weight (G_{OW}); gross growth efficiency (K_1); condition index (Ci); mortality rate (Z_{60}); depositions of protein (D_P), carbohydrates (D_C) and lipids (D_L) (mg per mussel);

TABLE 4.

Comparison of regression lines obtained for the growth in volume of juvenile mussels fed on different algal diets. *x*: time (days); *y*: mean Ln(Volume).

	d.f.	Σx^2	Σxy	Σy^2	Regression Coefficient	Deviations from Regression		
						d.f.	SS	MS
Within								
Ts	3	996.75	9.077	0.08463	0.00911	2	0.00197	0.00098
Dt	3	996.75	1.478	0.00528	0.00148	2	0.00309	0.00154
Pt	3	1010.00	4.667	0.02319	0.00462	2	0.00162	0.00081
TsDt	3	1010.00	8.293	0.06813	0.00821	2	0.00004	0.00002
TsPt	3	998.75	11.318	0.13452	0.01133	2	0.00626	0.00313
DtPt	3	998.75	2.973	0.00984	0.00298	2	0.00099	0.00050
						12	0.01397	0.00116
Pooled, *W*	18	6011.00	37.806	0.32559	0.00629	17	0.08781	0.00517
		Difference among slopes:				5	0.07384	0.01477
Comparison of residual variances: (Test de Barlett)				F = 1.77		$P > 0.05$		
Comparison of slopes:				F = 12.68(5,12)		$P < 0.01$		

final composition as percentages of DW, and ratios among chemical components. Matrix of correlation coefficients is presented in Table 7. Analysis were carried out without data of unfed mussels.

Depositions of protein and lipids were significantly correlated between them and with all growth rates, gross growth efficiency and condition index; carbohydrate depositions were correlated with none of these parameters.

Significative and positive relationships were also detected among lipids (as percentage of DW) with all growth rates, K_1, depositions of protein and lipids per mussel, and lipid:protein ratio. Ash contents (as % DW) were negative correlated with growth rates, K_1, and depositions of protein and lipids. Protein and carbohydrate levels were correlated with none of the parameters.

Lipid:protein ratio was correlated with volume growth rate, and lipids as %DW.

DISCUSSION

During the acclimation period, food ratio was gradually increased from low levels to a dietary level of 1.20 mg algal DW per mussel per day, 21% of dry body weight per day, or 10–15% of organic matter weight depending on the diet. Food rations of 10–20% of dry weight per day have been reported for 0.2 g mussels (Bayne et al. 1976). Food ration was increased throughout the experimental period up to 1.81 mg algal DW per mussel per day in all the diets. However, feeding rates were different throughout the experimental period because of the different increases in body weight for each diet. Thus final food ration varied between 22–27% of dry body weight (*Dunaliella* and *Dunaliella* + *Phaeodactylum* diets) and 7.5–13% (remaining diets).

The unfed control group was included to show that growth was not produced by any factor other than microalgal diets. Data obtained show that growths in length or volume did not occur in the unfed mussels. Therefore, growths observed in the other groups of mussels were indeed due to the microalgal diets used.

Length and volume growth rates were not constant throughout the experimental period. There were two periods clearly different in the growths in length and volume (Figs. 1, 2). In all diets, growth rates were higher during the first 18 days. Nielsen (1988) had observed this same behaviour in juvenile *M. edulis*, as the acute response (initial increase) and acclimation to increasing temperature. Environmental temperature in Arousa waters is 12–13°C in April, and the experiments were carried out at 18°C. Therefore this increase may provoke a response in the growth rate. However, mussels were acclimated to laboratory conditions during 8 days before beginning the experiments. In these experiments a new steady-state in growth rate was reached after day 18 (Figs. 1 and 2). A strong decrease in growth rate was also reported by Nielsen (1988) at 18°C. Other physiological parameters, as filtration rate, respiration rate or scope for activity show acclimation to temper-

TABLE 5.

Relative volume growth rate (*Gvr*) at days 18, 34, 46, and 60 of mussels fed on different algal diets.

Diet	Gvr_{18}	Gvr_{34}	Gvr_{46}	Gvr_{60}
Unfed	1	0	0	0
Ts	100	100	100	100
Dt	100	80	65	65
Pt	68	53	56	60
TsDt	107	95	99	99
TsPt	86	101	94	105
DtPt	87	76	69	64

TABLE 6.

Length (G_{60}, $\times 10^{-3}$), volume (Gv_{60}, $\times 10^{-3}$), dry flesh weight (G_{DW}, $\times 10^{-3}$) and organic weight (G_{OW} $\times 10^{-3}$) growth rates; mortality rate (Z_{60}), condition index (Ci), and gross growth efficiency (K_1) for mussels fed on different algal diets.

	G_{60}	Gv_{60}	G_{DW}	G_{OW}	Z_{60}	Ci	K_1
Ts	5.41	15.70	21.12	20.94	0.05	93.91	32.11
Dt	3.07	10.63	7.48	5.19	0.11	51.07	2.81
Pt	3.17	9.50	10.51	8.85	0.05	67.96	10.73
TsDt	5.19	15.56	20.71	20.63	0.11	95.93	31.59
TsPt	6.41	16.35	24.78	24.75	0.00	93.02	27.85
DtPt	3.93	10.23	13.78	14.85	0.36	69.42	9.47
Wild	–	–	–	–	–	69.13	–

TABLE 7.

Matrix of correlations among growth and biochemical parameters for mussels fed on different algal diets.

	G_{60}	Gv_{60}	G_{DW}	G_{OW}	Z_{60}	K_1	Ci	D_P	D_C	D_L	C/P	L/P	L/C
G_{60}	1.00												
Gv_{60}	.945*	1.00											
G_{DW}	.987**	.930**	1.00										
G_{OW}	.972**	.892**	.992**	1.00									
Z_{60}	−.340	−.431	−.293	−.178	1.00								
K_1	.899*	.932*	.937*	.910*	−.410	1.00							
Ci	.903*	.893*	.956*	.947*	−.277	.980**	1.00						
D_P	.895*	.935*	.932*	.899*	−.444	.998**	.977**	1.00					
D_C	.643	.744	.698	.711	.126	.747	.786	.745	1.00				
D_L	.946*	.978**	.961*	.940*	−.306	.964**	.959*	.964**	.824	1.00			
C/P	−.225	−.197	−.237	−.116	.848	−.345	−.309	−.369	.289	−.151	1.00		
L/P	.804	.917*	.815	.782	−.262	.850	−.216	.865	.905*	.932*	.135	1.00	
C/L	.350	.299	.375	.303	−.812	.457	−.020	.483	−.154	.279	−.544	.747	1.00
LIP (%)	.946*	.933*	.976**	.953*	.376	.972**	−.118	.977**	.744	.972**	.046	.990**	.801*
ASH (%)	−.945*	−.945*	−.982**	−.987**	−.164	−.986*	−.613	.991**	−.789	−.847	−.053	−.575	−.502

Minimum pairwise N of cases: 6; 1-tailed signif.: * .01 **.001.

ature between 10 and 20°C (reviewed by Bayne et al. 1976) and a new steady-state is established in two weeks after perturbance. However, growth is a more complex physiological process.

The effects of diets on growth were more evident during the second period. Two types of diets can be established in relation to their food value (Figs. 1, 2): a) diets that include *T. suecica* in their formulation, and b) *D. tertiolecta* diet, *P. tricornutum* diet and the mixed diet of them. Different food values were also observed in growths in weight, gross growth efficiencies, and condition indexes (Table 6), all these parameters being significantly correlated among them (Table 7). Mussels fed on *T. suecica* diets utilized more efficiently the ingested ration for growth (K_1 about 30%), and the condition indexes were almost 100, against 59.88 for freshly collected mussels of equivalent volume.

Shell-length growths of mussels fed with *T. suecica* diets were 0.10–0.14 mm day^{-1} during the first 20 days, decreasing to 0.04–0.05 mm d^{-1} from this day. These values are lower than other reported for mussels in nature: 0.10–0.34 mm d^{-1} for 26 mm mussels (Aguirre 1979), 0.24 mm d^{-1} for 18.2 mm mussels (Pérez and Roman, 1979), for mussels cultured on rafts in Arousa waters; 7–11 mm mo^{-1} from other studies in nature (reviewed by Jorgensen 1990). However, growths obtained in our experiments were similar to others obtained in laboratory (Jorgensen 1990). According to Jorgensen (1990) mussels growing at 0.34 mm d^{-1} exploit the whole potential for growth; thus, in our experiments mussels exploited ½ of the potential for growth during the first experimental period, but only ⅕ after day 18 of culture.

A synergistic effect was observed in mixed diets, mainly in those including *Tetraselmis*. Based on a linear relationship between cell concentration of suspension and growth rate (Strömgren and Cary 1984), if only additive effects among diets were true, relative growth rates of mixed diets would be similar to means of relative rates of monoalgal diets. Relative growth rates were higher than means of relative rates of monoalgal diets (Tables 3, 5). *Tetraselmis* + *Phaeodactylum* diet provided the highest growth rates, while *T. suecica* and *Tetraselmis* + *Dunaliella* supported similar growths. *P. tricornutum* had a food value higher than *Dunaliella*, and kept a constant relative growth rate in relation to *T. suecica* throughout the experimental period. Diets of *Dunaliella* and *Dunaliella* + *Phaeodactylum* supported a relative good growth only during the first experimental period, their relative growth rates declining throughout the experimental period.

Certain factors associated with algal cells have been suggested for explaining why some algal species are better than others as food source of molluscs, for example, cellular size, wall composition, digestibility, toxic metabolites and gross biochemical composition (Webb and Chu 1982). None of these characteristics alone has offered an entirely satisfactory explanation. Under the present conditions of culture, gross biochemical composition of the three used algae was not very different (Table 9), except for a higher

TABLE 8.

Biochemical composition, as mg per mussel, and relationships between components of mussels fed on different algal diets and wild mussels.

	DW	PRO	CHO	LIP	Ash	Cho/Pro	Lip/Pro	Lip/Cho
Ts	22.77	14.63	3.24	1.07	3.83	0.22	0.073	0.33
Dt	6.70	3.70	1.22	0.22	1.82	0.35	0.063	0.18
Pt	14.11	8.57	1.28	0.53	3.38	0.14	0.059	0.41
TsDt	24.78	14.53	4.99	1.21	4.06	0.34	0.083	0.24
TsPt	17.90	12.35	2.31	0.89	2.88	0.20	0.075	0.39
DtPt	8.04	4.88	2.02	0.30	1.83	0.41	0.061	0.15
Wild	3.50	1.85	0.41	0.29	0.51	0.30	0.154	0.54

DW, dry meat weight; PRO, protein; CHO, carbohydrates, and LIP, lipids.

TABLE 9.

Gross biochemical composition (% of dry weight) of *T. suecica*, *D. tertiolecta* and *P. tricornutum* in exponential growth phase.

	Protein	Carbohydrates	Lipids	RNA	Ash
T. suecica	21.20	4.69	9.95	5.80	26.49
D. tertiolecta	24.74	8.34	15.44	6.13	15.64
P. tricornutum	20.34	5.66	12.83	4.59	40.70

carbohydrate content for *D. tertiolecta* and a higher ash content for *P. tricornutum*.

The low nutritive value of *D. tertiolecta* for juvenile bivalves has already been reported (Walne 1970, Wikfors et al. 1984, Enright et al. 1986a). *T. suecica* and *D. tertiolecta* are of similar shape and size; the main difference is the presence of a medium rigid cell wall in *T. suecica* and the absence of a cell wall in *D. tertiolecta*, which presents a glycocalix-type envelope (Oliveira et al. 1980). The *Dunaliella* cell is more flexible than other microalgal cells; flexibility probably causing a higher resistance to breakage by the crystalline style. Moreover, it has been reported that the filtrates of *D. tertiolecta* cultures contain ectocrines that inhibit the filtration activity of *M. edulis* (inhibition dependent of concentration), but no *T. suecica* (Ward and Targett 1989).

Composition and balance of fatty acids of the diet has frequently been related with food value of microalgae (Langdon and Waldock 1981, Webb and Chu 1982, Enright et al. 1986b), mainly regarding their contents of polyunsaturated fatty acids. The low nutritive value of *D. tertiolecta* has been related with the absence of C20 and C22 polyunsaturated fatty acids, while *T. suecica* contains 20:5*w*3 (Langdon and Waldock 1981; Volkman et al. 1989) and *P. tricornutum* 20:5*w*3 and 22:6*w*3 (Siron et al. 1989).

However, analytical data obtained in our laboratory for these microalgae in the present culture conditions showed the presence of C20 and C22 polyunsaturated fatty acids in *D. tertiolecta* (Herrero et al. 1992). Thompson et al. (1990) studied the influence of irradiance on the fatty acid composition of different microalgae and they also found C20 and C22 polyunsaturated fatty acids in *D. tertiolecta* in similar conditions of irradiance that those used in our experiments.

Nevertheless, it must be taken into account the marked differences in fatty acid content and composition found for some species grown in different laboratories, that raises the question whether consistent results can be obtained for the same species cultured under similar conditions (Volkman et al. 1989).

Molluscs require a dietary source of arginine, histidine, methionine, cysteine, leucine, isoleucine, valine, lysine, tryptophan, threonine and proline (Boudreau 1985). All these aminoacids are supplied for the microalgae used in these experiments (Fábregas and Herrero 1985, Herrero et al. 1985). The amount of aminoacids are very similar in all of them, although *P. tricornutum* and *T. suecica* have significantly higher amounts of methionine (2.28 and 1.45 gr per 16 gr of N, respectively) and threonine (5.19 and 5.27 gr per 16 gr of N, respectively) than *D. tertiolecta* (0.80 gr per 16 gr of N of methionine and 2.58 gr per 16 gr of N of threonine). Vitamin content (Fábregas and Herrero 1990) and mineral composition (Fábregas and Herrero 1986) are very similar in *T. suecica* and *D. tertiolecta*, except for a higher content of tocopherol, Zn and Cu in *T. suecica*, and for β-carotene in *D. tertiolecta*.

The mixed diet of *T. suecica* and *P. tricornutum* is probably the best balanced diet regarding their biochemical composition, promoting the fastest growth of all tested diets.

T. suecica has the biggest cell size among the microalgae used in this work with the highest biomass per cell. For instance, four *P. tricornutum* cells yield the same organic matter as one *T. suecica* cell. These different organic matter:volume ratios act on the energetic cost of feeding. This fact may be particularly important when the food value of ingested organic matter is low. Laing and Millican (1986) reported that in the lowest food value diets for *O. edulis*, clearance rates and size-specific metabolic rates were higher, associated with lower organic growth of the animals. They suggested that it may represent an attempt by the spat to obtain the required amounts of essential nutrients from the low food value diets by increasing the filtration rate, leading to a greater metabolic demands on the assimilated ration, with less energy available for organic growth.

The biochemical composition of the mussels was also modified by the diet (Table 8). *D. tertiolecta* has the highest carbohydrate content of the three algae used (Table 9), and the inclusion of *D. tertiolecta* in the diet produced increasing carbohydrate levels.

From biochemical data, an alternate hypothesis explaining growth dynamics shown in Figs. 1 and 2 emerges. "Wild" mussels at the beginning of the experiment contain considerably more lipid (9%DW) than experimentally-fed mussels (3–5%DW) at the end of the experiment. This decrease in lipids as %DW was lower in diets supporting faster growth. Moreover, in diets of *Dunaliella* and *Dunaliella* + *Phaeodactylum* lipid contents as mg per mussels also decreased. The period of rapid initial growth may be supported by some stored lipid component, exhausted from day 18 in diets of *Dunaliella* and *Dunaliella* + *Phaeodactylum*, and that diets containing *T. suecica* must include in higher amounts.

Growth rates and gross growth efficiencies were significantly correlated with body lipids as percentages of dry flesh weight

TABLE 10.

Efficiencies of conversion of protein, carbohydrates and lipids offered in the diets by mussels fed on diets of *T. suecica*. Data correspond to the whole experimental time (60 days).

	Protein			Carbohydrates			Lipids		
Diet	Offer.	D_P	%	Offer.	D_C	%	Offer.	D_L	%
Ts	24.9	10.9	43.8	5.5	2.4	44	11.7	0.49	4.2
TsDt	23.1	10.4	44.9	6.4	4.1	61	12.5	0.56	4.5
TsPt	21.3	10.0	46.9	5.5	1.8	33	12.1	0.53	4.4

Offer., mg offered in diets; D_P, D_C, D_L, depositions of protein, carbohydrates, and lipids (mg per mussel), respectively.

(Table 7). Lipid:protein ratio was also higher in diets promoting faster growth. Therefore, mussels fed with diets that supported faster growth reached higher lipidic contents. This was also found by Laing and Millican (1986) with cultured *O. edulis* spat, and spat with higher lipid levels grew faster and with higher survival when planted out in the sea.

In the diets of higher food value, between 44 and 47% of the protein offered to mussels was deposited as body protein (Table 10), at a deposition rate of 0.17–0.18 mg d^{-1}, against a 33–64% of carbohydrates (0.03–0.07 mg d^{-1}) and 4.2–4.5% of lipids (0.009 mg d^{-1}).

Starved mussels (unfed group) had a high survival rate ($Z = 0$) during the first 20 days. Mortality increased to Z values of 0.43 at the end of the experiments (Table 6). During the starvation, the loss of dry flesh was 0.32 mg per day, or 64% of initial dry flesh. Riisgard and Randlöw (1981) and Strömgen and Cary (1984) found that *M. edulis* may have some shell growth when not fed or fed below maintenance levels. In our experiments losses per day were 0.17 mg d^{-1} of protein, 0.09 mg d^{-1} of carbohydrates and 0.03 mg d^{-1} of lipids. A preferred utilization of proteins as metabolic substrate has been observed for sexually mature mussels during winter (reviewed by Gabbot 1976), and an increasing catabolism of endogenous protein to support metabolic requirements at lower energy intakes for 10 mg dry weight mussels (Hawkins and Bayne 1991).

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INFLUENCE OF TEMPERATURE ON THE PHYSIOLOGY OF GROWTH IN *RUDITAPES DECUSSATUS* (L.) LARVAE

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ABSTRACT Larvae of the butterfish clam *Ruditapes decussatus* obtained from a wild stock were reared at 10, 16, 22 and 28°C from D-larval stage to metamorphosis. Growth in length and weight in addition to clearance and ingestion rates were studied. Growth efficiencies were calculated for each temperature. Allometric equations relating feeding rates to weight and length were obtained. Growth rate steadily increased with increasing temperature during the experimental interval. Larval life could be divided into an initial mixotrophic period (from D stage to 140–150 μm length) followed by an exotrophic period (from 140–150 μm length to metamorphosis), according to their feeding activity. The clearance rate (CR) in fully active feeding (exotrophic) larvae was an isometric function of the ash free dry weight (AFDW), CR (ml · h^{-1}) = 7.46 AFDW (mg). The effect of temperature on feeding rates was specially noticeable during the first few days of culture. Low temperatures (10°C) strongly inhibited feeding rates whereas high temperatures (28°C) yielded the maximum feeding activity. Gross growth efficiency ranged between 33 and 78%, increasing with increased temperature and decreasing with larval development.

KEY WORDS: Veneridae, clam, larva, physiological energetics, growth, temperature

INTRODUCTION

It is well known that increases in culture temperature shorten the larval period of many bivalves (e.g. *Mercenaria mercenaria* L., Loosanoff and Davis 1963, *Ostrea edulis* L., Walne 1965; *Mytilus edulis* L., Bayne 1965; etc.). From the physiological energetics standpoint, this increase in growth velocity at high temperatures can be explained by the particular effects of temperature on each component of the energy balance: ingestion, absorption, excretion and respiration. In fact, the scope for growth of an immature animal under certain environmental conditions is determined by the balance between energy gains and losses, expressed by the equations developed from Winberg (1960) and Ivlev (1966): G = I − F − N − R, A = I − F; where G is growth, I is ingestion, F is faecal loss, N is nitrogenous excretion, R is respiration and A is absorption, all terms usually expressed as rates. Limits to increased net energy gain in bivalve molluscs are set primarily by functional constraints on ingestive (I) and digestive (A) capacity, rather than by the metabolic costs (U + R) (Bayne et al. 1989).

Temperature, in addition to food concentration, is a main factor influencing the energy budget, often due to its effects upon the feeding activity, measured in bivalves as clearance and ingestion rates, and not the digestive activity (see review by Winter 1978). Several studies deal with the influence of temperature on the energy budget of juvenile or adult bivalves (reviewed by Newell and Branch 1980), but few examine larval development in general (Wilson 1980, Sprung 1984a,b,c,d, Crisp et al. 1985, MacDonald 1988). None of these address the Veneridae family, which includes species of commercial interest such as *Ruditapes decussatus*, an intertidal clam that naturally occurs inside a wide thermic range, from northern Europe to the south of Portugal and Morocco. The aim of the present investigation was to examine the energetics and the actual growth efficiencies in clam larvae in relation to larval size and temperature. The driving influence of food availability on these physiological parameters was the subject of a separate study (Pérez Camacho et al. 1991).

MATERIALS AND METHODS

The experiment was carried out in July 1990 using wild butterfish clams, *Ruditapes decussatus*, collected from the Galician Rias (NW Spain) and conditioned at 20°C as the parent stock. Spawning was induced thermically and by sperm addition. Oocytes with a mean diameter of 69.8 (±1.92) μm were obtained and fertilised with approximately 10 sperms per oocyte. They were then immediately transferred to 100 l flat bottom tanks for incubation with 1 μm filtered, u.v. sterilized sea water containing Chloramphenicol (8 mg l^{-1}) (Pérez and Román 1973).

After 48 hours, D-stage larvae were extracted and placed in 6 l plastic tanks, gently aerated and reared at a density of 10 (±1) ml^{-1}). Three experimental tanks and a control were placed into each one of 4 isothermic chambers at 10, 16, 22 and 28 (±1)°C.

The water was changed every two days using treated sea water acclimated to the experimental temperatures and food was added. The initial algal concentration was 100 *Isochrysis galbana* Parke cells μl^{-1} (2 mg l^{-1}). Suspended particle concentration was recorded before and after the water change by means of a TAII Coulter Counter fitted with a 100 μm aperture sampling tube. Larvae were fed daily after the 11th day due to the increase in the ingestion rates.

Clearance Rate (CR, μl, h^{-1}) and Ingestion Rate (IR, cells h^{-1}) were calculated following the equations: $CR = (V/nt)[\ln(c_0/c_1) - \ln(c_{0b}/c_{1b})]$ and $IR = (V/nt)[(c_0 - c_1) - ((c_{0b} - c_{1b})/2)]$, where V is the water volume, n is the number of larvae, t is feeding hours, c_0 and c_1 are the initial and final particle concentrations in the experimental chambers and c_{0b} and c_{1b} are those in the controls. Feeding rates were adjusted to the allometric equation: $FR = aW^b$, where FR is the feeding rate, W is the body weight, and a and b are fitted parameters.

On a weekly basis, a sample of 50 larvae from each tank was observed under a Nikon light microscope in order to measure length (antero-posterior axis) and to calculate the mortality. Dead larvae were sieved out and the volume of water was readjusted to maintain the initial density of 10 larvae ml^{-1}.

Samples of 1000 to 11000 larvae were dried on Whatman glass microfibre filters at 90°C until constant weight was reached. Organic matter content was estimated as weight lost after ashing at 450°C overnight. Number of larvae was calculated by counting at least five 1 ml aliquots in a Sedgewick-Rafter chamber under a microscope.

Gross growth efficiency (K_1) was calculated for each temperature from the growth and accumulated ingestion data, following the modified Ivlev (1966) equation: $K_1 = G/I$, where G is growth in organic biomass and I is ingestion in total organic weight of consumed algae.

Statistical procedures followed Snedecor and Cohran (1971) and Sokal and Rohlf (1980). Physiological rates were subjected to ANOVA to test significant differences. Multiple comparisons were performed using the Student-Newman-Keuls test. A multivariate approach was used to express clearance and ingestion rates as simultaneous functions of AFDW and temperature. Data were logarithmically (base *e*) transformed and fitted to multiple regression models using a backward stepping procedure which excluded variables that were not significant at the 5% confidence level. Statistical analyses were performed using the Statgraphics computer package.

RESULTS

Growth

Figures 1 and 2 show the length and weight growth of larvae reared under the different temperatures. At 28°C they reached a mean size of 273 μm and 3.6 μg in 17 days. In the same period at 22°C larvae reached 230 μm and 2.4 μg. At 16°C, growth was reduced to 207 μm and 1.9 μg in 20 days. Larvae reared at 10°C showed a poor growth rate until the 13th day, and this treatment was then abandoned due to the high mortality having reached only 122 μm and 0.4 μg. The effect of temperature on growth rate was almost linear although the thermic coefficient, Q_{10}, was greater in the lower temperature interval (9.25, 10–16°C) than those at higher temperatures (1.84, 16–22°C; 1.64, 22–28°C).

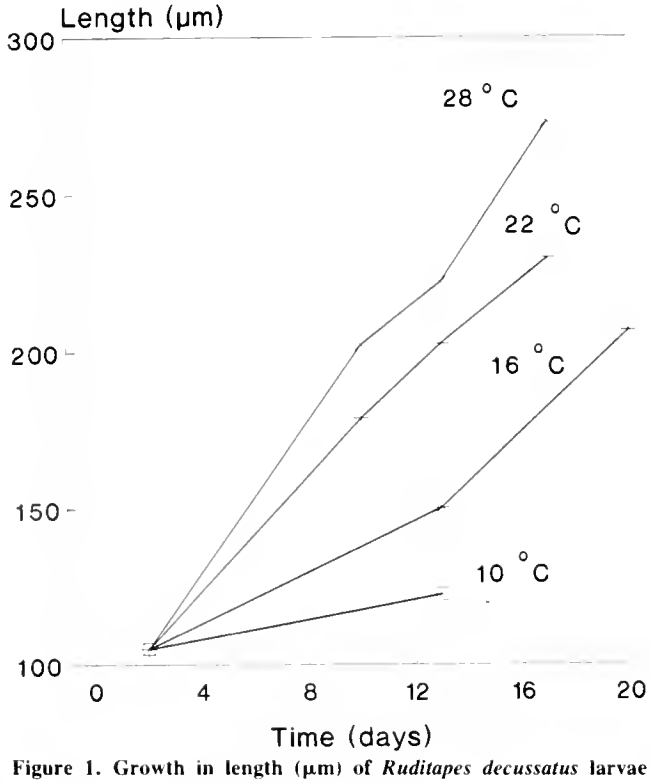

Figure 1. Growth in length (μm) of *Ruditapes decussatus* larvae reared at 28 (×), 22 (+), 16 (*) and 10°C. (□).

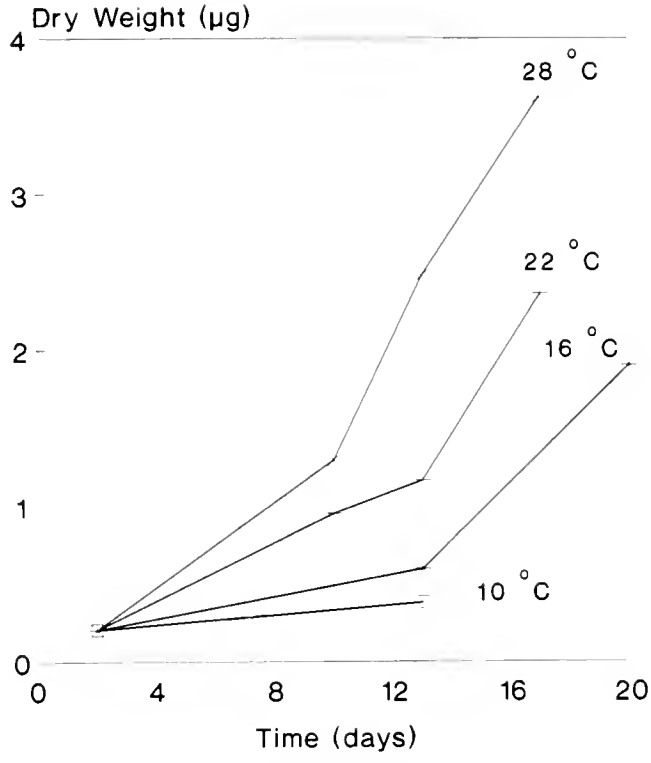

Figure 2. Dry weight (μg) increment of *Ruditapes decussatus* larvae reared at 28 (×), 22 (+), 16 (*) and 10°C. (□).

The growth in length at each temperature (Fig. 1) was approximately a straight line relationship whose slope is equivalent to the growth rate (GR, μm day^{-1}). Experimental data reflected a linear relationship between temperature (T) and GR over the 10 to 28°C range, as shown in the equation (ranges in parentheses represent the standard errors):

$$GR = -3.3(\pm 0.71) \pm 0.52(\pm 0.035)T;\ r = 0.995;\ n = 12$$

Dry weight (DW, μg) and ash-free dry weight (AFDW, μg) were related to length (L, μm), following the equations:

$$DW = 1.56.10^{-7}\ L^{3.03(\pm 0.109)};\ r = 0.993$$

$$AFDW = 0.66.10^{-7}\ L^{3.01\pm(0.112)};\ r = 0.992$$

An analysis of covariance (Snedecor and Cochran 1971) did not show significant differences in these weight:length relations between larvae cultivated at different temperatures.

The percentages of mortality, controlled weekly, were minimal at 16°C (5–16%). At 22 and 28°C, mortality was enhanced during the first week but then stabilized at 0–10%. Lastly, mortality was moderate during the first week at 10°C, but greatly increased later (>50%) and the culture was prematurely stopped.

Feeding Rates

Clearance rate (CR) markedly increased with size, from values of about 0.1–0.8 μl h^{-1} up to 10 μl h^{-1} in advanced develop-

mental stages. The power equations relating clearance to weight can be seen in Table 1. The great increase in feeding activity was not uniform but more pronounced during the first few days of larval life. Early straight-hinge or D-stage veligers have not yet fully developed their feeding activity. Therefore, a more accurate relationship between clearance rate and size can be achieved when only fully active larvae, longer than 140 μm, are considered. If early larvae of low clearance activity are excluded, the power equations obtained are those shown in Table 2. The slope is significantly lower ($p < 0.05$) at the higher temperature and there is a clear general trend towards greater slopes when temperature decreases. This means that temperature markedly affects clearance in small and medium larvae but the differences tend to diminish during development. When all the temperature data were pooled, CR ($\mu l\ h^{-1}$) proved to be an isometric function of total and ash free dry weights (μg): CR = 7.46 AFDW.

Early development of the *R. decussatus* clam was therefore divided into three different periods, following the pattern described by Gerdes (1983): P.I, from D-stage larva to 140 μm length; P.II, from 140 to the beginning of metamorphosis (240 μm) and P.III, metamorphosis and early postlarva (see further explanation below).

For the purpose of comparing the feeding activity of different larval sizes, specific clearance rates (SCR), per μg DW, were calculated. This is equivalent to a standardization of the CR to a larval size of 1 μg DW, halfway between larval sizes. Figure 3 describes the effect of temperature on SCR. An analysis of variance detected significant differences ($p = 0.0015$) in SCR between temperatures. The effect was almost linear even though the thermic coefficient, Q_{10}, was greater in the low temperature interval (5.08, 10 – 16°C) than those at higher temperatures (2.02, 16–22°C; 2.68, 22–28°C).

The effect of temperature on SCR was tested separately for each larval period (Table 3). In P.I this effect was highly significant ($p = 0.003$) and a test of multiple ranges detected two homogeneous groups: 10–16°C and 22–28°C. In P.II the effect was significant ($p = 0.020$) and the homogeneous groups were 16–22°C and 28°C.

The weight specific ingestion rates (SIR), were directly calculated from the daily decrease in suspended particle concentration, and thermal treatments were compared with an analysis of variance, which detected highly significant differences ($p = 0.0002$). Figure 4 plots the SIR as a function of temperature. As can be seen, compensation in ingestion occurred in the medium (16–22°C) and high (22–28°C) temperature intervals, with Q_{10} of 1.49 and 1.25 respectively. Low temperatures however had a very negative effect on the larval grazing activity (10–16°C, $Q_{10} = 3.59$).

The calculated weight specific ingestion rates are plotted against weight in Figure 5. According to the feeding activity, three different periods could be identified in the early development of the clam *Ruditapes decussatus*. The first period, P.I, ranges from D-stage larva until about 140 μm; the second, P.II, from this length to metamorphosis (near 240 μm), and P.III includes metamorphosis and early postlarva. In P.I feeding activity steadily increased, in response to the change from endotrophy to exotrophy (see below). In P.II specific ingestion did not show any clear increasing or decreasing trend, fluctuating on a steady value, except with larvae at 28°C, in which they distinctly decreased. Finally, algal uptake activity in postmetamorphic clams at 28°C were obtained, appearing as a prolongation of the second larval period.

Separating the larval periods described above, the pattern ob-

TABLE 1.

Parameters of the power equation expressing clearance rate (CR) as a function of ash-free dry weight (AFDW) including the whole range of larval sizes: p, probability level; *a*, intercept; *b*, slope; r, correlation coefficient. On the last line the parameters back-calculated for all the temperatures (T).

T (°C)	p	ln *a*	*b*	r
16	<0.001	2.74 ± 0.285	**1.99** ± 0.180	0.962
22	<0.001	2.11 ± 0.259	**1.26** ± 0.218	0.888
28	<0.001	2.02 ± 0.120	**0.87** ± 0.123	0.937
Pooled	<0.001	2.14 ± 0.157	**1.40** ± 0.123	0.896

$CR = a\ AFDW^b$.

TABLE 2.

Parameters of the power equation expressing clearance rate (CR) as a function of ash-free dry weight (AFDW) including only the larvae longer than 140 μm ("fully active").

T (°C)	p	ln *a*	*b*	r
16	<0.05	2.3 ± 0.63	**1.4** ± 0.55	0.786
22	<0.01	2.15 ± 0.146	**1.16** ± 0.156	0.966
28	<0.001	1.96 ± 0.067	**0.62** ± 0.090	0.942
Pooled	<0.001	2.01 ± 0.208	**1.00** ± 0.213	0.684

Symbols as in Table 1.
$CR = a\ AFDW^b$; length > 140 μm.

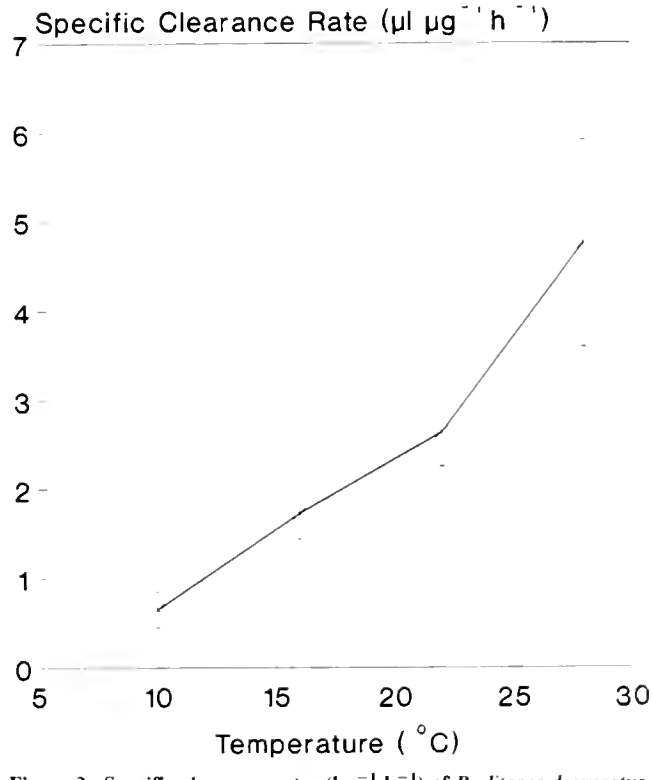

Figure 3. Specific clearance rates ($l\ g^{-1}\ h^{-1}$) of *Ruditapes decussatus* larvae as a function of rearing temperature.

TABLE 3.

Specific clearance rates (SCR, $\mu l\ g^{-1}\ h^{-1}$) of larvae reared at different temperatures (T, °C).

T (°C)	P.I SCR	n	P.II SCR	n	P.III SCR	n
10	0.65 ± 0.203	24				
16	1.04 ± 0.191	18	2.3 ± 0.40	21		
22	2.4 ± 0.49	6	2.7 ± 0.43	30		
28	2.52 ± 0.195	3	6.3 ± 1.80	21	2.59 ± 0.148	12
	p = 0.003**		p = 0.020*			

P.I, from D-stage larva to 140 μm length; P.II, from 140 μm to the beginning of metamorphosis (240 μm) and P.III, metamorphosis and early postlarva. **, $p < 0.01$; *, $p < 0.05$.

served with SCR was confirmed in SIR (Table 4). In P.I the thermal effect was highly significant ($p = 0.0099$) and the homogeneous groups were 10–16°C and 16–22–28°C, whereas in P.II the effect was much lower ($p = 0.077$; n.s.).

Both CR ($\mu l\ h^{-1}$) and IR (cells h^{-1}) could be expressed as a function of temperature (T°C) and ash free dry weight (AFDW, μg) by means of multiple regressions:

$$\text{Ln CR} = 2.2\ \text{Ln AFDW} + 0.58\ T - 0.032\ T^2 + 0.0007\ T^3 - 0.11\ \text{T.AFDW}\quad r = 0.906;\ p < 0.0001$$

where all the variables were significant with $p < 0.05$ for T^2 and T^3 and $p < 0.01$ otherwise, and,

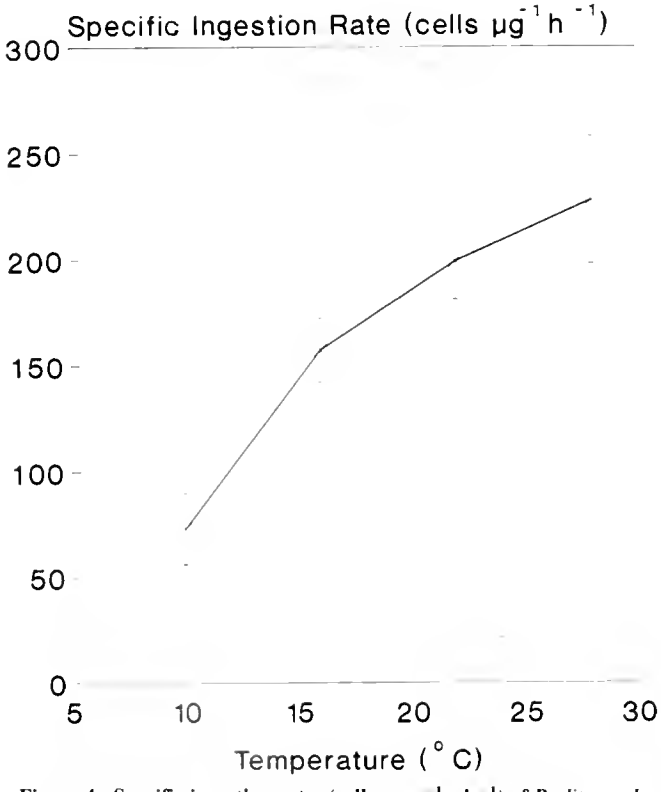

Figure 4. Specific ingestion rates (cells · $\mu g^{-1} \cdot h^{-1}$) of *Ruditapes decussatus* larvae as a function of rearing temperature.

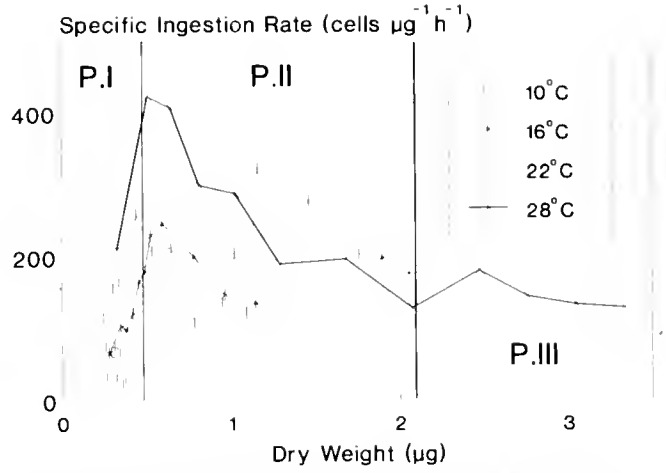

Figure 5. *Ruditapes decussatus*. Specific ingestion rates (cells $\mu g^{-1}\ h^{-1}$) at each rearing temperature (28 (×), 22 (+), 16 (*) y 10°C (□)) during the three trophic periods defined by Gerdes 1983: P.I., endotrophic period; P.II., exotrophic period; P.III., metamorphosis and early postlarva.

$$\text{Ln IR} = 1.49\ \text{Ln AFDW} + 1.09\ T - 0.056\ T^2 + 0.00095\ T^3 - 0.060\ \text{T.AFDW}\quad r = 0.996;\ p < 0.0001$$

where all variables were highly significant, with $p < 0.01$ for interaction and $p < 0.0001$ otherwise.

Nevertheless it must be assumed that the multiple regression models are best regarded as descriptive tools, but they are useless for predictions in situations where additional variables are exerting an effect (Newell and Branch 1980).

Growth Efficiency

Gross growth efficiency (K_1) ranged between 40 and 80%. The efficiency of conversion of the ingested food into organic biomass increased with rising temperatures. Larval development at 28, 22 and 16°C was completed with 73, 65 and 52% K_1, respectively. The K_1 is only 33% at 10°C in the early stages of development.

Table 5 shows the K_1 dividing the larval development into the previously mentioned periods. At the temperatures which supported rapid development (22 and 28°C), larvae underwent the first, mixotrophic, period in 5 days and the growth efficiencies were extremely high (75–80%). The second period (P.II),

TABLE 4.

Specific ingestion rates (SIR, cells $\mu g^{-1}\ h^{-1}$) of larvae reared at different temperatures (T, °C).

T (°C)	P.I SIR	n	P.II SIR	n	P.III SIR	n
10	73 ± 16.9	24				
16	122 ± 17.6	18	187 ± 16.4	21		
22	210 ± 51	6	198 ± 21.3	30		
28	211 ± 9.8	3	280 ± 42	21	147 ± 11.8	12
	p = 0.009**		p = 0.077 (n.s.)			

P.I, from D-stage larva to 140 μm length; P.II, from 140 μm to the beginning of metamorphosis (240 μm) and P.*III, metamorphosis and early postlarva. **, significant differences $p < 0.01$; n.s., not significant differences.

TABLE 5.

Gross growth efficiencies (%) at each temperature during the three trophic periods: P.I., endo and mixotrophic; P.II., exotrophic; and P.III., metomorphosis and early postlarva.

T (°C)	P.I (D-140 μm)	P.II (140–240 μm)	P.III (240–260 μm)
10	33	n.r.	n.r.
16	48	52	n.r.
22	77	65	n.r.
28	78	73	18

n.r., not recorded.

exotrophic, covers the 150–240 μm range in length and is characterized by a maximum and steady weight-specific ingestion rate which is the only energy source for metabolism. Growth efficiency varies between 50 and 70%, clearly increasing with high temperatures. When larvae undergo metamorphosis (about 240 μm), K_1 markedly decreases. Clams reared at 28°C, with 240–260 μm length, show a reduced K_1, 18%.

DISCUSSION

Growth

Growth in larval length (Figure 1) adjusted well to straight lines whose slope is the growth rate (GR, $\mu m \cdot day^{-1}$), as previously stated by Walne (1966). Experimental data reflected a linear relationship between temperature (T) and growth rate within the interval studied. This effect agrees with data from Pérez et al. (1977) with *Venerupis pullastra* larvae at 14, 18, 22 and 26°C and also data from Loosanoff and Davis (1963) with *Mercenaria mercenaria* between 18 and 30°C, as shown in Figure 6.

The optimal growth temperature of bivalve veliger larvae, has always appeared to be higher than those normally encountered in the natural environment and is also greater than the optimum for adults of the same species. For example, maximum larval growth was found at 31°C in *Cardium glaucum* (Bruguiere) (Kingston 1974), 30°C in *M. mercenaria* (Loosanoff and Davis 1963), 28°C in *R. decussatus* (present paper), 26°C in *V. pullastra* (Pérez et al. 1977), between 25 and 28°C in *Ostrea edulis* (Walne 1965; Davis and Calabresse 1969), between 17 and 22°C, depending on the geographical origin, in *Mytilus edulis* (Bayne 1965), and at 16°C in *Chlamys hastata* Sowerby (Hodgson and Bourne 1988).

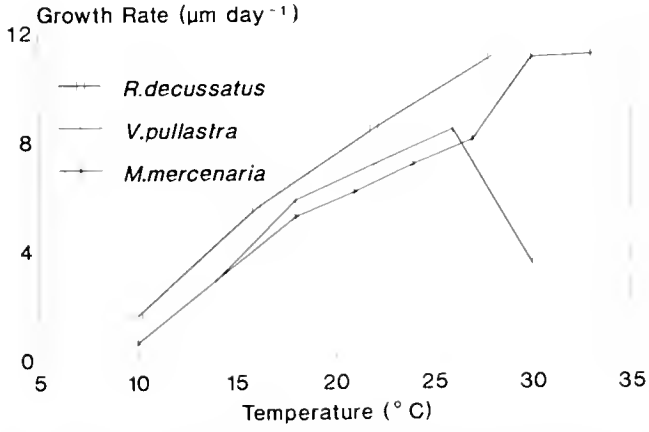

Figure 6. Growth rates ($\mu m\ day^{-1}$) of larvae of three Veneridae species as a function of rearing temperature. (□) *Ruditapes decussatus* (present paper); (+) *Venerupis pullastra* (data from Pérez et al. 1977); (*) *Mercenaria mercenaria* (data from Loosanoff and Davis 1963).

The relative organic content remained constant during larval development or even showed a slight decrease, as exponents in the allometric equations show (3.01 ≈ 3.03), in agreement with the intensive research undertaken by Lucas et al. (1986), and despite previous results by Holland and Spencer (1973) with *O. edulis* and those from Jespersen and Olsen (1982) with *M. edulis*. Sprung (1984a) and Lucas et al. (1986), found that the variation of relative organic content during mussel larval development followed a U-shaped line. They observed higher minimum values and almost constant patterns of organic content in the best fed batches. This appears to be true for *R. decussatus* larvae in the present paper and has been confirmed in another study (Pérez et al. 1991) under different environmental conditions. Therefore, the constancy of the organic content near 40% throughout the entire larval period appears to be a consistent feature in well fed bivalve veligers.

Feeding Rates

Feeding rates were measured under food levels designed to maximize actual larval growth. Initial algal concentration was 100 *I. galbana* cells μl^{-1} (2 mg l^{-1}), a food level universally used in the hatchery procedures and close to the natural availability in the Galician Rias (NW Spain) during the reproductive season of this species (1–2 mg l^{-1} total particulate matter and 0.5–1 mg l^{-1} particulated organic matter, Navarro et al. 1991). Although high algal concentrations, such as those used in the present paper, reduce the larval CR (e.g. Jespersen and Olsen 1982), maximum IR and rapid growth are obtained at these cell concentrations (Beiras 1992). Besides, considering the low efficiency of particle capture in veliger larvae (our own microscopic observations, see also Gallager 1988), CR is an unsuitable concept to describe feeding behaviour of mollusc larvae as it does not correspond to the total volume of water actually exploited by the larva.

Total ash free dry weight content is an accurate indicator of the feeding rate of veligers, as has been pointed out in oyster larvae (Beiras et al. 1990) because it excludes the inert parts of the larva both in the shell and in the visceral mass. Clearance and Ingestion have an almost isometric relationship with organic weight, however feeding rates are often expressed as a function of the total dry weight, which is easier to calculate. Both adjustments show equivalent b exponents when, as in the present experiment, the relative organic content of larvae remains constant during development.

The b exponents in equations shown in Table 1, considering the whole larval period, are substantially greater than those previously recorded in bivalve veligers and they suggest a much higher feeding activity per g in large larvae than in the smaller ones. Although larvae showed food in the gut from the D-stage stage (48 hours after fertilisation) they still have little algae uptake activity until they reach 140 μm size. When larvae smaller than 140 μm are included very high b exponents are found in the clearance allometric equations (Table 1). Therefore, it was more suitable to consider only the fully active larvae in the calculations of the weight dependent clearance rate equations (Table 2). By doing so, exponents are not significantly higher than 1 and are now comparable to previous data on mussel veligers: 0.85 (Riisgård et al. 1981); 0.82 (Jespersen and Olsen 1982); 0.77 (Sprung 1984b) and oyster veligers: 1.11 (Helm, quoted by Bayne 1983); 1.02 (Beiras et al. 1990).

Lucas et al. (1986) also established three different trophic periods in *M. edulis* larvae reared at 17–20°C. An initial endotrophic period of two days, in which energy costs are exclusively covered by the egg vitelline reserves; a transitional or mixotrophic period (6 days) which is distinguished by the rising importance of the feeding activity, and, thirdly, a subsequent exotrophic period supported entirely by the algal food. Moreover, Bayne (1965) stated that mussel larvae do not eat during their first 3 days of life.

Concerning the thermic effect, the clearance rate sustained high values at temperatures near 30°C, in accordance with data by Wilson (1980) and Beiras et al. (1990) both with *O. edulis*. In contrast, Crisp et al. (1985) found a decrease in clearance when larvae of the flat oyster reared at 20°C were exposed to higher temperatures, although this inhibition could be due to a short term larval response. On the other hand, low temperatures blocked larval feeding activity and larval growth consistently, despite copious food availability.

In adult molluscs Dehnel (quoted by Dame 1972) has found the greater Q_{10} at temperatures above and below habitual ones, suggesting a certain compensation ability of the effect of thermal variations, inside the natural environmental span, on physiological rates. Compared to adults, larvae seem to perform better at unusually high temperatures, at least with reference to physiological rates of energy gain and growth.

Growth Efficiency

The high growth efficiency of bivalve veliger larvae has been previously stated by Jørgensen (1952), Walne (1965) and Sprung (1984d) although the present values (Table 5) are among the highest found. Only 65% K_1 observed by Bayne (1975) in mussel larvae and 75% of maximum K_1 recorded by Crisp et al. (1985) in flat oyster larvae are of the same order.

The relationship between feeding level and fish growth have been widely studied and reviewed by Brett (1979). Optimum ration, corresponding to the maximum growth efficiency, is positively correlated with temperature. At low temperatures greater efficiencies are achieved at low rations and vice-versa. Above optimum ration the logarithms of growth efficiency decrease linearly (Paloheimo and Dickie 1966). In the present experiment, ingested rations seemed to be optimal at 28°C, as shown by the high K_1, but they were increasingly above the optimum ration at lower temperatures. This would explain the decrease in growth efficiency at lower temperatures.

From an energetics standpoint, higher K_1 can only be explained through an increase in absorption efficiency or a decrease in the metabolic costs. A decrease in the metabolic loss at high temperatures is extremely unlikely so the only possibility is a positive correlation between temperature and absorption efficiency. It is widely accepted that high temperatures enhance the permeability of biological membranes and so may facilitate the processes of absorption in the gut.

Little is known about the ontogenic changes in the physiological energetics of bivalves, particularly during the larval stages. An attempt to investigate the changes in growth efficiency during veliger development has been made in this study. In the initial mixotrophic period, from D-stage larva to about 150 μm, the egg reserves still play an important energetic role. At the temperatures which supported rapid development (22 and 28°C) larvae undergo this period in 5 days and the growth efficiencies are extremely high (75–80%) (see Table 5). Gut passage time of food should be high because feeding activity is considerably reduced, enabling a very efficient absorption. The second exotrophic period covers from 150 to 240 μm in length and is characterized by a maximum and steady weight-specific ingestion rate which is the only source of energy. Growth efficiency oscillates between 50 and 70% and clearly increases with high temperatures. When larvae undergo metamorphosis (about 240 μm) K_1 displays a sharp decline, which is in agreement with the temporary stop in biomass increase. Therefore clams reared at 28°C, with 240–260 μm length, show a K_1 reduced to 18%.

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MICROSCOPIC ANATOMY OF THE MANTLE OF THE PEARL OYSTER *PINCTADA MAZATLANICA* (HANLEY, 1856).

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ABSTRACT The microscopic anatomy of the mantle of *Pinctada mazatlanica* is described using histological and cytochemical techniques. The typical structure of the mantle of a bivalve was observed, with three folds in the marginal zone. Four different secretory cells were characterized: 1) large secretory cells, lightly basophilic, found in all the epithelia of the mantle which contain carbohydrates, acid proteins, sulfated acid mucopolysaccharides and calcium granules; 2) small secretory cells, highly basophilic, found only in the middle fold which secretes acid mucopolysaccharides; 3) acidophilic secretory cells, found in the periostracal groove and in the shell epithelium, which take part in protein synthesis; and 4) large acidophilic secretory cells found only in the central zone associated with glycogen synthesis. Lipids were found in the epithelia and pallial nerve. Carbonic anhydrase activity was found in the shell epithelium. Alkaline phosphatase activity was found in all epithelia, except the inner epithelium of the outer fold. The presence of specialized secretory cells, carbonic anhydrase and alkaline phosphatase in the outer mantle epithelium can be related to calcium deposition. These observations suggest that this epithelium is the most suitable as graft tissue in pearl culture.

KEY WORDS: Morphology, histochemistry, mantle, shell, pearl oyster, *Pinctada mazatlanica*

INTRODUCTION

In bivalves, the mantle plays an important role as an internal defense mechanism called "nacrezation" (Malek and Cheng 1974). This natural process has been used by man for the development of pearl farming (Tsujii 1960, Alagarswami 1970, Alagarswami and Qasim 1973, Shirai 1981, Coeroli and Mizuno 1985). The process of pearl formation is compared with shell formation because the pearl has the same chemical composition as the inner shell layer. All mollusks with shells can produce pearls, but only those with a nacreous inner layer form pearls of economic value (Robertson 1941).

To understand the characteristics of the tissue responsible for pearl formation, some researchers have made histological and histochemical studies of the mantle of the pearl oysters *Pinctada martensii* (Tsujii 1960) and *Pinctada maxima* (Dix 1972a,b). In Mexico there are two pearl oyster species which have not yet been studied in this way: *Pinctada mazatlanica* and *Pteria sterna*. This study is focused on *P. mazatlanica*, commonly known as "mother of pearl of the Gulf of California." The quality of the pearls produced by this species was a factor of great importance in the founding and colonization of the region (Cariño-Olvera and Cáceres-Martínez 1990, Monteforte and Cariño 1991). Due to over fishing, the abundance of the resource is limited and the population has declined. Knowledge of its biology, ecology, and physiology is also limited. There is no information about the microscopic anatomy of this species, except for the gonad (Sevilla 1969). In this study we have made histological and cytochemical analyses of the mantle tissue with the object of providing knowledge for later studies leading to the culture of pearls in Mexico.

MATERIAL AND METHODS

Samples of *P. mazatlanica* were collected at Isla Espiritu Santo, B.C.S., Mexico in November 1990 and February, June, August, October, and December 1991. The sample sites of the mantle for histological examination are shown in Figure 1.

For fixation, neutral 10% formalin, Zenker's mixture, Carnoy's mixture, absolute ethanol and ice-cold acetone were used, with the conventional process of dehydration and inclusion into paraffin and freezing cuttings (Lynch *et al.* 1972, Humason 1979). Sections of 6 to 8 μm were made and stained using the techniques shown in Table 1.

RESULTS

The typical morphology of a bivalve mantle was found in *P. mazatlanica* with three zones: the marginal, the pallial and the central (Fig. 2), and the mantle isthmus (Fig. 3). Table 2 shows the distribution and secretory function of the cells. In the marginal zone, mucus, periostracum and prismatic layer are secreted. In the outside of the pallial and central zones, the nacreous layer is formed.

Histology

Marginal Zone

This zone is surrounded by a single layer of columnar epithelium. In the connective tissue, there are longitudinal, radial, oblique, and transversal muscle fibers. Collagen fibers in blue were seen by both Gallego's trichromic and Masson's trichromic techniques and in red by the Van Gieson technique. Silver impregnation stained the collagen fibers dark red. Reticular and elastic fibers were not evident. In both inner and outer epithelia, large secretory cells (about 25 by 14 μm) are observed. These cells are globular, cytoplasm lightly basophilic, with a basophilic excentric nucleus ("B1" cells) (Fig. 5). In the epithelium of the periostracal groove and in the shell epithelium, granular acidophilic secretory cells (about 17 by 11.5 μm) were observed ("A1" cells) (Fig. 6). In the connective tissue there are granular and agranular leukocytes, fibroblasts, nerve tissue, and blood vessels (Fig. 2). The inner fold is the largest mantle fold. The surrounding epithelium is columnar, with long cells and basal nuclei. The epithelial cells'

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**COFAA Fellowship

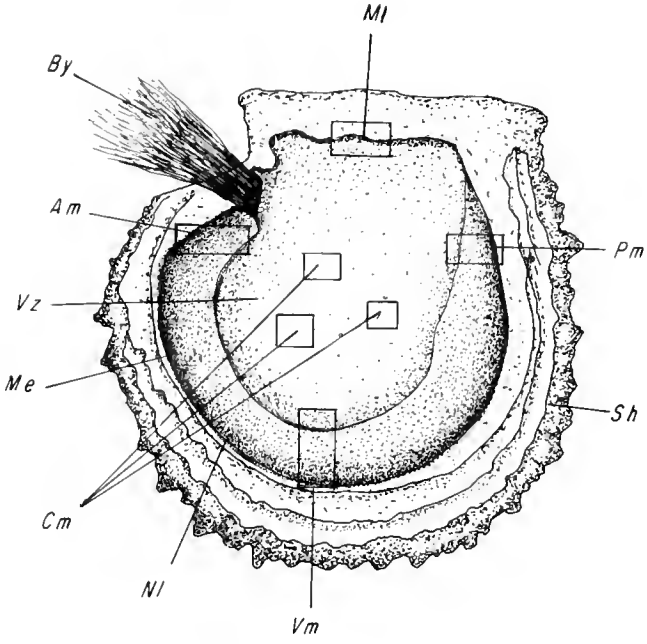

Figure 1. Right valve of *Pinctada mazatlanica*. Sampled zones in the mantle. Am, anterior mantle; By, byssus; Cm, central mantle; Me, mantle edge; NI, nacreous layer limit; Pm, posterior mantle; Sh, shell; Vm, ventral mantle; Vz, visceral zone. MI, mantle Isthmus.

cytoplasm contains melanin granules (Fig. 4). "B1" cells are observed in both inner and outer epithelia. The outer epithelium of the middle fold is ciliated columnar with short cells. The cytoplasm of the inner epithelial cells has melanin granules. "A1" cells were abundant in the bottom of the fold. There are numerous "B1" cells, mainly in the top of the fold. Small cells (about 6 to 10 μm in size) with highly basophilic cytoplasm ("B2" cells) were observed in this fold. An acidophilic secretion observed in the bottom of the fold (periostracal groove) corresponds to the organic material of the shell (conchiolin). The inner epithelium of outer fold, where the periostracal groove is located, is columnar with very long cells. The cytoplasm of these cells contains basophilic granules and basal nuclei. "A1" cells are observed in the inner epithelium (periostracal groove). These cells are less numerous in the outer epithelium. "B1" cells are also observed.

Pallial Zone

The outer epithelium is low columnar and the inner epithelium is ciliated columnar with melanin pigmented cytoplasm. The outer epithelium has more "B1" and "A1" cells than the inner one. Close to the marginal zone, the pallial nerve and the pallial artery are observed.

Central Zone

The outside consists of a simple low columnar epithelium with short cells and a simple cubic epithelium on the inside. In the outer epithelium "B1" and "A1" cells are observed. There are also large acidophilic cells (about 13 by 21 μm) with evident granules ("A2" cells) (Fig. 7).

Mantle Isthmus

The epithelium of the dorsal marginal mantle, also called mantle isthmus epithelium, is columnar, with very tall cells (about 25 to 50 μm) (Fig. 3). This region is characterized by the absence of secretory cells.

TABLE 1.

Techniques used for histological and cytochemical tests.

STAIN FOR	TECHNIQUE	FIXATION	CONTROL	REFERENCE
General morphology	Hematoxylin-Eosine	Neutral 10% formalin		Martoja and Martoja (1970), Lynch et al. (1972)
Collagen fibers	Van Gieson	Neutral 10% formalin		Martoja and Martoja (1970)
Collagen fibers	Gallego's Trichromic	Zenker and Carnoy Mixture		Martoja and Martoja (1970)
Collagen fibers	Masson's Trichromic	Zenker and Carnoy Mixture		Martoja and Martoja (1970)
Elastic fibers	Gallego's Elastic Fibers	Zenker and Carnoy Mixture	Mouse artery	Martoja and Martoja (1970)
Reticular fibers	Silver Impregnation	Neutral 10% formalin	Clam's connective tissue positive to reticular fibers	Lynch et al. (1972), Humason (1977)
Carbohydrates	Periodic Acid-Schiff	Zenker and Carnoy Mixture Absolute ethanol	Diastase glycogen removal Lillie's Acetylation Mouse kidney	Lynch et al. (1972), Humason (1977)
Acid mucopolysaccharides	Toluidine Blue	Zenker and Carnoy Mixture		Spannhof (1966), Lynch et al. (1972)
Acid mucopolysaccharides	Alcian Blue	Zenker and Carnoy Mixture		Spannhof (1966)
Glycogen	Best's Carmine	Absolute ethanol Carnoy Mixture	Diastase glycogen removal Mouse kidney	Spannhof (1966)
Proteins	Ninhydrin-Schiff	Neutral 10% formalin	Deamination Mouse kidney	Spannhof (1966), Humason (1977)
Acid proteins	Alkaline Toluidine Blue	Neutral 10% formalin		Garcia-Dominguez (1977)
Basic proteins	Solid Green	Neutral 10% formalin	Deamination	Spannhof (1966)
Lipids	Oil Red	Neutral 10% formalin		Humason (1977)
Lipids	Red Sudan III	Neutral 10% formalin		Humason (1977)
Lipids	Black Sudan B	Neutral 10% formalin		Humason (1977)
Lipids	Nile Blue	Neutral 10% formalin		Spannhof (1966)
Nucleic acids	Methyl Green-Pyronin	Neutral 10% formalin		Spannhof (1966), Humason (1977)
Nucleic acids	Gothard	Neutral 10% formalin	Removal of RNA by chlorhydric hydrolysis	Martoja and Martoja (1970)
Calcium	Von Kossa	Neutral 10% formalin	Decalcification with citrates buffer pH=4 Mouse partially decalcified femur	McGee-Russell (1957a b)
Calcium	Alizarine S	Neutral 10% formalin	Decalcification with citrates buffer pH=4 Mouse partially decalcified femur	McGee-Russell (1957a b)
Carbonic anhydrase	Kurata	Ice-cold Acetone	Incubation without substrate	Fand et al. (1958), Davenport (1960)
Alkaline phosphatase	Gomori-Takamatsu	Ice-cold Acetone	Incubation without substrate	Pearse (1980)
Melanin	Ferro-Ferricyanide	Neutral 10% formalin	Melanin removal with Peroxide	Humason (1977)

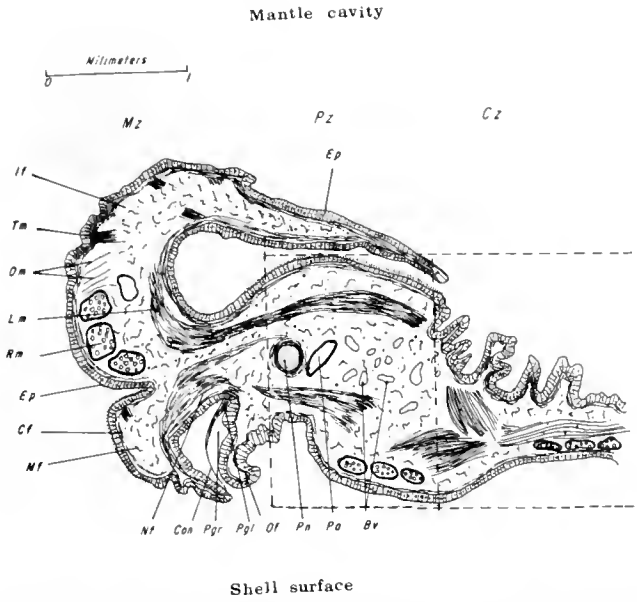

Figure 2. Transversal section of the mantle edge of *Pinctada mazatlanica*. Hematoxylin and eosine (35×). Bv, blood vessels; Cf, collagen fibers; Con, conchiolin; Cz, central zone; Ep, epithelium; If, inner fold; Lm, longitudinal muscles; Mf, middle fold; Mz, marginal zone; Nf, nerve fibres; Of, outer fold; Om, oblique muscles; Pa, pallial artery; Pgl, periostracal gland; Pgr, periostracal groove; Pn, pallial nerve; Pz, pallial zone; Rm, radial muscles; Tm, transversal muscles.

Cytochemistry

Table 3 shows the histochemical reactions in epithelia and cells. Carbohydrates were identified with a positive PAS reaction in epithelia, basal membranes, "B1" cells of the marginal zone, and "A2" cells. All the cells of the pallial zone were negative to the PAS reaction. "B1" cells, positive to PAS, were found mostly in the middle fold (Fig. 8). Glycogen was observed in "A2" cells and in some leukocytes of the connective tissue using PAS and Best's carmine techniques. "B1" cells of the three zones of the mantle were positive for acid mucopolysaccharides using the alcian blue techniques (Fig. 9). The periostracum conchiolin was also positive, but with less intensity. Epithelia with melanin were green with toluidine blue. Other epithelia showed beta metachromasia. Gamma metachromasia was observed in "B2" cells and with less intensity in periostracum conchiolin. Mantle proteins were observed in "A1" cells using ninhydrin-Schiff and solid green techniques. Acid proteins, stained with alkaline toluidine blue, were evident in all epithelia except the epithelium of the inner fold and inner epithelium of the middle fold. "B1" cells of the central zone showed strong metachromasia while the same cells of the middle fold were lightly metachromatic (Fig. 5). Ribonucleic acid was evident in the epithelia and "A1" cells. Lipids were observed in epithelia and slightly in "B1" cells. Calcium was present in "B1" cells, some leukocytes of connective tissue, and epithelia. Decalcified controls were negative to these techniques. A positive reaction for carbonic anhydrase was observed in the outer epithelium of the pallial zone (Fig. 10). Alkaline phosphatase was evident in the epithelia of the pallial zone, the inner fold, middle fold, and outer epithelium of the outer fold (Fig. 11).

DISCUSSION

We observed a morphological and chemical differentiation between the epithelial and secretory cells of the different zones of the mantle. The low columnar epithelium located in the space between middle and outer folds secretes the periostracum. "A1" cells take part in protein synthesis (Beedham 1958, Tsujii 1960, Timmermans 1969). The continuity of these cells with the newly formed periostracum is observed. The presence of "B1" cells in the nacre secretory epithelium of the mantle of *P. mazatlanica* indicates they are associated with shell formation. Other authors have related

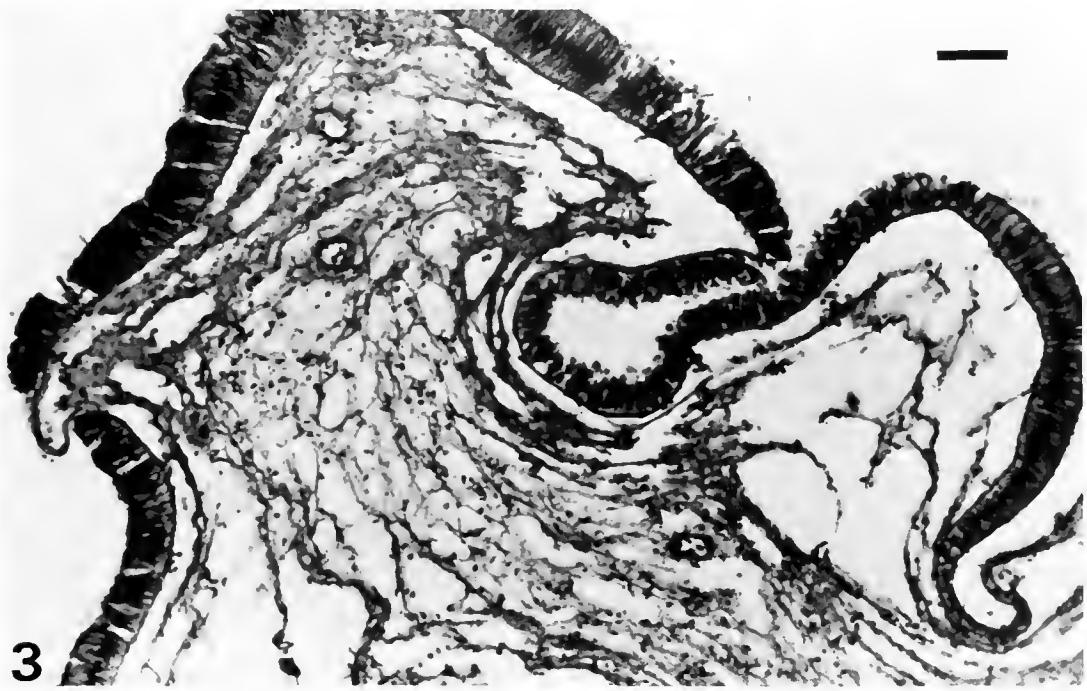

Figure 3. Mantle Isthmus of *Pinctada mazatlanica* Hematoxylin and eosine. (×256) Scale bar = 50 μm.

TABLE 2.

Distribution and secretory function of the cells in the mantle of *P. mazatlanica*.

	CELLS			
	A1	A2	B1	B2
MARGINAL ZONE				
Inner fold				
Inner side	-	-	M	-
Outer side	-	-	M	-
Middle fold				
Inner side	-	-	M	M
Outer side	P	-	P	P
Outer fold				
Inner side	P	-	P	-
Outer side	Pr	-	Pr	-
PALLIAL ZONE				
Inner side	-	-	M	-
Outer side	N	-	N	-
CENTRAL ZONE				
Inner side	-	-	-	-
outer side	N	N	N	-
MANTLE ISTHMUS	-	-	-	-

A1) Secretory acidophilic cells; A2) Acidophilic cells of the central zone; B1) Large secretory basophilic cells; B2) Small secretory basophilic cells of the middle fold; –, absent; P, periostracum secretion; M, mucous secretion; Pr, prismatic layer secretion; N, nacreous layer secretion.

excess calcium and acid mucopolysaccharides in mucous cells with calcium deposition (Tsujii 1960, Bevelander and Benzer 1948, Timmermans 1969, Dix 1972b). Mucous secretion of these cells takes part in the transfer of calcium salts from the mantle to the shell (Love and Frommhagen 1953, Beedham 1958). Morrison (1993) found basal invaginations in many of the epithelial cells of the shell surface of the mantle of *Crassostrea virginica*, and these invaginations are associated with ion transport. In the connective tissue of the gonad of *P. mazatlanica*, we observed amebocytes with calcium, which can be related to calcium transport (Tsujii 1960). The organic matrix of the nacreous layer of the shell has acid mucopolysaccharides and acid proteins, probably in a mucopolypeptide complex, secreted by "B1" cells of the central zone. "B1" cells of the inner and middle folds secrete mucous composed of carbohydrates (not glycogen) and possibly neutral mucopolysaccharides for the lubrication needed for the contraction of extension of the mantle folds. "B2" cells secrete a different type of acid mucopolysaccharide than "B1" cells. It is evident that mucus secreted by the middle fold is composed of more than one type of acid mucopolysaccharide. The organic matrix of the nacreous layer contains basic proteins secreted by "A1" cells and acid proteins and acid mucopolysaccharides both secreted by "B1" cells. As in *P. maxima* (Dix 1972b), in *P. mazatlanica* proteins were found in acidophilic cells (they were basic proteins) and acid mucopolysaccharides in basophilic cells.

Galtsoff (1964) and Dix (1972a) suggest the inner layer of the ligament is secreted by the columnar epithelium of the mantle isthmus. In *P. mazatlanica*, the ligament is composed of a mucopolypeptide complex. In this area, associated secretory cells are absent. Beedham (1958) suggested that this absence of glands may be related to the association between the epithelial cells and the inner ligament matrix. The tall epithelial cells form a secretory

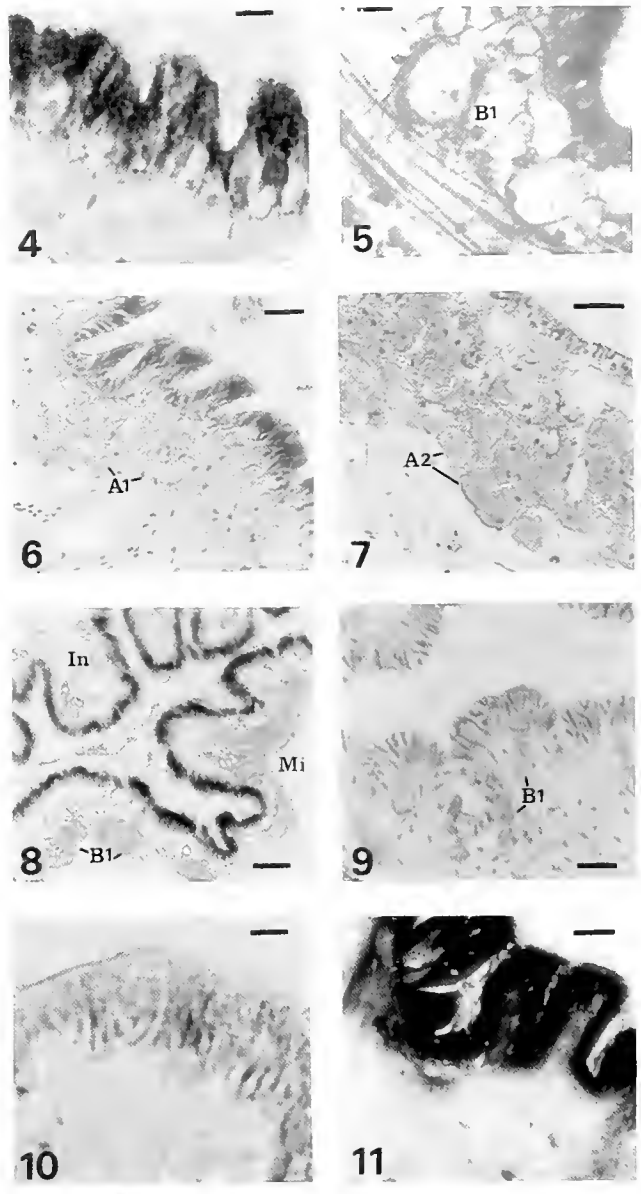

Figure 4. Marginal zone of the mantle of *Pinctada mazatlanica*. Inner epithelium of the middle fold. Hematoxylin and eosine. (×500) Scale bar = 5 μm.

Figure 5. Marginal zone of the mantle of *Pinctada mazatlanica*. Middle fold. "B1" cells. Alkaline toluidine blue. (×400) Scale bar = 10 μm.

Figure 6. Marginal zone of the mantle of *Pinctada mazatlanica*. Inner epithelium of the outer fold (periostracal groove). "A1" cells. Hematoxylin and eosine. (×160) Scale bar = 25 μm.

Figure 7. Central zone of the mantle of *Pinctada mazatlanica*. Outer epithelium. "A2" cells. Hematoxylin and eosine. (×160) Scale bar = 35 μm.

Figure 8. Marginal zone of the mantle of *Pinctada mazatlanica*. In, inner fold; Mi, middle fold. "B1" cells. Periodic acid-Schiff (PAS). (×256) Scale bar = 25 μm.

Figure 9. Pallial zone of the mantle of *Pinctada mazatlanica*. Outer epithelium. "B1" cells. Alcian blue. (×256) Scale bar = 25 μm.

Figure 10. Pallial zone of the mantle of *Pinctada mazatlanica*. Outer epithelium. Kurata's technique for carbonic anhydrase. (×500) Scale bar = 10 μm.

Figure 11. Pallial zone of the mantle of *Pinctada mazatlanica*. Outer epithelium. Gomori-Takamatsu technique for alkaline phosphatase. (×200) Scale bar = 40 μm.

TABLE 3.

Histochemical reactions in epithelia and cells of the mantle of *P. mazatlanica*

TECHNIQUES	CELLS				EPITHELIA		
	A1	*A2*	*B1*	*B2*	*INNER*	*OUTER*	*ISTHMUS*
PERIODIC ACID-SCHIFF (PAS)	–	+	+ + +	–	+ +	+ +	+
PAS + DIASTASE	–	–	+ + +	–	–	–	–
PAS + ACETYLATION	–	–		–	–	–	–
TOLUIDINE BLUE	–	–	–	+ +	+	+	+
ALCIAN BLUE	–	–	+ + +	–	+	–	+
BEST CARMINE	–	–	–	–	+	–	
BEST CARMINE + DIASTASE	–	+ +	–	–	–	–	
NINHYDRIN-SCHIFF	+ +	+	–	–	+	+	+
NINHYDRIN-SCHIFF + DEAMINATION	–	–	–	–	–	–	–
ALKALINE TOLUIDINE BLUE	–	–	+ +	–	+	+	+
SOLID GREEN	–	–	–	–	–	–	–
SOLID GREEN + DEAMINATION	–	–	–	–	–	–	–
GOTHARD	+ +	–	–	–	+	+ +	+
GOTHARD + HYDROLYSIS	–	–	–	–	–	–	
PYRONINE-METHYL GREEN	+ + +	–	–	–	+	+ +	+
RED SUDAN III	–	–	–	–	+	+	
BLACK SUDAN B	–	–	+	–	+	+	
OIL RED	–	–	–	–	+	+	
NILE BLUE	–	–	+	–	+	+	
VON KOSSA	–	–	+ +	–	+ + +	+ +	+
VON KOSSA + DECALCIFICATION	–	–	–	–		–	–
ALIZARIN S	–	–	+	–	+ +	+	+
ALIZARIN S + DECALCIFICATION	–	–	–	–	–	–	–
KURATA	–	–	–	–	–	+ +	–
KURATA - SUBSTRATE	–	–	–	–	–	–	–
GOMORI-TAKAMATSU	–	–	–	–	+ + +	+ + +	–
GOMORI-TAKAMATSU - SUBSTRATE	–	–	–	–	–	–	–
FERRO-FERRICYANIDE	–	–	–	–	+ + +	–	–

–, negative reaction; +, weak reaction; + +, moderate reaction; + + +, intense reaction.

surface which is able to produce the organic material for the inner ligament secretion.

The glycogen present in the "A2" cells of the central zone suggest that this may correspond to the "vesicular cells" of Galtsoff (1964) and Gabbott and Peek (1990) and the "parenchymal cells" of Combs (1959). Tsujii (1960) found these cells in the mantle of *Pinctada martensii*. These cells may store energy for the calcification process. In other bivalves of the Mytilidae Family, Lozada and Reyes (1981) believe that the glycogen of the mantle may also used for the reproductive process and probably is moved by amebocytes to the gonad during the spawning period. Gabbott and Peek (1990), however, believe that gametogenesis takes place

directly in the mantle. We observed some amebocytes with glycogen in the central mantle but we have no evidence of the transport of glycogen from the mantle to the gonad. There were no adipogranular cells (Gabbott and Peek, 1990) observed in *P. mazatlanica*. Probably, epithelial cells produce some lipids for the organic material of the shell. "B1" cells of *P. mazatlanica* may contain lipids joined to carbohydrates.

The nacreous shell layer epithelium of the mantle of *P. mazatlanica* is probably more efficient in calcium carbonate deposition because it is the only one in which we found carbonic anhydrase activity. This enzyme was found in the mantle and other tissues of many mollusks and plays an important role in the calcification process (Robertson 1941, Freeman and Wilbur 1948, Stolowski 1951, Kawai 1954; 1955, Wilbur and Jordey 1955, Freeman 1960, Tsujii 1960, Timmermans 1969). Although the reaction was negative in the marginal zone and the inner epithelium of pallial zone, this does not necessary mean the absence of the enzyme. The shell epithelium was positive in enzymatic activity in spite of some technique limitations, so enzymatic activity in that area must be high. This suggests the outer epithelium of the pallial zone is the most active area of calcium deposition. Alkaline phosphatase has been associated with the mineralization process in vertebrates, but in invertebrates the role of this enzyme is not clear. However, it has been associated with calcium deposition in mollusks (Maniagault 1939, Bevelander and Benzer 1948, Tsujii 1960, Timmermans 1969) or conchiolin secretion (Beedham 1958, Kado 1960). Because of its location in *P. mazatlanica's* mantle, this enzyme may be related to the calcification process rather than to the formation of the periostracum. The morphological and histochemical characteristics of the outer epithelium of the mantle, mainly the pallial zone, suggest a strong and continuous nacre deposition so, this epithelium could be the most suitable as graft tissue in pearl culture.

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GROWTH AND PHYSIOLOGICAL CONDITION OF THE JAPANESE PEARL OYSTER, *PINCTADA FUCATA MARTENSII* (DUNKER, 1850) IN OHMURA BAY, JAPAN

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ABSTRACT Growth and physiological condition of the Japanese pearl oyster, *Pinctada fucata martensii* were investigated from May to December of 1984 and 1985 in Ohmura Bay, Japan. Mean growth rates (whole weight) of one and two year old pearl oysters were 138–157 mg/day and 68–69 mg/day, respectively. These growth rates were comparatively low as compared with a good growth pearl farm. The slow growth of pearl oysters in the Ohmura Bay pearl farm have been caused by low food concentrations as indicated by phytopigment concentrations, which reflect food quantity.

Red tide occurrences (*Heterosigma* sp. or *Prorocentrum* sp.) in 1985 may have slowed the recovery of meat condition and glycogen stores in pearl oysters after spawning. It is also possible that the temperature stress (above 28–30°C) in the summer may have contributed to the decline of physiological condition and the cause of mortality of pearl oysters weakened by spawning.

KEY WORDS: Japanese pearl oyster, growth, mortality, condition index, filtration rate, glycogen, red tide

INTRODUCTION

Pearl oyster farming has been a very important industry in Japan, with about 170 million Japanese pearl oysters, *Pinctada fucata martensii* (Dunker 1850) cultured in 1991. This represents about 68 metric tons of pearls worth about 85 billion yen. There are many pearl oyster farming areas in Nagasaki Prefecture which supplied approximately 20% of the total Japanese pearl production in 1991. Ohmura Bay (Fig. 1) is one of the largest pearl oyster farming areas in Nagasaki Prefecture.

Recently, pearl oyster growth rates had declined in Ohmura Bay. It had been suggested that the poor growth rates in Ohmura Bay were caused by either poor food quantity and/or quality. The purpose of this study was to determine the effects of seasonal changes in environmental conditions of a pearl farm [water temperature, salinity, dissolved oxygen (DO), suspended solids (SS), particulate organic matter (POM), phytopigments and total carbon in SS] on growth, mortality and physiological conditions [glycogen content of adductor muscles, condition index and filtration rate] of pearl oysters. Phytopigments in the digestive diverticula were measured as indicators of the phytoplankton intake of pearl oysters (Numaguchi 1985).

MATERIALS AND METHODS

Pearl Farm Environmental Conditions

The study site was a pearl farm in an inlet of Ohmura Bay (Fig. 1), where the depth at mean low water (MLW) is about 12 to 13 m. The experiment was carried out from May to December in both 1984 and 1985.

Water temperature and salinity were measured using a salinometer (Type MC 5/2 Model, Electronic Switchger Limited, London, England), and DO was determined with an Oxygen Meter (Model 57, Yellow Springs Instrument Co., Inc., Ohio, USA). Measurements were made at least once each month, from surface to bottom at 1 m intervals.

SS, POM, total carbon in SS and phytopigments were measured as indicators of food availability. By means of a suction pump, sea water was collected at a depth of 2 m (the depth at which experimental pearl oysters were held), and filtered through a (pre-heated to 500°C) GF/C filter (Whatman Ltd, Maidstone, England). The filter was then dried at 110°C for 24 h (SS measurement) and ashed at 500°C for 2 h to determine the ash-free dry weight, which was regarded as POM (Itoh 1978b).

Chlorophyll *a* and phaeopigment concentrations (their sum is defined as phytopigments) were measured by the method of Strickland and Parsons (1968). Total carbon in SS was measured with C · H · N Corder (MT-3 Type, Yanagimoto Co Ltd, Kyoto, Japan). Duplicate or triplicate measurements were made for each sample.

Growth and Physiological Condition of Pearl Oysters

For both year classes (1 and 2), 50–60 pearl oysters were placed in each of 30 net baskets. Baskets were then suspended from rafts at a depth of 2 m at the study site. Pearl oysters and net baskets were cleaned monthly to remove fouling organisms.

At regular intervals (1 month or less), baskets were brought to the laboratory and 27 pearl oysters of each year class were randomly taken across all baskets and cleaned. Twenty of these pearl oysters were used for morphological measurements [shell length, whole weight, dry shell weight and dry (dried for 48 h at 110°C) meat weight]. Four pearl oysters from each year class were dissected and analysed for phytopigments in the digestive diverticula and glycogen in the adductor muscle. Three pearl oysters were used for filtration rate measurements. On each sampling occasion cumulative mortality was determined from 100 pearl oysters deployed in a separate net basket at the study site.

The condition index (the ratio of dry meat weight to dry shell weight) has been a suitable index for assessing the physiological condition (meat condition) of oysters (Brown and Hartwick 1988, Littlewood and Gordon 1988, Rainer and Mann 1992). The condition index was calculated according to the formula of Brown and Hartwick (1988) and the coefficient was used 100 in this experiments:

$$\text{Condition index} = (\text{dry meat weight}/\text{dry shell weight}) \times 100.$$

The digestive diverticula phytopigment (D D pigment) content

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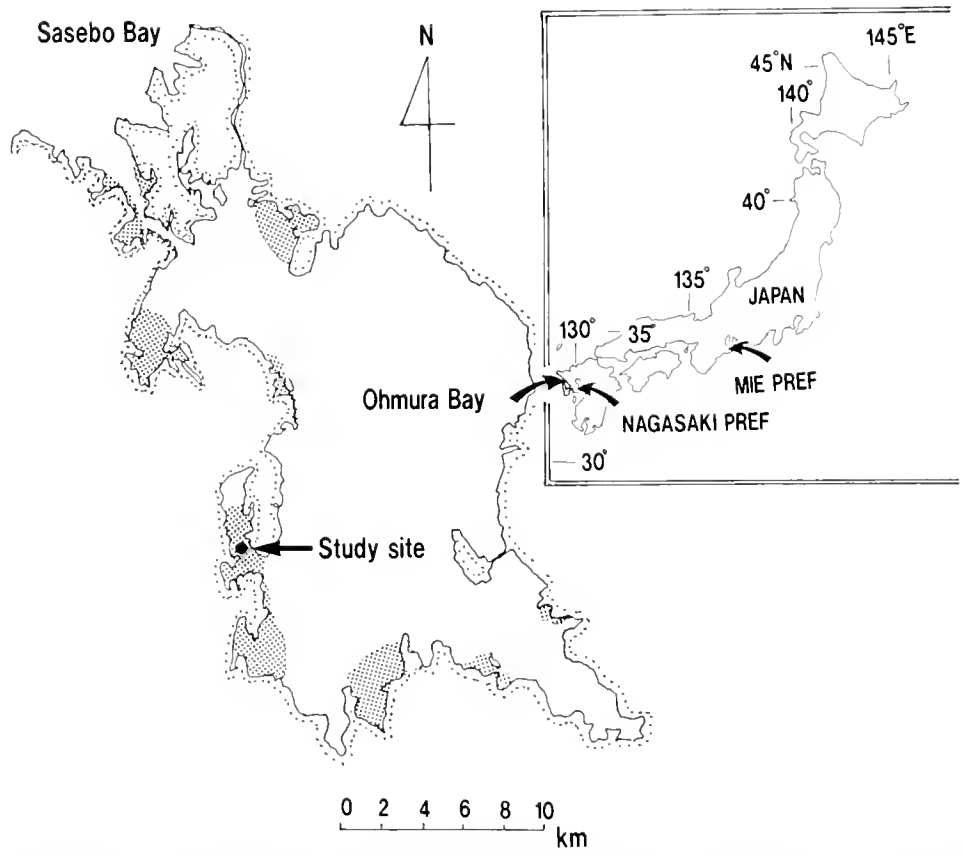

Figure 1. Map showing location of study site in Ohmura Bay, Nagasaki Prefecture, Japan. Shaded areas show commercial pearl farms in Ohmura Bay.

and adductor muscle glycogen concentration were determined immediately after sample collection according of the methods of Strickland and Parsons (1968) and Yoshikawa (1959) respectively. Filtration rates were measured using a method based on that of Jørgensen (1949). Three pearl oysters were pre-conditioned in a 10 l glass chamber for 24 h at a temperature ambient to that of sea water at the pearl farm at the sampling time. The specimens were then placed in another 10 l glass chamber and fed *Pavlova lutheri* (Droop) for 6–10 h (Walne 1972); Changes in algal cell concentration were measured with a Coulter Counter (Type-ZB, Coulter Electronics Inc, Hialeah, Florida, USA). The filtration rate was calculated as follows:

$$F = (\log C_0 - \log C_1)V/\log e \cdot t,$$

where: V is the volume of water available per animal and C_0 and C_1 are the cell concentrations at the beginning and end of the experiment, respectively.

RESULTS

Pearl Farm Environmental Conditions

Seasonal variation in water temperature, salinity, and DO concentration at 2 m depth in 1984 and 1985 are shown in Fig. 2. Both years water temperature increased sharply from May to August, and decreased almost linearly from September to December. In August of both years, water temperature reached a maximum of approximately 29 to 30°C. Salinities ranged 32–34‰ during the study period except for July 1985 when salinity dropped to 28‰ during a period of high rainfall. DO concentration varied from 4.5–6.9 ml l^{-1} at 2 m depth (Fig. 2).

Fig. 3 shows seasonal changes in SS, POM, total carbon in SS, and phytopigments at 2 m depth in 1984. SS values ranged from 2.8–6.7 mg l^{-1} with an average of 4.2 mg l^{-1}. POM values ranged from 0.8–4.2 mg l^{-1} with an average of 1.9 mg l^{-1}. Total carbon in SS ranged from 2.3–15.6% (average value: 7.5%) and the total carbon concentration in seawater was estimated to range from 100–866 μg l^{-1} with an average of 313 μg l^{-1}. Phytopigment concentrations ranged from approximately 1–4 μg l^{-1}, except for a peak in mid June (4.5 μg l^{-1}) and early October (7.1 μg l^{-1}).

Fig. 4 shows seasonal changes in SS, POM and phytopigments at 2 m depth in 1985. The values of SS ranged from 1.6–7.1 mg l^{-1} with an average of 3.4 mg l^{-1}. Seasonal changes of POM and SS were similar and the average value of POM was 2.0 mg l^{-1}. Phytopigments peaked in June (12.7 μg l^{-1}), August (30.6 μg l^{-1}), and October (40.2 μg l^{-1}). In 1985, a small red tide occurred in August and October, and the color of seawater in the pearl farm changed to brown. The actual color shade depended on the causative algal species. Algae that caused the red tide were *Heterosigma* sp. and *Prorocentrum* sp., which were dominant in early August and early October, respectively. The mean phytopigment value was 8.1 μg l^{-1}, while the mean values of SS, POM, and phytopigments during the period without red tides were 2.9 mg l^{-1}, 1.5 mg l^{-1}, and 3.1 μg l^{-1}, respectively.

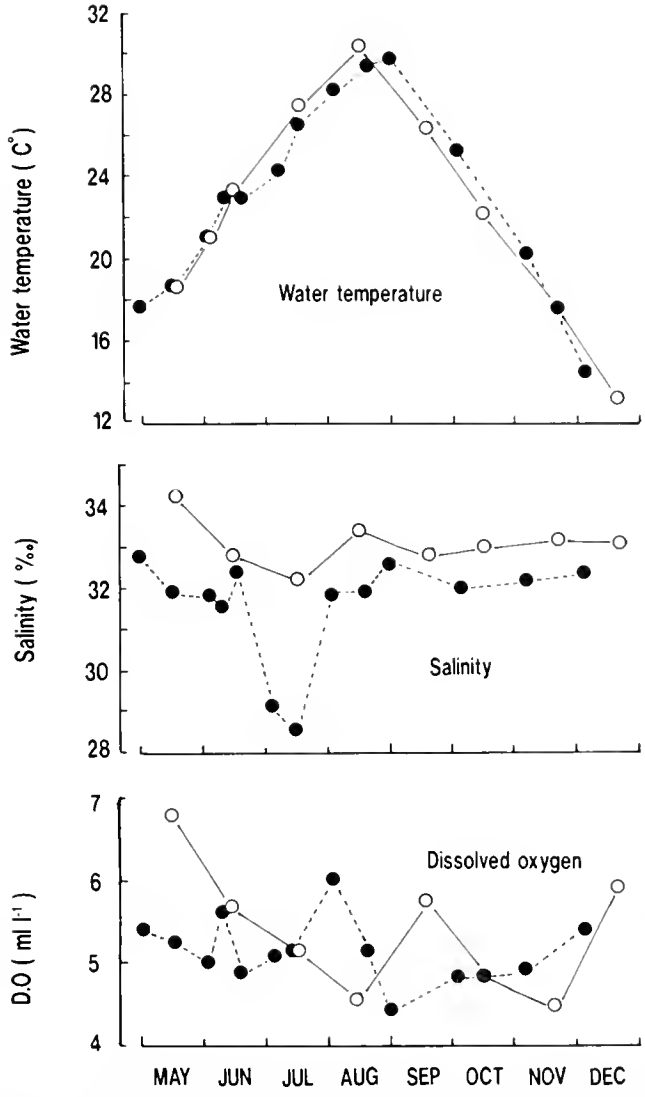

Figure 2. Seasonal changes in water temperature, salinity and dissolved oxygen (DO) at 2 m depth of the pearl farm in 1984 (○) and 1985 (●).

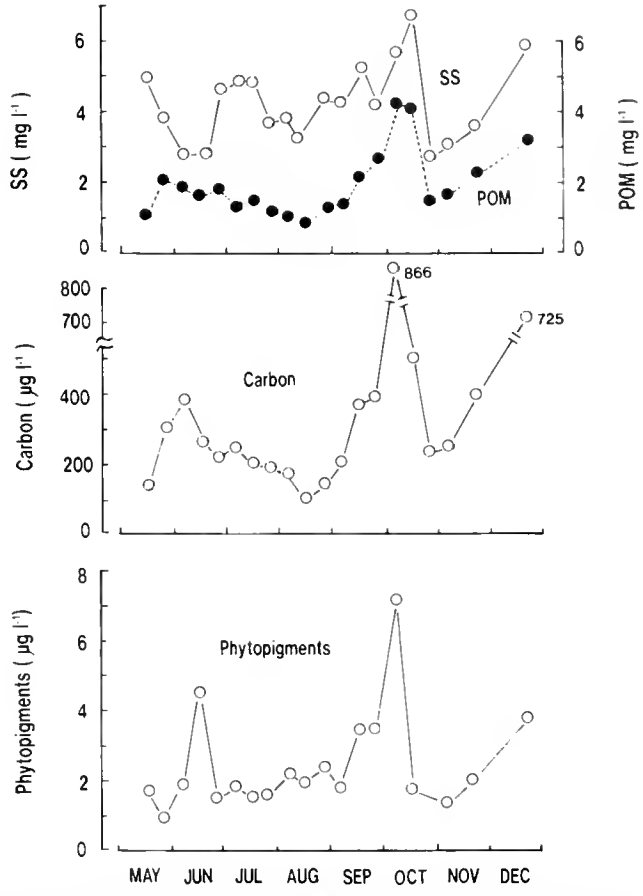

Figure 3. Seasonal changes in suspended solids (SS), particulate organic matter (POM), total carbon in SS, and phytopigments (chlorophyll *a* + phaeopigments) at 2 m depth of the pearl farm in 1984.

Growth and Mortality of Pearl Oysters

Growth curves (whole weight) of both one and two year old pearl oysters were almost the same for 1984 and 1985 (Fig. 5). One year old pearl oysters grew rapidly from June to November 1984 and 1985, but they did not grow from May to June both years. Seasonal growth of two year old pearl oysters was similar, but slower than that of one year old pearl oysters (Fig. 5). The growth rates (whole weight) of one year old pearl oysters were 157 mg/day in 1984 (218 days culture duration) and 138 mg/day in 1985 (190 days culture duration). In the case of two year old pearl oysters growth rates were 69 mg/day in 1984 (218 days culture duration) in 1984 and 68 mg/day in 1985 (190 days culture duration). The growth rate of one year old animals was higher in 1984 than 1985, but there was little difference in the growth rate of two year old pearl oysters between these two years.

Cumulative mortality of one year old pearl oysters was lower than that for two year old oysters (Fig. 6). Mortalities of one year old pearl oysters increased after June 1984 and after October 1985 and cumulative mortality at the end of each experiment were only 5% in 1984 and 6% in 1985. In the case of the two year old oysters, the mortality occurred from August to December and cumulative mortality at the end of each experiment were 14% for both study years (1984 and 1985).

Physiological Condition of Pearl Oysters

Patterns in seasonal changes of the condition index of one and two year old pearl oysters were different between 1984 and 1985 (Fig. 7). In 1984, the condition index of both age groups decreased from June to August, followed by a progressive increase from August to December except for the decrease in one year old pearl oysters in December. In 1985, the condition index of both age groups decreased from June to September, followed by an increase from October to November. It is noteworthy that changes in the condition index of both age groups showed a quite similar trend from the middle of June to early November 1985.

Seasonal changes in the adductor muscle glycogen concentration showed the same patterns in both age groups, but they were different between the two years (Fig. 8). In 1984, the glycogen concentration in one year old pearl oysters decreased from May to June, increased from June to September, decreased from October to November but increased again from November to December.

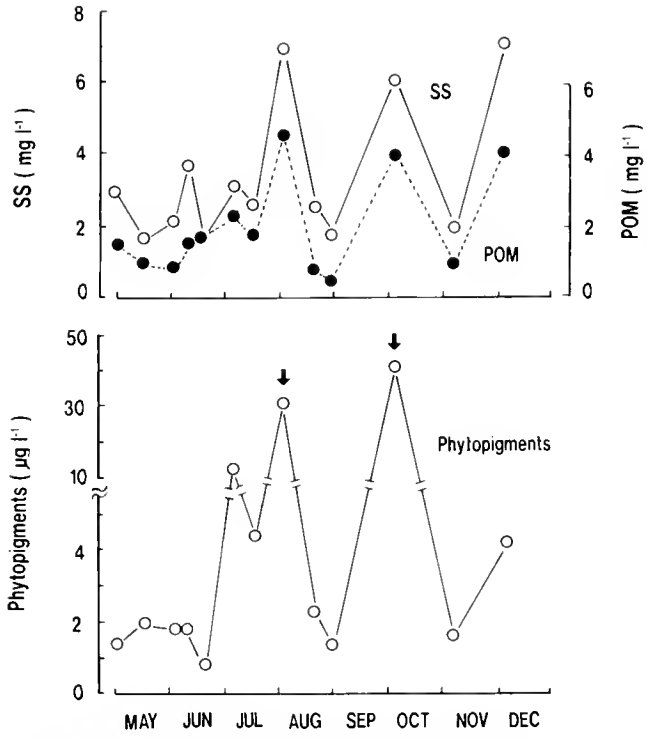

Figure 4. Seasonal changes in suspended solids (SS), particulate organic matter (POM) and phytopigments (chlorophyll *a* + phaeopigments) at 2 m depth of the pearl farm in 1985. Arrows mark periods of red tide bloom.

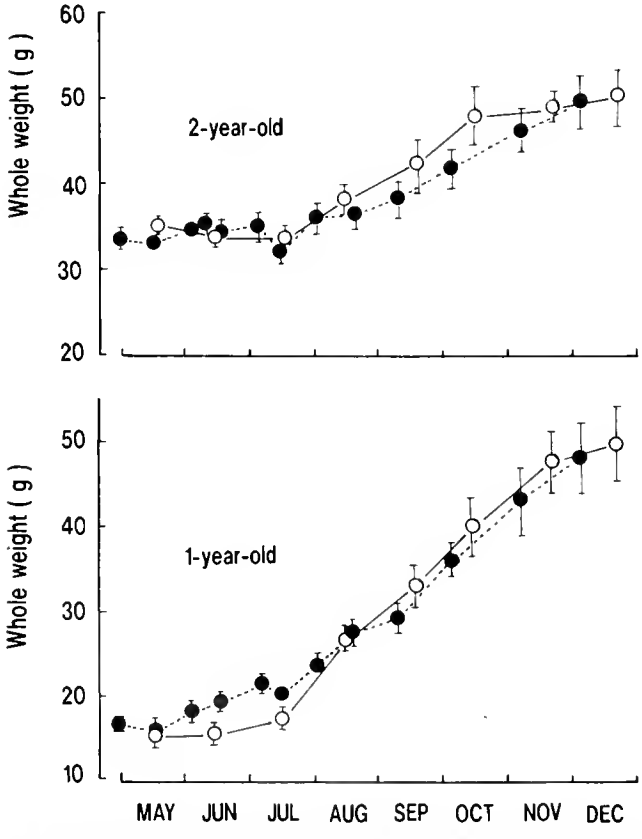

Figure 5. Seasonal changes in whole weight of 1 and 2 year old pearl oysters 1984 (○) and 1985 (●). Values shown are means ± 95% confidence intervals.

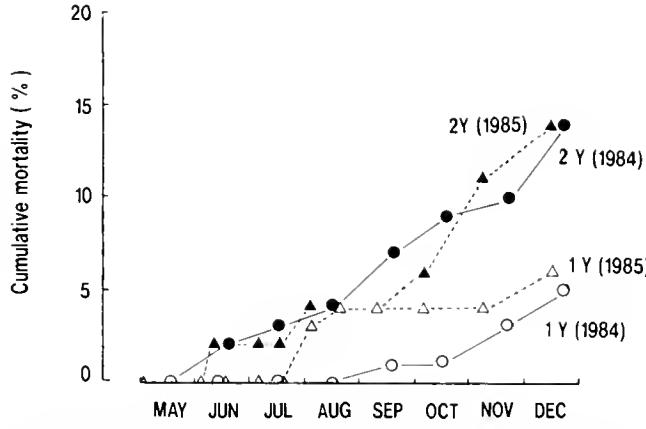

Figure 6. Cumulative mortality of pearl oysters [one (○) and two year old (●)] in 1984 and [one (△) and two year old (▲)] in 1985.

The pattern in two year old pearl oysters in 1984 was similar; glycogen concentration decreased from May to July but increased irregularly from July to December. In 1985, the glycogen concentration in both year classes of pearl oysters showed two peaks in May and June and decreased from June to October.

D D pigment content in one year old pearl oysters increased

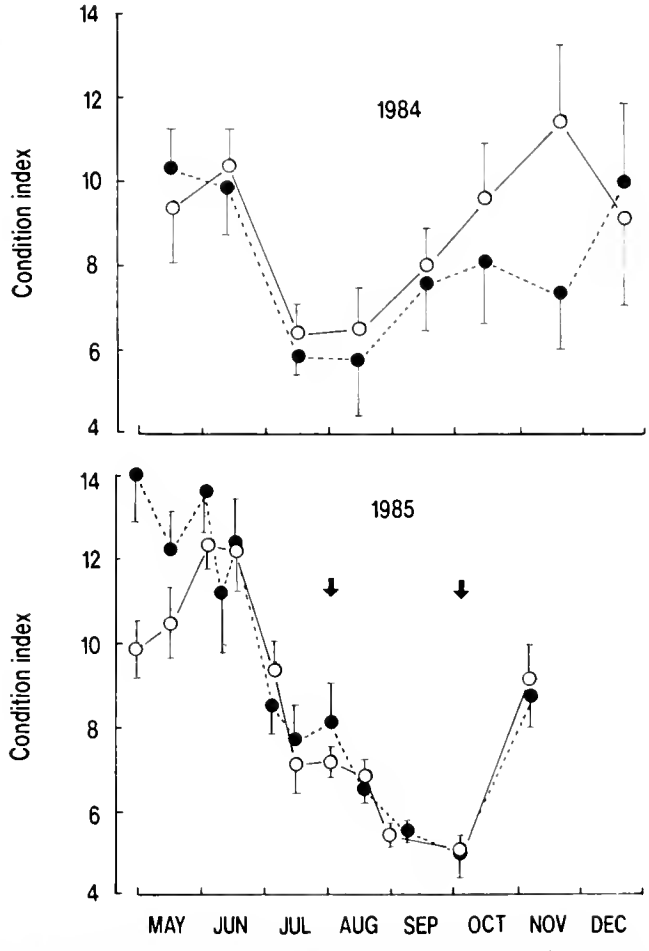

Figure 7. Seasonal changes in the condition index of one (○) and two year old (●) pearl oysters in 1984 and 1985. Values shown are means ± 95% confidence intervals. Arrows mark periods of red tide bloom.

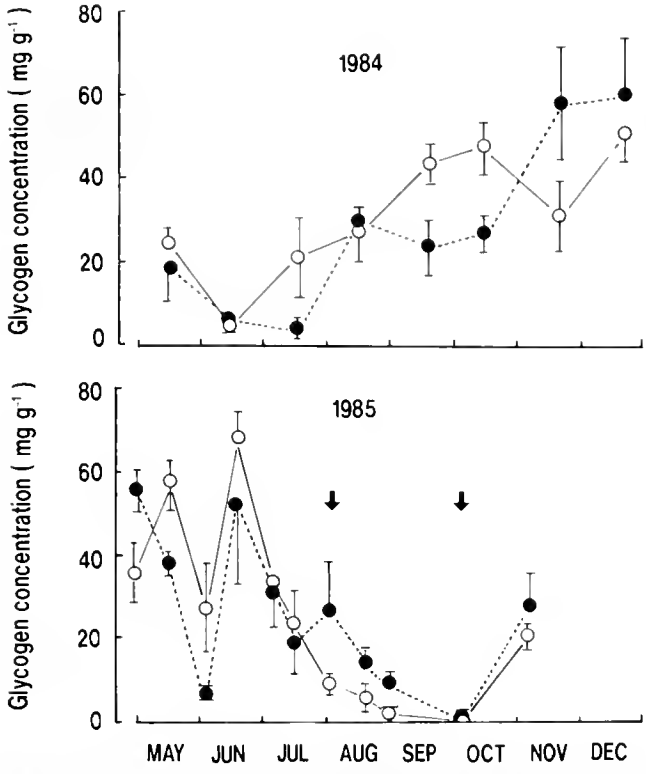

Figure 8. Seasonal changes in glycogen concentration of adductor muscles in one (○) and two year old (●) pearl oysters in 1984 and 1985. Values shown are means ± 95% confidence intervals. Arrows mark periods of red tide bloom.

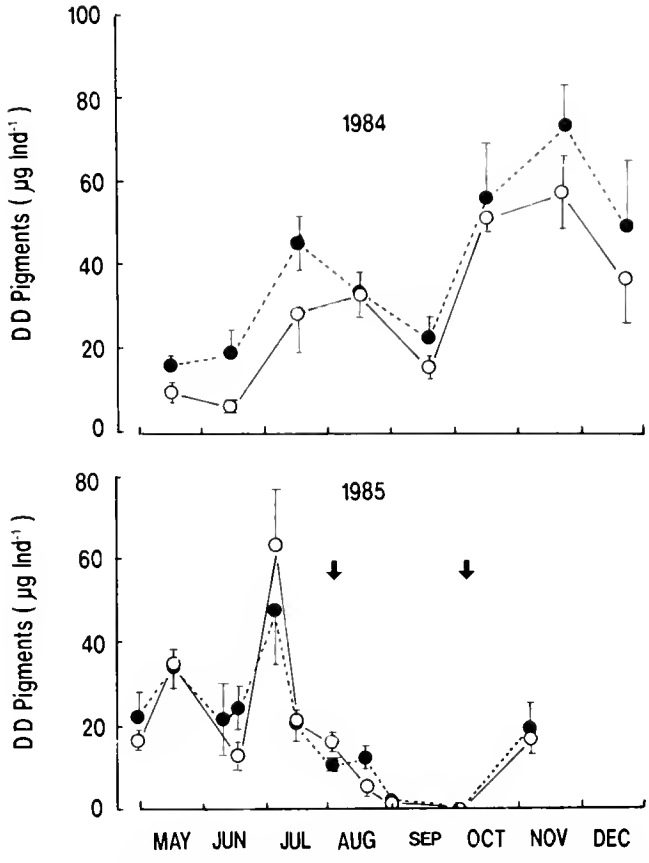

Figure 9. Seasonal changes in the digestive diverticula phytopigment (D D pigment) content of one (○) and two year old (●) pearl oysters in 1984 and 1985. Values shown are means ± 95% confidence intervals. Arrows mark periods of red tide bloom.

from June to August, decreased from August to September, increased from September to November in 1984 and decreased from November to December in 1984 (Fig. 9). In 1985, D D pigment content peaked in May and early July, decreased from May to June and from early July to early October and was at a very low level from the end of August to early October. Seasonal changes in D D pigment content for both 1984 and 1985 were similar for both year classes of pearl oysters.

Seasonal changes in the filtration rate of pearl oysters for 1984 and 1985 are shown in Fig. 10. During 1984, filtration rates increased from May to July and decreased July to December, however seasonal changes for 1985 were severely disrupted by the occurrence of red tides. Filtration rates dropped low in early August and early October 1985, but they increased sharply in August after the red tide had disappeared.

DISCUSSION

During 1984 and 1985 in this study, mortality of pearl oysters in Ohmura Bay was lower at 5–6% in one year old and 14% in two year old pearl oysters (Fig. 6). Neither salinity nor DO levels are likely to have been contributory causes to mortality in this study as these parameters were within the range of tolerance reported for pearl oysters (Yuki 1951, Kobayashi and Matsui 1953, Kobayashi 1955, Miyauchi 1962, Ota and Fukushima 1961, Katada 1958, 1959, Mori 1948, and Miyauchi and Irie 1966).

However, it is conceivable that continuous high water temperature (above 28–30°C) lowers the physiological condition of animals and increase mortality (Okino 1977). The critical upper temperature limit (Kobayashi and Tobata 1949, Kobayashi and Matsui 1953, Kobayashi 1955, 1957, Miyauchi 1962, Uemoto 1968, Okino 1977) was reached in August 1984 and 1985 (Fig. 2). Numaguchi (1994) demonstrated that filtration rates plunged dramatically with increasing water temperature from 28–31°C. This suggests that pearl oysters reduce their food intake when seawater temperature are high. The cumulative mortality of two year old pearl oysters reached only 2–3% until July, while mortality increased after August and reached 14% in December. It is possible that high water temperature (above 28–30°C) in the summer may have contributed to the mortality of pearl oysters.

In 1984 and 1985 growth rates (whole weight) of two year old pearl oysters were 69 and 68 mg/day, respectively. And average phytopigment concentrations were 2.5 and 3.1 μg/l, respectively. Seki (1972) reported higher growth rates for two year old pearl oysters of 109–121 mg/day, and higher average phytopigment concentrations of 4.0–5.1 μg/l from 1967 to 1969 at a pearl farm in Ago Bay of Mie Prefecture. As compared with the pearl farm in Ago Bay, the growth rates and phytopigment concentrations in Ohmura Bay pearl farm were both lower (Fig. 11). These results suggest differences in the overall growth rate of pearl oysters in Ago Bay and Ohmura Bay might be due to the differences in the amount of phytoplankton as food of pearl oysters, which is indicated by phytopigment concentrations, between these two locations.

Higher phytopigment concentrations (Fig. 4; 31–40 μg l^{-1}) were observed during the red tide in August and October 1985 in

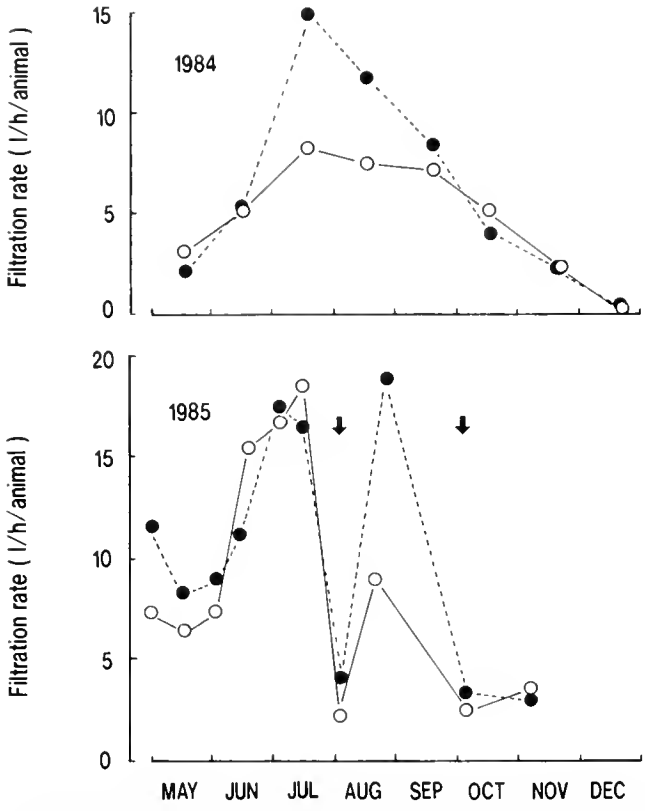

Figure 10. Seasonal changes in filtration rate of one (○) and two year old (●) pearl oysters in 1984 and 1985. Arrows mark periods of red tide bloom.

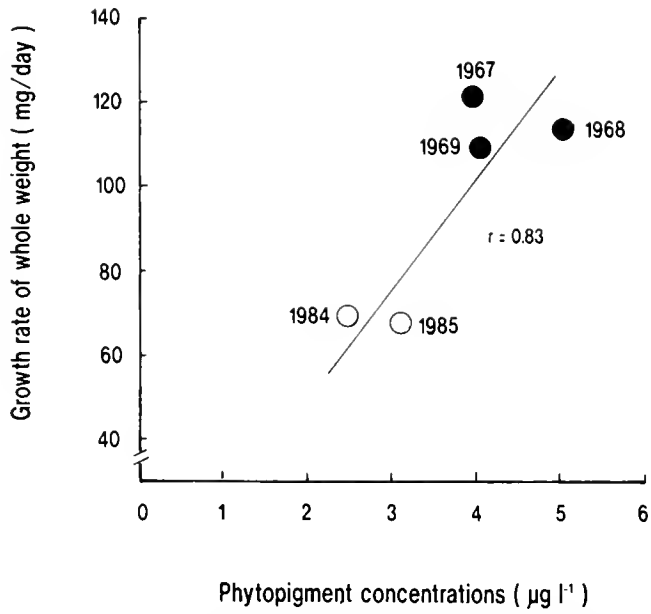

Figure 11. Relation between average phytopigment concentration of pearl farm and growth rates (whole weight) of two year old pearl oysters. Number of symbols show year of investigation. ●; Data of Ago Bay by Seki (1972), ○; Data of Ohmura Bay by present study.

the pearl farm of Ohmura Bay (Fig. 4). Reduction and recovery of the filtration rate of pearl oysters were observed during and after red tide blooms (Fig. 10). High concentrations of red tide and non-red tide phytoplankton have been reported to decrease the feeding rate of oysters (Loosanoff and Engle 1947), which can be measured by D D pigment content in pearl oysters (Numaguchi 1985). Many records of mortality due to red tide blooms has been described in bivalve culture (Widdows et al. 1979, Tracey 1988, Shumway 1990, Heinig and Campbell 1992). In this regard, the present study indicated that red tide organisms, *Heterosigma* sp. and *Prorocentrum* sp. reduced the feeding of pearl oysters, although they did not cause the immediate death of pearl oysters.

Both pearl oyster filtration rates (Fig. 10) and food intakes (as shown by the D D pigment content) were depressed by red tides as occurred in early August and early October 1985 (Fig. 4). This would have rather serious consequences for pearl oysters that need an adequate food supply after spawning, because much energy is consumed during reproductive activity, which causes exhaustion of the animals reserves (Itoh and Mizumoto 1978, Itoh 1978a).

The condition index of the pearl oysters decreased from June to August in 1984, and from early June to late September in 1985 (Fig. 7) and largely coincided with decreasing glycogen stores (Fig. 8), which are an energy source for the maturation and spawning of pearl oysters (Ashikaga 1949, 1951, Tanaka and Hatano 1952). The periods of decreasing condition index and glycogen stores overlapped the spawning season of pearl oysters (Tateishi and Adachi 1957, Uemoto 1958, Yagihara et al. 1959).

The results in this study suggest that the low growth rates of pearl oysters may have been caused by low food concentrations as indicated by phytopigment concentrations, which are a measure of phytoplankton biomass (Fig. 11). The red tide occurrence in 1985 may have slowed the recovery of meat condition (Fig. 7) and glycogen stores (Fig. 8) in pearl oysters after spawning. It is also possible that the temperature stress (Above 28–30°C) in the summer of 1984 and 1985 (Fig. 2) may have contributed to the decline of physiological condition and the cause of mortality of two year old oysters weakened by spawning.

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IN VITRO AND *IN VIVO* EFFECTS OF EIGHT CHEMOTHERAPEUTANTS ON THE OYSTER PARASITE *PERKINSUS MARINUS* (MACKIN, OWEN, AND COLLIER)

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ABSTRACT Eight therapeutants were tested for *in vitro* inhibition of *Perkinsus marinus* (Mackin, Owen, and Collier) enlargement and *in vivo* control of established infections. In addition, acute toxicity of six anticoccidials to oysters was determined. For *in vitro* experiments 0.2 ml aliquots of infected hemolymph were exposed to 5 concentrations (100 mg/l, 10 mg/l, 1 mg/l, 0.1 mg/l and 0.01 mg/l) of amprolium, arprinocid, cycloheximide, lasalocid, malachite green, monensin, sulfadimethoxine, and a potentiated sulfadimethoxine. Exposure lasted 1 day and was followed by incubation in fluid thioglycollate medium. Lasalocid and malachite green were the most effective compounds, showing significant anti-*P. marinus* activity at concentrations as low as 10 mg/l. Cycloheximide, monensin, and to a lesser extent sulfadimethoxine, were also effective but only at the highest concentration tested (100 mg/l). At concentrations lower than 10 mg/l, no compound tested had a significant effect on *P. marinus*. Lasalocid, monensin, and malachite green, were toxic to oysters at concentrations below 10 mg/l. The 96-hr LC50 for lasalocid was 0.59 mg/l. No median lethal dose was obtained for monensin or malachite green, but oyster mortality resulted from exposures ranging from 1 mg/l to 10 mg/l of either compound. In two *in vivo* experiments, infected oysters were exposed to amprolium, cycloheximide, malachite green, and sulfadimethoxine at various concentrations. Only cycloheximide was effective in reducing *P. marinus* infections. After 15 days of exposure to 10 mg/l of cycloheximide, weighted prevalence significantly declined from 3.78 in untreated controls to 2.10 in treated oysters. In addition, infections as measured by repeated hemolymph samples from individual oysters, significantly decreased after treatment. Extension of cycloheximide exposure to 30 days similarly reduced disease prevalence and weighted prevalence. Infections, however, were not completely eliminated even after 30 days of exposure to 10 mg/l of cycloheximide. Furthermore, infections progressed after treatment was discontinued as indicated by an increase in weighted prevalence from 0.71 at the end of treatment to 1.31 one month later.

KEY WORDS: *Perkinsus*, oyster, chemotherapeutants, Apicomplexa

INTRODUCTION

The oyster industry in Virginia has been in serious decline since an MSX epizootic caused large-scale mortality to oysters planted on private leased grounds in the lower portion of the Chesapeake Bay in 1960 (Burreson 1991, Haskin and Andrews 1988, Hargis and Haven 1988). Between 1960 and 1985 *Haplosporidium nelsoni*, the causative agent of MSX disease, was responsible for most of the disease-induced mortality in Chesapeake Bay oysters. However, since 1985, *Perkinsus marinus* (Mackin, Owen, and Collier) has gradually replaced *H. nelsoni* as the most important pathogen in Chesapeake Bay (Burreson 1991, Burreson and Andrews 1988, Andrews 1988a). In addition to disease, overharvesting and water quality deterioration have contributed to the decline of the oyster resource and industry (Hargis and Haven 1988). Nevertheless, disease-induced mortality and reduced plantings in private grounds, because of fear of high losses due to disease, have been cited as the primary factors involved in the rapid decrease in oyster landings during the 1960s and 1980s (Burreson, 1991).

P. marinus has proven to be extremely difficult to eradicate once it has invaded an area and no treatment for individual oysters is available. Low temperature and salinity has been shown to arrest infections, but not in a manner that could be applicable for treatment (Andrews 1988b). Unlike *H. nelsoni*, which can be eliminated by a 2 week exposure to salinities of 10 ppt or less (Ford 1985), *P. marinus* persists after 8 weeks of exposure to salinities as low as 6 ppt (Ragone and Burreson 1993). Control measures that could possibly prevent or reduce the impact of disease on oyster populations in Virginia include management strategies (Andrews and Ray 1988), breeding programs for increased disease resistance (Burreson 1991), and introduction of a more resistant oyster species (Mann et al. 1991). None of these strategies have been fully tested yet, and it is unlikely that their application would exclude the potential usefulness of chemotherapy to treat small lots of oysters that could be held in a bath regime. Applications of such an approach would include treatment of seed and broodstock oysters, as well as oysters held in trays or aquaria for use in aquaculture and research.

There have been only two studies that explored the effects of chemotherapeutics on *P. marinus*. At a time when the parasite was classified as a fungus, Ray (1966a) determined that among 12 antifungal antibiotics, cycloheximide was the most effective compound, completely inhibiting *P. marinus* enlargement when added at 25 mg/l to pieces of infected oyster tissues in seawater. That result prompted an investigation on the effect of cycloheximide on infected oysters (Ray 1966b), which showed that cycloheximide was effective in prolonging the life of *P. marinus*-infected oysters for several weeks longer than untreated controls. Higher concentrations or longer exposure times increased survival time and decreased infection intensities. Conclusions, however, were limited since sample sizes were small (N = 5 in most cases), and no statistical analysis was performed on the data.

The present study focuses on identifying efficacious anti-*P. marinus* compounds that could be used on oysters. The experimental approach was to test chemicals against *P. marinus* cells (*in vitro* experiments), and against infections in oysters (*in vivo* experiments). Chemicals were selected based on existing information indicating efficacy against *P. marinus*, efficacy against coccidians, potential for use in aquatic organisms, and safety considerations. A total of eight compounds were tested. Six of them (amprolium, arprinocid, lasalocid, monensin, sulfadimethoine, and potentiated sulfadimethoxine) are anticoccidials widely used

in the poultry industry. It was reasonable to expect that anticoccidial compounds would be effective in controlling *P. marinus* infections, since *P. marinus* has more recently been classified within the phylum Apicomplexa, which contains coccidians (Perkins 1976, Levin 1978). Indeed, chemicals effective against avian coccidiosis have been especially recommended for use in fish (Meyer and Schnick 1989), and oysters (Perkins 1979). Nevertheless, there is very little information on recommended doses for treatment of aquatic organisms, particularly bivalves. Therefore, *in vivo* studies of the anticoccidials included a toxicity experiment to determine possible lethal concentrations to oysters. In addition to anticoccidials, cycloheximide and malachite green were included at a later stage, after the toxicity study had been conducted. These latter compounds have the disadvantage of being potentially harmful to human health or non target organisms (Bower 1989, Meyer and Schnick 1989). However, they have successfully been used against aquatic pathogens and they constitute good reference standards. Cycloheximide has been used to control *Labyrinthuloides haliotidis*, a pathogen of juvenile abalone (Bower 1989), in addition to its use against *P. marinus*. Bower found that exposure of infected abalone to 1 mg/l of cycloheximide for 5 consecutive days reduced infection prevalence and abalone mortality; exposure to 100 mg/l for 10 days did not affect abalone survival. Malachite green has been included since it has been the most extensively used parasiticide of aquatic organisms (Meyer and Schnick 1989). Exposure of rainbow trout to 1.5 mg/l of malachite green for 1 day prevented the development of proliferative kidney disease (PKD) (Clifton-Hadly and Alderman 1987).

MATERIALS AND METHODS

In Vitro Experiments

Two experiments were conducted to examine the effect of chemicals on *P. marinus* enlargement in fluid thioglycollate medium (FTM). The first experiment consisted of exposing hemolymph from infected oysters to chemical baths containing 100 mg/l of active ingredients of amprolium, arprinocid, cycloheximide, lasalocid, malachite green, monensin, sulfadimethoxine, and potentiated sulfadimethoxine in 0.22 μm filtered York River water (FYRW) of 23 ppt salinity for one day. If 100 mg/l baths significantly inhibited the number of parasite cells enlarging to the prezoosporangia (hypnospore) stage, infected hemolymph was then exposed to 10 mg/l, 1 mg/l, 0.1 mg/l, and 0.01 mg/l baths during a second experiment.

The methodology was based on the hemolymph technique (Gauthier and Fisher 1990), modified to test for effects of chemicals on parasite cells. Infected hemolymph was withdrawn with a syringe from the adductor muscle sinuses of at least 10 oysters (selected from a heavily infected group) until a 10 ml stock was obtained. After mixing the stock, 0.2 ml aliquots were dispensed into 1.5 ml microcentrifuge tubes. Infected hemolymph aliquots were then exposed to 1 ml chemical baths for one day. After exposure to chemical baths, hemolymph aliquots were centrifuged at 265 × g for 15 minutes to concentrate parasite cells in pellets and discard cell free serum. The pellets, containing merozoites, meronts and schizonts of the parasite, were further washed in FYRW and then resuspended in 1 ml FTM containing 0.05 ml of penicillin and streptomycin (2500 units/ml). The inoculated media was incubated at 20°C in the dark for 5–7 days, then samples were centrifuged at 265 × g for 15 minutes and the supernatant was discarded. Pellets were resuspended in 1 ml 2 M NaOH for 15 minutes and then washed twice in distilled water. Pellets were then stained with 2 drops of Lugol's iodine working solution (Gauthier and Fisher 1990). After staining, the volume of each sample was raised to 1 ml with distilled water and dispensed into a separate well of a 24-well tissue culture plate. Enumeration of the total number of recognized prezoosporangia per well was made on an inverted scope at 100× magnification.

Chemical baths containing amprolium, sulfadimethoxine, cycloheximide, or malachite green were freshly prepared by dissolving 10 mg of the chemical in 10 ml of FYRW of 23 ppt salinity and then serially diluting in FYRW to the desired concentration. In the case of highly water insoluble compounds (arprinocid, lasalocid, monensin, potentiated sulfadimethoxine) 10 mg of the chemical was first dissolved in 1 ml of dimethylsulfoxide (DMSO) and then diluted in FYRW.

The experimental design consisted of triplicate treatments and included an untreated control group, and a solvent control group run at 1% DMSO (the highest concentration of solvent used). A one way ANOVA was used to examine differences in mean number of cells between treatments. When differences were detected, a Dunnett's test was performed to examine differences between individual treatments and controls.

Toxicity Experiment

For this experiment, *P. marinus*-free oysters (70 mm mean shell height, 32 g mean whole weight) were obtained from the Wye River in Maryland. After a 2 week acclimation to 25°C and 17 ppt in 1 μm FYRW, a sample of 25 oysters was taken for diagnosis of *P. marinus* in a combined sample of rectal, gill, and mantle tissue taken from each oyster following the method of Ray (1952). Subsequently, the remaining oysters were randomly assigned to aquaria for exposure to chemical baths.

The experimental design consisted of duplicate treatments of 5 oysters per 10 l bath. Oysters were exposed to 4 daily renewal baths of amprolium, arprinocid, lasalocid, monensin, potentiated sulfadimethoxine, or sulfadimethoxine at four concentrations: 0.01 mg/l, 0.1 mg/l, 1 mg/l, and 10 mg/l. In addition, there were 2 control baths, one untreated control and one solvent control at 0.1% DMSO. Stocks of chemical solutions were freshly prepared each day by dissolving 100 mg of active ingredients in 10 ml of DMSO and then adjusting to the desired concentration by dilution in FYRW. Sulfadimethoxine, a highly water soluble compound, was directly dissolved in FYRW.

Oysters were fed a daily ration of diatoms (*Chaetoceros calcitrans* and *Thallasiosira pseudonana*) and/or flagellates (*Isochrysis galbana*) at an approximate concentration of 5×10^8 to 5×10^9 cells/l. Chemical solutions were dispensed into aquaria, following the addition of food, using a 100 ml beaker. Aeration was continuously provided to aquaria. Aquaria were inspected daily, at the time of water change, for dead oysters. Oysters that remained open, after the tanks were emptied for water exchange, were considered dead. When appropriate, the concentration of exposure associated with a 50% mortality at 96 hr (LC50) was estimated by graphic and binomial methods (Gelber et al. 1985).

In Vivo Experiments

There were 2 experiments designed to determine the effect of chemicals on *P. marinus* infections in oysters. Experiment 1 was conducted to determine if amprolium, cycloheximide, malachite green, and/or sulfadimethoxine baths were effective in reducing *P.*

marinus infections. Adult oysters (>50 mm shell height), were collected from Point of Shoals in the James River in September 1992, cleaned of fouling organisms, individually labeled using a water proof marker, and subsequently maintained in a static tank filled with 1 μm FYRW at 20°C and 20 ppt for one week. Water was renewed daily. During that time, 0.3 ml hemolymph samples were withdrawn from each oyster and diagnosed for *P. marinus* following the hemolymph technique (Gauthier and Fisher 1990).

One hundred and eighty oysters, most with light infections, were selected and randomly assigned to 10 l aquaria in groups of ten. Concentrations of chemical baths ranged from 100 mg/l to 1 mg/l. Compounds considered safe, namely amprolium and sulfadimethoxine, were applied as 100 mg/l and 10 mg/l baths. Cycloheximide and malachite green were applied as 10 mg/l and 1 mg/l baths. Since oysters did not survive exposure to 10 mg/l or 1 mg/l of malachite green, another experiment was set up to test 0.1 mg/l and 0.01 mg/l.

All baths, including untreated controls, were tested in duplicate. Stock solutions of the chemicals were prepared immediately prior to use by adding 10 mg, 100 mg, or 1000 mg of active ingredients to approximately 10 ml of FYRW in 50 ml centrifuge tubes. Thorough mixing was accomplished with a vortex blender. There was no need for solvents during *in vivo* experiments since all compounds tested, including amprolium which is 20% water soluble, could be dissolved in FYRW. Daily rations of microalgae suspensions were added to each tube until the volume was raised to 50 ml. Then, the chemical-algae suspension was mixed as before and added to aquaria every other day for 2 weeks.

Dilution water consisted of 1 μm FYRW with a salinity of 22 ppt warmed to 20°C. Aeration was provided continuously. Aquaria were covered with sheets of Plexiglas to avoid possible loss of chemicals at the air interface. On days when chemicals were not added to aquaria, oysters were fed an algae diet as in the previous experiment.

After the 2 week treatment, *P. marinus* diagnosis was performed on a second hemolymph sample and on a tissue sample taken from each oyster. Tissue samples were assayed for *P. marinus* as previously described. Only tissue diagnosis was performed on oysters exposed to the lower concentrations of malachite green, during the follow-up experiment. Infection intensity in tissue samples was determined as described by Mackin (1962), and categorized as negative, light, moderate, or heavy. To calculate weighted prevalence, the following code numbers were assigned to the intensity (I) categories: 0 = negative, 1 = light, 3 = moderate, and 5 = heavy (Paynter and Burreson 1991). Weighted prevalence was calculated as the average value of infection intensity for number of oysters diagnosed in the treatment replicate (Σ In_I/N).

A one way ANOVA was used to examine differences in log transformed weighted prevalence between treatments. Differences in log transformed prezoosporangia counts were examined by a repeated measure ANOVA. A Dunnett's test followed to identify treatments that differed from controls. In addition, a paired *t*-test was used to compare pre-treatment and post-treatment abundance of *P. marinus* cells in the hemolymph samples of oysters from selected treatments.

In experiment 2 the ability of a 30 day cycloheximide exposure to reduce infections in oysters was tested. In addition, the subsequent progression of infections was monitored for 30 days after treatment. Two hundred and fifty oysters (66 ± 7 mm mean shell height) were collected from Point of Shoals in the James River in November 1992, cleaned and 25 oysters were sacrificed for *P. marinus* diagnosis. A second diagnostic sample was taken after 10 days of acclimation to 20°C. The diagnosis was performed on a combined tissue sample as previously described. Subsequently, the remainder of the oysters were labeled, and randomly assigned to one of four treatments: 30 day exposure to 10 mg/l cycloheximide bath, 30 day untreated control, 30 day exposure to 10 mg/l cycloheximide followed by 30 days without treatment, and 60 day untreated control. All treatments were tested in duplicate 25 l aquaria containing 25 oysters each. Chemicals were mixed with algae and added to aquaria, as in experiment 1, for 30 days. Oysters were fed an algae diet, and aeration was provided, as in previous experiments. Dilution water consisted of 1 μm FYRW with a salinity of 20 ppt heated to 20°C. Aquaria were covered as in the previous experiment. Disease prevalence and intensity were determined, as described before, for all treatment replicates at 30 days and 60 days. The effects of treatment and time on transformed prevalence and transformed weighted prevalence were examined by two way ANOVAS.

For statistical analysis of results from *in vitro* and *in vivo* experiments, data were transformed, if necessary, using SuperAnova software (Abacus Concepts Inc. 1989). Prezoosporangia count data and weighted prevalence data were log $(x + 1)$ transformed, and prevalence was arcsin transformed. Normality and homogeneity of variance of untransformed and transformed data were examined, using $SPSS^X$ software (SPSS Inc. 1986), by the Kolmogorov-Smirnov test and Cochran's C test respectively. Data used for subsequent analysis was significantly normal and homogeneous at a confidence level above 90%. ANOVA and Dunnett tests were performed using SuperANOVA software (Abacus Concepts Inc. 1989), *t*-tests were performed using Statview software (Abacus Concepts Inc. 1992).

Amprolium was obtained from MSD-AGVET, St. Louis, MO; lasalocid, sulfadimethoxine, and potentiated sulfadimethoxine from Roche Vitamins and Fine Chemicals, Nutley, NJ; lasalocid, monensin, and arprinocid from Lilly Research Laboratories, Greenfield, IN; cycloheximide from Sigma Chemical Company, St. Louis, MO; and malachite green from Argent Chemical Laboratories, Redmond, WA.

RESULTS

In Vitro Experiments

At 100 mg/l exposure, there was a significant ($P = 0.0001$) difference in prezoosporangia abundance among treatments (Fig. 1). Solvent controls had a higher abundance of prezoosporangia than untreated controls. Therefore, only untreated controls were included in the statistical analysis in order to give conservative results. Results of Dunnett's test indicate that 5 treatments: lasalocid, malachite green, cycloheximide, monensin, and sulfadimethoxine were associated with significantly lower prezoosporangia abundance ($P < 0.05$) than the untreated control. At 10 mg/l exposure, there was also a significant ($P = 0.0001$) difference in prezoosporangia abundance between treatments (Fig. 2). At this concentration, however, only lasalocid and malachite green were associated with a significantly ($P < 0.05$) lower prezoosporangia abundance than untreated controls. At concentrations lower than 10 mg/l, no significant ($P > 0.05$) difference was found among treatments.

Toxicity Experiment

Oysters ($N = 25$) from the baseline diagnostic sample were all negative for *P. marinus*. All solvent control oysters survived 96

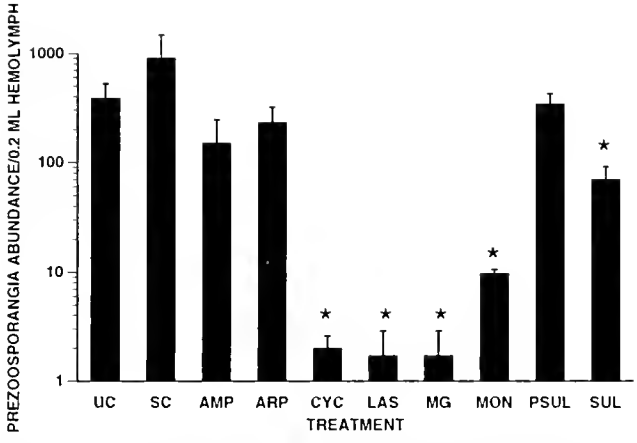

Figure 1. ***In vitro*** **experiment. Mean number of prezoosporangia after exposure to 100 mg/l chemical treatments averaged over 3 replicate 0.2 ml hemolymph samples. Abbreviations: UC = Untreated control, SC = Solvent control, AMP = Amprolium, ARP = Arprinocid, CYC = Cycloheximide, LAS = Lasalocid, MG = Malachite green, MON = Monensin, PSUL = Potentiated sulfadimethoxine, SUL = Sulfadimethoxine. * = Significantly lower ($P < 0.05$) than untreated control (using transformed data). Error bars = Standard errors.**

hour exposure to 0.1% DMSO. Among the chemicals tested in this experiment, only lasalocid and monensin were lethal to oysters. The LC50 for lasalocid was 0.51 mg/l as determined by the graphic method, and 0.59 mg/l with a 95% confidence interval ranging from 0.1 to 10 mg/l by the binomial method. Exposure to 1 mg/l and 10 mg/l of monensin for 96 hours resulted in 10% and 40% mortality respectively.

In Vivo Experiments

At the start of experiment 1, most oysters had light infections (<1000 *P. marinus* cells per 0.3 ml of hemolymph). Comparison of parasite cell abundance in hemolymph samples taken before and after treatment and among treated and untreated groups, revealed that only oysters exposed to cycloheximide had lower infections after treatment than they had before treatment (Fig. 3). Tissue samples showed an overall agreement with hemolymph samples, but intensity estimates were generally higher for tissue samples within each oyster. At the end of the 15 day period, weighted prevalence was significantly ($P < 0.05$) lower in oysters exposed to 10 mg/l of cycloheximide than in untreated controls. Average weighted prevalence was 2.10 and 3.78 respectively. In hemolymph samples, there was a significant ($P = 0.0151$) effect of treatment on prezoosporangia abundance averaged over time. The effect of time alone however, averaged over treatment, was not significant ($P = 0.8674$). Nevertheless, the pattern of change over time was different for different treatments, as indicated by the significant ($P = 0.0001$) effect of the interaction term. In agreement with results from tissue diagnosis, only oysters exposed to 10 mg/l of cycloheximide had a significantly lower ($P < 0.05$) number of prezoosporangia per unit hemolymph than untreated controls. There were no oysters surviving exposure to 10 mg/l or 1 mg/l of malachite green after 15 days. More than 50%, 21 in 40, of the oysters exposed to malachite green were dead after chemicals were renewed four times. During the follow-up experiment, all oysters survived exposure to 0.1 mg/l and 0.01 mg/l of malachite green, but no effect on disease prevalence or infection intensity was detected at those concentrations.

In experiment 2, the first diagnostic sample indicated a 68% prevalence of mostly light infections (weighted prevalence = 0.92) at the time oysters were collected. Ten days after acclimation to 20°C, prevalence rose to 95% and intensities had increased (weighted prevalence = 1.85). Following 30 days of exposure to cycloheximide, infection prevalence and weighted prevalence declined to 67% and 0.71 respectively (Figs. 4 and 5). At that time, oysters exposed to cyclohexmide had fewer and lighter infections than untreated control oysters. The magnitude of the decline in infections, as measured by the reduction in weighted prevalence

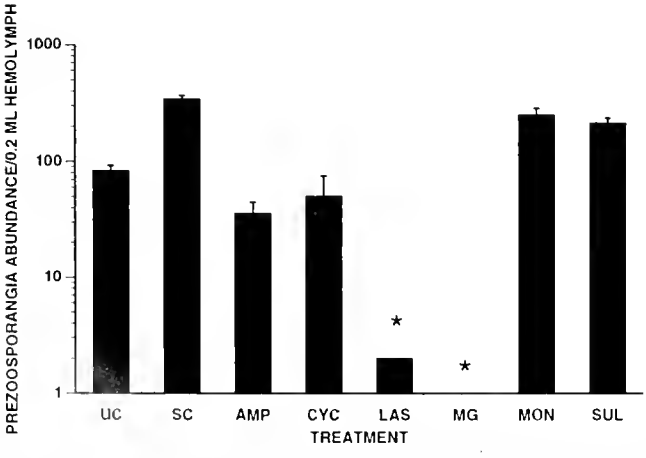

Figure 2. ***In vitro*** **experiment. Mean number of prezoosporangia after exposure to 10 mg/l chemical treatments averaged over 3 replicate 0.2 ml hemolymph samples. Abbreviations: UC = Untreated control, SC = Solvent control, AMP = Amprolium, CYC = Cycloheximide, LAS = Lasalocid, MG = Malachite green, MON = Monensin, SUL = Sulfadimethoxine. * = Significantly lower ($P < 0.05$) than untreated control. Error bars = Standard errors. No error bars shown when replicate samples were equal.**

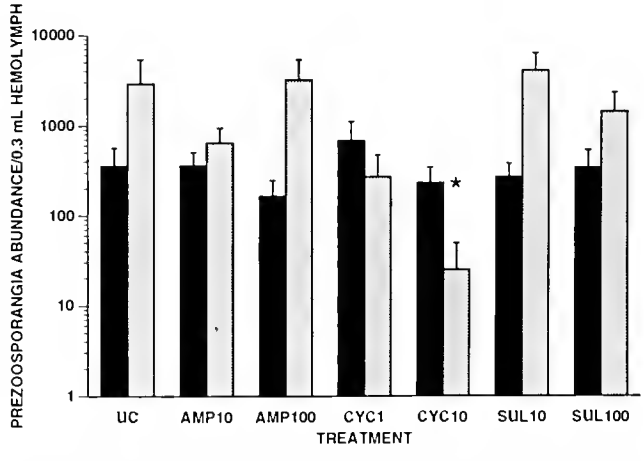

Figure 3. ***In vivo*** **experiment 1. Mean number of prezoosporangia, before and after treatment, per 0.3 ml hemolymph samples averaged over 16–20 oysters. Dark bars indicate pre-treatment values. Light bars indicate post-treatment values. Abbreviations: UC = Untreated control, AMP = Amprolium, CYC = Cycloheximide, SUL = Sulfadimethoxine. Numbers following acronyms indicate concentration of exposure in mg/l. One sample of the CYC1 group had more than 100,000 cells after treatment and was excluded from this chart. * = Final value significantly lower ($P < 0.05$) than initial value, and overall value significantly lower ($P < 0.05$) than untreated control.**

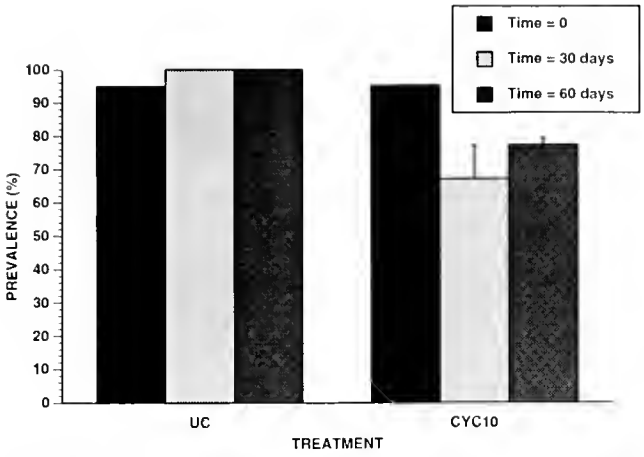

Figure 4. ***In vivo*** **experiment 2. Effect of treatment on prevalence. UC = Untreated control treatment, CYC10 = 10 mg/l cycloheximide treatment. The bar to the left of each group corresponds to the diagnostic sample (N = 25 oysters) taken immediately prior to the initiation of the experiment. Other bars indicate average of 2 replicate groups of 25 oysters. Error bars = Standard deviation. No error bars shown when replicate samples were equal.**

between treated oysters and untreated control oysters after the treatment period, in this experiment, was similar to the one in the previous experiment (i.e. a 1.50 decline as compared to a 1.68 decline, respectively). Two way ANOVAs indicated a significant effect of treatment on prevalence and weighted prevalence ($P = 0.0001$ and $P = 0.0011$ respectively). There was no significant ($P = 0.2350$) effect of time on prevalence (prevalence was already at the maximum at 30 days in controls), but there was a significant ($P = 0.0143$) effect of time on weighted prevalence as weighted prevalence rose both in control and treated oysters alike (Figs. 4 and 5). The interaction effect of treatment and time on prevalence and weighted prevalence was not significant ($P = 0.2350$ and $P = 0.9118$ respectively). There was no adverse effect of cycloheximide on oyster survival. Among 100 oysters treated, only 8 died as compared to 4 that died among 100 untreated controls. Even though sublethal effects were not measured, no overall differences in soft tissue appearance or wet tissue weight between oysters exposed to cycloheximide and untreated controls were observed.

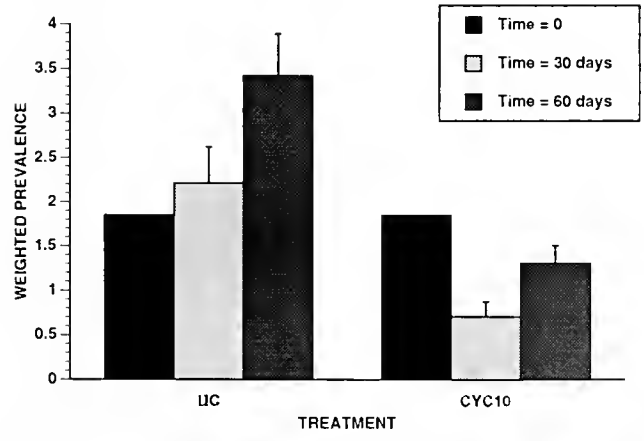

Figure 5. ***In vivo*** **experiment 2. Effect of treatment on weighted prevalence. UC = Untreated control treatment, CYC10 = 10 mg/l cycloheximide treatment. The bar to the left of each group corresponds to the diagnostic sample (N = 25 oysters) taken immediately prior to the initiation of the experiment. Other bars indicate average of 2 replicate groups of 25 oysters. Error bars indicate standard deviation. No error bars shown when replicate samples were equal.**

DISCUSSION

In Vitro Studies

Of the initial eight compounds tested, five (i.e. cycloheximide, lasalocid, malachite green, monensin, and sulfadimethoxine) showed efficacy in inhibiting *P. marinus* cells from enlarging in FTM. Malachite green and lasalocid, followed by cycloheximide, were the most effective compounds. We speculate that the mechanism by which chemicals inhibited *P. marinus* enlargement is related to the general mode of chemical action. Lasalocid, monensin, and malachite green may have been lethal to *P. marinus* cells. Polyether ionosphorus compounds (including lasalocid and monensin) are known to disrupt the electrochemical balance of ions, such as Na^+ and K^+, which are involved in maintaining the selective permeability of membranes thereby having a cytocidal effect (McDougald 1982). Malachite green is a respiratory enzyme poison known to destroy mitochondria (Alderman 1985). In the ease of cycloheximide, a protein synthesis inhibitor highly active against a large number of yeasts including fungal pathogens (Ennis and Lubin 1964), a cytostatic effect is more likely to have occurred in the short term.

Inhibition of *P. marinus* enlargement by cycloheximide has been reported by Ray (1966a). In Ray's study, exposure of infected tissues to 25 mg/l of cycloheximide in FTM completely inhibited prezoosporangia development. In this study, exposure of infected oyster hemolymph to 100 mg/l of cycloheximide in FYRW of 23 ppt salinity for 1 day drastically inhibited subsequent prezoosporangia development in FTM, and exposure to 10 mg/l resulted in some inhibition. Adding chemotherapeutants into FTM, as in Ray's (1966a) study, may result in greater inhibition of prezoosporangia development given the longer time of exposure in FTM (at least 5 days) as compared to the 1 day exposure used in the present investigation. Further comparison of results is complicated because Ray used infected tissues and categorized the degree of inhibition on an ordinal scale.

Perhaps incorporation of chemicals into the culture medium would be a better way for screening compounds *in vitro*, but the biochemical action of antimetabolite anticoccidials such as amprolium and sulfadimethoxine may be neutralized by aminoacids and vitamins present in the medium. Amprolium and sulfadimethoxine are analogs of thiamine and p-aminobenzoate respectively, and presence of the latter compounds inhibits the action of the referred anticoccidials (McDougald 1982).

To some extent, anticoccidials (particularly the polyether ionosphore lasalocid and monensin) showed activity against *P. marinus* as expected based on the proposed affinities of this parasite to the coccidians. However, the fact that compounds that are mostly considered antifungals (malachite green and cycloheximide) were even more effective, indicates that perhaps other antifungals should be tested.

It should be noted that no adverse effect of DMSO was detected on *P. marinus* cells. To the contrary, it appears that DMSO may have prevented cell deterioration, as suggested by the higher cell counts in the solvent control than in the untreated (FYRW) control. Control of microbial contamination by DMSO could account for the increased cell counts in solvent controls. This observation

is supported by the indication that DMSO may not only act as a solvent without interfering with parasite development or host cell integrity, but it may also sterilize *in vitro* assays (Ryley and Wilson 1976).

Toxicity Studies

Among the six anticoccidials tested in the toxicity experiment, only lasalocid and monensin caused oyster mortality when applied at 10 mg/l or 1 mg/l. Oyster mortality due to lasalocid and monensin exposure is not entirely surprising since both chemicals are polyether ionosphorus compounds known for their toxic effects associated with their mode of action (McDougald 1982). It should also be noted that there was no oyster mortality associated with exposure to 0.1% DMSO, suggesting that DMSO can be used as solvent at that concentration or at lower concentrations.

In addition to anticoccidials affecting oyster survival in the toxicity experiment, malachite green was lethal to oysters in experiment 1 (*in vivo*). Similar to concentrations of exposure of monensin and lasalocid, 10 m/l and 1 mg/l of malachite green resulted in oyster mortality while 0.1 mg/l and 0.01 mg/l did not. Even though experiment 1 was not designed to determine median lethal doses, results suggest that acute toxicity of malachite green to oysters may be in the range of that for most freshwater invertebrates (0.51 mg/l–3.45 mg/l) but lower than that for the Asiatic clam *Corbicula leana* (122 mg/l), as reported by Bills et al. (1977). In addition to its destructive action against mitochondria, malachite green is known to be teratogenic in laboratory animals, and its use in aquaculture in the US has not been approved by FDA (Meyer and Schnick 1989).

In Vivo Studies

Among all compounds tested, only cycloheximide was effective in controlling *P. marinus* infections, while not adversely affecting oyster survival. In contrast to exposure to low salinity (Ragone and Burreson 1993), infections did not merely stop progressing, but regressed significantly after exposure to cycloheximide. Both hemolymph and tissue samples confirmed reduction of infections, indicating that chemical exposure had some parasiticidal effect. Short term medication may arrest parasite development whereas long term medication may kill the parasite. This conclusion agrees with results reported for other antiparasitic drugs (McDougald 1982).

Tissue samples generally showed higher infection intensities than hemolymph samples suggesting perhaps that established infections in the tissues were not as easily affected by the chemical as parasites circulating in the hemolymph. Alternatively, higher parasite abundance in the tissues as compared to hemolymph may only be indicative of a difference in sensitivity of the two assays; the tissue diagnostic assay being more sensitive than the hemolymph one, as proposed by Burreson and Ragone (1993).

In agreement with Ray (1966b), experiment 2 showed that exposure of infected oysters to 10 mg/l of cycloheximide for 30 days results in a decrease in infection prevalence and intensity. Experiment 2 clearly defined the effect of cycloheximide on disease prevalence and weighted prevalence. Prevalence declined from 100% (in the untreated control group) to 67% (in the treated group) and weighted prevalence declined respectively from 2.21 to 0.71. The magnitude of decline in weighted prevalence after treatment was similar to experiment 1, suggesting that 15 more days of treatment were not enough to further reduce infections. However, the fact that several light infections of treated oysters comprised only a few cells may have prevented a greater effect from being detected. Thirty days after stopping treatment, prevalence rose to 77% and weighted prevalence increased to 1.31 in the treated group. A similar increase in weighted prevalence occurred in the untreated control group. The mode of action of cycloheximide, as an inhibitor of protein synthesis, probably accounts for the temporary regression of infections during treatment and subsequent recurrence of infections once treatment was removed. Cells that survive treatment may be metabolically inactive during the treatment period. Perhaps a better strategy to control *P. marinus* infections with cycloheximide would be to treat infected oysters for 1–2 weeks to kill metabolically active parasites, withdraw treatment for 1 week to allow "dormant" parasites to develop, followed by another cycloheximide treatment. This schedule could be repeated until infections were eliminated or greatly reduced.

In summary, cycloheximide baths of at least 10 mg/l renewed every other day for 2 weeks are necessary to reduce *P. marinus* infections in oysters held at 20°C and 20 ppt. Extension of treatment for 2 more weeks may not result in eradication of parasites. Infections may relapse once treatment is stopped and mortalities will most likely follow. Given its potential to cause harmful effects in humans, the use of cycloheximide is currently restricted to laboratory applications. All other uses have been canceled by EPA (Carl Grable, Environmental Protection Agency, pers. comm.).

In the future, other effective anti-*P. marinus* compounds that could be used on oysters may be found using the basic approach developed in this study. The recently developed *P. marinus* cell cultures (La Peyre et al. 1993) would facilitate screening of compounds *in vitro*. Studies that follow this line of research, however, should note that the potential applications of chemotherapy to *P. marinus*-infected oysters may be restricted to small lots of oysters, for example important broodstock. It is unlikely that this approach would be practical for aquaculture situations where oysters must be grown "in the field." In nature, disease pressure to *P. marinus* is probably continuous during the warm months, and oysters would be re-exposed to infections after treatment. Furthermore, additional studies concerning the persistence of the chemical in the oyster and in the environment will be necessary to comply with regulatory requirements.

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GROWTH AND MORTALITY OF EASTERN OYSTERS, *CRASSOSTREA VIRGINICA* (GMELIN, 1791), AND PACIFIC OYSTERS, *CRASSOSTREA GIGAS* (THUNBERG, 1793) UNDER CHALLENGE FROM THE PARASITE, *PERKINSUS MARINUS*

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ABSTRACT Stocks of oysters *Crassostrea virginica* and *C. gigas* were produced in the Virginia Institute of Marine Science (VIMS) hatchery in April 1991 and grown in two quarantined flumes receiving water from the York River, VA. From July 1991 through November 1992, growth and mortality of both species were compared. Also, beginning in June 1992 one flume (containing one group of each species) was "dosed" (oysters infected with *P. marinus* added) and the other flume remained "undosed" (no infected oysters added). Disease prevalence and intensity, mortality, and shell height were compared both within (between undosed and dosed groups) and between species. Mean shell height of *C. gigas* was significantly greater ($P \leq 0.05$) than that of *C. virginica* in all but four monthly samples. In November 1992 (at age 19 months), mean shell height of *C. gigas* was 55 mm and that of *C. virginica* was 41 mm. Cumulative mortality of *C. gigas* was 70% compared to 59% for *C. virginica*. Mortality of *C. gigas* occurred primarily in April–May and September 1992 in conjunction with salinity below 20 ppt; mortality of *C. virginica* was greatest in September–November 1992 in conjunction with infection by *P. marinus*. Prevalence and intensity of *P. marinus* infections were greater in *C. virginica* than in *C. gigas*. The dosed *C. virginica* group had 100% prevalence with heavy infections by August 1992; maximum prevalence in the dosed *C. gigas* group was 80% with only 1 heavy infection throughout the study. There were no differences in mean shell height between dosed and undosed groups of either species. Mortality in the dosed *C. virginica* group was significantly greater ($P \leq 0.05$) than in the undosed group in September and October 1992. Thus even though *C. gigas* is more tolerant of *P. marinus* and grows faster than *C. virginica*, a high non-disease mortality may be indicative of a lack of adaptability of *C. gigas* to the environmental factors prevailing in lower Chesapeake Bay.

KEY WORDS: oysters, disease, growth, mortality, *C. virginica*, *C. gigas*, *P. marinus*

INTRODUCTION

Landings of the eastern oyster, *Crassostrea virginica*, along the east coast of the United States have been drastically curtailed by two pathogenic parasites, *Haplosporidium nelsoni* and *Perkinsus marinus* (see Andrews 1988, Haskin and Andrews 1988). In the case of *H. nelsoni*, selective breeding has resulted in strains of oysters that are considerably more tolerant of the disease than non-selected groups (Ford and Haskin 1987). In addition, oysters inhabiting areas of salinity below 12 ppt are protected from the effects of the disease (Haskin and Ford 1982, Ford 1985). The utilization of selected strains and careful monitoring of the salinity and disease prevalence of growing areas represent viable strategies for managing *H. nelsoni* infections (Ford and Haskin 1988).

Unfortunately, oysters selected for tolerance to *H. nelsoni* are not tolerant of *P. marinus* (Burreson 1991). *P. marinus*, being tolerant of a wide range of salinity (Ragone and Burreson 1993), is currently epizootic throughout both Chesapeake Bay and Delaware Bay (Burreson and Andrews 1988, Andrews 1988, Ford 1992). In addition, triploid oysters are as susceptible to *P. marinus* infection as diploids (Barber and Mann 1991). Thus there is no obvious management tool to counteract the effects of this disease, and as a result, oyster landings in the mid-Atlantic continue to decline.

One possible strategy for enhancing oyster production in this region is the utilization of a non-indigenous species, the Pacific oyster, *Crassostrea gigas* (Mann et al. 1991). Much speculation has been offered as to the potential impact that introduction of this species would have on the existing fishery and ecosystem of Chesapeake Bay (Newell 1989, Mann et al. 1991). The only analogies, however, upon which to base predictions of what might happen if *C. gigas* becomes established in the mid-Atlantic come from the West Coast of the U.S., France, New Zealand, and Australia (Ayres 1991, Chew 1991; Dinimani 1991, Goulletquer and Heral 1991). In no previous introduction of *C. gigas*, however, has *C. virginica* been the native species, so prediction of potential species interactions is impossible. It is also difficult to predict survival, reproduction, recruitment, and ultimate geographic distribution of an introduced population merely by comparing prevailing environmental conditions with those in areas where *C. gigas* has been successfully introduced.

This study attempts to provide information relevant to these questions, given the constraints of following the International Council for the Exploration of the Seas (I.C.E.S.) protocol to prevent unwanted introductions. It was previously demonstrated that *C. gigas* is considerably more tolerant of *P. marinus* than *C. virginica* (Meyers et al. 1991). Little is known, however, about other physiological factors that might relate to the suitability of *C. gigas* as an alternate source of oyster production in the mid-Atlantic region. Toward that end, this study was designed as an inter- and intra-species comparison of growth and mortality, under minimum and maximum challenge from *P. marinus*, the major factor presently limiting oyster production in Chesapeake Bay (Burreson and Andrews 1988).

METHODS AND MATERIALS

Broodstock of *C. virginica* was obtained from Nansemond Ridge, James River, VA. *C. gigas* broodstock was offspring of oysters imported from Washington state in 1989 (see Shpigel et al.

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1992). Broodstocks were conditioned in the Virginia Institute of Marine Science (VIMS) hatchery and spawned separately. *C. virginica* broodstock (10 males, 5 females) was spawned 9 April 1991 and *C. gigas* broodstock (12 males, 3 females) was spawned 16 April 1991. Larvae were reared in 400 gal tanks. Water was changed every other day and a mixed diet of *Isochrysis galbana* (Tahitian), *Thalassiosira pseudonana* (3H), *T. weissflogii*, and *Chaetoceros calcitrans* was added 1–2 times per day (Barber and Mann 1991). Eyed larvae were allowed to settle on crushed oyster shell, prior to transfer to upwellers. All water that came in contact with *C. gigas* in the hatchery was chlorinated and dechlorinated prior to release; water leaving upwellers containing *C. gigas* was released on land.

In July 1991, 600 oysters of each species were placed into each of two Nestier trays (total of four trays). One tray of each species was put into each of two flumes, receiving water from the York River, VA at one end and draining from a standpipe at the other end into a settling pond. Water flow into each flume was maintained as consistently as possible (about 20 l/min). Trays within each flume were rotated weekly to reduce the effect of uneven food availability within flumes. Both flumes were drained and flushed as needed to remove fouling organisms and biodeposits. Water temperature in the flumes was recorded (mercury thermometer) several times per week and salinity was continuously monitored from the VIMS pier on the York River. Weekly means for both were calculated from the daily readings.

Beginning in July 1991, and continuing through June 1992, mean shell height was obtained by measuring 100 randomly selected individuals (50 from each tray) of each species on a monthly basis. Mean shell heights of both species were compared for each sampling date using *t*-tests (Zar 1974). Counts of dead oysters were obtained when shell measurements were taken. Finite mortality rates were calculated for each species as the number of oysters that died over each monthly interval divided by the number of oysters alive at the beginning of the interval; cumulative mortality was calculated for each species as the sum of instantaneous mortality rates ($\log_e$ finite rate) (Krebs 1972).

After measuring shell height and counting dead oysters in June 1992, the number of oysters in each group was equalized at 450 oysters for subsequent experimentation. For *C. gigas*, this meant adding some oysters (reared and maintained in the same manner as the original oysters) and for *C. virginica*, this meant removing some oysters. In addition, several dozen live oysters infected with *P. marinus*, collected from Wreck Shoal, James River, VA were then added to one of the flumes to infect one group of each species. These are referred to as the "dosed" groups. The "undosed" groups in the other flume were subject only to potential infective agents entering via the influent. The goal of this approach was to have the "dosed" oysters receive a maximal *P. marinus* challenge and the "undosed" oysters a minimal *P. marinus* challenge.

From July 1992 through November 1992, the shell heights of 50 oysters from each group of both species were measured, and the number of dead oysters was counted on a monthly basis. Mean shell height for all four groups was examined as a function of both species and disease treatment with two-way ANOVA (Zar 1974). Finite mortality rates were calculated for each group as the number of oysters dying within a monthly interval expressed as a percentage of the number of live oysters at the beginning of the period (Krebs 1972). Mortality for dosed and undosed groups of both species was compared using contingency table analysis (Zar 1974, p. 296). Cumulative mortality continued to be calculated, as described above, combining both dosed and undosed groups.

In June, July, August, September, and October 1992, small pieces of gill and rectal tissue were removed from 10 oysters from each group for determination of *P. marinus* prevalence, using the thioglycollate technique (Ray 1952). Disease intensity was reported as light (L), moderate (M), or heavy (H), based on the relative abundance of prezoosporangia found in the thioglycollate cultures (Barber and Mann 1991).

RESULTS

Mean temperature in the flumes ranged from a low of 4.9°C in January 1992 to a high of 29.5°C in July 1991 (Fig. 1). Temperature generally decreased from September 1991 to February 1992 and increased from March to August 1992. Mean salinity in the York River ranged from a low of 17.6 ppt in May 1992 to a high of 23.9 ppt in November 1991 (Fig. 1). Salinity below 20 ppt was recorded from April to July 1992 and again in September 1992.

Mean shell height of *C. gigas* was significantly greater ($P \leq 0.05$) than mean shell height of *C. virginica* in all but four of the months sampled (Fig. 2). Growth in both species was greatest in the fall and spring months and least in the winter and summer months. By November 1991, *C. gigas* had attained a mean shell height that was about 10 mm greater than that of *C. virginica;* this differential was maintained throughout the study. In November 1992, at age 1.5 years, *C. gigas* averaged 55.3 mm in shell height while *C. virginica* averaged 41.2 mm in shell height.

There were clear differences between oyster species in both cumulative mortality and the times at which greatest mortality occurred (Fig. 3). Monthly mortality rates were greatest for *C. virginica* in September (21%), October (31%), and November (14%) 1992. Monthly mortality rates for *C. gigas* were greatest during two periods, April (12%)–May (22%) and September (24%) 1992. At the end of the study in November 1992, percent cumulative mortality of *C. gigas* was 70% and that of *C. virginica* was 59%.

P. marinus was not detected in *C. virginica* in June 1992, prior to dosing (Fig. 4). The dosed group had a 30% prevalence in July; this increased to 100% in August, September, and October. *P. marinus* was first detected in the undosed group in September (78% prevalence) and again in October (100% prevalence). Infection intensity increased rapidly in the dosed group, as heavy infections were seen in the dosed group in August (2), September (3), and October (5); no heavy infections occurred in the undosed group (Table 1). Thus *P. marinus* became readily established and

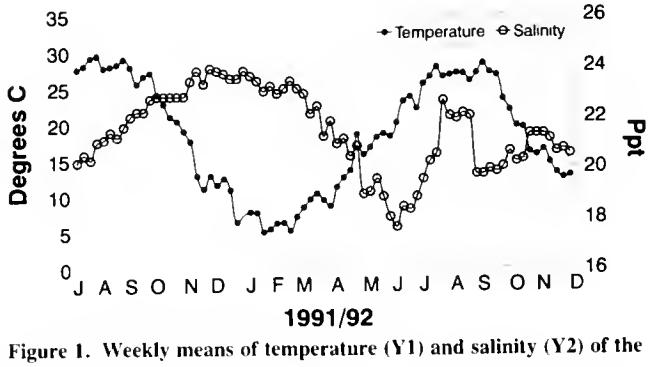


Figure 1. Weekly means of temperature (Y1) and salinity (Y2) of the York River, VA entering the flumes.

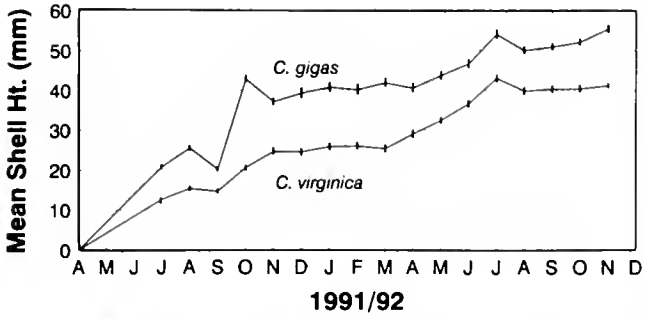
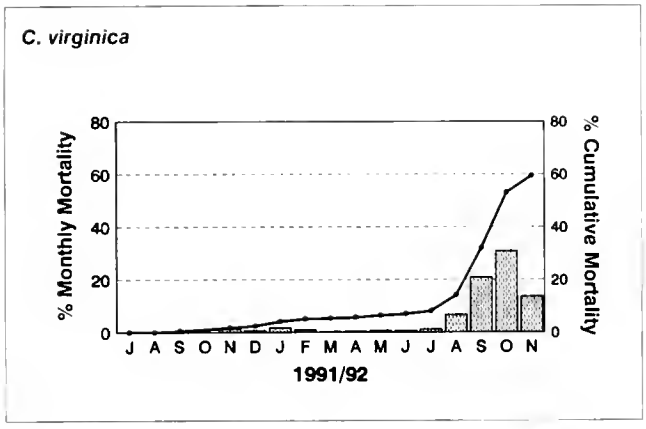


Figure 2. Mean (±1SE) shell height of *C. virginica* and *C. gigas* from July 1991 to November 1992. Means after July 1992 include undosed and dosed groups (n = 100).

infections progressed rapidly in the dosed *C. virginica* group. In the undosed group, infection occurred later (probably coming into the flumes via the influent) and did not progress to advanced stages, even by the end of the study.

In June 1992, no *P. marinus* was found in *C. gigas*, prior to dosing (Fig. 4). The dosed *C. gigas* group had a 20% prevalence in July, followed by an 80% prevalence in both August and September and a 70% prevalence in October. In the undosed group, prevalence was 50% in September, but only 10% in October. All infected oysters in both *C. gigas* groups had light infections, except for one heavy infection which was found in the dosed group in August (Table 1). Thus *C. gigas* became infected with *P. marinus*, but prevalences were lower than those seen in *C. virginica* and actually decreased (in both dosed and undosed groups) between September and October. At the same time, all but one infection was light, indicating that once infected, progression of the disease in *C. gigas* was limited compared to *C. virginica*.



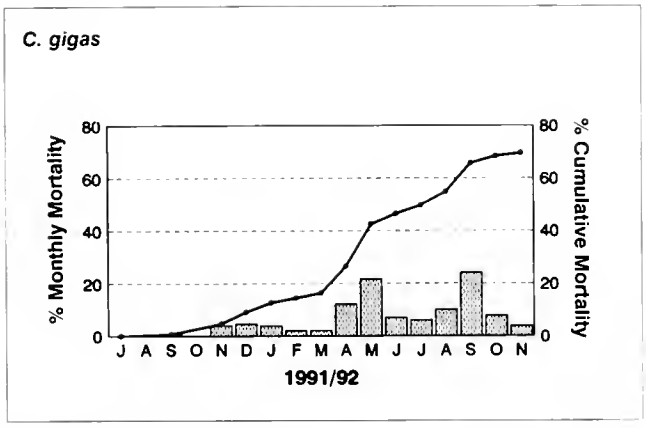
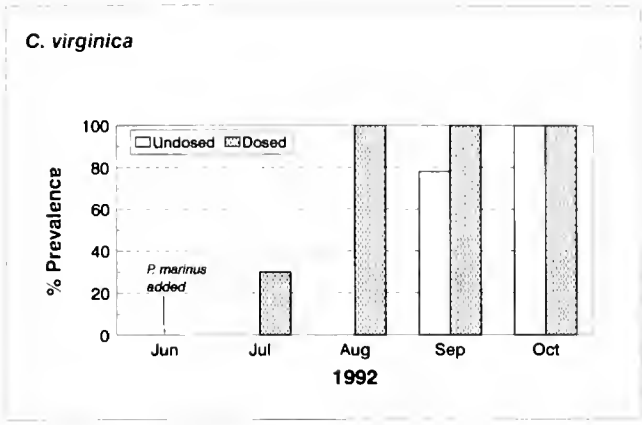


Figure 3. Percent monthly mortality (Y1) and percent cumulative mortality (Y2) of *C. virginica* and *C. gigas* from July 1991 to November 1992. Undosed and dosed groups of each species were combined after June 1992.



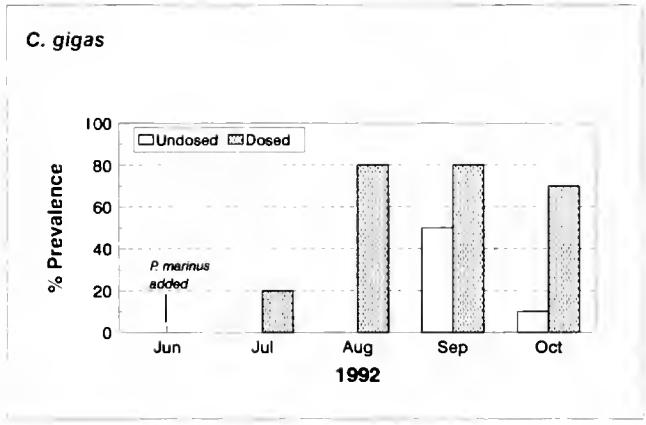


Figure 4. Percent prevalence of *Perkinsus marinus* in undosed and dosed flumes in months June to October, 1992. Dosed groups were challenged with addition of infected oysters in June; undosed groups exposed only to *P. marinus* entering flume via influent.

From July through November 1992, mean shell height was significantly related ($P \leq 0.05$) to species but not to exposure to *P. marinus* (Fig. 5). Thus shell height of *C. gigas* was greater than that of *C. virginica* in all months and no difference in shell height was detected between dosed and undosed groups of either species. It should also be noted, however, that no increase in mean shell height of either species occurred over this time period.

Percent monthly mortality of *C. virginica* in July, August and November 1992 was similar for dosed and undosed groups; in September and October 1992, however, mortality was significantly greater ($P \leq 0.05$) in the dosed groups than in the undosed groups (Fig. 6). In September, mortality for *C. virginica* was 24%

TABLE 1.

Intensity of *P. marinus* infections in undosed and dosed groups of *C. virginica* and *C. gigas* from June through October 1992.

	C. virginica				*C. gigas*			
Date	**n**	**L**	**M**	**H**	**n**	**L**	**M**	**H**
June	10	0	0	0	10	0	0	0
July								
Undosed	10	0	0	0	10	0	0	0
Dosed	10	3	0	0	10	2	0	0
August								
Undosed	10	0	0	0	10	0	0	0
Dosed	10	5	3	2	10	7	0	1
September								
Undosed	9	7	0	0	4	2	0	0
Dosed	8	4	1	3	5	4	0	0
October								
Undosed	10	9	1	0	10	1	0	0
Dosed	9	2	2	5	10	7	0	0

L = light; M = moderate; H = heavy.

in the dosed group and 17% in the undosed group; in October, dosed mortality was 41% and undosed mortality was 21%. For *C. gigas*, percent monthly mortality was similar in dosed and undosed groups in July, August, September, and November; in October, however, mortality in the dosed group (11%) was significantly greater ($P \leq 0.05$) than in the undosed group (4%) (Fig. 6).

DISCUSSION

P. marinus infections became established in the dosed groups of both species of oysters within one month of being introduced. The undosed groups of both species became infected (to a lesser extent) three months after the dosed groups. Undoubtedly these infections resulted from infective stages of *P. marinus* entering the flume via the influent water. Infections in the undosed groups, however, never developed into heavy infections in either species, indicating that either too few infective stages got into the flume or the ones that did get in arrived too late in the summer to result in advanced cases of the disease. Thus the goal of attaining two contrasting levels of disease challenge was achieved.

This study confirms previous work demonstrating the relative tolerance of these two oyster species to intense *P. marinus* challenge (Meyers et al. 1991). *C. virginica* had 100% prevalence and heavy (fatal) infections within two months of exposure to the parasite. *C. gigas*, on the other hand, although becoming infected with *P. marinus*, had a lower maximum prevalence (80%) that actually decreased over time. Only 1 heavy infection was found in the *C. gigas* groups; all other infections were light. Obviously, *C. gigas*, even though susceptible to initial infection, is somehow able to inhibit development of the disease and even reduce infection prevalence. Differences in potential defense mechanisms between these two species are discussed by La Peyre (1993).

By age six months, *C. gigas* had attained an approximate 10 mm shell height advantage over *C. virginica*. This statistically significant size advantage was maintained throughout the remainder of the 19 month study period. Of interest is the fact that both species had very similar growth patterns. Most growth occurred in the spring and fall; almost no growth took place in the winter and

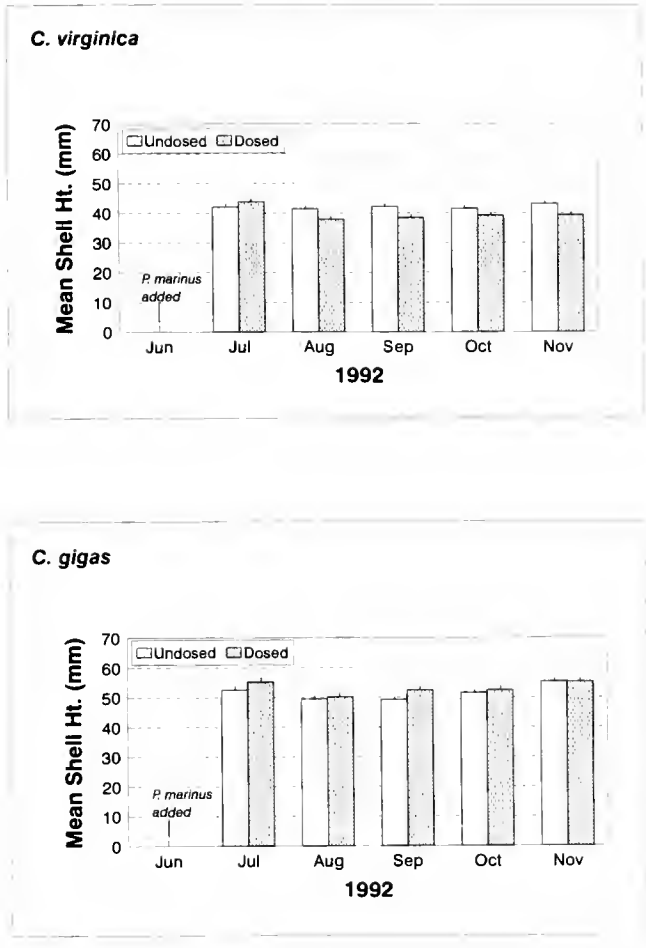


Figure 5. Mean shell height (+1SE) of undosed and dosed groups of *C. virginica* and *C. gigas* in months July to November 1992, after *P. marinus* was added to the dosed flume (n = 50).

summer, probably reflective of similar temperature tolerances of the two species (Galtsoff 1964, Mann et al. 1991).

Growth rate of *C. virginica* in this experiment, however, was lower than that observed in trays directly in the York River by Barber and Mann (1991). After 18 months in the flumes, mean shell height of *C. virginica* was 41.2 mm, but after 18 months *in situ*, mean shell height was 64.6 mm (Barber and Mann 1991). Presumably the growth rate of *C. gigas* was similarly less than maximal in the flumes. Lower growth overall in the flumes was most likely the result of limited food availability.

Since little growth occurred in either species over the summer months of July through September, both in 1991 and 1992, it is not surprising that there was no relationship between shell height (growth) and disease level. A previous study has shown that growth in *C. virginica* is inhibited by *P. marinus* (Paynter and Burreson 1991). Presumably, if this study had continued until the following spring when growth would have again commenced, differences would have become apparent. During August and September 1992, there was a major bloom of the dinoflagellate, *Cochlodinium* sp., in the York River. Although not directly toxic to oysters, this species has questionable nutritional value (Luckenbach et al. 1993), and may have contributed at least indirectly to the lack of growth (and increased mortality) seen in both species at this time.

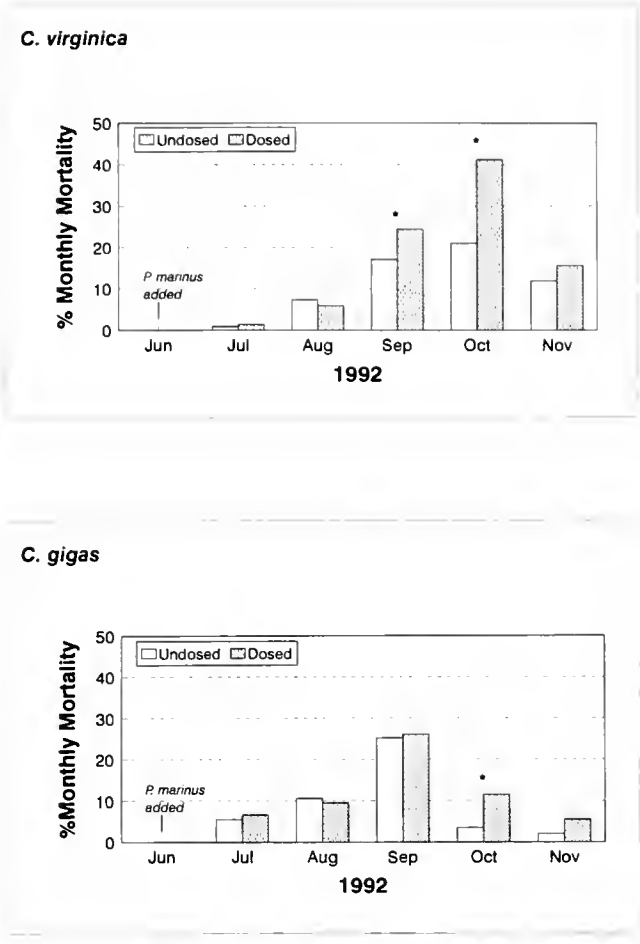


Figure 6. Percent monthly mortality of undosed and dosed groups of *C. virginica* and *C. gigas* from July to November 1992, after *P. marinus* was added to the dosed flume. Asterisks (*) indicate significant differences ($P \leq 0.05$) between groups.

Over the 19 month study period, cumulative mortality of *C. gigas* was 70%, compared to 59% for *C. virginica*. Greatest mortality of *C. gigas* occurred during two periods, April–May of 1991 and September 1992, while most mortality of *C. virginica* took place from September–November 1992. These differences in mortality patterns between the two species are related to the most likely causes of mortality.

As reviewed by Mann et al. (1991), the optimal salinity for growth and spawning of *C. gigas* is 20–35 ppt; the optimal salinity for *C. virginica* is 5–30 ppt (Galtsoff, 1964). In this study, salinity was below 20 ppt for an extended period, including April–May 1991. Thus the most obvious explanation for the mortality of *C. gigas* during April–May 1991 is a lack of tolerance to salinity below 20 ppt. The physiological stress experienced by *C. gigas* due to unfavorable salinity may have been exacerbated by processes related to gametogenesis. March to May is the period of gonadal maturation and spawning in *C. gigas* in this location (Barber unpublished data). In Japan and Washington State, mortalities of *C. gigas* have been associated with periods of maximal gametogenic activity (Perdue et al. 1981, Beattie et al. 1988). Also, food coming into the flumes may have been insufficient to support both growth and gametogenesis. Thus there were several possible physiological stressors contributing to the mortality of *C. gigas* in April and May 1992, but salinity appears to be the primary factor.

The second period of mortality of *C. gigas* was in September 1992, but given the facts that infection by *P. marinus* was light and that mortality was similar in both dosed and undosed groups, it is unlikely that this mortality was related to *P. marinus*. September 1992 was another period during which salinity in the York River dropped below 20 ppt. It is possible that less than optimal salinity, combined with the bloom of *Cochlodinium*, was the primary factor causing mortality. Even though mortality in the dosed *C. gigas* group was significantly greater than in the undosed group in October 1992, it was about half that observed in September, and given the light intensities of infected oysters, probably not disease related.

Unlike *C. gigas*, mortality in *C. virginica*, greatest in September, October and November 1992, was more closely related to prevalence and intensity of *P. marinus* infections. Appreciable mortality did not occur in *C. virginica* until after the *P. marinus* became established in the flumes. Mortality in the dosed *C. virginica* group was significantly greater than in the undosed group in both September and October in conjunction with the development of moderate and heavy infections. The relatively high mortality that also occurred in the undosed groups in September and October, in spite of a lack of advanced *P. marinus* infections, could have been related to effects of the *Cochlodinium* bloom. Thus it is likely this bloom was indirectly responsible for the mortalities seen in August and September in the dosed *C. gigas* group as well as the undosed groups of both species. Note that mortality in these groups was reduced in November, after the bloom had subsided and water temperature had declined to below 20°C.

Even though *C. gigas* is considerably more tolerant of *P. marinus* and grows faster than *C. virginica*, other factors may ultimately determine the suitability of *C. gigas* as an alternate oyster species on the mid-Atlantic coast of the U.S. It is not known to what extent overall growth and mortality was related to effects of the flumes and the *Cochlodinium* bloom encountered during this study. It is clear, however, that under identical conditions, mortality of *C. gigas* is greater than that of *C. virginica*, and not related to *P. marinus*. Evidence is presented here that *C. gigas* is more susceptible to mortality at salinity below 20 ppt, especially if this occurs during periods of gonadal maturation. The disease resistance exhibited by *C. gigas* is of little importance if significant mortality occurs by other means prior to attainment of market size. The high non-disease mortality experienced by *C. gigas* may be indicative of a lack of adaptation to the environmental conditions prevailing in the mid-Atlantic, particularly lower Chesapeake Bay, and may explain why populations have not become established on the east coast of the U.S. in spite of several historical opportunities.

ACKNOWLEDGMENTS

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OYSTER SERUM AGGLUTININS AND RESISTANCE TO PROTOZOAN PARASITES

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ABSTRACT Serum agglutinins or lectins are reported to be induced in marine molluscs by exposure to bacteria and may enhance bacterial clearance from the host; however, there is a little information on possible relationships between lectins and protozoan parasites of molluscs. Two protozoans, *Haplosporidium nelsoni* and *Perkinsus marinus*, cause mortality of eastern oysters, *Crassostrea virginica*. We tested the hypothesis that if hemolymph agglutinins or other hemolymph proteins are important in the defense against these parasites, oysters with high "baseline" (pre-exposure) levels, or oysters that produce these substances after challenge, should have lower parasite burdens and survive longer than animals without these characteristics. In May 1990, individually labelled oysters were placed in Chesapeake Bay, MD, where they were exposed primarily to *P. marinus*, and in Delaware Bay, NJ, where they were exposed to both parasites. Changes in parasite densities, total protein, and agglutination titers were followed by repeated sampling of hemolymph from individual animals over a year. Oysters in Delaware Bay were affected initially by *H. nelsoni*, but mortalities from both parasites reduced survival to only 10% by September 1990. In Chesapeake Bay, mortalities were attributed to *P. marinus* only and survival was 36% by May 1991. There was no correlation between survival time and individual protein concentrations in samples from both locations collected during the pre-exposure or post-exposure/pre-mortality periods. The high mortality rate in Delaware Bay precluded hemolymph sampling after September 1990, but continued collections in Chesapeake Bay showed that protein concentrations eventually became inversely correlated with parasite burdens and survival time. This happened, however, only after most oysters had systemic *P. marinus* infections and were beginning to die. Agglutination titers were not correlated with parasite densities or survival time. We conclude that the serum agglutinins tested play no role in defense against either *H. nelsoni* or *P. marinus*, and that differences in total protein were related to pathology rather than disease resistance.

KEY WORDS: eastern oyster, *Crassostrea virginica*, *Perkinsus marinus*, *Haplosporidium nelsoni*, defense mechanisms, lectins, agglutinins, hemolymph protein

INTRODUCTION

Agglutinating substances, or lectins, that recognize and opsonize foreign material for phagocytosis by hemocytes, are considered important defense molecules in the hemolymph of invertebrates, including marine bivalves (Renwrantz 1983, Olafsen 1988). In a number of species, experimental challenge with bacteria has resulted in elevated agglutination titers, which may, in turn, be associated with clearance of the bacteria from hemolymph. Olafsen et al. (1992) have suggested that there exists a "baseline level" of lectins in marine bivalves, which can be enhanced by exposure to bacteria and can facilitate their immobilization and removal from the host. There is little information, however, on the effect of protozoan pathogens on serum agglutinins.

Eastern oysters, *Crassostrea virginica* (Gmelin), are susceptible to two protozoan parasites, *Haplosporidium nelsoni* (Haskin et al. 1966) and *Perkinsus marinus* (Mackin et al. 1950), the causative agents of MSX and Dermo diseases, respectively. Several studies have reported changes in levels of agglutinating molecules in the hemolymph of oysters infected by *H. nelsoni* (Kanaley and Ford 1990, Ling 1990, Chintala and Fisher 1991). Selectively bred strains of oysters, highly resistant to MSX disease (Haskin and Ford 1979), had significantly higher agglutination titers for the bacterium *Vibrio cholerae* during autumn months than did a susceptible stock (Chintala and Fisher 1991). It was suggested that the differences might be related to a defense mechanism; however, the resistant and susceptible groups were obtained from different geographic locations before the experiment and it is also possible that the measured differences were related to their previous habitat or to nondefense associated genetic differences.

Agglutinins are among the protein components of invertebrate hemolymph, although their proportional representation varies and other elements, such as lytic enzymes and antimicrobial factors (Chu 1988), may also be important in defense. In fact, total serum protein concentrations often vary with the health of an organism, although it is not clear whether this is caused by a response to infection or a disease-associated loss of metabolites. The relationship of total hemolymph proteins to MSX and Dermo diseases is

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also unclear. Concentrations have been found to decrease in relation to *H. nelsoni* infection intensity (Ford 1986), but not to *P. marinus* intensity (Chu and LaPeyre 1989).

In the past it has been difficult to evaluate potential defense mechanisms against *H. nelsoni* or *P. marinus* in relation to the disease process because diagnosis required sacrifice of the oysters in question. Recently, detection of *H. nelsoni* (Ford and Kanaley 1988) and *P. marinus* (Gauthier and Fisher 1990) in hemolymph from living oysters has been shown to be an effective diagnostic technique. Now, by repeated withdrawal of hemolymph from experimental oysters, it is possible to monitor changes in disease intensity and hemolymph biochemicals from the same oysters over time. Using this technique, we tested the hypothesis that if hemolymph agglutinins or other proteins are important in the defense against the protozoans *H. nelsoni* or *P. marinus*, eastern oysters with high "baseline" levels before exposure to the parasites, or those that responded to challenge by producing these substances, should have lower parasite burdens and survive longer than animals without these traits.

MATERIALS AND METHODS

There are currently no confirmed strains of oysters known to be resistant to Dermo disease and it was not possible to obtain strains resistant or susceptible to MSX disease that had been reared under common conditions, had not been exposed to known disease agents, and were also large enough to bleed repeatedly (2- to 3-years-old). We therefore decided to use a single stock of oysters and to compare individuals rather than strains. Experimental oysters were obtained in April 1990 from Long Island Sound, where MSX disease was rare and Dermo disease was absent. These oysters were held for one month prior to deployment to eliminate mortality due to dredging stress. Previous studies had demonstrated that this wild stock was susceptible to both diseases, although there was a large range among individuals in the onset and development of infections, and in subsequent survival time, all presumably related to innate resistance (Valiulis 1973, Ford 1986).

In May 1990, 200 oysters, which ranged from 77 to 129 mm shell height, were individually labelled and notched at the posterior shell margin adjacent to the adductor muscle. An initial hemolymph sample was collected from all 200 oysters as described below immediately before the oysters were deployed in wire-mesh trays. Half were held intertidally in lower Delaware Bay at the Rutgers University Cape Shore Laboratory (CS oysters) where MSX disease is enzootic. The other half were hung from a dock at the University of Maryland-Department of Natural Resources hatchery at Deal Island, Maryland on the Eastern Shore of the Chesapeake Bay (DI oysters). Both MSX and Dermo diseases have been reported at this location (Chintala and Fisher 1991), although *P. marinus* had predominated during the year before the present study (Ford unpublished). After deployment in May 1990, the oysters were examined at 1- to 2-wk intervals during the summer and monthly during the winter to clean the trays and remove dead animals. Dead or dying oysters were diagnosed for *P. marinus* infections by fluid thioglycollate culture of rectal and mantle tissue and for *H. nelsoni* infections by tissue slide histology. Over the following 12 months, hemolymph was collected on 5 occasions from all surviving oysters. Based on previous long-term studies of infection and mortality patterns for both parasites (Ford and Haskin 1982, Andrews 1988), the sampling schedule was divided into 3 periods: 1) Period 1—pre-exposure (May 1990), 2) Period 2—post-exposure/pre-mortality (July 1990), and 3) Period 3—rapidly proliferating infections causing mortality (September, October, and November 1990 and May 1991).

Hemolymph Collection

A 300-μL sample of hemolymph was withdrawn from the adductor muscle of each oyster with a 26-G needle and syringe. All but one drop (used for detection of *H. nelsoni* plasmodia, see next section) was placed into a microfuge tube and spun at 500 × g for 5 mins. The serum (cell-free supernatant) was pipetted into a second microcentrifuge tube for determination of total protein concentration and agglutination titers for *V. cholerae* CA401, horse erythrocytes (RBCs), and human RBCs. Beginning with the September 1990 collection, the pellet was analyzed for *P. marinus* as described below.

Haplosporidium nelsoni Prevalence and Intensity

The drop of hemolymph was scanned at 20× using an inverted microscope and the relative abundance of *H. nelsoni* plasmodia scored as described in Ford and Kanaley (1988).

Perkinsus marinus Prevalence and Intensity

Fluid thioglycollate medium (1 mL) and Pen-Strep (0.1 mL) were added to the hemocyte pellet, which was vortexed to resuspend the cells into the medium. The tubes were incubated at room temperature in the dark for at least 7 d. After incubation, the tubes were recentrifuged and the thioglycollate medium removed. The pellet was resuspended in 0.5 mL of 2 M NaOH, recentrifuged, rinsed twice in distilled water, and placed in the wells of a microtiter plate. Two drops of Lugol's iodine were added to each well and the *P. marinus* hypnospores were allowed to settle for 2–3 h. The number of hypnospores was then counted as described in Gauthier and Fisher (1990).

Total Serum Protein Concentrations

Total protein in a 10-μL subsample of the serum was determined by the Bradford method (1976) using a bovine gamma globulin standard.

Agglutination Assays

Vibrio cholerae, a bacterium, and erythrocytes from 2 vertebrate species (human and horse) were chosen for assays because previous studies documented high agglutination titers for each in oyster hemolymph (Tamplin and Fisher 1989, Chintala and Fisher 1991, Fisher and DiNuzzo 1991). Agglutination of *Vibrio cholerae* CA401 was tested in round-bottom microtiter plates after a serial two-fold dilution of serum with filtered seawater at ambient salinity (Tamplin and Fisher 1989). Bacterial concentrations were adjusted to approximately 3×10^8 (via standard McFarland nephelometer units) and added 1:1 to the diluted serum (Chintala and Fisher 1991). Plates were read 24 h later. The agglutination titer was recorded as the reciprocal of the highest dilution where agglutination was observed.

The remainder of the serum was treated with 0.02% sodium azide and shipped to the University of Texas Medical Branch at Galveston (UTMB) where agglutination titers were measured within one week according to the method of Fisher and DiNuzzo

(1991) using horse and human RBCs. No RBC agglutination assays were performed in May 1991. Human RBCs (type O Rh^+) were obtained from the UTMB Blood Bank diluted 450:63 in an anticoagulant, CPDA-1. Horse RBCs were purchased from Cocalico Biologicals, Inc.[1] (Reamstown, PA) diluted 1:1 in Alsever's solution. Two percent suspensions of lightly packed RBCs were added 1:1 to dilutions of oyster serum and wells were examined for agglutination within 3 h.

Statistical Comparisons

To evaluate the significance of serum agglutinin and total protein levels to parasite concentrations and oyster survival times, we analyzed data in two ways. In the first, we separated oysters that survived until May 1991 (survival group) from those that died during the experiment (mortality group). Mean agglutinin titers, protein concentrations, and *P. marinus* densities of the two groups were compared by *t*-tests on individual sampling dates. In the second, we regressed protein concentrations and agglutination titers for each oyster, at each sampling date, against the total survival time for that individual. For CS oysters, these tests were made for hemolymph components measured during Periods 1 and 2 only. Changes over time in individual oyster hemolymph components were examined with a repeated measures analysis of variance for randomized complete block design. Agglutination statistics were compiled with $\log_2$ titer values; *P. marinus* concentrations were $\log_{10}$ transformed before analysis. The level of significance in all tests was $\alpha = 0.05$.

RESULTS

Disease Prevalence and Oyster Survival Times

In contrast to the expected pattern, *P. marinus*, as well as *H. nelsoni*, was abundant in Delaware Bay during the study period (Ford 1992). Mortality in the CS oysters was very heavy from the end of July to the middle of September, by which time only 10% of the oysters survived (Fig. 1). Twenty gapers were collected during this period. Eighteen were infected by both parasites, but until the end of August, when survival was about 50%, *H. nelsoni* infections were much heavier than *P. marinus* infections. Thereafter, infections of both parasites were equally advanced. Hemolymph sampling of CS oysters was discontinued after September 1990 and by November, total survival was only 4%. Because of small numbers, detailed statistical analyses of the data were of limited value. Thus, unless specified, all results presented below refer to DI oysters only.

A few DI oysters with very light *H. nelsoni* infections were observed in the autumn. There was no indication that they had intensified until May, 1991, when the experiment ended. No *H. nelsoni* infected gapers were found. By September 1990, however, *P. marinus* prevalence was approximately 80% with a mean of 10^3 hypnospores mL^{-1} hemolymph. In October, 96% of all oysters were patently infected. Mortalities began in late August (Fig. 1), and prevalence and intensity of *P. marinus* remained high throughout the fall (Fig. 2). In May 1991, 36% of the DI oysters were still alive. The mean survival time of DI oysters was 251 days, nearly three times longer than the 94 days recorded for CS oysters.

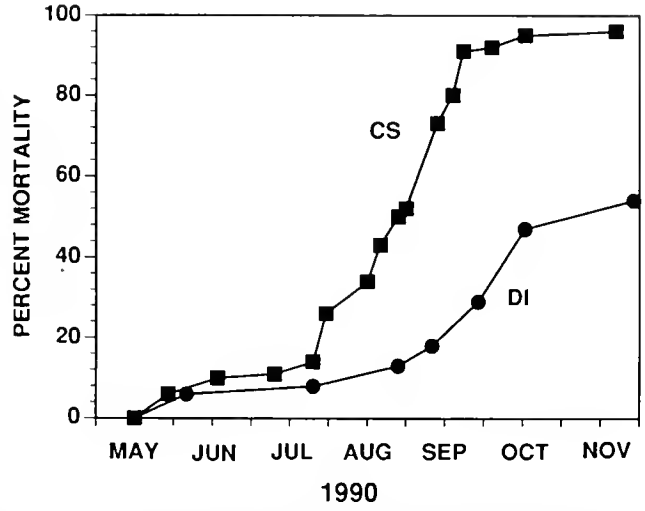

Figure 1. Cumulative mortalities of oysters exposed to *Perkinsus marinus* and *Haplosporidium nelsoni* in Delaware Bay at Cape Shore (CS), NJ and in Chesapeake Bay at Deal Island (DI), MD during 1990.

At each sampling during the fall of 1990, oysters that survived until the following May had at least an order of magnitude fewer circulating *P. marinus* cells ($p < 0.05$) than those that died during the study (Fig. 2). Although nearly all oysters in the survival group had patent infections, most individuals had fewer than $10^{3.5}$ *P. marinus* cells at any sampling date, whereas those in the mortality group had more than $10^{3.5}$ cells. Parasite densities in most survivors increased slightly from September to November but then

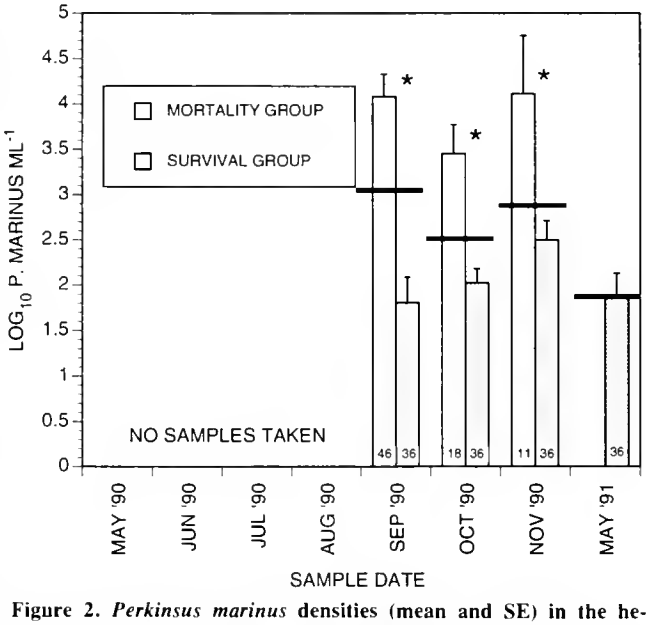

Figure 2. *Perkinsus marinus* densities (mean and SE) in the hemolymph of oysters held at Deal Island, Maryland. Survival Group = those oysters that were still living at the end of the study in May 1991; Mortality Group = those oysters that died before May 1991. Mean of all animals is denoted by horizontal bar. Asterisk denotes significant difference between groups at $\alpha = 0.05$. N is shown at bottom of histogram.

[1]Mention of commercial products or companies does not constitute endorsement or recommendation for use by the U.S. Environmental Protection Agency.

decreased between November and the following May, when the mean *P. marinus* density was $10^{1.9\pm0.26}$ (Fig. 2). Infection intensity of *P. marinus* in September, October, and November 1990 was significantly and negatively correlated with survival time (Table 1, I), although parasite numbers explained only ⅓ of the variation in survival time at these sampling dates.

Serum Protein Concentrations

Initial (May 1990) protein concentrations were about 3 mg mL^{-1} and remained unchanged in the July sample (Fig. 3). There were no differences between mortality and survival groups in May and July 1990 samples, but in September, protein concentrations began to increase sharply. By October, concentrations in the survival group had increased nearly 5-fold and in the mortality group about 3.3-fold, to 15 and 10 mg mL^{-1}, respectively. Concentrations in both groups decreased in November, especially in the mortality group. The survival groups exhibited significantly higher protein concentrations than the mortality group from September through November.

Total serum protein concentrations of individual oysters sampled in May and July 1990 were not correlated with survival time, but in September, October and November, they were significantly and positively correlated with survival (Table 1, II). Further, the amount of variation in survival time explained by serum protein increased 7-fold from 9.8% in September to 49.7% in November. There was a significant negative correlation between protein levels for all oysters and *P. marinus* densities in October and November 1990 samples (Table 1, III). This relationship did not exist for survival-group oysters considered alone (Table 1, IV). In CS oysters, there were no significant correlations between serum protein concentrations measured in May or July 1990 and survival time during late July and August 1990, when *H. nelsoni* was primarily associated with death, or during September 1990, when both *H. nelsoni* and *P. marinus* caused mortality.

Serum Agglutination Titers

Agglutination titers of *V. cholerae* CA401 showed a significant decline during the experiment (Fig. 4). Titers for agglutination of horse RBCs (HR) also changed significantly with time, decreasing in early autumn and then increasing in November (Fig. 5). There were no differences between survival and mortality groups in either assay. At each sampling date, titers of HR and *V. cholerae* were positively and significantly correlated.

Agglutination titers for human RBCs (HM) also decreased through September. They increased again in October and November in the survival group, but not in the mortality group. Titers in the survival group were significantly higher than in the mortality group in November, but there was no significant correlation between survival time and agglutination titer for individual oysters at any sampling period. Agglutination of HM was significantly correlated to the agglutination of *V. cholerae* only during May, October and November, 1990. There was no correlation of any agglutination titer to hemolymph protein concentration or intensity of *P. marinus*. In CS oysters, neither agglutination titers nor serum protein concentrations measured in May or July 1990 were associated with survival time during late July and August 1990, when *H. nelsoni* was primarily associated with death, or during September 1990, when both *H. nelsoni* and *P. marinus* caused mortality.

DISCUSSION

Evidence is mounting that humoral and/or hemocyte surface-bound lectins of marine molluscs facilitate the immobilization and removal of invading bacteria (Olafsen 1988, Olafsen et al. 1992). The only previous study to examine a similar relationship between

TABLE 1.

Regression statistics relating survival time, serum protein concentrations, and circulating *Perkinsus marinus* densities measured for individual oysters deployed in the Chesapeake Bay at Deal Island, MD during 1990.

Analysis	Equation ($y=$)	r^2	N	p
I. Survival in days (y) vs Log_{10} *P. marinus* mL^{-1} (x) for samples collected in				
September 1990	$-39.756x + 418.255$	0.354	82	0.0001
October 1990	$-44.635x + 478.285$	0.331	54	0.0001
November 1990	$-24.294x + 468.553$	0.344	47	0.0001
II. Survival in days (y) vs serum protein in mg mL^{-1} (x) for samples collected in				
May 1990	$0.816x + 248.918$	8.65×10^{-5}	100	0.9276
July 1990	$-1.815x + 275.749$	1.96×10^{-4}	92	0.8959
September 1990	$19.490x + 171.761$	0.098	82	0.0046
October 1990	$12.249x + 212.406$	0.328	54	0.0001
November 1990	$10.638x + 292.721$	0.497	47	0.0001
III. Serum protein in mg mL^{-1} (y) vs Log_{10} *P. marinus* mL^{-1} (x) of all oysters collected in				
September 1990	$-0.158x + 6.633$	0.022	82	0.1841
October 1990	$-1.841x + 17.609$	0.286	54	0.0001
November 1990	$-1.657x + 14.792$	0.358	47	0.0001
May 1991	$-0.143x + 6.127$	0.006	36	0.6638
IV. Serum protein in mg mL^{-1} (y) vs Log_{10} *P. marinus* mL^{-1} (x) of survival group oysters only, collected in				
September 1990	$-0.083x + 6.722$	0.005	36	0.6796
October 1990	$-0.901x + 16.386$	0.065	36	0.1342
November 1990	$-0.693x + 13.226$	0.073	36	0.1120
May 1991	$-0.143x + 6.127$	0.006	36	0.6638

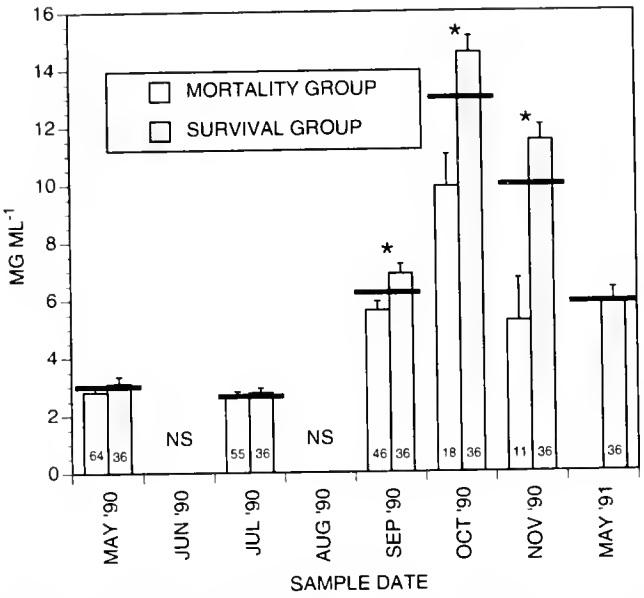

Figure 3. Protein concentrations (mean and SE) in the hemolymph of oysters held at Deal Island, Maryland. Survival Group = those oysters that were still living at the end of the study in May 1991; Mortality Group = those oysters that died before May 1991. Mean of all animals is denoted by horizontal bar. Asterisk denotes significant difference between groups at $\alpha = 0.05$. N is shown at bottom of histogram. NS denotes no sample taken.

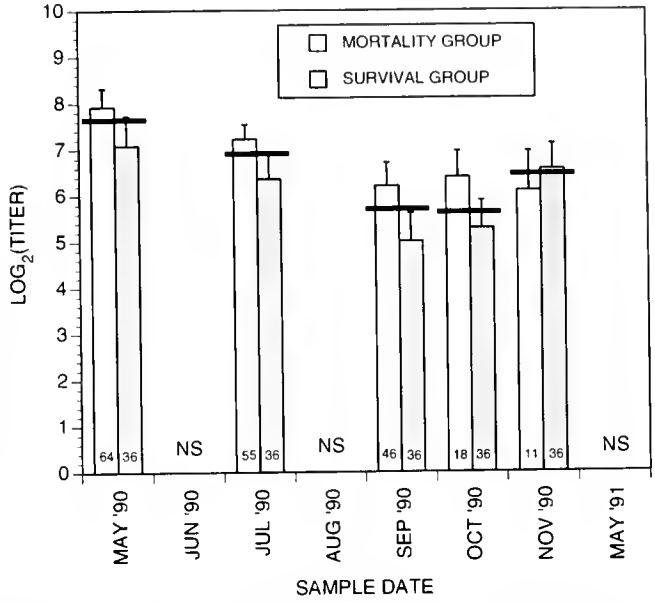

Figure 5. Agglutination titers for the horse RBCs (mean and SE) in the hemolymph of oysters held at Deal Island, Maryland. Survival Group = those oysters that were still living at the end of the study in May 1991; Mortality Group = those oysters that died before May 1991. Mean of all animals is denoted by horizontal bar. N is shown at bottom of histogram. NS denotes no sample taken.

molluscs and a protozoan found that, after challenge by *Haplosporidium nelsoni* (MSX), a resistant host strain of eastern oysters had higher agglutination titers for *Vibrio cholerae* than did the susceptible stock (Chintala and Fisher 1991), although it was not clear whether the lectin played a role in defense.

The present study examined the relationships of parasite burden and survival time to hemolymph agglutinins and total protein measured in individual oysters at three periods during the course of MSX and Dermo disease development: 1) pre-expo-

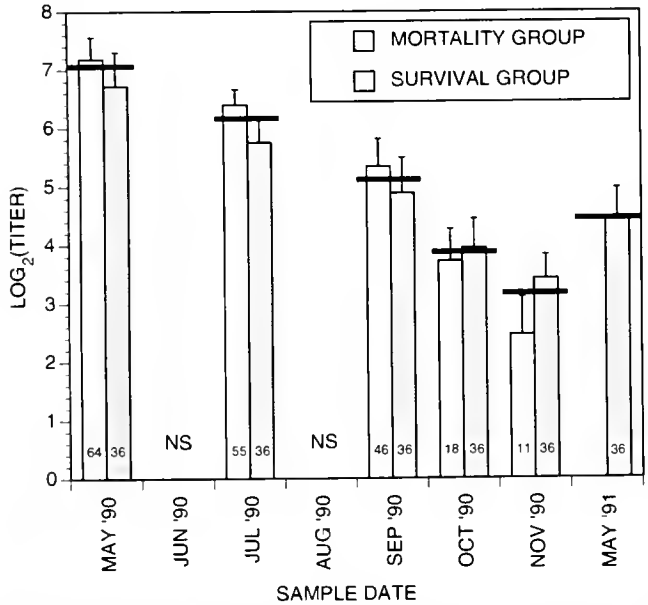

Figure 4. Agglutination titers for *Vibrio cholerae* CA401 (mean and SE) in the hemolymph of oysters held at Deal Island, Maryland. Survival Group = those oysters that were still living at the end of the study in May 1991; Mortality Group = those oysters that died before May 1991. Mean of all animals is denoted by horizontal bar. N is shown at bottom of histogram. NS denotes no sample taken.

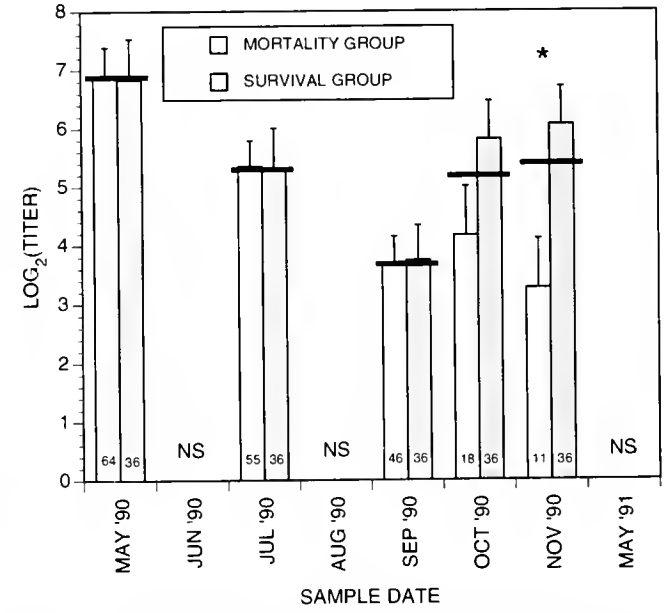

Figure 6. Agglutination titers for human RBCs (mean and SE) in the hemolymph of oysters held at Deal Island, Maryland. Survival Group = those oysters that were still living at the end of the study in May 1991; Mortality Group = those oysters that died before May 1991. Mean of all animals is denoted by horizontal bar. Asterisk denotes significant difference between groups at $\alpha = 0.05$. N is shown at bottom of histogram. NS denotes no sample taken.

sure, 2) post-exposure/pre-mortality, and 3) proliferating-infection/mortality. Our hypothesis was that if agglutinins or other hemolymph proteins are important in defense against either *H. nelsoni* or *P. marinus*, eastern oysters with high levels in Periods 1 or 2 should have lower parasite burdens and survive longer than animals without these traits. If high agglutinins, high total serum proteins, or both are important defense mechanisms against either of these pathogens, we would expect them to be present in the individuals that survive in any unselected population as well as in future offspring (i.e. resistant strains).

The experimental design was intended to examine the hypothesis separately for the two parasites by exposing oysters to *H. nelsoni* in Delaware Bay (CS) and to *P. marinus* (primarily) in Chesapeake Bay (DI). This strategy worked for DI where *P. marinus* densities were clearly correlated with mortality and *H. nelsoni* did not appear until the end of the experiment. Both parasites, however, were abundant at CS during the study and caused very high mortalities. The relative intensity of parasites in CS gapers indicated that *H. nelsoni* was the principal cause of oyster deaths through August, after which *P. marinus* became equally important. Despite the necessarily abbreviated sampling of CS oysters, it was clear that there was no relationship between serum protein concentrations or agglutination titers measured in Periods 1 or 2 and subsequent survival time of animals exposed to both pathogens.

Similarly, there was no evidence that agglutination titers or total protein levels in Periods 1 or 2 were related to survival rates of DI oysters, which were killed primarily by *P. marinus*. Following a seasonal pattern described earlier (Ford 1986), total serum protein increased during the late summer and autumn, coinciding with Period 3 when *P. marinus* had intensified and was causing deaths. The increase was found in all oysters, but in contrast with Periods 1 and 2 samples, there was a clear correlation of higher protein concentrations with longer survival. This relationship became stronger with time, and by November, total protein concentration explained nearly half of the variance in survival. The abundance of *P. marinus* was the link between protein and survival: high parasite burdens were associated with short survival times and low protein concentrations.

The lack of any significant correlation between pre-exposure serum agglutinin or protein levels and survival times of oysters exposed to both *H. nelsoni* and *P. marinus* indicates that high "nonanticipatory" or "baseline" (Olafsen 1992) levels of these substances did not confer protection against the two protozoans. Also, there was no evidence that initial infection (Period 2) by the pathogens induced higher concentrations or titers that might be protective. Protein concentrations did change in relation to infection intensities and survival time, but only after infections were already well-developed. Lower concentrations were associated with heavier infections when all oysters were considered; however, there was no correlation, at any sampling date, between protein and infection intensity when the survival group was considered alone. Circulating parasite densities in the survival group were at least an order of magnitude lower than in the mortality group, implying that protein concentration decreased in response to infection intensity only after a threshold was reached in parasite numbers or their pathological consequences, or both. This is similar to the finding for *H. nelsoni* parasitized oysters in which hemolymph protein concentrations were depressed in relation to infection intensity only after infections were well established and tends to support the argument that the changes were due to pathological depletion of circulating proteins in parasitized oysters (Ford 1986). The report by Chu and LaPeyre (1989) that serum protein levels were not related to *P. marinus* intensities may indicate that their experimental oysters had relatively light infections.

Interestingly, despite significant infection-related changes in total protein, agglutination titers were altered relatively little during Period 3. In only one sample was an agglutination titer for the survival group different from that of the mortality group (HM titer for November 1990) and none of the correlations between titers and survival times for individual oysters was significant. Although circulating agglutinins or lectins are part of the hemolymph protein complement, there is no agreement as to what proportion they constitute. For instance, Acton et al. (1969) indicated that adsorbing oyster hemolymph with sheep RBCs removed 98% of its protein content, whereas McDade and Tripp (1967), also using sheep RBCs, reported a loss of only 11%. We did not examine this question directly. However, because we found no covariance between any of the agglutination titers and protein concentrations, it is reasonable to conclude that the agglutinins did not constitute a very large fraction of the serum protein complement.

Phagocytosis of *H. nelsoni* by oyster hemocytes is rare in both resistant and susceptible oysters (Ford et al. 1993). In contrast, oyster hemocytes avidly ingest *P. marinus*, but are not always able to destroy it intracellularly (Mackin 1951; R. Gustafsen, Rutgers University, personal communication 1993). These reports may help explain our findings as they suggest that differential phagocytic rates, in which opsonizing lectins might play a role, do not appear to be involved in resistance to either disease agent, although they might affect disposal, through diapedesis, of ingested *P. marinus*. Although the lectins tested here are widespread among molluscs and showed high titers in previous studies (Tamplin and Fisher 1989, Chintala and Fisher 1991, Fisher and DiNuzzo 1991, Olafsen et al. 1992), they appear to play no role in defense against *H. nelsoni* or *P. marinus*.

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CHARACTERIZATION OF OVERWINTERING INFECTIONS OF *PERKINSUS MARINUS* (APICOMPLEXA) IN CHESAPEAKE BAY OYSTERS

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ABSTRACT To determine the nature and abundance of overwintering *P. marinus* infections, infected oysters (*Crassostrea virginica*) collected from the upper James River, VA, were placed in a tray and suspended from a pier in the lower York River, VA in November 1991. Every six weeks through May 1992 oysters (n = 25) were removed from the tray, examined for *P. marinus* by hemolymph culture in fluid thioglycollate medium (FTM), gradually warmed in individual containers to 25°C and held for one month. After the incubation period, which permitted the development of very light and/or cryptic parasite stages to detectable levels, the oysters were reanalyzed for *P. marinus* by both hemolymph and tissue cultures in FTM. A second group of 25 oysters from the tray was sacrificed at the initiation of each incubation, diagnosed using FTM cultures of hemolymph and tissue, and examined for cryptic stages using immunoassays. On the basis of FTM assays, prevalence of *P. marinus* gradually declined from 100% in November 1991 to 32% in May 1992. Incubation of oysters at 25°C always resulted in an increase of *P. marinus* prevalence and intensity, suggesting that the parasite was more abundant than initial FTM cultures indicated. Immunoassay diagnosis revealed infections in many of the oysters diagnosed as negative by FTM cultures. Most infections detected by immunoassay were comprised of individual *P. marinus* meronts within hemocytes in the midgut epithelial lining. Previously unidentified cryptic stages were not observed. *Perkinsus marinus* appears to overwinter at very low intensities in a high proportion of oysters. Comparison of *P. marinus* prevalence and intensity in transplanted oysters maintained in the York River to that in oysters monitored at the original James River collection site suggests that salinity may greatly influence overwintering infections. Infection intensity and prevalence declined earlier and to a greater extent at the James River site (4–12 ppt) than at the York River location (19–23 ppt). It appears that the synergistic effect of low temperature and low salinity may be more important in regulating *P. marinus* epizootics than either factor acting alone.

KEY WORDS: immunoassay, parasite, prevalence, intensity, oyster, salinity, temperature

INTRODUCTION

Perkinsus marinus (Mackin, Owen and Collier 1950), a protozoan parasite of the eastern oyster, *Crassostrea virginica* (Gmelin 1791), is distributed along the southeastern coast of the United States and in the Gulf of Mexico (Andrews 1988, Andrews and Ray 1988). This subtropical distribution implies the importance of temperature as a regulating factor and temperature is believed to be the most important factor affecting the geographic distribution and activity of *P. marinus* (Ray 1954, Andrews and Hewatt 1957, Mackin 1962, Quick and Mackin 1971, Andrews 1988). The influence of temperature on the activity of *P. marinus* has been well documented. Multiplication and virulence of the parasite are highest at temperatures exceeding 20°C (Ray 1954, Andrews and Hewatt 1957, Mackin 1962, Quick and Mackin 1971, Andrews 1988). Temperatures below 15°C retard *P. marinus* infectivity and infection progression (Ray 1954).

Minimum winter temperature is believed to control the northern limit of the geographic distribution of *P. marinus* and variations in seasonal and annual temperature patterns between northern and southern waters result in important regional differences in the seasonal cycle of the pathogen. Typically, *P. marinus* is present in southern areas at high prevalences throughout the year, declining only slightly during winter months (Andrews and Ray 1988, Soniat and Gauthier 1989, Crosby and Roberts 1990). In contrast, epizootics in the Chesapeake Bay display a more dramatic seasonal periodicity in which peak prevalences in late summer and fall are followed by a precipitous decline during the late winter-early spring months. In late spring as temperature consistently exceeds 20°C the pathogen begins multiplying, new infections are acquired, and prevalence once again increases (Andrews 1988, Andrews and Hewatt 1957).

The scarcity of overwintering infections of *P. marinus* in the Chesapeake Bay has long puzzled investigators. Frequently, oyster grounds having 100% prevalence in the fall exhibit late winter prevalences as low as 0%. The lack of positive diagnoses for *P. marinus* using tissue cultured in fluid thioglycollate medium (FTM) in late winter prompted Andrews and Hewatt (1957) to place oysters collected in winter from an enzootic area having 0% prevalence at the time of collection into warm water aquaria (23 to 28°C) in an effort to detect overwintering infections. Three groups of 15 or fewer oysters were warmed for periods of 60 to 100 days. After warming, *P. marinus* was detected in nearly all of the oysters, but since the oysters were not isolated from one another it was impossible to separate overwintering infections from proximity infections which may have been acquired during the warming period. Based on the time-distribution of deaths the authors speculated that initially only three oysters were infected. This work suggests that some prepatent infections that are not easily disclosed by routine diagnosis using FTM may be present in oysters during the winter months. However, the nature and abundance of overwintering infections of *P. marinus* and their relative contribution to subsequent summer prevalences remains unknown. The objective of this investigation was first to determine the nature of overwintering infections, that is, to determine whether overwintering infections are comprised of typical *P. marinus* cell types or cryptic stages which do not respond to FTM. The second objective was to determine the proportion of oysters that carry overwintering infections and the intensity of such infections in individual oysters.

MATERIALS AND METHODS

Oyster Collection and Experimental Design

Perkinsus marinus-infected oysters (n = 275) were collected from Point of Shoals, James River, Virginia on 13 November

1991. "Uninfected" control oysters (n = 275) were collected on 31 October 1991 from Ross' Rock, Rappahannock River, Virginia. This group served as a control for new infections acquired in either field or laboratory conditions during the investigation. Historically, *P. marinus* had not been detected in oysters sampled in spring and fall from this location, but in recent years Ross' Rock oysters maintained in the laboratory at high salinity and temperature have exhibited infections, suggesting that the parasite may have actually been present at low levels. While probably not parasite free, Ross' Rock has the lowest prevalence of any oyster bed in Virginia and hence was the best local source for native control oysters. Following collection, the oysters were transported to VIMS, cleaned of fouling organisms and placed in separate 400-L aerated tanks containing 15°C filtered (1.0 μm) York River water that was diluted to 15 ppt with fresh tap water. The oysters were maintained at 15°C and 15 ppt until the initiation of the experiment on 18 November 1991. Water was changed once a week and the oysters were fed algae paste (0.1 g/oyster) daily.

On 18 November 1991, 50 oysters were randomly sampled from each group and diagnosed for *P. marinus* using both hemolymph and tissue FTM cultures. This sample was taken for the purpose of comparing diagnostic techniques and determining *P. marinus* prevalence and intensity at the initiation of the investigation. The remaining 225 oysters from each group were placed in separate mesh trays (120 × 60 × 14 cm) and suspended in the York River from a pier at the Virginia Institute of Marine Science (VIMS), Gloucester Point, Virginia. Transplantation of the oyster population to the York River made it logistically easier to deploy control oysters, and closely monitor oysters and environmental parameters. At the time of deployment water temperature was 12°C and the salinity was 24 ppt. Daily mean temperature and salinity data were obtained from the VIMS York River monitoring program. Means are based on measurements recorded at six minute intervals by a metering system.

Perkinsus marinus prevalence and intensity in the infected oyster population, James River tray group (JRT), was assessed approximately every six weeks from November through May. On each date (November 18, 1991; January 6, 1992; February 25, 1992; April 3, 1992; and May 19, 1992) two groups of 25 oysters were removed from the tray. The first group was sacrificed on the sample date, diagnosed using both hemolymph and tissue FTM cultures, and preserved in Davidson's AFA for later analysis using routine histology and immunoassay. Oysters in the second group were cleaned, individually labeled, notched, and a hemolymph sample was withdrawn for *P. marinus* diagnosis. The oysters were then placed in individual 1-L plastic containers containing aerated, ambient 1.0 μm-filtered York River water, gradually warmed (1–2°C per day) to 25°C and incubated for 30 days. Filtration of ambient water served to remove infective particles from the ambient water. While we cannot be certain that all infective cells were removed, we have successfully maintained uninfected oysters in previous investigations using the same filtration method. During the warm-up period water was changed and the oysters were fed daily. The incubation presumably permitted the development to detectable levels of very light and/or cryptic parasite stages which might not be detected by tissue or hemolymph assays. Isolation of oysters in individual containers removed the possibility of parasite transmission between oysters during the warm-up period. After the incubation period the oysters were diagnosed for *P. marinus* using both hemolymph and tissue assays. Rappahannock River tray control oysters (RRT) (n = 25) were also sampled and warmed as described above.

As part of the VIMS oyster disease monitoring program native Point of Shoal, James River (JRN) oysters were monitored monthly for *P. marinus*. Each month 25 oysters were collected, sacrificed, and examined for *P. marinus* using tissue FTM cultures. Sample dates and ambient water conditions measured at the time of collection are shown in Table 1. While not originally intended to be a part of this investigation, observed differences in disease patterns between the JRN monitoring samples and JRT samples during the course of this investigation led us to further analyze JRN oysters collected in March and May for prepatent infections. In addition to diagnosis by FTM tissue culture, JRN oysters collected in March and May were preserved for immunoassay diagnosis of *P. marinus* and 25 additional oysters were collected, diagnosed for *P. marinus* using hemolymph assays, and warmed as described above.

Diagnosis of P. marinus

Tissue diagnosis of *P. marinus* was by culture of rectal, mantle, and gill tissue in fluid thioglycollate medium (FTM), as described by Ray (1954). Infections were categorized as negative, light, moderate and heavy (Ray 1954) and assigned numerical values of 0, 1, 3, and 5, respectively (Mackin 1962). The numerical values for all individuals examined were summed and divided by the total number of individuals examined for the determination of weighted prevalence. Weighted prevalence is the same measure as weighted incidence described by Mackin (1962). The terminology has been changed here based on definitions of prevalence and incidence presented by Margolis et al. (1982). Incidence relates to new cases of infection appearing in a population within a given time and prevalence relates to the number of individuals infected. Given this distinction, we believe that the term weighted prevalence is more appropriate than weighted incidence.

A modification of the method described by Gauthier and Fisher (1990) was used for hemolymph diagnosis. Notches were cut in oyster shells posterior to the adductor muscle using a lapidary saw. A 300-μL hemolymph sample was withdrawn from the adductor muscle sinus using a 3-cc disposable syringe with a 23-gauge needle. The hemolymph was added to a microcentrifuge tube containing 1.0 mL of FTM fortified with 500 units of penicillin and streptomycin. Cultures were incubated in the dark at 27°C for 5–7 days. Following incubation the samples were centrifuged at 400 × g for 10 minutes. The supernatant was removed and the pellet was resuspended in 1.0 mL of 2 M NaOH. The samples were then incubated at room temperature for 30 minutes. Following incubation the samples were washed twice with distilled water and finally

TABLE 1.

James River native oyster (JRN) sample dates and temperature and salinity at time of collection.

Sample date	Temperature (°C)	Salinity (ppt)
13 November 1991	11.0	12
22 January 1992	4.5	10
18 February 1992	7.5	12
17 March 1992	10.5	5
14 April 1992	16.0	7
14 May 1992	21.0	4

resuspended in 1.0 mL distilled water and stained with 50 µL Lugol's stain (1:6 dilution). The samples were gently mixed with a pipette and transferred to 24-well culture plates. A Zeiss inverted microscope was used to examine the samples and infections were categorized as light (1–200 cells/well), moderate (200–15,000 cells/well), and heavy (>15,000 cells per well). Weighted prevalence was determined as described above.

Immunoassays and histological examinations were conducted on selected individuals in an effort to detect prepatent infections and possible cryptic states that were not revealed by FTM cultures. It was assumed that immunoassays would highlight rare infections and that if cryptic stages of *P. marinus* were present, they would have been antigenically similar to known stages and recognizable by the polyclonal antibody. Since our main objective was to detect prepatent infections and possible cryptic stages, not to compare techniques, immunoassays were only conducted on oysters that were diagnosed as negative by both tissue and hemolymph FTM cultures. The oysters selected for analysis by immunoassay and histology included 4 JRT oysters sampled in April, 14 JRT oysters sampled in May, 21 JRN oysters sampled in March, and 19 JRN oysters sampled in May. The primary antibody used in the immunoassays was polyclonal, rabbit anti-*P. marinus* raised against hypnospores (supplied by C. F. Dungan, Cooperative Oxford Laboratory, Maryland). Production and specificity of the antibody is described by Dungan and Roberson (1993). Biocell goat anti-rabbit IgG gold probe and Biocell light microscopy silver enhancement reagents were utilized to detect and visualize specific binding of the primary antibody (Goldmark Biologicals, Phillipsburg, New Jersey). Briefly, oysters preserved in Davidson's AFA were processed for paraffin histology following standard techniques. Three consecutive 5-µm sections were affixed to separate glass slides, dewaxed in xylene, and hydrated in a graded ethanol series to water. One of the three slides was stained with Harris' hematoxylin and eosin. The remaining two slides were washed in running tap water and phosphate buffered saline (PBS) and blocked for 30 min with 10% v/v normal goat serum in phosphate buffered saline containing 1.0% bovine serum albumin (PBSA). One of the two slides was incubated for 30 min in a 1:100 dilution of primary antibody in PBSA. The other slide, serving as a negative control, was incubated for 30 minutes in a 1:100 dilution of normal rabbit serum in PBSA. The slides were then washed in PBS and incubated for 1 hr in a 1:100 dilution of affinity purified goat anti-rabbit IgG coated onto 5-nm colloidal gold particles in PBSA. After thorough washing in PBS and distilled water, the bound colloidal gold particles were visualized with silver enhancement reagents that produced a brown black color. The slides were then washed in distilled water, counter-stained with fast green, dehydrated in ethanol, cleared in xylene and covered with a coverglass.

RESULTS

Perkinsus marinus prevalence in James River oysters examined at the initiation of the experiment was 100%. Tissue and hemolymph assays for the detection of *P. marinus* closely corresponded. Tissue diagnosis revealed 8 heavy, 9 moderate, and 33 light infections while diagnosis by hemolymph assay found 5 heavy infections, 6 moderate infections, 36 light infections, and 3 oysters diagnosed as negative. Initial prevalence of *P. marinus* in the Rappahannock River oysters was 8% based on tissue diagnosis and 2% based on hemolymph diagnosis. All infections were light.

During the course of the study, water temperature at the York River transplant site declined from 12°C in November, at the time of deployment, to a low of 3.8°C in mid-February (Fig. 1). Water temperature during the winter months was relatively warm, generally 1–3°C above the long term (1947–1991) average. Salinity ranged from 18.8–23.0 ppt and the mean weekly salinity was 22.1 ppt (S.D. = 1.66).

On the basis of tissue FTM diagnosis of sacrificed oysters, *P. marinus* prevalence in JRT oysters gradually declined during the winter months to 32% in May (Fig. 2). Weighted prevalence followed a similar trend declining from 2.0 in November to 0.56 in May (Fig. 2). Hemolymph assays for *P. marinus* prevalence closely corresponded with tissue diagnosis in November, January and May samples of sacrificed oysters, but were less sensitive in February and April analyses (Fig. 2). In most instances false negatives by hemolymph diagnoses were categorized as very light to light infections by tissue cultures.

In every sample of warmed JRT oysters, prevalence determined after the 30 day incubation period at 25°C was higher than that determined by hemolymph assays prior to incubation (Fig. 3). In November, January, and February *P. marinus* prevalence was >90% prior to warming and increased to 100% after warming. More striking differences in prevalence before and after incubation were observed in April and May. Prevalence increased from 36% to 52% in April and from 36% to 76% in May, based on hemolymph assays. Post warm-up prevalence based on tissue culture diagnosis was 71% in April and 100% in May. Post-incubation determinations of prevalence by hemolymph assays were similar to tissue diagnoses in November through February and lower than tissue diagnoses in April and May (Fig. 3). Weighted prevalence after warming was always higher than before warming. A decline in weighted prevalence, both initial and final, was observed from November through April (Fig. 3).

Some oyster mortality occurred during all five incubations. Mortalities during November, January, February, April, and May incubations were respectively, 36, 20, 12, 4, and 32 percent. Twenty-one of the 26 dead oysters were examined for *P. marinus*. Fifteen of those had moderate to heavy infections, 4 had light infections, and two were uninfected. Both uninfected oysters and one of the oysters with a light infection were found in May.

Rappahannock River tray (RRT) control oysters that were warmed in November exhibited a relatively high prevalence of *P. marinus*, 54%, after warm-up. Post warm-up *P. marinus* preva-

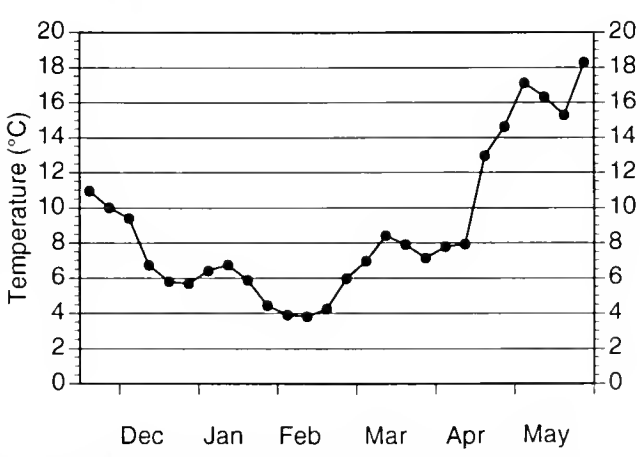

Figure 1. Mean weekly temperature at the VIMS pier York River site from mid-November 1991 through May 1992.

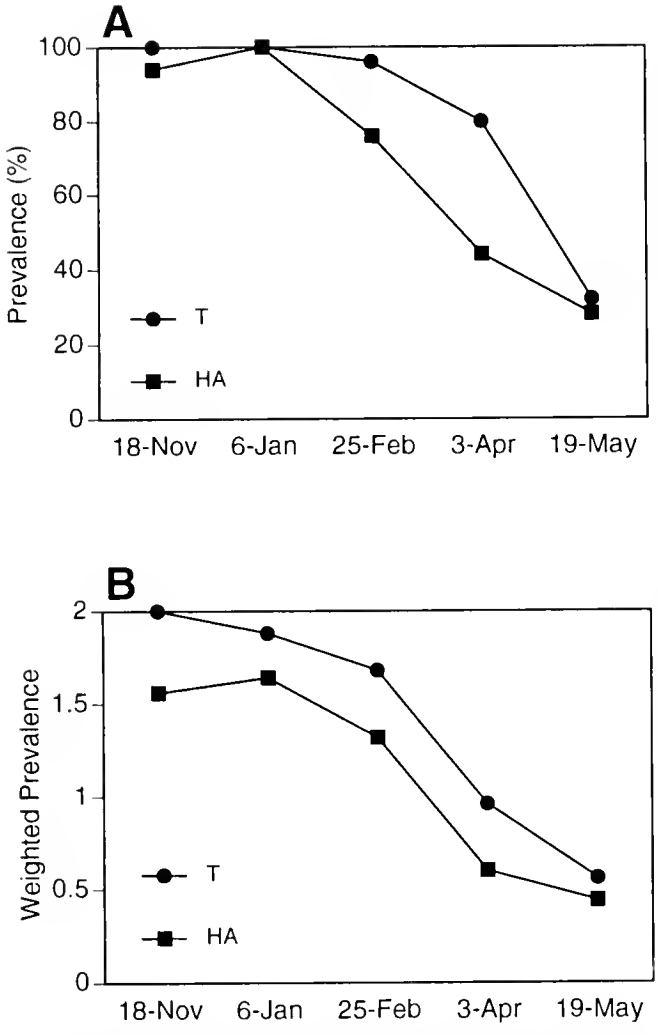

Figure 2. ***Perkinsus marinus*** **prevalence (A) and weighted prevalence (B) in sacrificed James River tray oysters (JRT). Diagnoses were made using both hemolymph (HA) and tissue culture (T) FTM assays. Sample dates (November 1991–May 1992) are shown on the *x*-axis. Sample size = 25.**

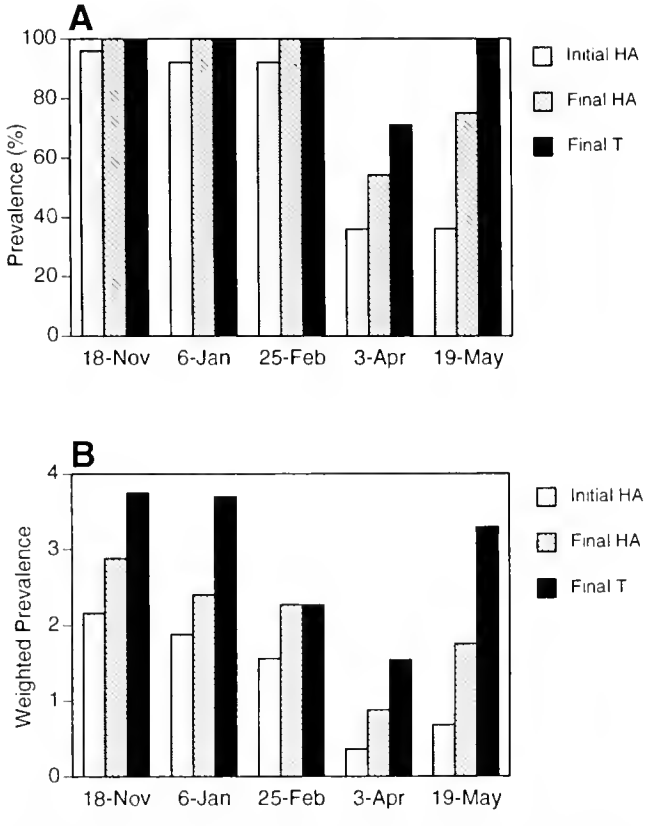

Figure 3. ***Perkinsus marinus*** **prevalence (A) and weighted prevalence (B) in incubated James River tray oysters (JRT) before (initial) and after (final) warm up. Diagnosis before warming was by hemolymph assay (HA). Post warm-up diagnosis was by hemolymph assay (HA) and tissue culture (T). Dates shown indicate date initial samples were taken and warming was initiated.**

lence in subsequent warmed groups did not exceed 9% (Fig. 4). The lowest post warm-up prevalence of *P. marinus* in the RRT group was observed in April, at which time no infections were detected. Weighted prevalence paralleled prevalence, increasing from initial pre-warm levels as a result of the incubation. The largest increase was observed in November; in subsequent warm-ups final weighted prevalence did not exceed 0.4, reflecting low prevalence and light intensity of infections. Mortality of RRT oysters during the warming period was 0% in November, January, and April, 4% in February and 12% in May. *Perkinsus marinus* was not detected in oysters dying in May. A heavy infection was observed in the oyster that died in February.

The prevalence of *P. marinus* in oysters collected at Point of Shoals in the James River and moved to the York River (JRT) was much different than prevalence in native oysters sampled directly from Point of Shoals in the James River (JRN) during the winter (Fig. 5). The prevalence declined much more rapidly in oysters at Point of Shoals than in the tray oysters. Water temperatures were similar at the two locations but salinity greatly differed. Point of Shoals salinity ranged from 4 ppt to 12 ppt while salinity at the York River site varied from 18.8 ppt to 23 ppt. Prevalence in JRN oysters that were warmed in March increased from 16%, based on pre-warm hemolymph assay, to 30.5% after warming. In May prevalence in warmed JRN oysters increased from 12% to 88%.

Immunoassays disclosed infections in many oysters that were diagnosed as negative by both tissue and hemolymph FTM cultures (Table 2). Immunoassays did not reveal a previously unrecognized cryptic stage of the parasite nor did they reveal high intensities of *P. marinus* cells overwintering in the oysters. Infections detected by immunoassays were typically comprised of single or small clusters of *P. marinus* cells within phagosomes of hemocytes in the epithelial lining of the digestive tract, usually the stomach (Fig. 6A,B). Often only one or two cells were observed in an entire preparation. Although the colloidal gold/silver stain assay obscures internal morphology to some degree, *P. marinus* cells appeared to be typical small, coccoid meronts as described by Perkins (1993). Occasionally, a localized positive antibody reaction was observed in stomach epithelium where no identifiable *P. marinus* cell was present. The reaction appeared to be a concentration of the silver stain precipitate; similar areas were not observed in negative control slides. Also observed was a positive antibody reaction inside hemocytes located in connective tissue, usually adjacent to the digestive tract (Table 2). Intact *P. marinus* cells were rarely observed in such instances, rather hemocytes contained a more or less diffuse aggregation of silver stain pre-

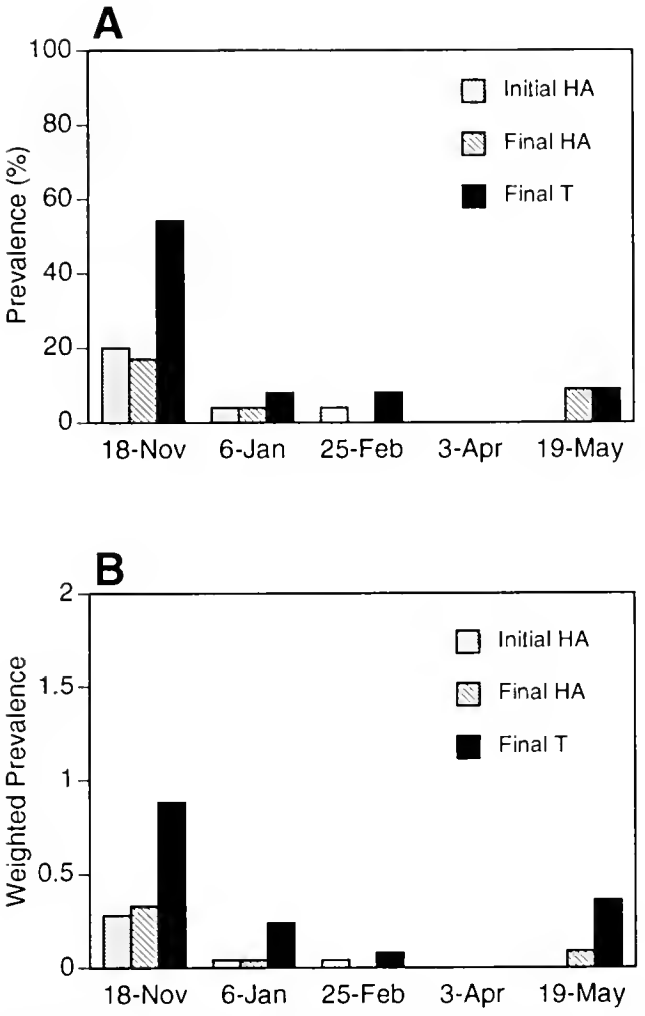

Figure 4. *Perkinsus marinus* **prevalence (A) and weighted prevalence (B) in incubated Rappahannock River tray oysters (RRT) before (initial) and after (final) warm up. Diagnosis before warming was by hemolymph assay (HA). Post warm-up diagnosis was by hemolymph assay (HA) and tissue culture (T). Dates shown indicate date initial samples were taken and warming was initiated.**

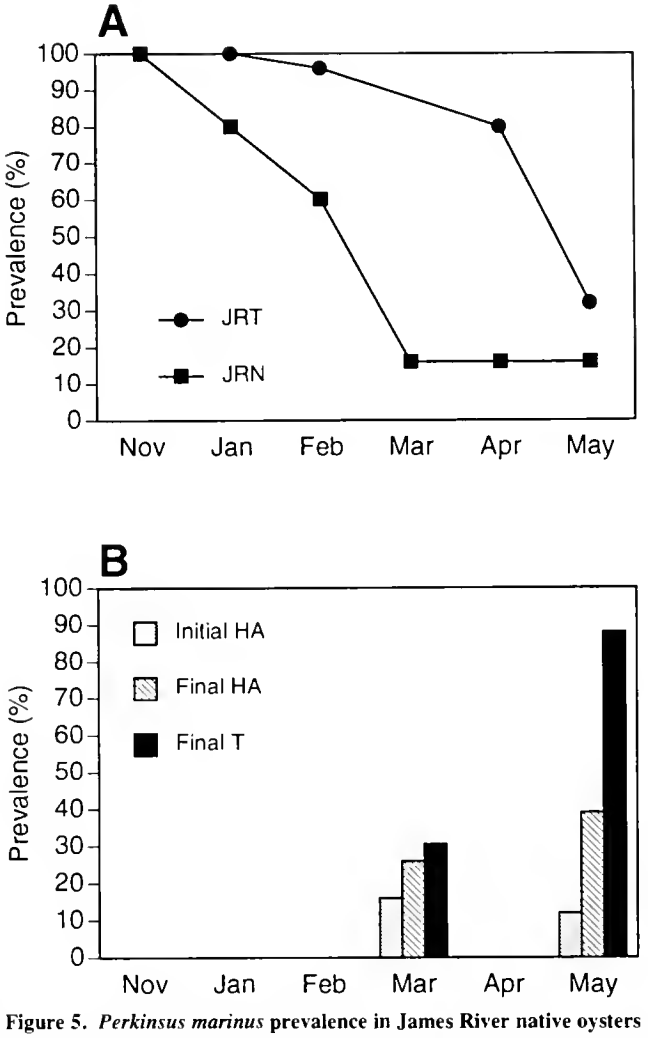

Figure 5. *Perkinsus marinus* **prevalence in James River native oysters (JRN) sampled directly from oyster grounds in comparison to that in James River tray oysters (JRT) maintained at the York River (A). Prevalence is based on tissue cultures of 25 oysters. Prevalence of JRN warmed oysters before (initial) and after warm up (final) is shown in B. Diagnosis before warming was by hemolymph assay (HA). Post warm-up diagnosis was by hemolymph assay (HA) and tissue culture (T).**

cipitate (Fig. 7A). In most oysters where this type of positive reaction was observed, a similar, but much less intense reaction was evident in the negative control section (Fig. 7B).

DISCUSSION

Annual epizootics of *P. marinus* in the Chesapeake Bay are believed to be initiated by oysters carrying overwintering infections from the previous fall. These overwintering cases develop into severe infections by August and cause a second generation of infections that are fatal by September (Andrews 1988). Andrews (1988) suggested that most light infections disappear in early winter while oysters carrying advanced infections are likely to die during the winter stress period. He speculated that rare survivors of advanced infections may be the carriers of overwintering infections. The results of the present investigation indicate that the proportion of oysters carrying overwintering infections is not limited to the rare survivors of advanced infections and may actually include 70–100% of the surviving oysters that were infected in the fall.

The ability to detect overwintering infections of *P. marinus* was enhanced by incubating oysters sampled during the winter at temperatures favorable to the multiplication of the parasite prior to diagnosis. Routine diagnosis based on oyster tissue incubated in FTM revealed a gradual decline in *P. marinus* during the winter and spring. However, all incubated JRT groups, with the exception of those warmed in April, had prevalences of 100% after warming. Because warmed oysters were held in individual containers in 1.0-μm filtered water it seems unlikely that observed increases in infections were the result of newly acquired infections during the warming period. Since *P. marinus* is enzootic at the York River site it is possible that new infections may have been acquired during the field exposure period and intensified during incubation. However, prevalence in the RRT control groups warmed in winter and spring remained very low after warming suggesting that new infections, if acquired, accounted for less than 10% of the observed increase in prevalence. Thus, it is most probable that the increase in prevalence during incubation reflects

TABLE 2.

Number of oysters examined and diagnosed as positive for *P. marinus* by immunoassay (IA) and frequency of positive immunoassay reactions observed within hemocytes located in connective tissue and epithelial linings of the digestive tract.

Sample Date	Sample Group	Number Examined by IA	Number Positive by IA	Epithelial Lining Digestive Tract	Connective Tissue
17 Mar	JRN	21	21	18	3
19 May	JRN	19	3	0	3
3 Apr	JRT	4	3	3	0
19 May	JRT	14	7	0	7

All immunoassays were performed on oysters diagnosed as negative for *P. marinus* by both tissue and hemolymph culture in FTM.
JRN = James River natives sampled directly from oyster ground; JRT = James River oysters maintained in trays at York River site.

the development of overwintering infections that were present but not disclosed by FTM assays prior to warming because of their light intensity.

Immunoassays also suggested that *P. marinus* was present in a higher proportion of oysters than FTM diagnosis indicated, supporting the results of the warming experiments, and suggesting

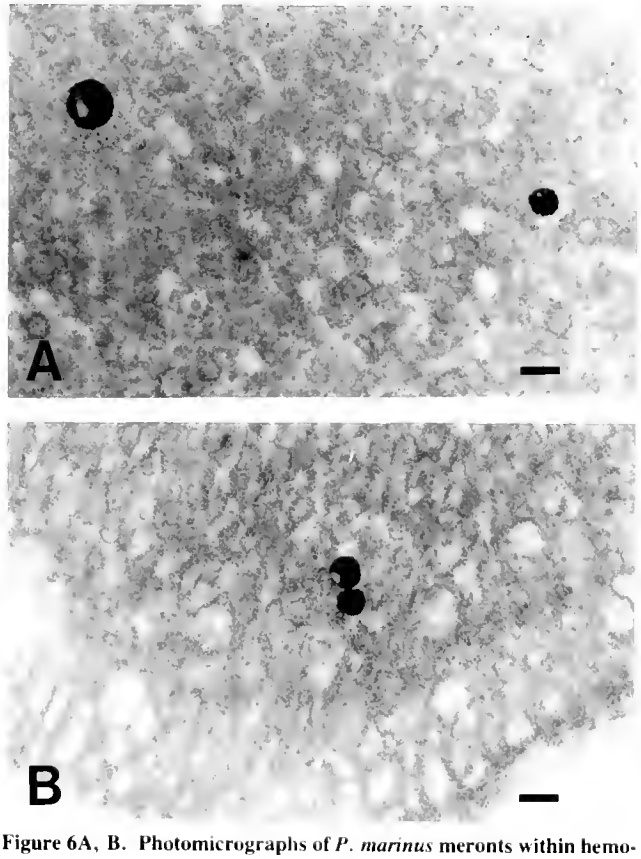

Figure 6A, B. Photomicrographs of *P. marinus* meronts within hemocytes in the gut epithelium of separate oysters visualized with colloidal gold immunoassay using anti-*P. marinus* antibody and fast green counter-stain. Oysters are from the 17 March James River native (JRN) sample. Scale bars = 5 μm.

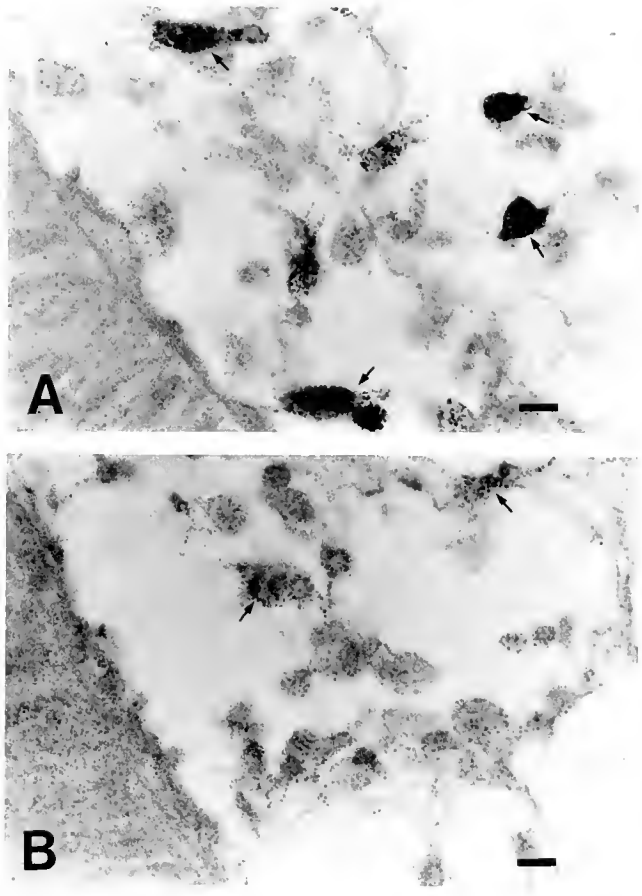

Figure 7. Photomicrographs of oyster hemocytes from the 19 May James River tray (JRT) sample. A: Positive immunoassay reaction using anti-*P. marinus* antibody in oyster hemocytes (arrows) adjacent to gut epithelium. B: Weak positive immunoassay reaction using normal rabbit serum (negative control) in oyster hemocytes (arrows) from the same oyster and area as in A. Scale bars = 5 μm.

that *P. marinus* overwinters at very low intensities in a high proportion of oysters. Immunoassays did not reveal the presence of previously unrecognized *P. marinus* cell types or "cryptic stages" but did detect very light infections of *P. marinus* in many oysters that were diagnosed as negative by FTM cultures of tissue and hemolymph. In most instances, particularly in March and April samples, typical *P. marinus* meronts were observed within hemocytes located in epithelial cells of the midgut.

The restriction of overwintering *P. marinus* meronts to hemocytes in the gut epithelium may be related to parasite expulsion involving the transport of parasite cells to the gut for elimination. Andrews and Hewatt (1957) and Ray (1954) suggested that at temperatures below 20°C, the metabolic activity of the parasite slows enabling the host to combat infections more effectively. The transport of foreign particles by oyster hemocytes to epithelial layers, including the lumen of the gut, and their subsequent elimination has been well documented (Stauber 1950, Tripp 1958, 1960, Feng and Feng 1974). Tripp (1958) suggested that oysters may eliminate *P. marinus* in this manner. Feng and Feng (1974) observed phagocytosis and migration of oyster hemocytes at temperatures as low as 6°C so it would be possible for elimination of *P. marinus* to occur during the winter months. The continuous decline in *P. marinus* intensity in the oysters sacrificed in this

study from November through April may reflect the elimination of *P. marinus* via host defense processes. In May a strong positive reaction to the antibody was observed in hemocytes located in connective tissue, although few intact *P. marinus* cells were observed. It is possible that the antibody was reacting with soluble antigen or noncellular particulate matter from degraded *P. marinus* cells. The antibody utilized is known to react with soluble and noncellular particulate substances in and around *P. marinus* lesions (Dungan and Roberson 1993) and intracellular killing of *P. marinus* meronts by hemocytes at low temperature (15°C) has been observed (La Peyre et al. 1992). Perhaps by May each year hemocytes have eliminated or degraded most of the *P. marinus* cells. Unfortunately, hemocytes in negative control slides, in which the primary antibody was substituted with normal rabbit serum, also reacted to the silver stain, but to a lesser degree. Although the reaction in the control slides was less intense than in the treatment sections, the presence of any positive reaction at all in negative control slides prevents us from concluding with certainty that the reaction observed in the section exposed to the antibody was a specific reaction for *P. marinus*. Additional research on the nature of the positive antibody reaction in oyster hemocytes is needed.

While expulsion or degradation of the pathogen by the host may have been occurring during the winter months, high *P. marinus* prevalences after warming indicate that the pathogen was not completely eliminated. The increase in weighted prevalence during the incubation period suggests that even very light infections can increase to lethal levels in a 30 day period when temperature exceeds 25°C.

Correspondence between hemolymph and tissue assays for *P. marinus* was variable. The two techniques closely corresponded in November, January, and May; however, during late winter when lighter infections prevailed, hemolymph assays were less sensitive than tissue cultures. In February and April, 20% and 45% respectively of the examined oysters were falsely diagnosed as uninfected using the hemolymph assay. This result suggests that there may be a seasonal pattern associated with the abundance of *P. marinus* in oyster hemolymph and contradicts the results of Gauthier and Fisher (1990) which suggested that the hemolymph assay technique is more sensitive than the tissue culture method. Differences in technique and season may account for the lower assay sensitivity found in the present investigation. Gauthier and Fisher (1990) conducted their comparison using oysters collected from the Gulf of Mexico in the late spring and summer when *P. marinus* infection intensities are relatively high. In addition, they used only mantle for tissue analysis and 1.0 ml of hemolymph. In this investigation a smaller volume of hemolymph (0.3 ml) and a combination of mantle, gill, and rectal tissue was utilized.

Perkinsus marinus prevalence in oysters collected at Point of Shoals in the James River and moved to the lower York River greatly differed from prevalence in Point of Shoals oysters collected directly from the site during the winter. The prevalence and intensity declined much more rapidly in oysters at Point of Shoals (JRN) than in the tray oysters (JRT). Further, prevalence of JRN oysters warmed in March did not exceed 40%, remaining much lower than JRT oysters warmed in February and April. *Perkinsus marinus* prevalence in JRN oysters warmed in May (88%) was higher than in March; however, since there were no in situ uninfected controls at Point of Shoals it is impossible to determine whether this increase in prevalence is a result of newly acquired infections or prepatent overwintering infections. There are undoubtedly many differences between the James River and York River locations, but the most prominent is salinity. Salinity at the James River site ranged from 4 ppt to 12 ppt, while the salinity at the York River tray site varied from 18.8 ppt to 23 ppt. Such differences in salinity may be largely responsible for the differences in prevalence and intensity of *P. marinus* between the two sites. Salinity at the James River location from November through February was 10–12 ppt. Prevalence steadily declined during this time, but a more pronounced decrease was observed from February to March as increased river flow caused a reduction in salinity to 5 ppt. Ragone and Burreson (1993) did not see a decline in prevalence in oysters exposed to salinities as low as 6 ppt for a period of eight weeks at temperatures above 20°C. The decline in prevalence observed in James River oysters in the present investigation may be a result of the synergistic effect of low temperature and low salinity. Although some degree of parasite expulsion may occur at cold temperature and salinities of 18–23 ppt, as observed in the JRT oyster population, elimination was enhanced at salinities below 12 ppt as observed in the JRN population. Although this study was not designed to examine the effect of temperature and salinity on prevalence and intensity of *P. marinus* infections, results suggest that a combination of low temperature and low salinity has more effect on reducing the abundance of *P. marinus* than either factor acting alone.

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CHEMICAL INHIBITION OF *PERKINSUS MARINUS* IN TWO *IN VITRO* CULTURE SYSTEMS

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ABSTRACT A rapid diagnostic test for oyster parasites, recently developed at the Cooperative Oxford Laboratory, utilizes Ray's fluid thioglycollate culture medium (RFTM) in polystyrene tissue culture plates to detect *Perkinsus marinus* cells in oyster hemolymph samples. This test was modified to serve as an *in vitro* assay system to detect compounds that exhibit inhibitory activity toward the cellular transformation and enlargement of *P. marinus* in RFTM. From an array of 42 compounds tested, the assay system detected 14 organic chemicals and 2 inorganic salts that inhibited hypnospore development. Cellular changes in treated *P. marinus* are described, and trypan blue vital staining confirmed that certain cellular changes resulted in death of the enlarging hypnospores.

The recent success of *in vitro* propagation of *P. marinus* at the Cooperative Oxford Laboratory, using the methods of LaPeyre et al. (1993), provided a second system for screening chemotherapeutic agents for their ability to kill or inhibit vegetative *P. marinus* cells. The 31 organic compounds tested contained 15 chemical formulations which inhibited the proliferation of *P. marinus* cells in the complete culture medium.

INTRODUCTION

Several of the most severe diseases of mollusks are caused by primitive Protozoa belonging to the phylum Apicomplexa (Levine 1978), yet there are only a few published reports (Ray 1966a,b, Sindermann 1990) of attempts to use chemical treatment for oyster diseases. In contrast, there are numerous compounds and therapeutic regimes for treatment of protozoan diseases of humans (Gilman et al. 1985), veterinary animals (Booth and McDonald 1985), fish, and crustaceans (Sindermann 1977). Some of these disease entities are Protozoa that have morphological similarities with the Apicomplexa.

Information found in pharmacology texts on therapeutic chemicals for Protozoa provides some insight as to which compounds may be effective against various life stages of *Perkinsus marinus*, the causative agent of perkinsiasis, a lethal disease in the American oyster, *Crassostrea virginica* (Mackin et al. 1950). These texts also contain information on the functional mechanisms of therapeutic compounds that inhibit disease entities, and many compounds are known to have specific reactive sites in Protozoa. For example, some chemicals are amino acid analogs; others inhibit cell wall synthesis. Information on the mode of action of reactive compounds may be very valuable in developing a better understanding of the physiology and life functions of *P. marinus*, an oyster pathogen that is causing extensive mortality in Chesapeake Bay oysters.

Salts of bivalent and trivalent elements have been used in veterinary medicine for the treatment of superficial abrasions and localized skin lesions caused by bacteria, fungi, and Protozoa (Booth and McDonald 1985). There are occasional references to the use of heavy metal salts in treatment of systemic infections by Protozoa. Some of the compounds reported to be reactive against Protozoa were included in this experimental consideration.

Ray (1952) devised a modified fluid thioglycollate medium (RFTM) and test procedure that permitted the trophozoites and sporangia of *P. marinus* found in oyster tissue to develop into enlarged prezoosporangia or hypnospores. This test has been the primary tool for describing levels of *P. marinus* infections in oysters (Sindermann 1990). During development of this procedure, Ray (1966a) tested several antibiotics for inhibition of bacteria, fungi, and other Protozoa frequently found in oyster tissue. Three of these antibiotics—amphotericin B, streptimidone, and cycloheximide—were found to be inhibitory to the survival and development of *P. marinus* cells (hypnospores) in RFTM. In a subsequent paper, Ray (1966b) evaluated the possibility of using cycloheximide to treat oysters infected with *P. marinus* so that they could be maintained under laboratory conditions without succumbing to perkinsiasis, or "Dermo" disease as it was known at that time. After several weeks of treatment, prevalence and intensity of *P. marinus* declined. This concept could be of value today since *P. marinus* epizootics have now reached a level in the Chesapeake Bay where it is impossible to hold oysters in the laboratory under summer water conditions without them succumbing to "Dermo" disease.

The primary purpose of this study was to develop information necessary for the *in vivo* treatment of oysters afflicted with *P. marinus*. Two easily conceived methods of controlling the effects of perkinsiasis would apply to aquaculture operations. Brood stock used in oyster hatcheries to produce seed oysters could be treated with chemicals prior to spawning to reduce parasite load and allow them to divert their physiological performance toward the production of viable gametes. The second use would be to treat lightly infected seed oysters to eliminate *P. marinus* prior to planting them in a disease-free environment.

METHODS

The *in vitro* assay for *P. marinus* hypnospore development utilized in this study is an adaptation of the hemanalysis technique (Gauthier and Fisher 1990) currently in use at the Cooperative Oxford Laboratory (Howard and Smith 1983). In this procedure, oysters are drilled at the valve margins adjacent to the adductor muscle and placed into recirculating aquaria for 24 hours prior to sampling. A hemolymph sample is taken from the adductor muscle sinus using a 5.0-ml sterile syringe fitted with an 18-gauge needle. Hemolymph from oysters known to contain heavy infestations of *P. marinus* was distributed into the sterile wells of a 24-well polystyrene tissue culture plate. A hemolymph sample of 0.5 ml was added to each well containing 1.3 ml of RFTM (Ray 1966c), supplemented with 400 units of mycostatin and 2.5 mg of chloramphenicol in 0.1 ml of sterile distilled water. In the test procedure, 0.1 ml of the test chemical suspended in triple-distilled

deionized water was added. Control wells received 0.1 ml of triple-distilled deionized water. Resulting dilutions of test chemicals in assay wells were 1, 2, 5, 10, 20, 50, 100, or 200 ppm. Tissue culture plates were incubated at room temperature and observed with an inverted microscope for the development of enlarged hypnospores (prezoosporangia) from the *P. marinus* cells (a mixture of trophozoites and sporangia) found in the circulating hemolymph of heavily infected oysters. During incubation in RFTM, enlarged hypnospores were observed for abnormalities in structure or for reduction in size. At least three control wells for the hemolymph extracted from each oyster were located in the tissue culture plates for convenient comparison between hypnospores in wells with and without chemicals.

The optimum time to observe cellular changes in test wells appeared to be 5 days after inoculation, at which time no further changes were seen. At day 5, a 0.5-ml aliquot of enlarged hypnospores was aspirated from the bottom of the wells and placed onto a clean polystyrene tissue culture slide. One ml of 0.001% (w/v) trypan blue in filtered sea water was added to the hypnospores as a dye exclusion test to detect living cells. After 5 days incubation, this preparation showed differential staining of live and dead cells.

The recent success of *in vitro* propagation of *P. marinus* at the Oxford Laboratory using the culture procedures reported by LaPeyre et al. (1993) provided a second assay system for evaluating chemotherapeutic agents for their ability to kill or inhibit vegetative meronts of *P. marinus*. The propagation technique for *P. marinus* isolates (CRTW-3HE) used tissue culture flasks and a complete growth medium, JLODRP-1.[1] This medium consists of inorganic salts, trace elements, amino acids, carbohydrates, vitamins, nucleic acid derivatives, lipids, proteins, and other compounds. For the exact composition of the media and its preparation procedures, see LaPeyre et al. (1993). The medium ingredients were dissolved in culture grade water obtained from a Milli-Q UF[1] water purification system. Osmolality of the medium was adjusted to 650 M osm/kg. The pH of the medium was maintained between 7.0 and 7.6.

Perkinsus marinus cells were removed from culture flasks, rinsed twice in culture medium, and resuspended in new culture medium. Aliquots were removed and placed into the wells of a 24-well polystyrene tissue culture plate to produce approximately 1×10^5 cells per well. Culture medium was added to create a total volume of 0.9 ml in the well. In the assay procedure, 0.1 ml of the test chemical suspended in sterile triple-distilled deionized water was added to the well. Serial dilutions of the test chemical in sterile triple-distilled deionized water were added to the wells to produce a final concentration of 200, 150, 25, 20, 10, 5, 2, and 1 ppm concentration of test chemicals. Control wells in the same polystyrene tissue culture plate contained *P. marinus* cells, and culture medium to a final volume of 0.9 ml, to which was added 0.1 ml of sterile triple-distilled deionized water. A control for the deionized water effects consisted of the aliquot cells and growth culture medium added to final concentration of 1.0 ml. Culture plates were incubated in a temperature-controlled incubator at 28°C.

Microscopic examination of culture wells was conducted with an inverted Olympus OM2[1] microscope. The relative abundance of *P. marinus* cells per field in the control wells was compared to cell abundance in wells to which chemotherapeutic agents had been added. Cell concentrations of 1×10^5 cells in the polystyrene wells yielded approximately 20 to 40 meronts and/or schizonts per field (20×). Populations of *P. marinus* cells in control wells doubled in 3 to 5 days after inoculation. A qualitative scoring system compared the cell concentrations in the treated wells to that in control wells. If cell concentrations were equal in the control and treated wells, no observed response was recorded. If the concentration of cells in the treated wells was less than the control well, a positive drug effect was recorded.

Between 10 and 14 days of incubation, *P. marinus* cells in control wells formed a continuous sheet across the bottom. In wells where chemicals were effective in deterring vegetative development, a concentration of cells similar to that at inoculation was observed. In some specific wells, a delay was noted to occur in *P. marinus* cell development. The phenomenon was scored as a "doubtful" (±) drug effect. However, after 14 days, cell concentrations in the "doubtful" wells approached those observed in the control wells.

Test Compounds

The chemicals used in the chemotherapeutic evaluation consisted of inorganic salts, as well as organic compounds. These compounds and manufacturers are listed in Table 5.

RESULTS AND DISCUSSION

In the thioglycollate culture system, microscopic observation of the enlarging hypnospores in test wells with chemical compounds revealed a gradation in cellular changes from lysis of the cell wall to no detectable change. Lysis or disintegration of the hypnospores occurred at the highest test concentrations of several test compounds. At lower concentrations, hypnospore cytoplasm and cell wall developed a granular appearance, suggesting denaturation of vacuole membranes or protein wall components. In some instances, the hypnospore enlargement process appeared to be inhibited by chemicals, and smaller hypnospores were produced in the thioglycollate culture system. Occasionally, compounds produced a crenated or irregular-shaped hypnospore, with distorted cytoplasm and an irregular membrane inside the hypnospore wall.

Most of the inorganic compounds listed in Table 1 failed to produce changes in hypnospore development. Notable exceptions were potassium permanganate, which caused cell lysis at 200 ppm and a granular appearance and smaller size hypnospores at 100 and 50 ppm, and cupric hydroxide, which produced smaller hypnospores and granular appearance at the two highest concentrations. Trypan blue staining confirmed that both compounds killed hypnospores at 200 ppm, but not at lower concentrations. Potassium permanganate is a strong oxidant, and cupric hydroxide is an alkaline formulation that may have altered the cell membranes. Other cupric compounds and other bivalent and trivalent ions had little effect on hypnospore development in this test system.

The thioglycollate test system revealed changes in hypnospore development for 15 of 31 organic test compounds known to be reactive against Protozoa, bacteria, and/or fungi (Table 2). Several compounds caused lysis or rupture of the hypnospore at high concentrations, and then showed a gradation of impact at lower con-

[1]The mention of trade names does not imply endorsement by the Maryland Department of Natural Resources.

TABLE 1.

Inorganic compounds tested for suppression of development of *Perkinsus marinus* hypnospores in *in vitro* concentration.

Compound		200 ppm		100 ppm		50 ppm		20 ppm	
		Response	Stained	Response	Stained	Response	Stained	Response	Stained
$ZnSO_4$	Zinc sulfate	±GS	10	–	10	–		–	
$NiSO_4$	Nickel sulfate	–		–		–		–	
$SnCl_2$	Stannous chloride	–		–		–		–	
$Zn(C_2H_3O_2)_2$	Zinc acetate	±GS	15	±GS	10	–	15	–	
$CuCl_2$	Cupric chloride	–		–		–		–	
$Cu(OH)_2$	Cupric hydroxide	SG	40	SG	15	±G	10	–	
$CuSO_4$	Cupric sulfate	±SG	15	–	15	–	10	–	
$AgNO_3$	Silver nitrate	±S	15	–	15	–	15	–	
$KMnO_4$	Potassium permanganate	R	100	GS	25	±S	25	±S	15
KIO_3	Potassium iodate	–		–		–		–	
$CO(NO_3)_2$	Cobaltous nitrate	–		–		–		–	
Controls			5–15						

G = granular appearance of surface and interior membranes; S = smaller hypnospores than control; R = ruptured wall.

centrations. Trypan blue vital staining confirmed that most of the observed morphological changes were lethal to the hypnospores. There were two notable exceptions—monteban and avatec—that appeared to cause no cellular changes in the test wells, but there were differences in the percentage of treated hypnospores stained as compared with the untreated controls. Metronidazole treatment resulted in the development of smaller hypnospores but trypan blue indicated that the cells were viable.

The exposure of *P. marinus* cells growing in complete growth medium to 10 inorganic salts and 31 organic compounds was observed for impact on cell population proliferation. Results recorded as growth suppression (+), delayed population development (±), or no response (−) are shown in Table 3 for the inorganic test compounds, and Table 4 for the organic test compounds. Silver nitrate was the only inorganic compound that showed an inhibitory effect on *P. marinus* proliferation. The concentration at which inhibition occurred (200 ppm) is relatively high and would be toxic to any living cell growing in tissue culture medium. This concentration of silver nitrate was also active against hypnospore development in thioglycollate test.

Thirteen (13) organic compounds exhibited inhibitory activity while two compounds caused only a delay in *P. marinus* proliferation. *Perkinsus marinus* cells in culture plate wells containing organic compounds that suppressed proliferation remained intact and appeared morphologically normal. Attempts to use a vital stain on *P. marinus* cells in wells which exhibited a depressed proliferation response yielded variable results. Staining of cells taken from control wells also produced a mixture of stained and unstained cells; therefore, a determination of whether the test chemicals had suppressed proliferation or actually killed the cells could not be made.

A modification of the thioglycollate culture technique origi-

TABLE 2.

Characteristics of *Perkinsus marinus* hypnospore development in response to selected organic compounds.

Compound	Concentration Tested						
	200 ppm	100 ppm	50 ppm	20 ppm	10 ppm	5 ppm	2 ppm
Avatec	L	GC*	GS*	*	·	·	·
Robenz	L	GS*	G*	–	·	·	·
Biocox	L	GS*	G*	–	·	·	·
Monteban	G*	G*	*	*	·	·	·
Coban	G*	G*	–	–	·	·	·
Monensin	L	L	L	S*	S*	–	·
Quinine sulfate	C*	C*	CS*	S*	–	–	·
Nitrofurazone	GS*	GS*	S*	–	·	·	·
Acriflavine	L	L	L	L	S*	–	–
Metronidazole	S	S	–	–	·	·	·
Malachite green	L	L	L	L	GS*	S*	–
Mertect	G*	G*	S	–	–	·	·
Captan	L	G*	G	–	–	·	·
Benomyl	SG*	SG*	G	–	–	·	·
Cycloheximide	G*	G*	G*	–	–	·	·

L = lysis of cell wall; G = granular appearance of cell wall and membranes; S = smaller size than control; C = crenated cell wall; * = hypnospores stained by 0.001% trypan blue; · = concentration not tested.

TABLE 3.

Inorganic compounds tested for suppression of development of *Perkinsus marinus* in culture medium.

Compound		Concentration 200 ppm	100 ppm	50 ppm	20 ppm
$ZnSO_4$	Zinc sulfate	–	–	–	–
$NiSO_4$	Nickel sulfate	–	–	–	–
$SnCl_2$	Stannous chloride	–	–	–	–
$Zn(C_2H_3O_2)_2$	Zinc acetate	–	–	–	–
$CuCl_2$	Cupric chloride	ppt	–	–	–
$CuSO_4$	Cupric sulfate	ppt	±	–	–
$AgNO_3$	Silver nitrate	+	±	±	–
$KMnO_4$	Potassium permanganate	–	–	–	–
KIO_3	Potassium iodate	–	–	–	–
$CO(NO_3)_2$	Cobaltous nitrate	±	–	–	–

± = delayed development; + = suppressed proliferation; – = no observed response; ppt = precipitated medium.

TABLE 4.

Organic compounds tested for growth suppression of *Perkinsus marinus* in culture medium.

Compound	Concentration Tested 200 ppm	100 ppm	50 ppm	20 ppm	10 ppm
Avatec	+	–	–	–	·
Robenz	–	–	–	–	·
Biocox	–	–	–	–	·
Monteban	+	+	+	–	·
Coban	+	+	–	–	·
Monensin	+	+	+	–	·
Quinine sulfate	–	–	–	·	·
Nitrofurazone	–	–	–	·	·
Acriflavine	+	+	–	–	–
Metronidazole	–	–	–	–	·
Malachite green	+	+	+	+	–
Mertect	±	–	–	–	·
Captan	+	+	+	+	–
Benomyl	+	±	–	–	·
Stenoral	±	–	–	–	·
Amprolium	–	–	–	·	·
Biomast	+	+	±	–	–
Griseofulvin	+	+	–	–	–
Chloraquine	–	–	–	·	·
Trimethoprim	–	–	–	·	·
Triple sulfa	–	–	–	·	·
Sulfamethazine	–	–	·	·	·
Sulfamerazine	–	–	·	·	·
Nicarb	–	–	·	·	·
Cycloheximide	+	+	+	±	–
Nizoral	–	–	–	–	·

± = delayed development; + = suppressed proliferation; – = no observed response; · = concentration not tested.

nally described by Ray (1966c) and the cell culture procedure for *P. marinus* in complete growth medium have provided an indication that some organic compounds and inorganic salts may be inhibitory to specific life stages of *P. marinus* organisms found in oyster hemolymph. Both *in vitro* tests showed greater reactivity of these compounds at higher concentrations, with a gradation to no impact at lower concentrations. Therefore, an estimate of the minimum effective inhibitory concentration and relative reactivity was obtained from the tests. Several of the endpoint concentrations for inhibitory activity observed in the *in vitro* tests correspond with the recommended dosages of the organic compounds that are known to have therapeutic value against Protozoa in humans and domestic animals.

Table 5 is a summary of information on each compound tested. Listed are the trade name, the chemical name, manufacturer, and the minimum effective inhibitory concentration as determined by the two tests. Compounds that failed to show any detectable alteration in the hypnospore enlargement process or did not completely inhibit *P. marinus* cell proliferation in growth media are indicated as "NR" or "no observed reaction."

Six compounds (monteban, coban, monensin, malachite green, benomyl, and cycloheximide) exhibited equivalent minimal inhibitory concentrations in the two test systems (Table 5). Four of the remaining compounds required higher concentrations to inhibit population proliferation of vegetative cells in complete growth medium than to prevent hypnospore development in the thioglycollate test system. Captan was the only compound that showed greater inhibition of the vegetative cells in growth medium, compared to the hypnospore proliferation enlargement response. Three compounds (robenz, quinine, and nitrofurazone) showed activity only against hypnospore development in RFTM and four compounds had an inhibitory effect on vegetative cell proliferation, but not on hypnospore development.

Several of the compounds tested (avatec, biocox, coban, cycloheximide, and monensin) are products purified from cultures of streptomyces (fungal) species. The derived drugs are able to form ionophore complexes with sodium and potassium ions in the developing parasites. The complexes render parasite membranes permeable to potassium and sodium ions. As a result, certain mitochondrial functions are inhibited (Booth and McDonald 1985). This group of compounds is used primarily in the poultry industry for treatment of coccidiosis caused by *Eimeria* species. The products used in the tests reported in this paper were commercial formulations adsorbed to finely ground rice hulls, for use in poultry feed.

Robenz is a guanidine derivative and is very effective against six species of *Eimeria* at a 33 ppm tissue level in chickens. The drug allows intracellular development of *Eimeria* up to the first multinucleate schizont, but prevents the formation of merozoites (Booth and McDonald 1985). Robenz was very effective in the laboratory against coccidiosis in chickens, but the parasites developed drug resistance to it after extensive use in the field.

Corid, or amprolium, destroys the first generation schizont in *Eimeria tenella*. The mode of action of amprolium is a thiamine antagonist in parasite metabolism. There were no observed responses to this compound by *P. marinus*. Dosages of 140 mg/kg of body weight are effective against clinical coccidiosis in cattle caused by *E. boris* and *E. zuernii* (Booth and McDonald 1985).

Nitrofurazone provides control of coccidiosis in lambs and goats at a dose of 70 mg/kg body weight. This is very close to the minimum effective concentration found in the *in vitro* test for *P. marinus* hypnospore development.

Metronidazole is a very successful drug for the treatment of human giardiasis, trichomoniasis, and hepatic amoebiasis, but had

TABLE 5.

Organic compounds tested for suppression of *Perkinsus marinus* development.

Trade Name	Compound	Manufacturer	Minimum Effective Concentration Against Hypnospores	Minimum Effective Concentration Against Cultured Meronts
Avatec	Lasalocid	Roche	100 ppm	200 ppm
Robenz	Robenidine	Cyanamid	100 ppm	50 ppm
Biocox	Salinomycin	Agribio	100 ppm	1000 ppm
Triple sulfa	Sulfamethazine 40% Sulfamerazine 40% Sulfaquinoxaline 20%	Russell	NR	NR
Monteban	Navasin	Lilly	50 ppm	50 ppm
Stenoral	Halomiginon	Hoechist	NR	±200 ppm
Cygro	Maduramigain ammonium	Cyanamid	NR	200 ppm (lysis)
SQ	Sulf-Q-Nox	Russell	NR	NR
Nicarb	Nitrocarbazin	Merck	NR	NR
Coban	Monensin	Lilly	200 ppm	100 ppm
Monensin		Sigma	10 ppm	50 ppm
3-Nitro-W	Monosodium 3-nitro-4-hydroxyphenylarsenate	Sigma	NR	NR
Quinine sulfate		Fisher	50 ppm	NR
Nitrofurazone	5-nitro-2-furaldehyde semi-carbazone	Sigma	100 ppm	NR
Acriflavine		Kodak	10 ppm	100 ppm
Metronidazole		–	NR	NR
Sulfamethazine		Sigma	NR	NR
Sulfamerazine		Sigma	NR	NR
Malachite green		Fisher	10 ppm	20 ppm
Corid	Amprolium	Merck	NR	NR
Mertect 340F	2-(4-thiazolylbenzimidazole)	Merck	100 ppm	500 ppm
Captan	N-trichloromethylthio-4-cyclohexene 1-2-dicarboximide	Dragon	100 ppm	20 ppm
Benomyl	Methyl 1-butylcarbamoyl	Dragon	100 ppm	200 ppm
Ferbam	Ferric dimethyl dithiocarbamate	Dragon	NR	NR
Maneb	Manganese ethylene bisdithiocarbamate	Dragon	NR	NR
Biomast			NR	±50 ppm
Griseofulvin	Grifulvin	Ortho	NR	100 ppm
Chloraquine phosphate	Sigma	NR	NR	
Trimethoprim	2,4-diamino-5 (3,4,5-trimelhoxybenzyl) pyrimidine	Roche	NR	NR
Cycloheximide	$C_{15}H_{23}NO_4$	Sigma	±50 ppm	50 ppm
Nizoral	Ketoconazole	Janssen	NR	NR

no effect on *P. marinus* cells. Acriflavine is used to treat *Babesia equi*, and shows activity against other species of *Babesia* in large animals and humans. Malachite green is a widely used water-soluble compound at concentrations of 2 to 10 ppm against sporozoan and fungal infections in fish (Sindermann 1977). This concentration compares favorably with the observed results of the *in vitro* inhibition tests of *P. marinus* cells. However, malachite green is extremely reactive to all organic compounds and is rapidly absorbed by non-target organic compounds; therefore, it must be administered in a procedure that will allow direct contact with *P. marinus* cells.

One group of compounds that was not active against the development of *P. marinus* hypnospores *in vitro* were the sulfonamides (sulf-q-nox, triple sulfa, sulfamerazine, and sulfamethazine). The pharmacological activity of sulfonamides has been studied extensively. They primarily interfere with paraminobenzoic acid synthesis in the folic acid pathway, and therefore are most active against stages of parasites that form genetic material—for example, in the development of second generation schizonts in *Eimeria* spp. The lack of reaction in this study and in the reports by Ray (1966a, b) suggests that enlargement of *P. marinus* trophozoites and sporangia into prezoosporangia does not involve the nucleic acid synthesis. However, it is possible that sulfonamides may be effective by *in vivo* administration where proliferative

stages are involved in the growth of the parasite population within host oysters.

The *in vitro* hypnospore development test utilized hemolymph from oysters heavily infected with *P. marinus*. The circulating pathogen cells were primarily trophozoites or meronts, many of which had been phagocytized by an oyster hemocyte. Another predominant form of the parasite was a sporangium, or mother cell, which contained between 2 to 32 cells that were developed by karyokinesis, but had not yet undergone cytokinesis to form a coccoid trophozoite (Mackin and Boswell 1956). Both of these forms appear to have the capability of enlarging into a prezoosporangium or hypnospore once they are placed into RFTM (Ray 1966a, Perkins 1988). During the process of enlargement in RFTM, a trophozoite or sporangium with a diameter range of 2 to 10 μm changes to a cell as large as 40 μm. Obviously, an array of metabolic activities must be undertaken by the *P. marinus* cells to change their shape and form. The most pronounced activity would be karyokinesis and then cytokinesis of a sporangium to form a trophozoite. Once trophozoites are formed, the necessary cellular enlargement process to become a presporangium or hypnospore should require some expenditure of metabolic energy. In the process of hypnospore enlargement, nuclear material and some of the cytoplasm become indistinct (Mackin 1962). The nuclear material will reappear as the prezoosporangia develop zoospores inside the cell wall. Any of these metabolic activities of *P. marinus* could have been compromised by the presence of the reactive chemical compounds in the *in vitro* test.

The *in vitro* tests used in this study did not provide an opportunity to examine whether the test compounds were active against *P. marinus* zoospores, or formation of zoospores within the zoosporangium. This step in the life cycle of *P. marinus* appears to occur in a low nutrient seawater medium and is reported not to occur in the thioglycollate test medium (Perkins 1988) or in the proliferation of meronts in complete growth medium (LaPeyre et al. 1993).

The *in vitro* screening procedure has evaluated an array of compounds, some of which were utilized in *in vivo* experiments to determine if an inhibition of *P. marinus* cell development can be achieved in living oysters. The effects of the test chemicals on *P. marinus* cells in individual oysters were easily monitored by the hemanalysis technique, using the circulating hemolymph of the treated oysters at various times after exposure to an array of dosages of selected chemicals. To date, none of the chemicals that exhibited inhibition of *P. marinus* in the *in vitro* systems has been observed to alter the prevalence or intensity of *P. marinus* in living oysters. The use of the minimal inhibitory concentration of chemicals in a test bath and by direct injection induced acute mortality in heavily infected oysters. However, these types of *in vivo* studies may still define a chemotherapeutic regime (dose, duration, compound, route of application) that will destroy *P. marinus* cells in the oyster, thereby enhancing the survivability of treated oysters placed in aquaculture systems and in the natural environment.

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SIZE-DEPENDENT MORTALITY IN HATCHERY-REARED POPULATIONS OF OYSTERS, *CRASSOSTREA VIRGINICA*, Gmelin 1791, AFFECTED BY JUVENILE OYSTER DISEASE.*

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ABSTRACT Recurring mortalities of hatchery reared juvenile oysters in the Damariscotta River, Maine, caused by juvenile oyster disease (JOD), prompted a monitoring program to track growth and survival of juvenile eastern oysters, *Crassostrea virginica*, during the summer of 1993. Three cohorts of oysters ranging in initial mean shell height from 12.1 to 25.9 mm were deployed on July 16 in growout trays adjacent to a commercial shellfish nursery operation. Mortalities commenced August 18 and continued beyond September 21, 1993 in all three cohorts. Dying oysters displayed cupping of the left valve, unequal shell growth with the left valve outgrowing the right, tissue emaciation, mantle retraction and conchiolin deposition on the inner shell surfaces. Cumulative mortality was highly correlated to mean cohort size (shell height). The smallest size class (Cohort 3) suffered the highest cumulative mortality (56.2%); the largest size class (Cohort 1) experienced relatively low cumulative mortality (13.6%). The results of this study suggest that the impacts of JOD can be minimized by attaining mean shell height of >25 mm prior to mid-July (requires early spawning) or by deploying seed after mid-August (requires overwintering).

KEY WORDS: *Crassostrea virginica*, juvenile oysters, disease, size dependent mortality

INTRODUCTION

Over the past two decades, the Damariscotta River, Maine has emerged as a center for commercial culture of eastern oysters (*Crassostrea virginica*) north of Cape Cod. Favorable growing conditions and proximity to hatchery-reared spat have contributed to the growth of this industry; six commercial oyster farms now exist along a 7-km stretch of the upper river (Hidu et al. 1981). Hatchery-reared oysters are grown in surface trays during the first growing season (June–November) and then planted on leased beds for growout to market size.

The future of eastern oyster culture in Maine has been threatened in recent years by several epizootics of unexplained Juvenile Oyster Disease (JOD) (see Bricelj et al. 1992). Heavy losses (40 – >90%) of juvenile oysters in the summers of 1988 and 1989 were experienced by most growers in the Damariscotta River (Davis, unpublished data). One commercial grower reported moderate (20–30%) mortalities in mid-September of 1991 (R. Clime pers. comm.). Histological examination of these oysters showed limited signs of mantle lesions (S. E. Ford pers. comm.). Oyster farms in southern New England and Long Island, New York have experienced similar outbreaks since 1989, and given the greater magnitude of oyster farming in those regions, the consequences have been more severe (Rask 1990, 1992, Bricelj et al. 1992, Relyea 1992).

Diseased oysters typically exhibit cupping of the left valve, which also outgrows the right valve, mantle lesions and conchiolin deposition on the inner shell surfaces, and reduced condition indices and shell growth before death (Bricelj et al. 1992). Other commercially reared bivalves including European oysters (*Ostrea edulis*, Linnaeus) and hard clams (*Mercenaria mercenaria*, Linnaeus) have not shown these symptoms when reared near diseased eastern oysters (Bricelj et al. 1992, Davis unpublished data).

Efforts to identify the etiologic agent for JOD outbreaks are ongoing, and as yet, no consensus has been reached among researchers. Several possible agents have been proposed. Bricelj and coworkers (1992) observed blooms of dinoflagellates (*Gymnodinium sanguineum*, Hirasaka) at the time of the epizootics and development of mantle lesions, and suggested there may be a causal relationship, although recent feeding experiments with this dinoflagellate failed to cause JOD symptoms in juvenile oysters (Wickfors and Smolowitz 1994). Bacterial (*Vibrio* spp.) infestations were seen in some mantle lesions, although it wasn't clear whether they were primary or secondary invaders (Bricelj et al. 1992). Farley and coworkers hypothesized that protistan parasites (*Ulceratus spraguei*) described as "small round intracellular bodies" may be responsible for mortalities (Farley et al. 1992, Farley and Lewis 1993). Lewis and Farley (1993) also reported being able to transmit JOD to healthy oysters in the laboratory.

There is a general consensus that mortality caused by JOD is related to size and that oysters above 25 mm shell height are less susceptible to the disease (Bricelj et al. 1992, Farley and Lewis 1993, Rask 1992, Relyea 1992, Lee et al. 1994). The goal of this study was to monitor growth and survival of three cohorts of eastern oysters and thus document the effect of size and age on susceptibility to JOD. Weekly shell height measurements of healthy, live JOD affected and dead oysters provides further insight into the role of size-dependence on JOD induced mortality. Understanding this relationship may provide growers with strategies for reducing losses of juvenile oysters to JOD.

MATERIALS AND METHODS

Stocks

Growth, mortality, and environmental conditions were monitored from July 16 to November 4, 1993, for three size classes of

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oysters in the Damariscotta River, Lincoln County, Maine, USA (44°00′53″N, 69°32′57″W). All cohorts were spawned at Mook Sea Farm, Inc., Damariscotta, Maine, a commercial shellfish hatchery supplying much of the oyster seed to the local growers. Cohort 1 was spawned in June 1992. The history of the broodstock is not known. The cohort was reared at commercial densities (17,000–1,700 · m^{-2}) on a nursery lease from late July through November 1992. Mortality during this first growing season was low (<5%). The oysters were stored over the winter of 1992–93 in the Damariscotta River and made available for this study in July 1993. Over wintering mortality was also negligible. The cohort had an initial mean shell height (±SE) of 25.9 (0.54, n = 50) mm on July 16, 1993. Cohort 2 was spawned April 14, 1993 from a line of oysters having undergone one generation of selection for rapid growth (Davis et al. 1990). The mean shell height (±SE) was 16.2 (0.29, n = 50) mm upon deployment for this study. Cohort 3 was also created from the fast growing line on April 29, 1993, although with different parents. Its mean shell height (±SE) was 12.1 (0.32, n = 50) mm on July 16. Highly significant differences ($p \leq 0.01$) in mean shell height occurred among each of the three cohorts at the onset of this study. Cohorts 2 and 3 left the hatchery in mid-June and were reared in upwellers or window screen trays near the nursery site prior to use in this study. All groups appeared healthy with no signs of JOM upon deployment at the study site on July 16.

All cohorts were reared in screened floating trays beside a commercial shellfish nursery (Pemaquid Oyster Co., Inc.). Trays consisted of 30 cm × 76 cm mesh envelopes supported by 35 cm × 80 cm rectangular frames of 1/2″ (12.7 mm) diameter PVC pipe. These assemblies were contained in 46 cm × 81 cm × 9 cm extruded polyethylene cages (12.7 mm mesh). Mesh envelopes were made of either 1 mm fiberglass window screen or 4.5 mm polyethylene netting. Cohort 1 was initially stocked in the 4.5 mm mesh envelope. Cohorts 2 and 3 were initially stocked in window screen envelopes on July 16 and transferred to the larger mesh August 4 when all individuals were large enough to be retained. Cohort 1 initially had 400 individuals per tray (1,700 · m^{-2}). Cohorts 2 and 3 each started with approximately 500 individuals per tray (2,200 · m^{-2}). Twenty-five oysters per cohort were randomly removed at each sampling period to provide samples for future histological studies. Densities were further reduced by the removal of dead oysters.

Sampling Protocol

All cohorts were sampled for growth and mortality approximately once a week from July 16 through September 21. A final census and growth assessment was taken November 4. At each sampling period, all oysters were examined for mortality and general health. Oysters displaying external signs of JOD (deformed left valve outgrowing the right) were measured starting August 26. Counts of disarticulated valves and intact gaping valves were recorded and expressed as percent mortality. Due to the periodic removal of live oysters for future histological studies, cumulative mortality (CM) was determined using instantaneous mortality rates (i) for each sampling period where $CM = 1 - e^{\Sigma i}$ (Krebs 1972). Confirmation of non-gaping (i.e., valves stuck together) live oysters was achieved by gently pinching the umbo (anterior to the hinge) which would separate the valves of moribund or recently dead individuals.

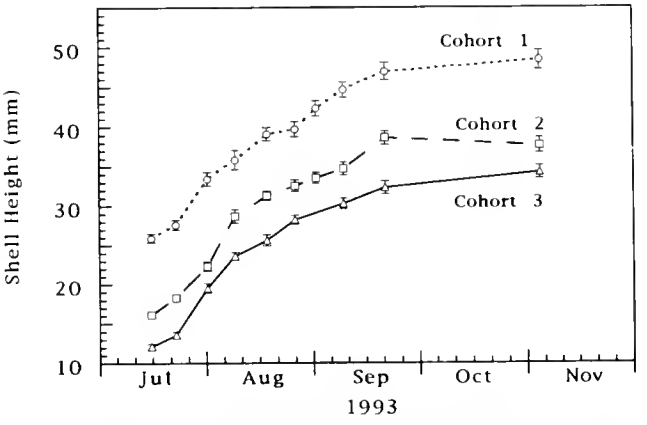


Figure 1. Growth of healthy oysters in three size cohorts during the 1993 study period in the upper Damariscotta River, Maine. Error bars indicate one standard error. All means are significantly different ($p \leq 0.05$) from one another except for cohorts 2 and 3 on November 4 ($p = 0.23$).

Shell height (SH) measurements of "healthy" oysters were taken with digital calipers (±0.1 mm) from 50 (25 on August 2 and August 9) randomly chosen individuals per cohort. Oysters were considered healthy if they had a uniform growth edge, were not gaping and did not display the characteristic shell deformities which typify the presence of JOD. Similar measurements were also taken of all (50 measurements when n > 50) live oysters having shell abnormalities (termed "deformed" in Table 1 and Fig. 4) from August 26 onward and all boxes (dead oysters) from August 18 onward. Live weights of 50 healthy individuals per cohort were measured with an electronic balance (±0.1 g).

Surface water temperature was recorded twice daily with a datalogger and weekly means were calculated. Surface salinity was measured weekly with an optical refractometer.

Statistical analysis was done with the SYSTAT statistical package (Systat 1992). Analysis of variance was used to test for significant differences in SH among cohorts. The Tukey-Kramer HSD *post hoc* test for mean separation was used to further discriminate differences among cohort means.

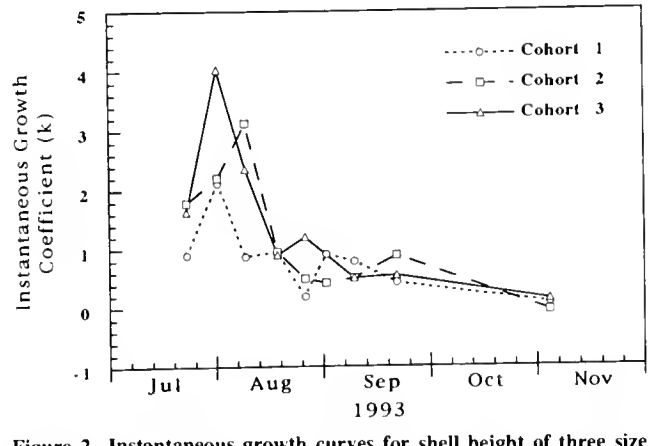


Figure 2. Instantaneous growth curves for shell height of three size classes of oysters. The instantaneous growth coefficient k_{SH} is defined as $\{[\ln(SH_{t+1}) - \ln(SH_t)]/[(t+1) - t]\} * 100$, where SH is shell height (mm), t is the sampling date (days).

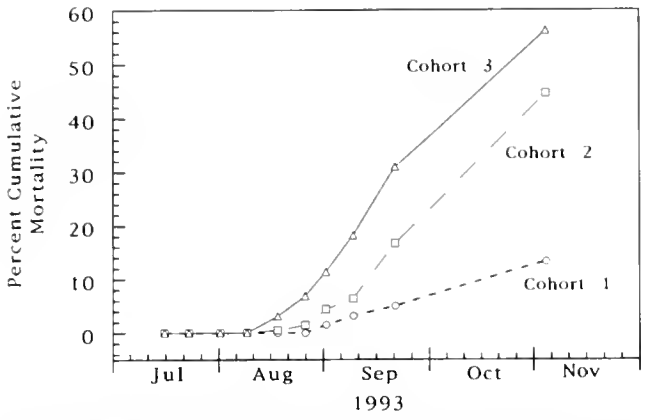


Figure 3. Mean percent cumulative mortality for three size cohorts of oysters.

RESULTS

Growth

Growth patterns of healthy oysters in the three cohorts were similar over the study period (Fig. 1). Healthy individuals in Cohorts 1–3 had final mean shell heights (±SE) of 48.5 (1.21), 37.6 (0.97) and 34.2 (0.82) mm, respectively. Corresponding final mean live weights (±SE) were 17.3 (0.95), 8.76 (0.64) and 6.74 (0.45) g. Significant differences ($p \leq 0.05$) in mean shell height of healthy individuals occurred between all cohorts for all sampling dates except November 4, when Cohorts 2 and 3 did not differ significantly from one another ($p = 0.23$). Maximum instantaneous growth coefficients (k) for Cohorts 1–3 were 2.12, 3.13 and 4.03, respectively, and occurred between July 21 and August 9 (Fig. 2). Although the slowest growth occurred in the final sampling interval (September 21–November 4), the precipitous drop in k values in mid-August corresponds with the onset of mortality and shell anomalies (Fig. 3). Growth continued throughout the rest of the summer, but at a much lower rate ($k \leq 1.0$).

Mortalities and Shell Deformities

Mortality of oysters occurred following the period of rapid growth in July (Fig. 2). All dead oysters displayed cupping and a reduction of shell growth in the right valve. Eighty percent of dead oysters showed conchiolin deposits (brown rings) on the inner valve surfaces. Mantle retraction and tissue emaciation were evident in moribund individuals.

Mortalities of the oldest and largest size class (Cohort 1) were first observed on September 2 and continued beyond September 21 (Table 1). Cumulative mortality was 13.6% by November 4 (Fig. 3). At the onset of mortality, mean shell height (±SE) for "healthy", deformed, and dead oysters were 42.3 (0.97), 31.9 (0.62, n = 38) and 27.0 (1.07, n = 4) mm respectively (Fig. 4). By the end of the sampling period (November 4) the mean SH (±SE) of dead individuals had increased to 35.0 (1.61, n = 16) mm although this increase in size was not significantly different ($p = 0.072$) from the September 2 sample mean. Shell deformities were first observed on August 26 and peaked on September 9 at 24.1% (Table 1). Size (SH) of live, but deformed oysters did not change significantly ($p \leq 0.05$) over the sampling period nor did they significantly differ in size from dead oysters on any of the sampling dates. Both groups were always significantly smaller in SH than normal oysters ($p \leq 0.05$ in both cases).

Cohort 2 experienced mortalities from August 18 through November 4 with greatest losses between September 9 and 21 (Table 1). Cumulative mortality by November 4 was 44.7% (Fig. 3). Shell deformities were first seen on August 26 and peaked by September 9 at 15.5% (Table 1). At the onset of mortality, normal and dead oysters had mean (±SE) shell heights of 31.3 (0.57) and 16.1 (1.35, n = 2) mm respectively (Fig. 4) and by November 4, SH (±SE) of dead oysters had increased significantly ($p \leq 0.01$) to 29.0 (0.72, n = 50) mm. The size (SH (±SE)) of deformed live oysters ranged from 22.5 (0.69) mm on September 2 to 30.1 (1.95) mm on November 4 (Fig. 4).

The smallest oysters (Cohort 3) suffered the highest mortalities of the three size classes. Mortalities, first observed on August 18, continued throughout the sampling period, reaching 56.2% cumulative mortality on November 4 (Table 1). Shell heights of the normal and dead oysters averaged (±SE) 25.6 (0.68) and 13.4 (0.79, n = 14) mm, respectively, at the beginning of the mortality period. Despite the rapid rate of mortality in this group, the average size of dead oysters increased significantly ($p \leq 0.05$) in conjunction with the mortality, ending with a mean size (±SE) of 25.3 (0.58, n = 50) mm (Fig. 6). Shell deformities, first recorded on August 26 (11.6%), had a maximum prevalence on September 21 (28.0%). Size (±SE) of deformed oysters increased from 20.0 (0.48, n = 44) mm on August 26 to 27.0 (1.15, n = 8) mm on November 4 (Fig. 4).

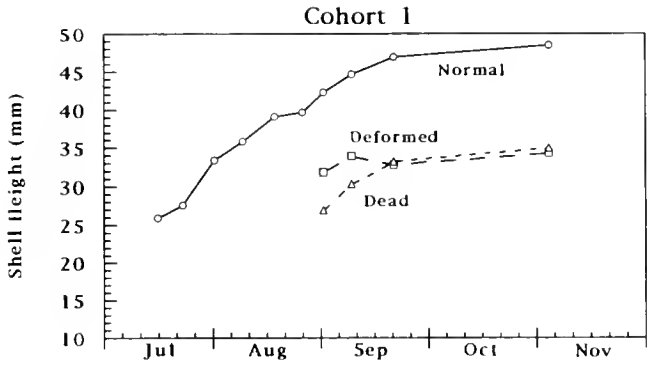
The onset of mortality in all three cohorts occurred when surface water temperature was 21–22.4°C (Fig. 5). Mortalities were observed throughout September and probably into October when water temperature was rapidly declining. Salinity was somewhat variable in the early summer 28–30‰, but remained constant at 30–31‰ from mid-July onward (Fig. 5).

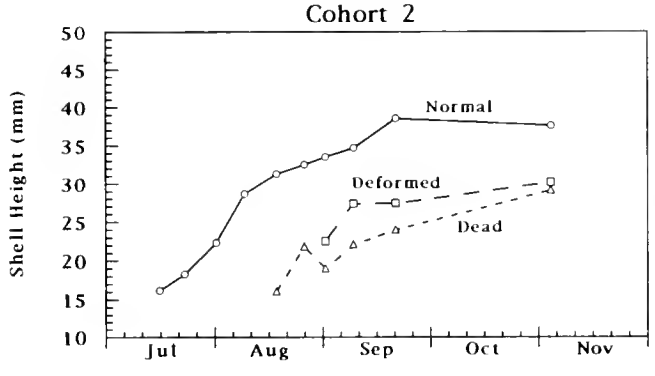
TABLE 1.

Counts and (percentages) of live oysters with deformed shells and dead oysters over the study period for three cohorts of oysters.

	Deformed Shell # (%)			Dead # (%)		
Date/Cohort	1	2	3	1	2	3
8/18	–	–	–	0	2 (0.5)	14 (3.1)
8/26	15 (5.1)	4 (1.0)	46 (11.6)	0	4 (1.0)	16 (3.9)
9/2	39 (14.7)	36 (9.9)	69 (19.4)	4 (1.5)	11 (2.9)	18 (4.8)
9/9	57 (24.1)	51 (15.5)	62 (18.8)	4 (1.7)	7 (2.1)	23 (7.7)
9/21	35 (17.0)	32 (11.6)	59 (28.0)	4 (1.9)	34 (11.0)	39 (15.6)
11/4	29 (17.5)	7 (4.3)	8 (6.9)	16 (8.8)	83 (33.6)	67 (36.6)

No mortalities were observed prior to August 18.





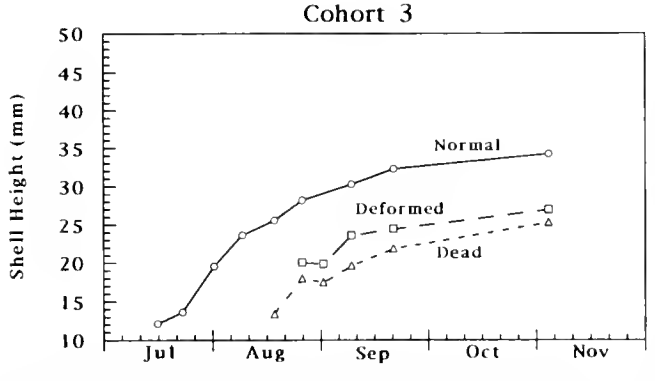


Figure 4. Changes in shell height of normal, deformed and dead oysters for three size classes during the study period.

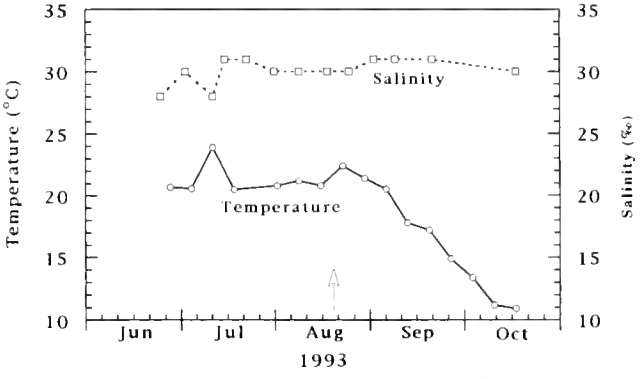


Figure 5. Temporal changes in surface water temperature (°C) and salinity (‰) throughout the study period. Arrow marks onset of mortality.

DISCUSSION

Juvenile oyster disease occurred in the Damariscotta River in 1993, although the mortality rate and thus impact on commercial growers was not as severe as in prior years. The three size classes (initial SH range = 12.1–25.9 mm) suffered mortalities from mid-August until after late September. All signs associated with mortality (valve cupping, unequal shell growth & abnormal conchiolin deposits), which are indicative of this disease (Bricelj et al. 1992), were also found here. Due to a reduced sampling effort after September 21, we were unable to determine precisely when the mortality stopped. Tissues found in dead oysters on November 4 had mostly decayed, indicating that the mortalities occurred in the early part of the final sampling interval (Fig. 3).

In spite of the apparent correlation between shell deformity and mortality, it isn't clear whether all deformed individuals eventually succumbed to JOD or whether recovery later occurred. In Cohorts 2 and 3, prevalence of deformed oysters declined greatly from mid-September onward, while mortalities continued to mount. This may indicate that all deformed individuals eventually died or, less likely, deformed oysters recovered and previously normal individuals made up the new mortalities. Tagging individuals would allow one to track their eventual fate and resolve this quandary.

Temperature and salinity were generally normal for the Damariscotta River in the summer months except for a short period of unusually warm weather in early July (Fig. 6). The salinity in the Damariscotta River is somewhat higher than those described in the study by Bricelj et al. (1992) in Oyster Bay, New York. Lewis and Farley's experimental work on the effect of salinity on JOD focused on salinities ≤26‰ (Lewis and Farley 1994); thus this study documents the occurrence of JOD in the upper end of the salinity range (28–31‰) for eastern oysters. Although Lewis and Farley (1994) noted a reduction of mortality at lower salinity, that is not a viable management option in the Damariscotta River.

Other researchers have reported that JOD primarily affects oyster between 6 and 30 mm shell height (Bricelj et al. 1992, Farley and Lewis 1993, Rask 1992, Relyea 1992, Lee et al. 1994). We found that oysters in the largest size class (Cohort 1) had a mean shell height (±SE) of 35.0 mm when they died, although mortalities were at a reduced rate compared to smaller cohorts (Fig. 6). Interestingly, size of dead oysters increased as mortalities progressed for all three cohorts (Fig. 4). Oysters may have been exposed to JOD for a prolonged period, thus explaining the increase in SH as the epizootic progressed. Alternatively, some individuals may resist the disease and continue to grow before succumbing.

This study supports other findings that JOM is inversely related to oyster size and age. The smallest (and youngest) size/age class (Cohort 3, SH = 12.1–34.2 mm) had the highest cumulative mortality (56.2%) while the largest (and oldest) size/age class (Cohort 1, SH = 25.9–48.4 mm) had the lowest cumulative mortality (13.3%). Regardless of the etiology of JOD, it is apparent that larger (older) oysters are more tolerant of the disease than smaller (younger) ones within the size range of oysters we exam-

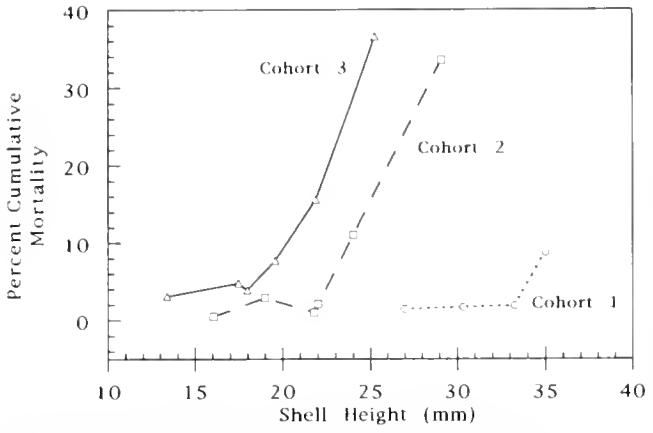


Figure 6. Time series relating shell height of dead oysters to percent cumulative mortality for three size classes of oysters.

ined. The relatively late (September 2) onset of mortality in the largest (oldest) group (Cohort 1) supports this assertion.

The mortality period in this study was more prolonged than that in the Oyster Bay, New York study as described by Bricelj et al. (1992). In their study, juvenile oysters succumbed to the disease over a 2–4 week period, whereas oysters in this study continued to perish over a 1–2 month period. The outbreak of mortalities occurred about one month later than in the Oyster Bay (mid-July) epizootic. Water temperatures were similar at the time of the outbreaks (20–23°C) and in both cases, first mortalities occurred after a 6–7 week period of water temperatures greater than 20°C and following periods of rapid growth.

In spite of the 1–2 month period of mortality seen in this study, there is evidence to suggest that there is a defined period of exposure after which high survival of even small seed can be attained. Two commercially reared groups (A and B) of juvenile oysters in close proximity to the study site were examined for end-of-season size and mortality. Groups A and B were spawned in mid-June and July 1, respectively, from different broodstock in different hatcheries. The parental lineage of Group A is unknown, while Group B arose from the same genetic stocks as Cohorts 2 and 3. Group A was deployed as 1 mm seed in upwellers adjacent to the study site in mid-July and then reared in window screen trays (densities = 17,000 · m^{-2}) at the study site from July 28 until November 19. Group B was deployed as 2–3 mm seed at the same site over a 17 day period starting August 18. It too was reared under commercial growing conditions similar to Group A. Striking differences were seen in both groups when sampled November 19 to estimate end-of-year size and mortality. Group A suffered 48.6% cumulative mortality with an 18.1 (SE = 0.54, n = 50) mm mean size of dead animals. The remaining healthy oysters had attained a mean SH (±SE) of 29.9 (0.86, n = 49) mm. Group B suffered 2% mortality with a mean SH (±SE) of 6.9 (0.23, n = 100) mm. The differences in survival between the two groups may be because 1) Group B was deployed after the exposure period for JOD; or 2) a lower size/age refuge exists which improves the survivability of very small oysters when challenged by JOD. Either scenario might explain why Cohort 1 used in this study experienced low mortality in 1992 (although year-to-year variability of JOD intensity can't be excluded as a possible reason).

The inverse relationship between JOD and oyster size suggests two possible management strategies for oyster growers. The first would be to maximize the size of seed (>25 mm) before the presumed JOD exposure period (mid-July–mid-August). This could be done by spawning as early in the year as possible to maximize growth in the early growing season. Spawning would be ideally timed so that deployment of 2–3 mm seed would be coincidental with the late spring plankton bloom and water temperatures above 8–10°C. Growth rate could also be improved through genetic selection and the use of triploid oysters. The second strategy would be to spawn animals late enough to deploy seed after the threat of exposure to JOD (late August) as was demonstrated in commercial Group B and Cohort 1 in the 1992 growing season, although when this late season spawning scenario was employed in 1992 at the F. M. Flower company in Oyster Bay, New York, high JOD induced mortalities were still observed (Relyea 1994). Small size at the end of the growing season would preclude bottom planting that year, so over wintering would be necessary and subsequent tray culture for a portion or all of the following growing season. Cohort 1 is an example of this second strategy. Aquaculturists will have to weigh the costs and benefits of these two strategies and choose the approach most suitable to their operation. For example, managers at the F. M. Flower & Son oyster farm have tried variations of both strategies and found the first to be superior for their operation (Relyea 1994).

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We thank Miranda Grace and Roshan Mazhar for assistance in processing samples, and the Pemaquid Oyster Co., Inc. for use of their nursery lease and the donation of seed oysters. This work was partially funded by the Maine Aquaculture Innovation Center through Grant No. 93-09.

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IN VITRO CULTURE AND MAINTENANCE OF *HAPLOSPORIDIUM NELSONI* (HASKIN, STAUBER AND MACKIN, 1966) SPRAGUE 1978 (MSX)

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ABSTRACT A considerable body of information concerning the relationship of the pathogen *Haplosporidium nelsoni* (MSX) and its eastern oyster host *Crassostrea virginica* (Gmelin 1791) has accumulated from the study of stained and sectioned specimens. Studies utilizing long-term *in vitro* culture and maintenance of this parasite could also contribute to understanding its cellular physiology, genetics, life cycle, and host interaction/specificity. Utilizing standard sterile techniques and a medium consisting of a 25% *Perkinsus marinus* medium (Kleinschuster and Swink 1993), 35% sterile oyster hemolymph, and 40% sterile sea water, cultures of *H. nelsoni* were maintained for several weeks allowing observation of several transitional stages of the parasite *in vitro*.

KEY WORDS: *Haplosporidium nelsoni*, MSX, *Crassostrea virginica*, cell culture

INTRODUCTION

It has long been recognized that the pathogenic protozoan *Haplosporidium nelsoni* (MSX) is one of the etiologic agents responsible for major mortalities in populations of the eastern oyster, *Crassostrea virginica*, occurring on the east coast of the United States (Haskin et al. 1966, Andrews and Wood 1967, Ford and Haskin 1982). Considerable information concerning progressive profiles of tissue, organ, and systemic infections has been obtained utilizing fixed, stained, and sectioned material (Farley 1968, Ford and Haskin 1987, Barber et al. 1988, Ford and Kanaley 1988). However, a more complete understanding of many aspects of the parasite's reproduction, cellular biology, mechanisms of invasion and transmission would be forthcoming if the organism could be cultured *in vitro* for extended periods. We report herein the partial attainment of this requisite as well as observations of several transitional stages of the parasite while in culture.

MATERIALS AND METHODS

Susceptible oysters were exposed to *H. nelsoni* in lower Delaware Bay and maintained at the Cape Shore and Bivalve facilities of the Haskin Shellfish Research Laboratory. Diagnosis of *H. nelsoni* infected specimens was by hemolymph sampling (Ford and Kanaley 1988).

Plasmodial stages of *H. nelsoni* were obtained by aspiration of blood sinuses in the adductor muscle of infected oysters. Hemolymph containing parasites and hemocytes was transferred to 25 cm^2 T-flasks containing 1–2 mL sterile sea water and appropriate antimicrobics (penicillin 200 U mL^{-1}; streptomycin 0.2 mg mL^{-1}; and amphotericin B 0.25 μg mL^{-1}) and held at room temperature for 3 h.

Enrichment of Parasites

Enrichment of parasites, and elimination of a large percentage of undesirable elements, primarily hemocytes, was obtained using two different media. Parasites tended to either detach from the culture flask spontaneously after initial seeding with infected hemolymph or would firmly attach to the culture vessels. In the former case, parasites could easily be "panned" (Ford et al. 1990) and poured off into clean flasks, leaving behind the bulk of the hemocytes still attached to the culture flasks. This procedure could be repeated several times with increasingly "clean" cultures.

Alternatively, when the parasites were firmly adhered to the culture flasks, the hemocytes could be induced to detach from the culture vessel by the substitution of the seeding medium with 100% osmotically corrected (750–800 mOsm) fetal calf serum (FBS) for a few hours at room temperature. Once detached from the culture flasks, the hemocytes were decanted and fresh medium added to the parasite cultures. This method also tended to more tightly adhere parasites to the plastic substrate.

Culture Methods

A variety of nutritional sources and mixtures thereof were used in an attempt to establish and optimize long-term culture of *H. nelsoni*, including autologous hemolymph, fetal calf serum, trypsinized oyster exudate, trypsinized oyster homogenate, SeruMax (Sigma Chemical Co.), liver infusion broth, and protease peptone. Many of these mixtures at various concentrations (all osmotically corrected) would support *H. nelsoni* culture to some degree. However, the most effective medium consisted of a mixture of 25% *Perkinsus marinus* medium (Kleinschuster and Swink 1993), 35% sterile oyster hemolymph and 40% sterile sea water. The pH of the medium was adjusted to 7.4 with 0.1N NaOH and cultures were maintained with antibiotics (penicillin 100 U mL^{-1} and streptomycin 0.1 mg mL^{-1}). Contamination by unidentified flagellates and/or zoospores was controlled by *gentle* rinsing of the cultures every two days with sterile sea water followed by addition of fresh medium. When contamination was not apparent, the culture medium was changed every three days. All observation and photography of live cultures utilized phase microscopy.

Confirmation that cultured organisms were *H. nelsoni* was obtained by routine histological techniques. The bottoms of plastic T-flasks containing cultured organisms were cut out, fixed in Davidson's medium, stained for one minute with 0.5% methylene blue, and mounted in glycerin. Short-term cultures were produced on glass slides, fixed in Davidson's, stained with Weigert's hematoxylin, dehydrated and mounted in Permount.

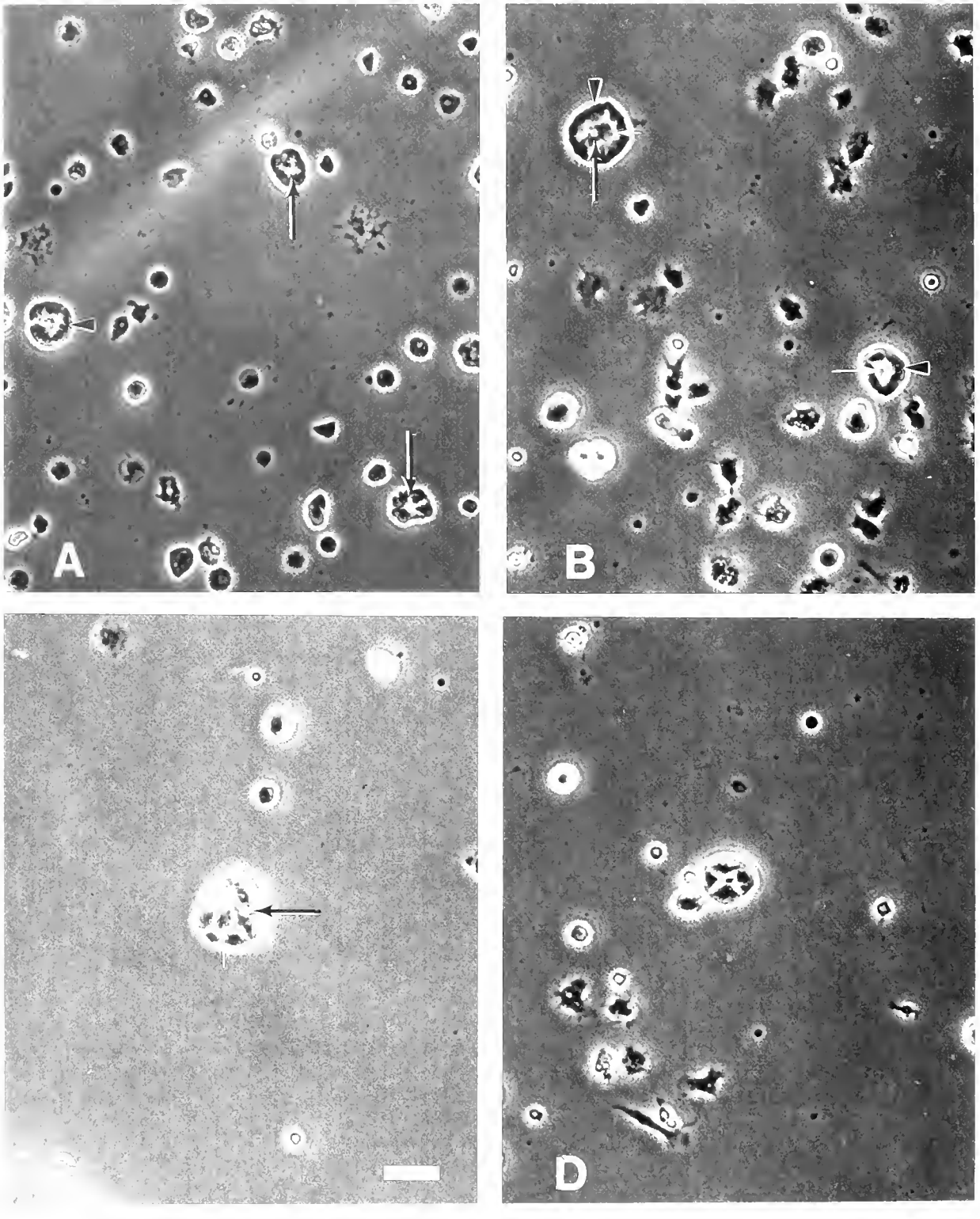

Figure 1. *In vitro* culture of *Haplosporidium nelsoni* at the earliest identifiable stage of development in this study. Photomicrographs A and B represent typical circular and flattened forms; Figures C and D represent folded forms. Arrowheads indicate perimeter, while arrows indicate central structure, black arrows indicate small clear area associated with the central structure (residuum). Scale bar = 100 μm.

RESULTS

Phase contrast photomicrographs of *H. nelsoni* in culture are seen in Figures 1–8. Under these conditions, optical birefringence of culture contents (cells and debris) is proportional to the 3-dimensional "roundness" of the objects, i.e. flattened objects appear dark. The following descriptions result from observations of numerous cultures over time. A consistent pattern of transitional

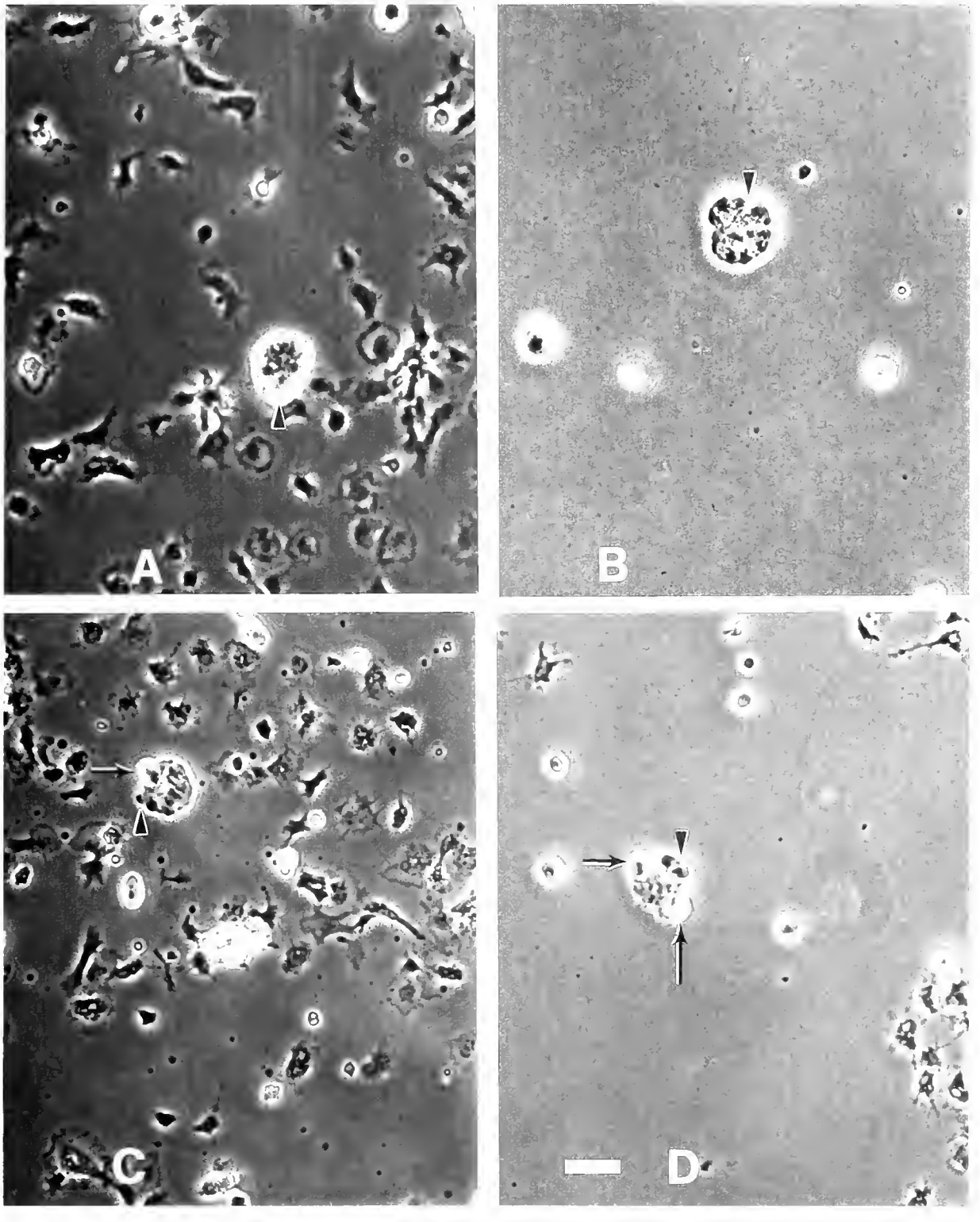

Figure 2. ***In vitro*** **development of** ***Haplosporidium nelsoni*****. Notice suggestion of indentations in the perimeter (A–D, arrowheads) and initiation of apparent fragmentation (C and D, black arrows). Scale bar = 100 μm.**

forms was seen and they are presented in the sequence observed 1) apparent plasmotomy, 2) vegetative budding, and 3) quiescence. Although these were the dominant stages, it must be noted that at any one time a particular culture exhibited asynchronous budding and different stages of parasite development. Consequently the earliest identifiable stage of plasmotomy in this study is represented in Figure 1A–D, earlier stages were not identified.

Most parasites at this early stage appeared generally flattened and circular with a definite perimeter and central structure termed a residuum (Fig. 1A–D). In many instances, the perimeter ap-

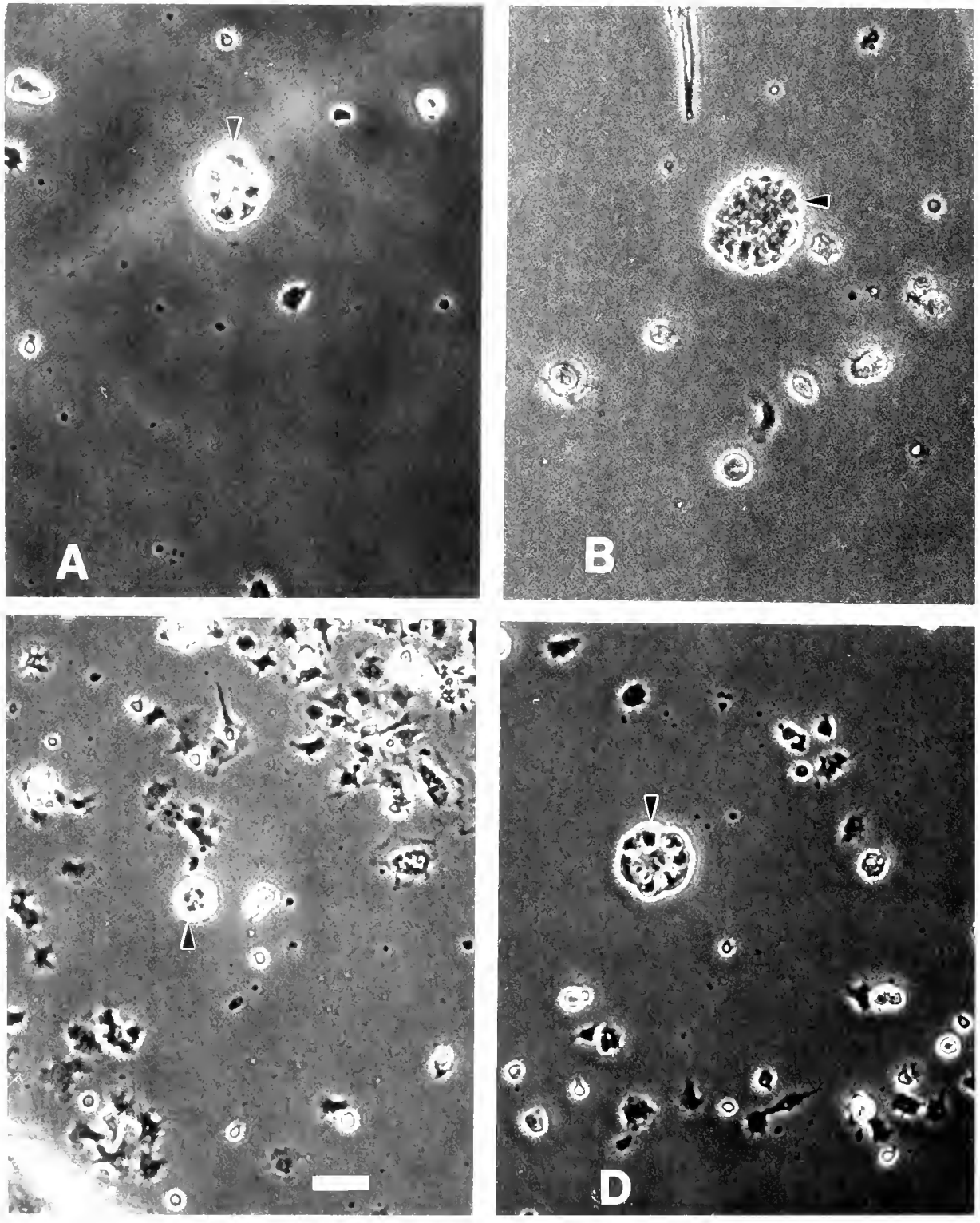

Figure 3. *In vitro* development of *Haplosporidium nelsoni*. **Notice symmetry and rosette formation of the plasmodium. Scale bar = 100 µm.**

peared to be "folded" and "unfolded" (Fig. 1C, D). These "folded" parasites, generally "unfolded" as they transformed into the more circular form commonly seen. A small clear area associated with the residuum was also often seen and persisted throughout plasmotomy (Fig. 1A–C).

A later stage of parasite development occurred with the apparent loss of perimeter integrity and the beginning of radial segmentation (Fig. 2A–D). In some instances, segmentation was followed by fragmentation (plasmotomy) of the parasite (Fig. 2C, D).

Figure 3A–D represents a later stage of Figure 2A–D. As seen, radial symmetry of the plasmodium, resulting in a "rosette" pattern, was pronounced. Subsequent fragmentation resulted in each

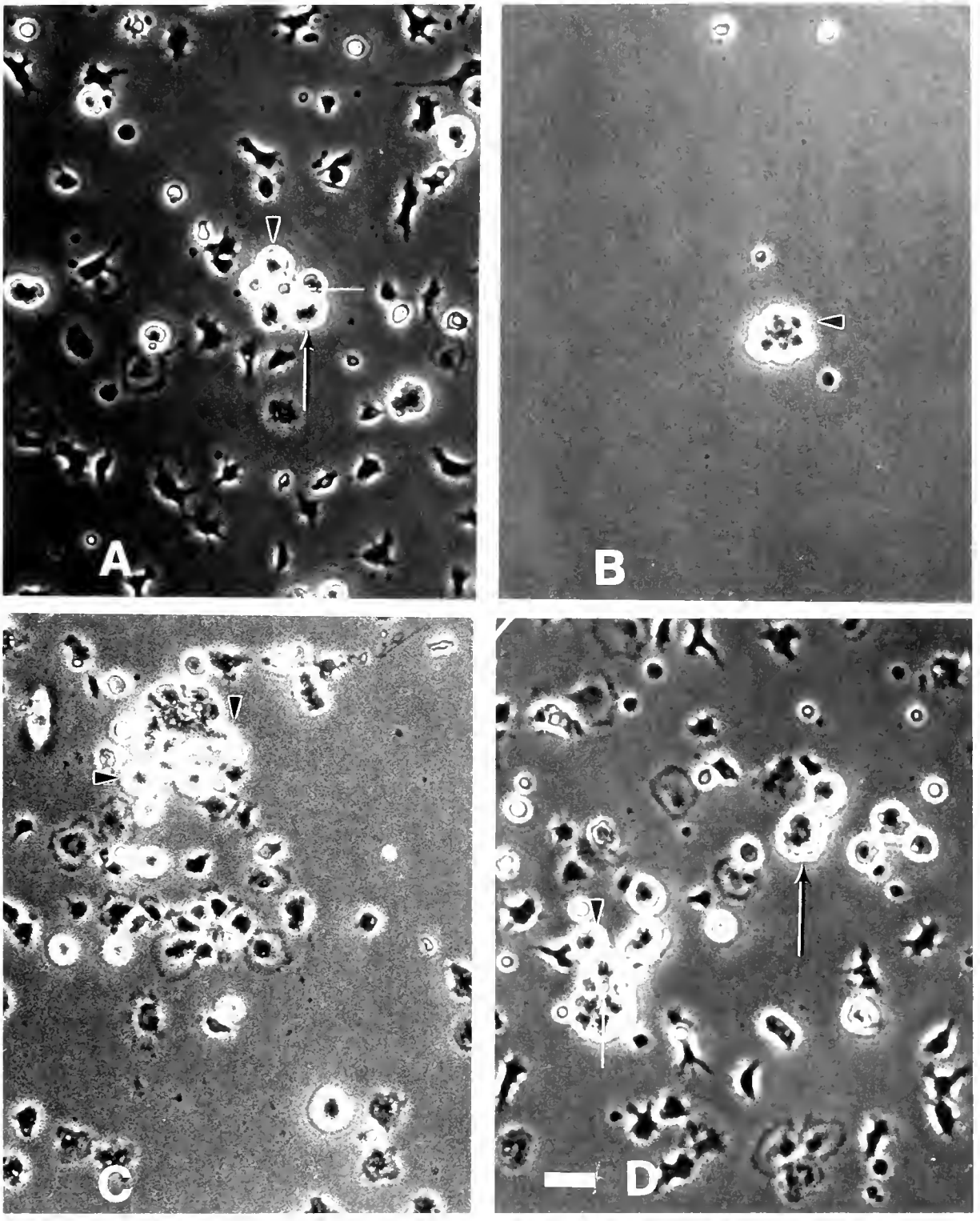

Figure 4. ***In vitro*** **development of** ***Haplosporidium nelsoni*** **showing symmetrical (A and B, arrowheads) and asymmetrical (C and D, arrowheads) fragmentation. Notice serrated and irregular cell membranes (A, D, E, and H, black arrows) and clear central areas (residua) (A, E, and H, white arrows). Scale bar = 100 μm.**

"segment" of the parasite becoming an individual, independent plasmodium (Fig. 4A–H). Fragmentation of plasmodia was either radially symmetrical (Fig. 4A, B), or irregular and asymmetrical (Fig. 4C, D). In either case, newly formed daughter cells exhibited irregular shapes and "serrated" cell membranes. Also apparent in many cases was the small, persistent clear area seen in earlier stages (Fig. 4A, D, E, H).

In the following stage, the daughter cells appeared to lose their

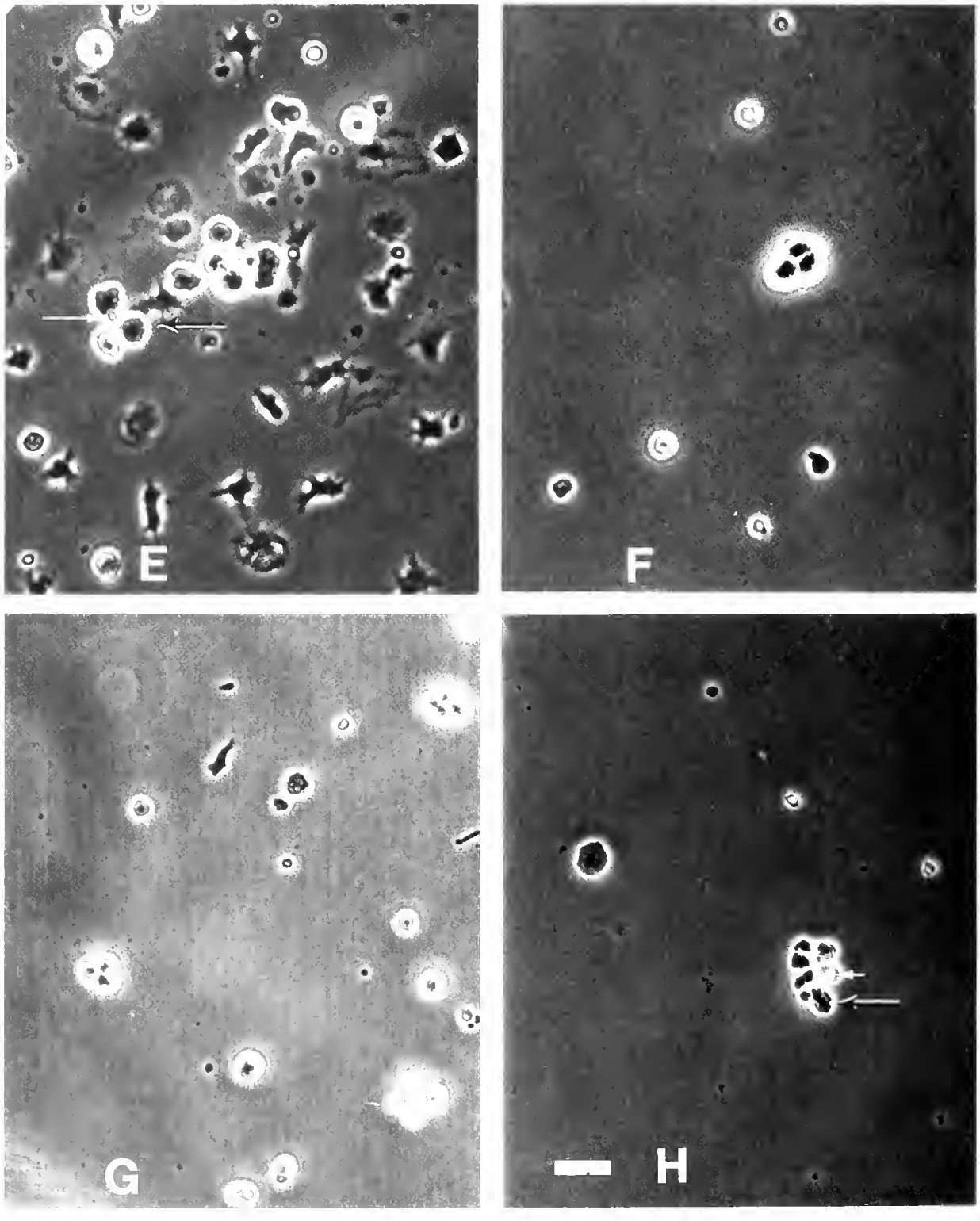

Figure 4. Continued.

serrated membrane and irregular shape and to assume the more rounded, birefringent, lobose shape typical of hemolymph-borne *H. nelsoni* (Fig. 5A-D).

Following a period of increase in size, the newly formed daughter cells exhibited a budding-type of replication preceded by exuviation, which resulted in much debris in the culture flasks (Fig. 6A-C). At this stage, adherence of *H. nelsoni* was considerable, i.e. the cells shown were not "floating." Occasionally, large, ovoid and very birefringent cells were seen which did not replicate. Interestingly, exuviation and budding could not be demonstrated at all times, but occurred primarily in cultures established during the summer months.

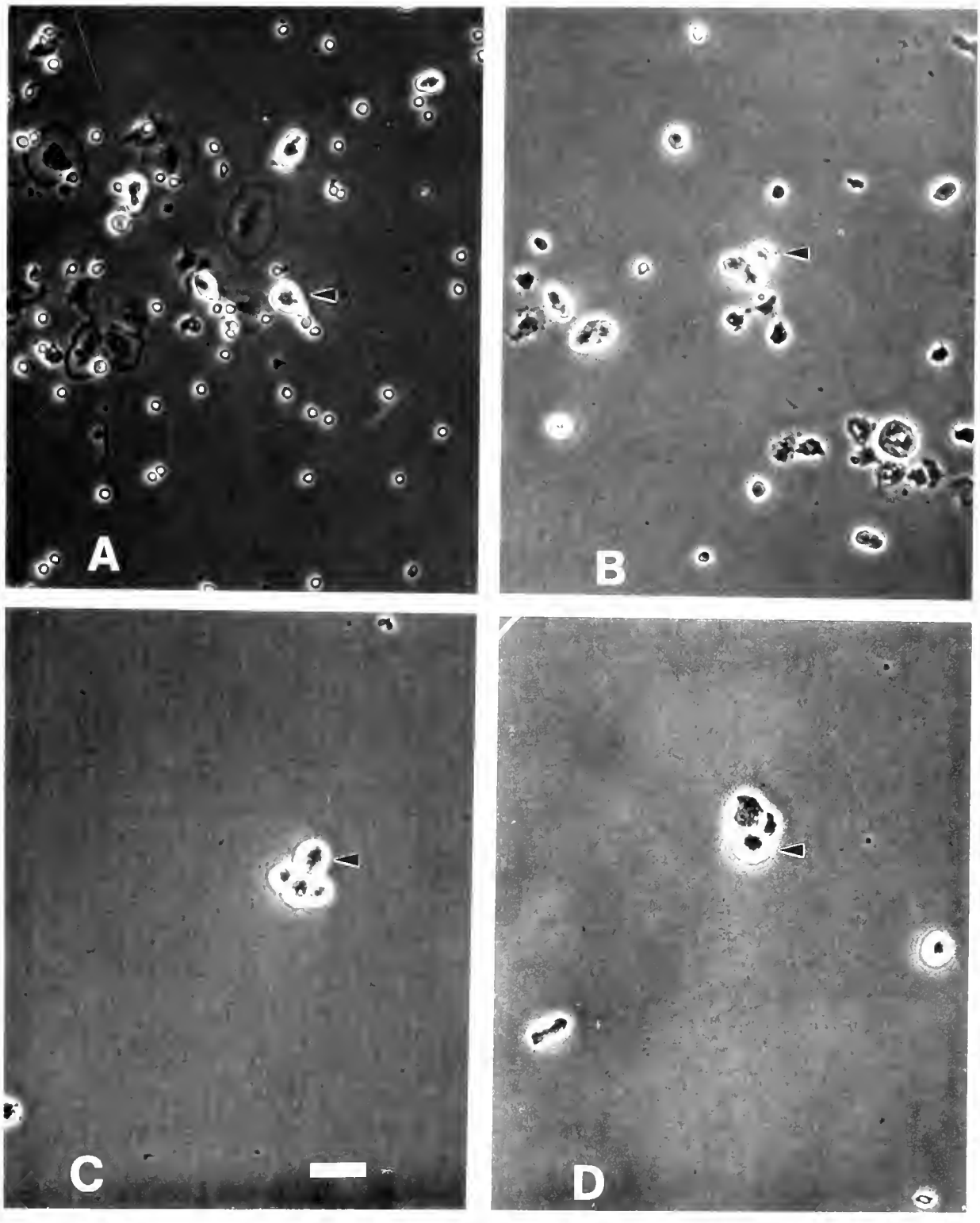

Figure 5. ***In vitro*** **development of** ***Haplosporidium nelsoni*****. Notice loss of irregular serrated shapes and assumption of typical lobose plasmodial forms (A–D, arrowheads). Scale bar = 100 μm.**

Following exuviation and replication, daughter cells began to decrease in size (Fig. 6) and occasionally divided (Fig. 7A, B). When these events did not occur, cells resulting from plasmotomy (Fig. 5) also decreased in size and occasionally divided (Fig. 7E). In each instance, many cells detached from the culture vessels while others remained attached to the plastic substrate and exhibited rounding and coalescence of cytoplasm reminiscent of cytoplasmic features consistent with plasmotomy (Fig. 7B, C, F, F, G). Parasites subsequently became inactive and or senescent, although most contained several nuclei. After this, it became dif

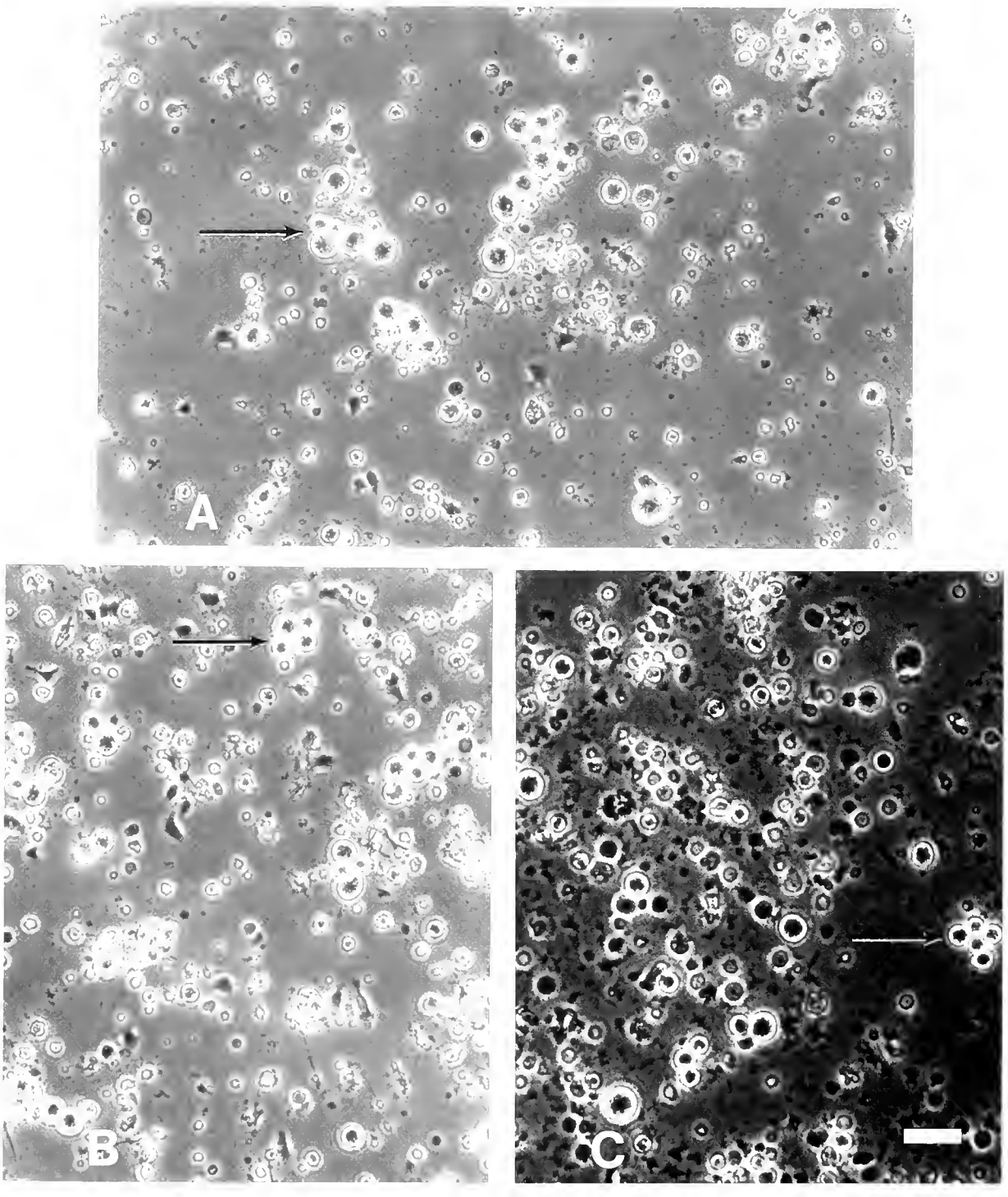

Figure 6. ***In vitro*** **development of** ***Haplosporidium nelsoni*****. Notice budding-type replication by plasmodia (A–C, black arrows). Cellular debris from exuviation is evident. Scale bar = 100 μm.**

ficult to identify parasites with confidence and further descriptions were not attempted.

Cytological examination of cultured, fixed, and whole-mounted parasites at all stages described herein revealed multinucleated organisms with each nucleus possessing an eccentric endosome and a Kernstab (Fig. 8A–D).

Additional forms of parasite replication are seen in Figures 9–11. These forms were rarely seen in culture, but produced viable daughter cells. Of particular interest is Figure 9 which appears to represent a germinal center with daughter cells at various stages of development. The different stages are still attached to the germinal center and demonstrate "serrated" membranes similar to these seen in daughter cells resulting from plasmotomy (Fig. 4). The stages are numbered 1–5 with 1 being presumed most mature (i.e. largest) and 5 being the least mature (i.e. smallest). Figure 10 may represent large developing plasmodia

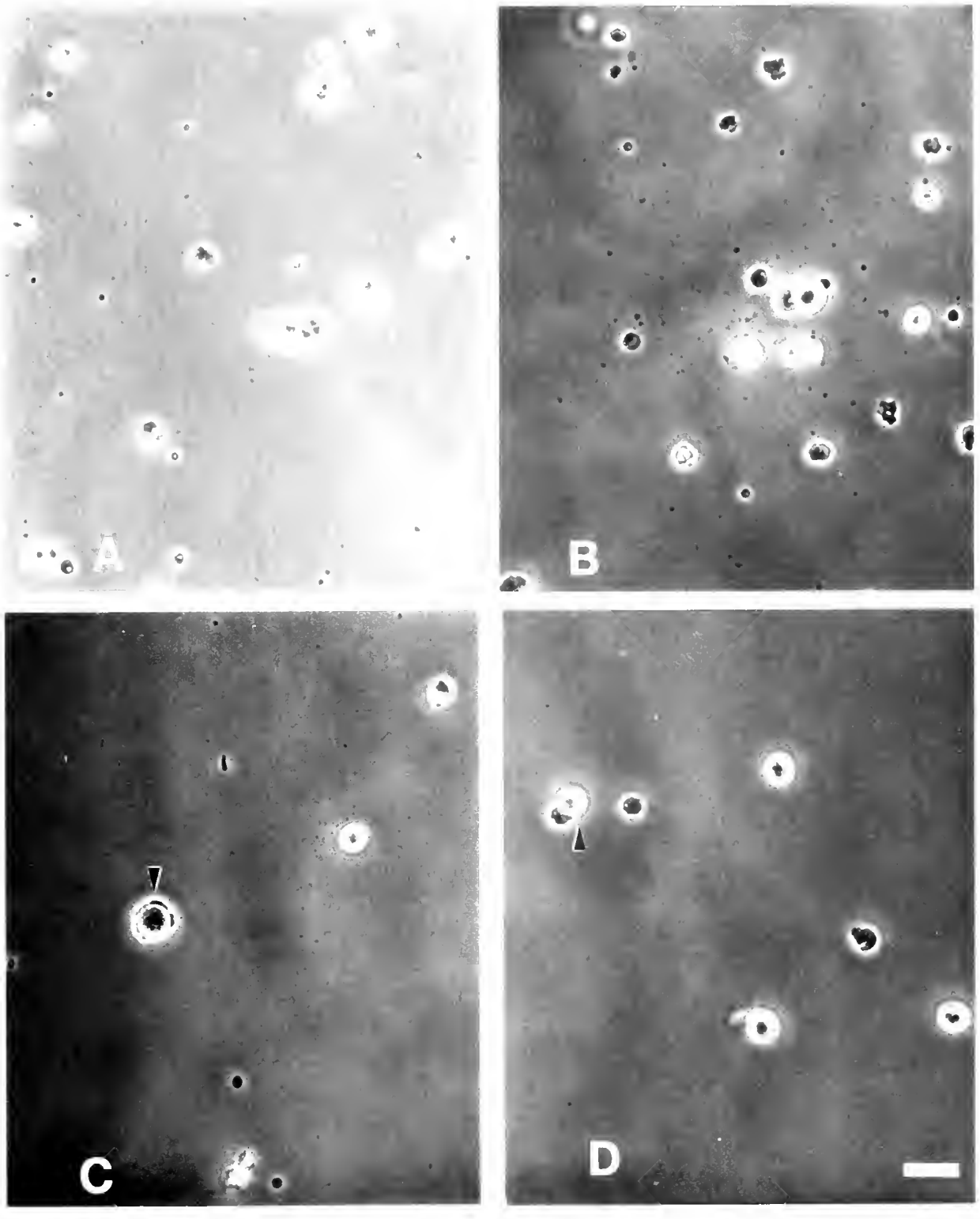

Figure 7. ***In vitro*** **development of** ***Haplosporidium nelsoni*****. Notice reduction in size, apparent duplication (A, B, and F), and cells exhibiting "rounding" with continued attachment to plastic substrate (C, D, E, and G, arrowheads). Scale bar = 100 μm.**

just prior to plasmotomy and Figure 11 represents plasmotomy of a very large plasmodium.

DISCUSSION

Techniques for the long-term *in vitro* culture and maintenance of *Haplosporidium nelsoni* afford new opportunities for the biological investigation of this parasite. It is hoped that continued refinement and improvement of these techniques will result in a concomitant increase in these insights.

In the present study, long-term (up to 5 weeks) observations confirm the suggestions of Farley (1967) and Burreson et al. (1988) that plasmotomy plays a key role in the life cycle of this

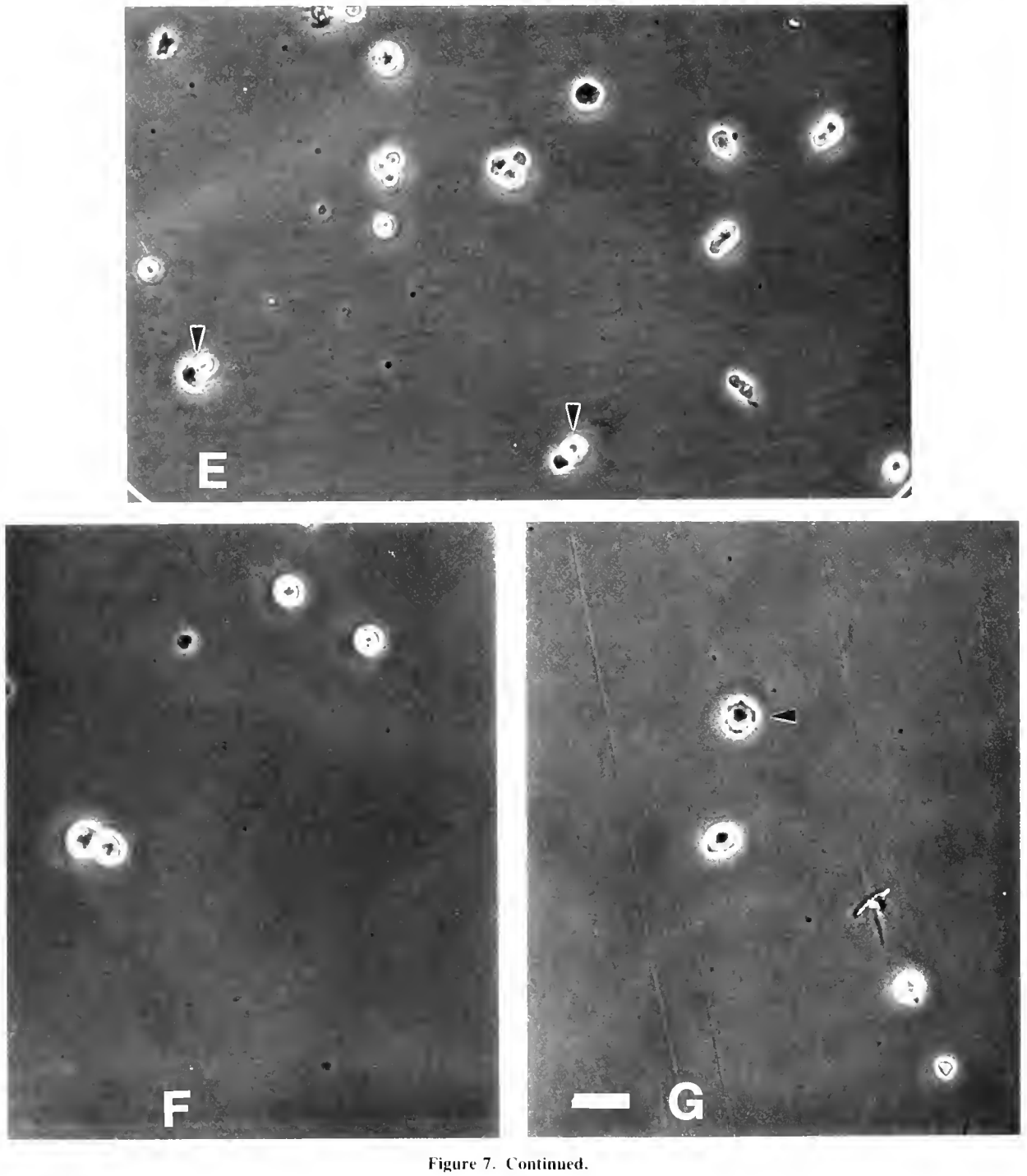

Figure 7. Continued.

parasite. Additionally, the apparent decline of activity of the organisms, following a period of considerable reproductive activity, suggest the possibility that the culture conditions were not adequate to support additional development or that oysters are not a "normal" host for *H. nelsoni* (i.e. do not provide the suitable environment for life cycle completion) and that an intermediate or alternate (reservoir) host exists (Haskin and Andrews 1988).

It must also be noted that the temporal developmental fate of any particular culture appeared to be dependent on unknown factors, although the physiological condition of the host was probably paramount. In some instances, events depicted in Figures 1–7 took 10 days in culture, while the same sequence in other cultures took up to 5 weeks. Whereas a seasonal pattern was evident in some between-culture differences (i.e. the post-plasmotomy exuviation and budding), others were not associated with time of year. No net increase in the number of organisms per culture flask could be claimed at the end of the culture period due to the inadvertent loss of organisms when the cultures were rinsed. As with all *in vitro* culture technology, it is not known to what extent, if any, perturbations of the organism were introduced by the techniques employed

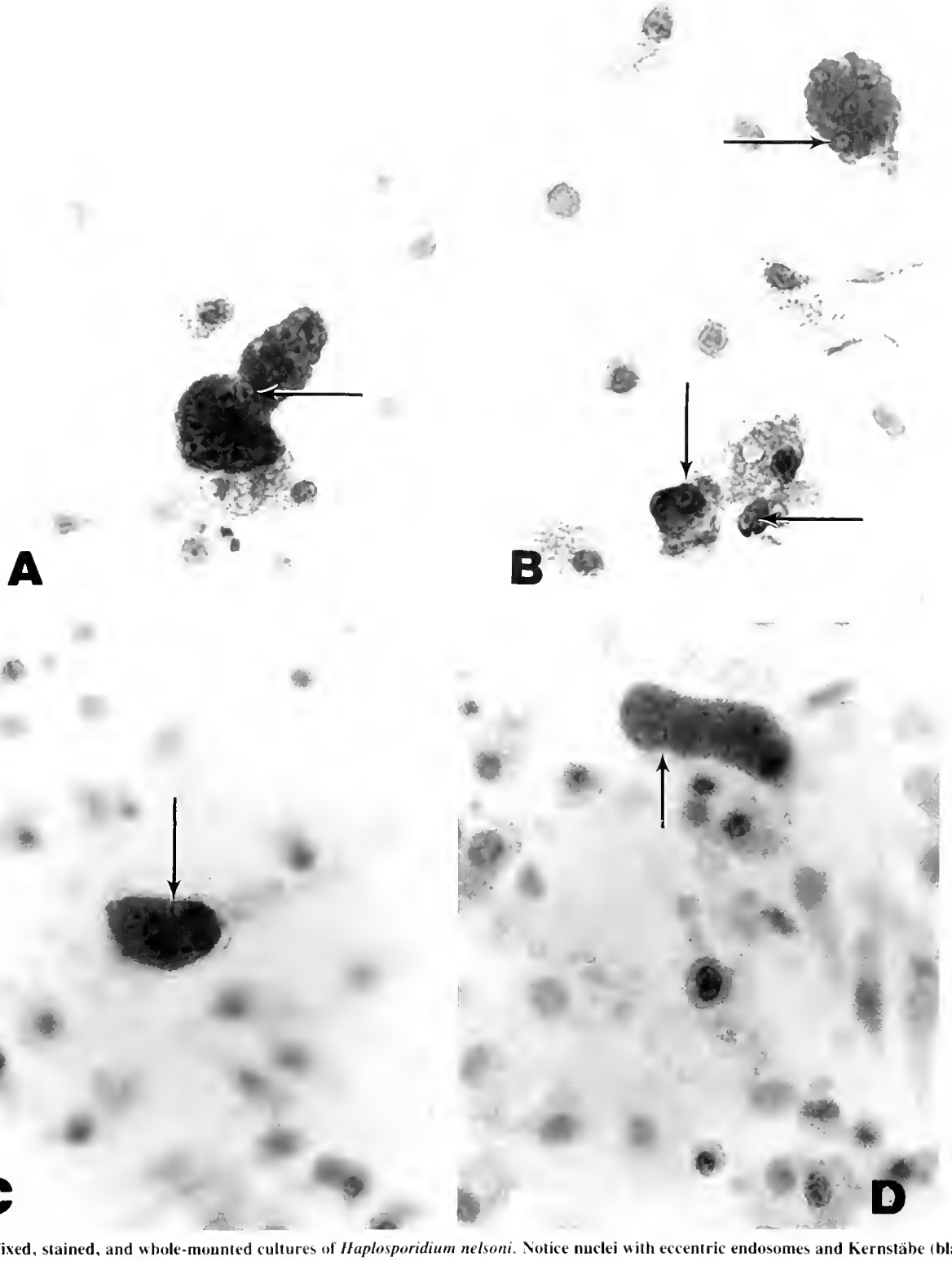

Figure 8. Fixed, stained, and whole-mounted cultures of *Haplosporidium nelsoni*. Notice nuclei with eccentric endosomes and Kernstäbe (black arrows). Scale bar = 12.5 μm.

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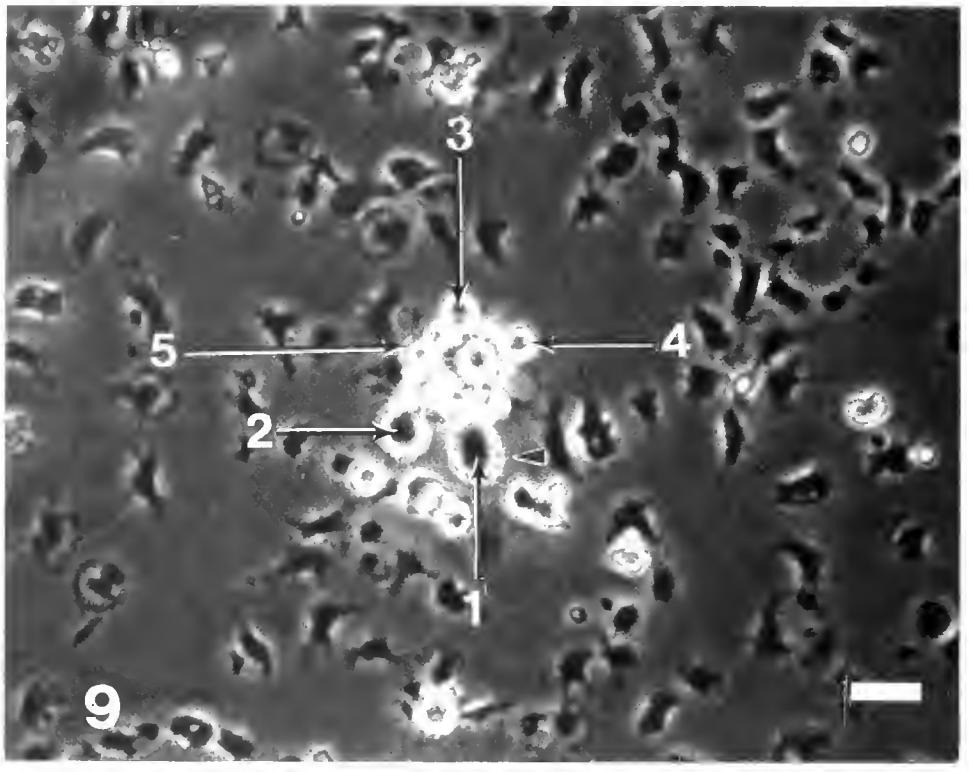

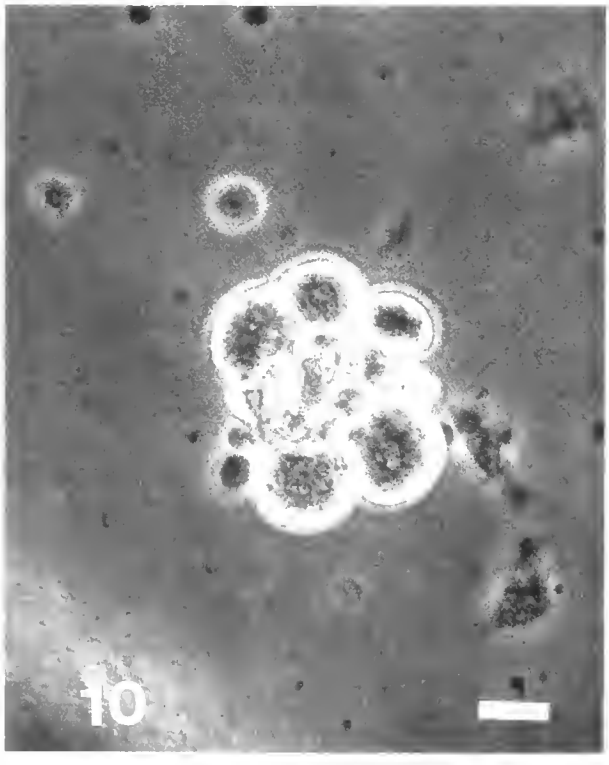

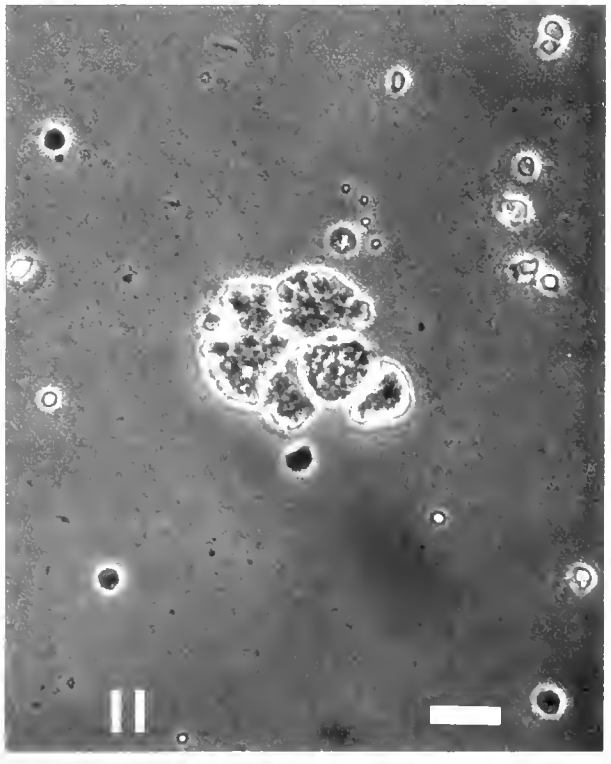

Figures 9, 10, and 11. Various forms of *Haplosporidium nelsoni* replication *in vitro*. Notice in Figure 9 different forms of daughter cells attached to apparent germinal center; number 1 is presumed most mature (largest) and number 5 is least mature (smallest). Notice serrated membrane of daughter cells (arrowheads). Figure 10 may represent mature plasmodia preceding plasmotomy and Figure 11 represents plasmotomy of a very large plasmodium. Figure 9 and 10, scale bar = 50 μm; Figure 11, scale bar = 100 μm.

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REPRODUCTIVE ACTIVITY OF OYSTERS, *CRASSOSTREA VIRGINICA* (GMELIN, 1791) IN THE JAMES RIVER, VIRGINIA, DURING 1987–1988

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ABSTRACT Reproductive activity in oysters, *Crassostrea virginica* Gmelin, in the James River, Virginia, was examined for 1987 from weekly estimates for fecundity and egg viability in oysters collected from Wreck Shoal, and for 1988 from weekly estimates of fecundity, egg viability, gonad volume fraction, gonad thickness, and mean egg size in oysters collected from Horsehead Reef. Maximum and mean fecundity values from Wreck Shoal oysters were higher than from Horsehead oysters. No relationship was evident between fecundity and egg viability at Horsehead Reef. A strong temporal relationship was observed between egg viability and peak oyster settlement in the James in both years of the study as estimated by off-bottom settlement substrates. In 1987 highest viability occurred from late June through mid August with peak settlement occurring from mid June through late August. In 1988 viable eggs were recorded from late July through the end of August; major settlement occurred from early August through mid September. Fishery independent estimates of oyster population abundance on Horsehead Reef, when combined with concurrent egg production and viability data, illustrate the losses that occur during the early life history stages of oysters in this location.

KEY WORDS: Oyster, *Crassostrea virginica*, James River, fecundity, gonad volume fraction, eggs, oyster settlement

INTRODUCTION

The James River, Virginia has served as the focal point for the Virginia oyster industry for over a century, being the source of the majority of seed oysters that were transplanted for grow-out to locations within the Virginia portion of the Chesapeake Bay and much further afield in the Middle Atlantic states. It has been the site of continuing investigations of oyster distribution in relation to bottom type (Baylor 1894, Moore 1911, Loosanoff 1931, Haven et al. 1981a, Andrews 1982, Haven and Whitcomb 1983), larval biology and settlement (Loosanoff 1931, Andrews 1951, 1954, Wood and Hargis 1971, Andrews 1979, Haven and Fritz 1985, Andrews 1983, Mann 1988), larval dispersal in relation to circulation (Pritchard 1953, Ruzecki and Moncure 1968, Ruzecki and Hargis 1988), disease impact (Andrews 1954, 1962, 1968, Burreson 1986, 1990) and a series of unpublished qualitative annual surveys of oyster resources by location (Virginia Institute of Marine Science Library Archive). Given the ecological importance (see Mann et al. 1991) and commercial value (see Haven et al. 1981b) of oysters originating from the James River it is surprising that comparatively little effort has been devoted to quantitative examination of the relationship between spawning and recruitment in the James River. Previously, Cox and Mann (1992) compared temporal and spatial variation in oyster fecundity in the James River; however, these were not coupled with estimates of egg viability or standing stock in an attempt to further estimate long term changes in egg production by James River oyster populations. This relationship becomes increasingly critical as both disease and commercial exploitation maintain significant pressures on the resource.

As part of a continuing effort to develop improved quantitative descriptions of the relationships between oyster standing stock, egg production, and recruitment in the James River this report describes studies to estimate temporal changes in fecundity and egg viability in oysters, *Crassostrea virginica* Gmelin, in surviving populations in the lower salinity regions of the river as described by Haven and Fritz (1985). Our initial plan was to focus a long term effort on Wreck Shoal, (Fig. 1, see also Haven and Whitcomb 1983), a large and commercially important oyster reef that has been implicated as an important broodstock area in the James River ecosystem by the works of Ruzecki and Hargis (1989) and Cox and Mann (1992); however, severe losses of oysters to disease (*Haplosporidium nelsoni* and *Perkinsus marinus*) at the Wreck Shoal site prevented further sampling at this site in 1988. Therefore, 1988 samples were collected from Horsehead Reef. Horsehead is one of the few reefs in the James River to have suffered minimal losses to disease, although low levels of *P. marinus* have been recorded in recent years (Burreson 1990). Horsehead reef is currently the site of intense fishing activity during the commercial season, from October 1 through May 31. The boundaries of this reef are described in Haven et al. (1981a), and Haven and Whitcomb (1983). In 1988 reproductive studies were supplemented by fishery independent estimates of population size to facilitate calculation of total egg production from oysters on Horsehead reef. Thus, Horsehead offers a suitable site for examination of seasonal reproductive activity in a defined oyster population as described here.

MATERIALS AND METHODS

Oysters were collected in both years of the study with a 60 cm-wide dredge having 7.5-cm teeth. In 1987 oysters were collected from Wreck Shoal at weekly intervals from June 25 to October 8. Dredging continued until 80 oysters with heights (maximum dimension from the hinge to the opposite margin) greater than 76 mm, the effective minimum size for commercial exploitation at that time, were collected. In the laboratory, oysters were opened and sex determined by microscopic examination of gonad smears. Sex ratio was compared to unity using chi-squared analysis. The first 10 females opened were used for fecundity and egg viability determination as described below. On all sampling occasions water temperature was recorded at the surface and bottom using a mercury thermometer, and water samples were collected

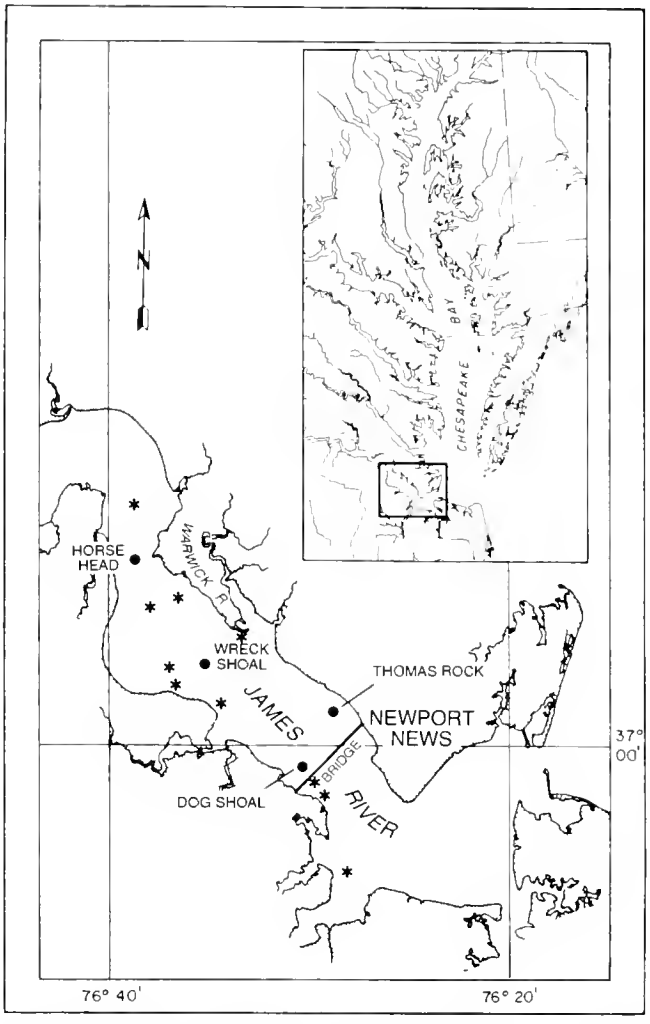

Figure 1. Location of collection sites in the James River, Virginia. Wreck Shoal and Horsehead are used in the present study. In addition, Dog Shoal and Thomas Rock were used by Cox and Mann (1992). These four, plus remaining sites market with * were used for monitoring spatfall.

using a Niskin bottle for subsequent estimation of salinity using a Beckman RS-10 induction salinometer.

Oysters were collected from Horsehead in 1988 at weekly intervals from June 15 through September 28. Dredging continued until 100 oysters were collected. Temperature and salinity measurements were also made in 1988. In the laboratory the oysters were opened, sex ratio determined, and that ratio compared to unity using chi-squared analysis. The first ten females were used to determine fecundity and viability, the second ten were fixed for subsequent histology and determination of gonad volume fraction (GVF), gonad thickness, and egg volume.

Fecundity, for the current study, is defined as the number of mature eggs contained in a single oyster. Separation of the gonadal mass from the visceral mass is impractical in oysters complicating direct estimation of gonad size for indirect estimation of fecundity. Histological procedures, such as employed by Morales-Alamo and Mann (1990), provide details concerning both stage of development and gonad volume fraction (GVF) but require multiple sections per specimen and are similarly impractical in rapid processing of a large number of specimens. Immunological techniques (Choi et al. 1993) provide direct estimates of egg protein and have promise for future use; however, they were not available at the time of this study. Fecundity estimates were made using the method of Cox and Mann (1992). Whole, wet tissue of individual oysters was homogenized using a commercial blender. The homogenate was washed through a 500-μm sieve to remove large debris, and eggs were retained on further washing through a 25-μm sieve. Eggs were washed into a calibrated glass cylinder and the cylinder contents made up to a known volume with 1 μm filtered sea water. The cylinder contents were thoroughly mixed and subsamples removed for counting of eggs using a Sedgewick Rafter cell under low power magnification on a compound microscope. Triplicate counts were made. Fecundity estimates were obtained from proportional volumes of cylinder contents and subsamples. Such estimates suffer the limitation of the assumption that all eggs retained on the sieve are amenable to fertilization. Prudent choice of sieve size is important, but clearly a compromise. Retained material may include immature eggs causing an overestimate of fecundity. Alternatively, eggs damaged during homogenization may pass through the sieve resulting in underestimation of fecundity. The relative magnitude of these errors was not assessed.

Egg viability was determined as follows. Three aliquots, each of 5,000 eggs, were removed from eggs isolated in the fecundity estimation procedure. Each aliquot was transferred to a 100-ml beaker and made up to 50-ml volume, resulting in a egg concentration of 100 eggs/ml. Sperm were isolated after homogenization of male oysters from the same source and repeated passage of homogenate through a 20-μm sieve. Sperm was added to the egg suspension to give a final concentration of 3×10^5 sperm/ml. All fertilizations were generally effected within one hour of isolation of gametes. Although this procedure is typical of research hatchery operations for naturally spawned eggs (see Castagna, Gibbons and Kurkowski, in press) the current method uses effectively stripped eggs. Stephano and Gould (1988) reported that stripped eggs of *Crassostrea gigas* are more susceptible to polyspermy than naturally spawned eggs, especially when fertilization is attempted within one hour after isolation suggesting that the current method may underestimate viability because of losses to polyspermy; however, we have observed no such polyspermy effects with *C. virginica* in pilot hatchery systems (Shaffer and Mann unpublished observations). Forty five minutes after addition of sperm, the sperm and egg mixture, now containing fertilized and developed eggs, was gently sieved through a 20-μm sieve to remove excess sperm and the filtrate returned to 100-ml beakers containing 50 ml of filtered sea water. The eggs were left to develop overnight, and three subsamples were subsequently removed to count the first-shelled veliger larvae present. In 1987, data were recorded only as percentage of cultures containing active veliger larvae 24 hours after the addition of sperm; the number of verligers per culture was not recorded. In 1988, the procedure was improved and egg viability was estimated as the number of active verligers per 1000 eggs. Losses of eggs and larvae due to handling during the washing procedure in 1988 were assumed to be negligible.

Oysters used for histological examination were fixed in Davidson's AFA fixative for 24h; at the end of that period transverse cuts were made through the visceral region, anterior and posterior to the junction of the gills and palps, to obtain a body segment approximately 6 mm thick. This segment was held in Davidson's fixative until it was embedded in paraffin after being dehydrated and cleared in an alcohol:xylene series. The time sequence for this

procedure was closely controlled to insure that any changes in egg size caused by fixation and dehydration were consistent throughout the study. Sections of 6μm thickness were subsequently cut, mounted on glass slides, stained in Harris' hematoxylin and eosin Y, and examined microscopically. Ten oysters were processed from each sampling date. On seven dates, however, measurements were made on only 8 or 9 oysters because the others were found to be either males or hermaphrodites or were heavily infected with the trematode *Bucephalus* sp., which compromised gonadal development.

Gonad volume fraction was measured by point-count volumetry using a square grid on a reticule mounted in the microscope eyepiece (Chalkey 1943, Weibel et al. 1966, Bayne et al. 1978). Four GVF measurements were made on each oyster at gonad locations selected at random from among eight regions around the circumference of the section. Up to eight counts were made in instances when the number of eggs in an oyster was extremely low. Estimates of statistical parameters were made after arcsine transformations to normalize data.

Gonadal thickness is described in terms of the total number of grid points included in computation of GVF as an estimate of gonad width in the section, and will be referred to as gonadal width throughout the text. Where gonad width exceeded maximum grid size (121, the maximum number of intersection points in the grid) width was measured by moving the grid toward the center of the section and adding to the count the number of points needed to reach the inside margin of the gonad. This procedure was required on most of the oysters collected on July 6 and August 3, and in one or two of the oysters on each of five other dates. Gonad width measurements were weighted for differences in total oyster cross-sectional area, by dividing the number of grid points by the cross-sectional area. Statistical comparisons of GVF and gonad width between sampling dates were made using the non-parametric Mann-Whitney test (Olson 1988) because variances were heterogeneous.

Egg size was measured for 4–8 animals from each date of collection as the cross sectional area of individuals egg, in histological preparations used for determination of GVF. Preparations were examined using a digitizing video imaging system (International Imaging Systems model 75) attached to a compound microscope (Olympus model BH-2). Five eggs were measured per field of view in 10 randomly chosen fields (total of 50 measurements) for each oyster section examined. The only criterion for selection of eggs to be measured was the presence of a large, distinct nucleus. Areas were converted to nominal diameter values (the diameter of a circle with the same area), and comparisons between sampling dates effected using the non-parametric Mann-Whitney test. Nominal diameters were used for comparison purposes only and are not to be interpreted as representing the actual diameter of the eggs.

Temporal and spatial changes in settlement of oysters throughout the James River were monitored in both 1987 and 1988 as part of a long term, continuing program. Methods were similar to those described by Haven and Fritz (1985). Shellstrings were deployed above the bottom at weekly intervals between June and October at the 14 stations (Fig. 1). A shellstring consisted of 12 oyster shells of similar size (about 76 mm in height) drilled through the center and strung on a piece of heavy gauge wire. After a one-week exposure, the number of spat attached to the smooth surface (underside) of the center ten shells was counted with the aid of a dissecting microscope. This number was then divided by 10 to obtain the number of spat per shell for that time interval. Weekly sampling allowed examination of settlement trends over the course of the summer at the various locations. Comparison between years were made by adding weekly values of spat per shell for the entire settlement period.

Fishery independent estimates of oyster populations on Horsehead were made using divers and quadrat collections from preselected sites. Ideally, samples would be collected from sites randomly selected from a uniform grid overlaying the reef as defined by the boundaries illustrated in Haven and Whitcomb (1983). Development of such a grid was practically unreasonable using LORAN given the temporal drift in LORAN signals and the requirement to repeatedly locate grid coordinates with accuracy over extended time periods. Marking a large number of grid coordinates with buoys was complicated by extensive boat traffic in the study area. As an alternative the latitude and longitude of a large number of sites were identified that could be approximately located by LORAN then fixed with accuracy by triangulation on known landmarks, these being either navigational buoys, prominent coastal features or both. Sampling stations were then chosen by designating site numbers and choosing stations for sampling from random number tables. Collections were made on September 22, 1988 by divers from quadrats (1 m^2). All material within the quadrat was retained. Samples were examined for total volume of oyster and shell collected, total numbers of oysters and size-frequency distribution, by 10 mm height intervals starting at 10 mm, of those oysters. Biomass estimates (wet tissue weight) were made from these values using mean individual data for oysters collected from Horsehead in July–August, 1979 (Haven and Morales-Alamo, unpublished). The number of quadrats required to provide estimates of oyster density with the smallest variation was determined by plotting the number of collections examined versus the standard error of the mean of the number of oysters found. Little decrease in standard error was observed when more than ten samples were included. Therefore twelve quadrat samples were collected on each date.

RESULTS

Data for oyster reproductive activity at Wreck Shoal (1987) and Horsehead (1988) are summarized in Table 1 and Table 2, respectively. In both years temperature gradually increased to a maximum in excess of 29.0°C in late July through mid August, after which temperature decreased to approximately 22.0°C by the end of the annual study period. Salinity was consistently higher at Wreck Shoal in 1987 than at Horsehead in 1988. Wreck Shoal values were generally between 16 and 20 ppt, with lower values on June 25, July 16 and September 24. Horsehead values were lower, generally increasing from below 7 ppt in June 1988 to above 15 ppt by mid August 1988. A value of 21.30 ppt was recorded during a period of consistently low precipitation in mid September 1988.

Percentage of female oysters in each sample was highly variable throughout the study period in both years, varying from 3% to 73% at Wreck Shoal in 1987 and 19% to 62% at Horsehead in 1988. All 1987 samples except July 2 were significantly different ($P < 0.01$) from 50%, a value corresponding to a sex ratio of 1:1. On three dates in 1988 the percentage of females in the sample was significantly higher ($P < 0.05$) than 50%; however, on seven dates the percentage was significantly lower ($P < 0.05$ or 0.01, see Table 2) than 50% indicating a preponderance of male oysters.

TABLE 1.

Summary of 1987 field studies at Wreck Shoal.

A	B	°C	ppt	F ± s.e. ($\times 10^6$)	V′	R%	Spat
6/25	6/20–26	26.2	14.0	0.64 ± 0.13	33	26**	0.3
7/2	6/27–7/3	25.5	19.3	1.25 ± 0.25	10	40	1.0
7/9	7/4–10	27.0	15.2	2.28 ± 0.39	70	29**	17.9
7/16	7/11–17	28.5	10.7	1.05 ± 0.09	60	65**	379.7
7/23	7/18–24	28.5	17.5	0.85 ± 0.18	100	73**	706.7
7/30	7/25–31	29.0	18.9	2.76 ± 1.10	30	73**	611.9
8/6	8/1–7	29.5	19.2	0.13 ± 0.04	80	29**	261.1
8/13	8/8–14	29.5	18.1	0.51 ± 0.28	20	23**	88.6
8/20	8/15–21	28.0	20.9	5.97*	0	21**	44.3
8/27	8/22–28	27.5	18.3	0.54 ± 0.20	0	32**	13.9
9/3	8/29–9/4	25.8	19.8	0.15 ± 0.05	0	29**	16.9
9/10	9/5–11	25.5	17.5	1.20 ± 0.43	40	23**	10.5
9/17	9/12–18	25.8	19.9	0.66 ± 0.44	17	19**	27.8
9/24	9/19–25	26.5	7.2	0.28 ± 0.12	0	15**	14.4
10/1	9/26–10/2	24.0	16.2	0.79*	0	3**	2.3
10/8	n.d.	22.5	13.9	1.77*	0	5**	n.d.

Date A is collection of dredge samples for fecundity and sex ratio estimation.
Date B is exposure period for shellstrings to estimate spat settlement.
Temperature (°C) and salinity (ppt) values are for bottom water collected on date A at site of dredge collections.
Mean fecundity (F ± s.e., $\times 10^6$, n = 10 except * when n = 1).
Viability estimates (V′) are percentage of individual oysters having eggs that produce veliger larvae.
Percentage female ($R = [f/f + m] \times 100\%$, n = 80, ** indicates significantly different from 50%, $P < 0.01$). Spatfall values are totals of data from fourteen stations (see Fig. 1), each station being included as spat per shell for the given time interval.
n.d. indicates no data collected.

Low percentage female values were consistently observed at the beginning and end of the study periods each year. Percentage of females at Wreck Shoal in 1987 was low, between 19% and 32%, on most dates. High percentages were found only between July 16 and July 30; however, no consistent pattern of variation was observed during the July-September period of either year. During the early July–mid September period in 1987, the percentage of female oysters increased gradually to 73% (July 23–30), but subsequently decreased to consistently <32% (August 6 onwards). In contrast, percentage of females demonstrated greater temporal stability at Horsehead in 1988, varying in the range 36–62% during the period June 22–September 14.

Mean fecundity values for the 1987 collections from Wreck Shoal were consistently higher by approximately one order of magnitude than the 1988 collections from Horsehead. 1987 values varied in the range 0.13–2.76 $\times 10^6$ (excluding the single value for August 20, 1987) with individual values as high as 12.04 $\times 10^6$ being observed on July 30, 1987. 1988 mean fecundity values were in the range 0.14–2.91 $\times 10^5$ with a maximum individual value of 8.1 $\times 10^5$. High variability in fecundity values within a sample of oysters of comparable size was observed at both sites suggesting asynchrony in gametogenesis within a population. Large temporal changes in mean fecundity, especially decreases over short time intervals suggesting spawning activity, were also observed with minimum individual fecundity values of $<10^4$ being recorded in both years.

Viable eggs were consistently found at Wreck Shoal from June 25, 1987 through August 13, 1987, with all individuals producing viable eggs on July 23, 1987. Viable eggs were again observed on September 10 and 24, 1987. In 1988, viable eggs were only observed at Horsehead during the period July 20–August 31. Viability was generally low with population means in the range 1.3–8.7 larvae/1000 eggs and a maximum individual value of 33.3 larvae/1000 eggs. Individual oysters devoid of viable eggs were observed on all sampling dates in 1988.

Oysters used for GVF estimates of 1988 Horsehead collections had shell heights ranging from 55.5 to 88.5 mm. Although this did not include representation from small size classes present in abundance in June and early July collections (see Table 3) it adequately represented the larger animals present throughout the June through September study period. Trends in fecundity, egg size, GVF, and gonad width did not match date for date through the season (Table 2). There was, however, a general trend in which peaks or valleys in means of the individual parameters deviated from each other by only one week. The common tread was characterized by statistically significant ($P \leq 0.05$) decreases in mean values with respect to the prior values on June 22, July 13, July 27 (except for gonad width), and August 17. Statistically significant increases with respect to the prior values ($P \leq 0.05$) occurred on June 29, July 6 (except for gonad width), and August 3; no detectable change was evident in gonad width between June 29, when a high value was attained, and July 27, because of high variability in the data.

These common trends suggest at least two separate major spawning periods at Horsehead in 1988; July 13 through 27, and August 3 through 24. A spawning event in late June is also suggested by statistically significant reductions in GVF and egg size on June 22 and June 29, respectively, in comparison to prior values. Decreasing mean values were also recorded for gonad width and fecundity in that period but associated variance values were high. September data were characterized in most instances by high variations which obscured any possible differences between dates for mean fecundity and GVF values. Egg size decreased significantly between August 10 and 24, and between August 31 and September 14, but increased again by September 28. A sig-

TABLE 2.

Summary of 1988 field studies at Horsehead Reef.

A	B	°C	ppt	F ± s.e. ($\times 10^5$)	V'' ± s.e.	GVF ± s.e. (n)	GW ± s.e.	D ± s.e. (μm, n)	R%	spat
6/15	6/13–6/19	24.5	6.65	1.11 ± 0.41	0	0.62 ± 0.05 (8)	1.61 ± 0.18	34.2 ± 0.2 (5)	18**	0.0
6/22	6/20–26	26.9	6.55	0.30 ± 0.09	0	0.48 ± 0.07 (10)*	1.29 ± 0.20*	34.0 ± 0.3 (5)	41*	0.0
6/29	6/27–7/3	n.d.	n.d.	0.14 ± 0.03	0	0.64 ± 0.03 (8)*	2.33 ± 0.38	31.4 ± 0.2 (8)*	40*	0.2
7/6	7/4–10	25.0	8.93	0.85 ± 0.19	0	0.75 ± 0.02 (8)*	2.08 ± 0.16	35.2 ± 0.2 (5)*	60*	5.9
7/13	7/11–17	27.1	10.48	1.48 ± 0.79	0	0.68 ± 0.03 (10)*	2.52 ± 0.20	33.2 ± 0.2 (6)*	41*	1.8
7/20	7/18–24	27.9	8.46	0.81 ± 0.20	7.3 ± 3.4	0.69 ± 0.03 (9)	2.15 ± 0.26	33.1 ± 0.2 (5)	62*	9.2
7/27	7/25–31	28.6	14.03	0.10 ± 0.02	8.7 ± 4.3	0.61 ± 0.04 (10)*	2.02 ± 0.25	30.8 ± 0.2 (5)*	52	5.8
8/3	8/1–7	29.0	9.75	2.91 ± 0.48	1.3 ± 1.0	0.76 ± 0.03 (9)*	3.42 ± 0.43*	33.5 ± 0.2 (5)*	55	38.2
8/10	8/8–14	29.5	12.58	0.86 ± 0.24	6.3 ± 2.7	0.54 ± 0.02 (10)*	2.42 ± 0.42*	34.8 ± 0.2 (7)*	51	53.7
8/17	8/15–21	29.4	12.02	1.10 ± 0.49	4.7 ± 1.7	0.44 ± 0.03 (9)*	2.27 ± 0.28	33.8 ± 0.2 (6)*	60*	4.7
8/24	8/22–28	26.4	15.47	1.85 ± 0.83	0	0.49 ± 0.6 (10)	1.53 ± 0.20*	31.4 ± 0.2 (5)*	49	10.7
8/31	8/29–9/4	25.7	14.22	1.53 ± 0.51	2.3 ± 1.6	0.44 ± 0.06 (10)	1.96 ± 0.20	34.5 ± 0.1 (5)*	45	21.6
9/7	9/5–11	23.5	16.18	0.97 ± 0.41	0	0.33 ± 0.09 (10)	1.82 ± 0.40	33.6 ± 0.2 (5)*	36**	18.3
9/14	9/12–18	23.7	21.30	2.47 ± 0.82	0	0.29 ± 0.10 (10)	1.07 ± 0.39	33.3 ± 0.1 (5)	45	11.0
9/21	9/19–25	24.0	14.80	0.96 ± 1.26	0	0.23 ± 0.08 (10)	1.24 ± 0.27	34.3 ± 0.2 (4)*	19**	2.1
9/28	9/26–10/2	22.0	n.d.	2.09 ± 0.20	0	0.30 ± 0.12 (9)	0.66 ± 0.23	34.5 ± 0.2 (5)	20**	0.6

Date A is collection of dredge samples for fecundity and sex ratio estimation.
Date B is exposure period for shellstrings to estimate spat settlement.
Temperature (°C) and salinity (ppt) values are for bottom water collected on date A at site of dredge collections. Adult fecundity (F ± s.e., $\times 10^5$, n = 10), and Viability (V) values are from live dredge collections. Viability (V'') values are numbers of veliger larvae obtained per 1000 eggs in 50 ml cultures (see Methods).
Gonad Volume Fraction (GVF ± s.e., n in parentheses).
GW is Point Count Data (Gonad Width, GW ± s.e., n as for GVF).
Nominal Egg Size (D ± s.e. μm, n in parentheses is number of oysters examined, see Methods).
Percentage Female Oysters (R = $[f/f + m] \times 100\%$, n = 100) values are from histological preparations.
* Adjacent to a GVF, GW or D value indicates a significant difference ($P < 0.05$) from the preceeding value.
* or ** adjacent to a R% value indicates a significant difference from 50% at either the $P < 0.05$ or $P < 0.01$ level, respectively.
Spatfall values are totals of data from fourteen stations (see Fig. 1), each station being included as spat per shell for the given time interval.
n.d. indicates no data collected.

nificant decrease in gonad width was observed between August 3 and 24, and August 31 and September 28.

Distinct spat settlement events in July and August were observed at Horsehead, but not at Wreck Shoal in 1987 (unpublished V.I.M.S. monitoring reports and Tables 1 and 2). By contrast, both sites exhibited July and August settlements in 1988 with the earlier event being atypical of the river-wide mean value for the James River. Cumulative values of all stations (see Fig. 1) at each sampling period indicate that overall settlement was higher throughout 1987 than 1988, with highest values occurring between July 11 and August 21, 1987 and settlement continuing at decreased levels through September 25, 1987. Horsehead and Wreck Shoal were the only stations in the James River that showed major spatfall peaks in July 1988 although high values were observed at most of the stations in August 1988. Settlement events were observed both upstream and downstream of the sampling sites in September 1988 resulting in modest river-wide values despite low values at Horsehead and Wreck Shoal.

Diver collections in September 1988 (Table 3) contained oysters of all size classes between 10 and 100 mm height with largest numbers in the 30–39.9 and 50–69.9 size classes; however, individuals of size less than 50 mm constituted only 11.3% of the biomass estimated as wet tissue.

DISCUSSION

The James River has long been a focal point of the Virginia oyster industry, providing settlements of oysters on an annual basis (Haven and Fritz 1985). Small "seed" oysters were removed from the James in vast quantities for transplant and growth to market size (now >63 mm in height) at locations throughout the Virginia portion of the Chesapeake Bay. Continuing encroachment of *P. marinus* and *H. nelsoni* into the James in the past three decades has decimated oyster reefs below Wreck Shoal (Fig. 1) with associated loss of spawning oysters (see comments in Ruzecki and Hargis 1989). This encroachment, together with a gyre-like circulation in the lower James (Ruzecki and Hargis 1989, Mann 1988), and a lack of significant oyster resources in the lower Chesapeake Bay adjacent to the James, suggests that the James River oyster resource is now an isolated, self-sustaining population and that the brood stock for this population probably resides upriver of Wreck Shoal in a salinity environment not conducive to high prevalence of *P. marinus* and *H. nelsoni*. Oyster fecundity data from our stations in the James River, including Wreck Shoal and Horsehead, during the summer of 1986 illustrate a decrease in mean fecundity in upstream collections with lowest values at Horsehead (Cox and Mann, 1992). Mean weekly values extracted from Cox and Mann (1992) and presented in Figure 2 illustrate a trend of decreasing fecundity in oysters in the Wreck Shoal-Horsehead region between 1986 and 1988.

There are marked quantitative differences in fecundity between 1987 collections from Wreck Shoal and 1988 collections from Horsehead. These are single year and site observations, therefore we cannot definitively state whether the differences are site related, reflect interannual variation, or a progressive detrimental effect of *H. nelsoni* and *P. marinus*. Settlement data (Tables 1 and

TABLE 3.

Cumulative size class distribution data of Horsehead oysters from twelve quadrat collections made on September 22, 1988.

Height (mm)	n	w (gm)	W (gm)	%
<9.9	0			0
10–19.9	10	0.53	5.3	0.1
20–29.9	104	0.77	80.1	1.3
30–39.9	246	1.29	317.3	5.2
40–49.9	129	2.20	283.8	4.7
50–59.9	233	4.08	950.6	15.6
60–69.9	320	6.36	2035.2	33.4
70–79.9	171	9.29	1588.6	26.0
80–89.9	58	12.02	697.2	11.4
90–99.9	12	12.02	144.2	2.4
TOTAL	1283		6102.3	
Mean m^{-2}	107		508.5	
Total	6.45×10^7		3.07×10^5	

No animals <9.9 mm were recorded. n is the cumulative number of oysters in each size class within all 12 quadrats of 1 m^2 each. w is the mean wet tissue weight per individual (data from Haven and Morales-Alamo, unpublished studies of oysters collected from Horsehead in July–August, 1979). Size classes from >80 mm use the same value of w. W = n × w. % is the percentage of the total biomass (as wet tissue weight) in the size class. Total values are based on the area estimate of 603,062 m (Haven and Whitcomb 1973).

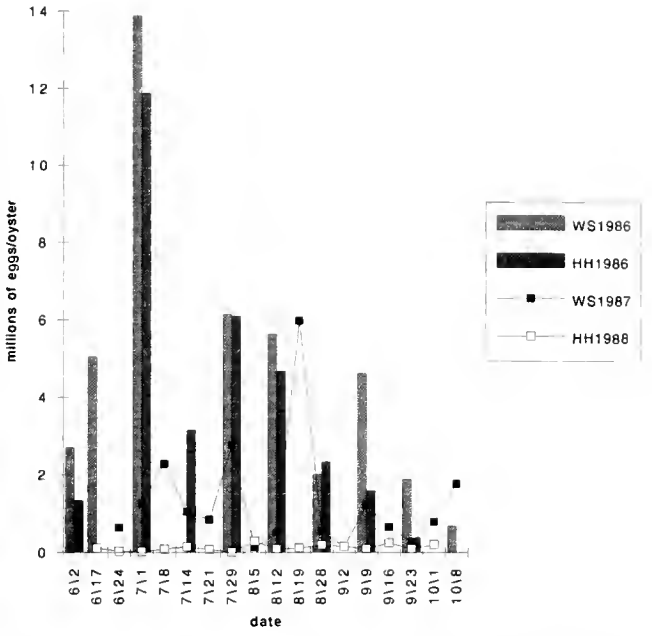

Figure 2. Fecundity of oysters at Wreck Shoal and Horsehead, James River, 1986–1988. 1986 data (bars) are recalculated from Cox and Mann (1992). 1987 and 1988 data (connected symbols) are from Tables 1 and 2 of this study.

2) show a marked decrease in settlement in 1988 coincident with low river flow, unusually high mid-summer salinities in the upper river, and a general upstream movement of the boundary of infective activity (Dr. E. M. Burreson, Virginia Institute of Marine Science, unpublished data). *H. nelsoni* has been shown to significantly reduce fecundity in *Crassostrea virginica* (Barber et al. 1988), and while it was recorded at Wreck Shoal it was very infrequent at Horsehead, typically less than 5% of animals examined, and then at low intensity. *P. marinus* was both more abundant and intense than *H. nelsoni* at Horsehead. The quantitative impact of *P. marinus* on fecundity is poorly understood, but the current observations suggest this as a subject worthy of further examination.

There was no consistent relationship between egg viability and fecundity estimates in 1988 collections from Horsehead (Table 2). The observed coincidence of high fecundity and low viability may be caused by overestimate of the former through counting of immature eggs, that is a methodological error, or production of uniformly inadequate eggs caused by physiological or nutritional stress of the adults (Gallager and Mann 1986, Gallager et al. 1986). Fecunity values alone may have limited value as indicators of reproductive potential in a location of consistently low salinity, such as Horsehead. Concurrent direct examination of other indicators, such as egg viability, would appear advisable. Cox and Mann (1992) present estimates of temporal variation in fecundity at four stations in the James River, including Wreck Shoal and Horsehead, during the summer of 1986 for oysters in the size range of 22 to 122 mm, with the majority between 30 and 105 mm. Oysters in 1986 Horsehead collections were more abundant but also smaller than at other stations. They also included individuals that were smaller than the size range of 55.5 to 88.5 mm used in the present study for GVF estimates for 1988 Horsehead collections. Observed differences in percentage of female oysters present may have been related to this size difference. In 1986 collections, male oysters were consistently more abundant than females at all stations. With the exception of July 16–30 collections in 1987, Wreck Shoal oysters also exhibited a preponderance of males (Table 1). By contrast, 1988 collections at Horsehead approached sexual parity throughout most of the study period.

GVF measurements were made on histological preparations of female oysters collected from Horsehead in 1965 and 1970 for disease diagnosis and stored in the VIMS histology archives. Measurements were also made on oysters collected in 1984 for evaluation of their gametogenic condition (Morales-Alamo and Mann 1989). Individual oyster size data were not available for these preparations; however, all oysters collected for these studies exceeded the effective market size of 75 mm height at the time of collection. Historical trends in gonad volume fraction values obtained from Horsehead female oysters collected in July and August of earlier years were significantly higher ($P < 0.05$) than those recorded in 1988. Mean GVF values in July and August 1988 were less than 0.60, compared to values of 0.80 ($n = 13$) and 0.76 ($n = 15$) for the same months in 1965, 0.77 ($n = 9$) for July 1970, and 0.73 ($n = 29$) and 0.88 ($n = 30$) for July and August of 1984.

A generalized trend with time was common to weekly 1988 values for fecundity, egg size, GVF and gonad width, and suggested a minor peak in gametogenic activity in June followed by two major peaks in the first half of both July and August. The major peaks were apparently followed by spawning events in the second half of July and August respectively. Settlement data (Table 2) illustrates settlement at all sites throughout the lower James River including Wreck Shoal and Horsehead. Asynchronous spatial settlement has been observed (Haven and Fritz, 1985); however, the synchrony of spawning and settlement events are unresolved. Settlement events exhibited two predominant peaks in mid July and August, and may be associated after a reasonable time interval to represent larval development, with the June and mid July spawnings. Likewise, minor settlement events at Horsehead between August 31 and September 14 could be associated with

August spawning. The quantitative and temporal contribution to the observed settlement of spawnings originating from oysters at locations in the James other than Horsehead Reef is unknown.

Spawning activity could not be related to specific conditions of temperature or salinity. Cox and Mann (1992) reported temporal changes in fecundity as indicators of spawning activity at four stations in 1986, using decreases in mean fecundity in successive samplings as indicators of spawning and variability about the mean as an indicator of synchrony within the population. Spawning at Wreck Shoal began July 1 and July 29 in 1986, with minor indication of a second spawning event in Wreck Shoal oysters at the beginning of September. Spawnings in 1986 occurred during periods of increasing temperature and salinity; however, this pattern was not consistent for 1987 spawnings where decreases in mean fecundity were recorded after collections on July 9, July 30, August 20 (only one individual), and September 10. A marked decrease in standard error values on July 16 and August 6 support spawnings immediately prior to these dates. There was a mass spawning at Horsehead at the beginning of July 1986 concurrent with a marked increase in both temperature and salinity. A second increase in fecundity in late July was followed by a protracted spawning which persisted into September as both temperature and salinity decreased from maximum summer values. All 1988 spawnings occurred during periods of increasing temperature, but were inconsistent with respect to salinity change (Table 2). There is an increasing volume of literature relating spawning activity of bivalves to food availability (Nelson 1955, 1957, Breese and Robinson 1981, Starr et al. 1990). We do not, however, have a detailed time course of data describing such changes in the James River and are therefore unable to investigate this possibility in the present study.

Fecundity estimates for 1988 Horsehead collections (Table 2) were made from animals in the size range 55.5 to 88.5 mm. Using size distribution data for September 1988 from Table 3 and including all individuals >60 mm and one half of the individuals in the 50–59.9 mm size class results in an estimated density of 56.5 oysters m^{-2}. This represents 80.1% of the estimated biomass. The product of areal density, reef area (603,062 m^2 from Haven and Whitcomb, 1983), and egg production corrected for viability and sex ratio (mean value of $\{F \times V'' .10^{-3} \times R.10^{-2}\}$ from Table 2 for the six occasions when viable eggs were found), provides an estimate of first-shelled veliger larvae production from Horsehead, that is 0.98×10^4 m^{-2} or 5.90×10^9 in total, during the summer of 1988. This value assumes a single spawning. In comparison it is notable that survival to **all** size classes >10 mm, representing a number of years of cumulative recruitment, is 107 m^{-2} (Table 3). The fate of larvae originating at Horsehead is unknown; they contribute to the pool which supplies the James River in total. While many in this pool are undoubtedly lost to tidal flushing and mortality, the remainder represent the primary larval supply for settlement in the James River. Cumulative estimates of seasonal settlement by station (data recalculated from Tables 1 and 2, not shown) indicate that Horsehead sustained lower settlement than other stations in the James, an observation consistent with previous observations for the period 1963–1980 (Haven and Fritz 1985, Table 4). Even when considering this situation a simple comparison of areal larval production with juvenile survival illustrates the enormous numerical losses that must occur during the early life history of oysters in the James. Roegner (1989) also observed high mortalities in early post-settlement stages of oysters which, after settling on prepared substrates in the laboratory, were immediately transferred to field locations at various intertidal exposures. In all subtidal exposures mortality approached or usually exceeded 90%, often much higher, within four weeks after settlement. Estimates of areal density of oysters on other reefs in the James, together with total reef area remaining unaffected by disease are not available; however, the present focus of the commercial fishing industry on Horsehead reef supports the argument that this location may represent a substantial proportion of the total broodstock remaining in the James at this time. Prudent conservation of this resource is warranted.

Estimation of fecundity and egg viability values have clear value in both management of commercial fisheries and ecological modelling. To date, efforts to construct quantitative life cycle budgets for oyster communities which include the larval phase have been limited for obvious methodological problems which, as illustrated here, have not yet been pursued to submission. These are, however, research areas that must be addressed if we are to use current modelling techniques in practical application.

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MODELING OYSTER POPULATIONS II. ADULT SIZE AND REPRODUCTIVE EFFORT

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ABSTRACT A time-dependent model of energy flow in post-settlement oyster populations is used to examine the factors that influence adult size and reproductive effort in a particular habitat, Galveston Bay, Texas, and in habitats that extend from Laguna Madre, Texas to Chesapeake Bay. The simulated populations show that adult size and reproductive effort are determined by the allocation of net production to somatic or reproductive tissue development and the rate of food acquisition, both of which are temperature dependent. For similar food conditions, increased temperature reduces the allocation of net production to somatic tissue and increases the rate of food acquisition. This temperature effect, however, is mediated by changes in food supply. Within the Gulf of Mexico, oyster size declines from north to south because increased temperature decreases the allocation of net production to somatic growth. An increase in food supply generally results in increased size as more energy is used in somatic growth; however, at low latitudes, as food supply increases, adult size decreases because the allocation of more net production to reproduction outweighs the effect of increased rates of food acquisition. Variations in temperature and food supply affect reproductive effort more than adult size because the rate of energy flow through the oyster is higher in warmer months when most net production is allocated to reproduction and small changes in temperature substantially change the spawning season. The wide range of reproductive effort expected from small changes in temperature and food supply suggest that comparisons of adult size and reproductive effort between oyster populations can only be made within the context of a complete environmental analysis of food supply and associated physical parameters and an energy flow model.

INTRODUCTION

Populations of any species tend to have a characteristic mean adult size, which is defined as the size reached by the average surviving adult individual in the dominant cohort. When the characteristic adult size is considerably below that characteristic of the population, the population is described as stunted (Hallam 1965). Stunting is generally considered to result from suboptimal conditions such as extreme environments or low food resources.

In the Gulf of Mexico, populations of the American oyster (*Crassostrea virginica*) exhibit a latitudinal gradient in characteristic adult size (Fig. 1, Table 1). Mean adult size decreases with decreasing latitude on the eastern and western coasts of the Gulf. At the extremes of this distribution, most oysters fail to reach the standard size limit of 7.6 cm that is required for commerical exploitation (e.g. Hofstetter 1977, Berrigan 1990). The nearly complete restriction of the Gulf of Mexico oyster fishery to the northern Gulf is the practical result of this trend. Additionally, year-to-year variations in mean adult oyster size show similar variations throughout the Gulf of Mexico (Wilson et al. 1992). That is, the characteristic adult oyster size increases or decreases uniformly among the many populations in the Gulf. Variation in age cannot be completely excluded as a contributor to these trends; however, the annual mortality in oyster populations from predators and disease exceeds 75% throughout the Gulf of Mexico (e.g. Butler 1953a, Moore and Trent 1971, Powell et al. 1992a) and fished and unfished populations were included in the analysis. Accordingly, the oyster populations sampled in the Gulf of Mexico were composed primarily of individuals that were one to two years in age (Wilson et al. 1992). Hence, size rather than age accounts for the trends seen in these populations.

The similar trends on both sides of the Gulf of Mexico in oyster size with latitude and the year-to-year variability in mean adult size suggest that one or more climatic variables limit oyster size. The correlation with latitude suggests temperature as a likely variable. From a physiological perspective, temperature may affect adult size by regulating the division of net production into somatic and reproductive tissue growth and by regulating the relative rates of filtration and respiration. As temperature increases, more net production is allocated to reproduction. Filtration and respiration rates also increase, but the rate of increase in filtration rate is greater (Powell et al. 1992b). Therefore, a complex interaction of temperature with oyster physiology may place an upper limit on adult size.

Related to adult size is the concept of reproductive senility (Peterson 1983) in which fecundity per unit biomass declines at large size or old age. The existence of reproductive senility in oysters remains to be determined. However, respiration rate rises faster than filtration rate with increasing body size (Klinck et al. 1992, Powell et al. 1992b). The different scaling of respiration and filtration with body size suggests that the scope for growth in oysters must eventually be curtailed at large size which will result in declining fecundity per unit biomass (Powell et al. 1992b). Consequently, populations of lower characteristic size may spawn more per unit biomass.

The objectives of this study are to investigate processes that contribute to variation in the characteristic adult size of oyster populations within a particular habitat and over a latitudinal gradient in temperature and to address the possible influence of reproductive senility in oyster populations. These objectives are addressed using an energy flow model (Fig. 2) developed for post-settlement oyster populations. A series of simulations are presented for Galveston Bay, Texas that consider the effect of variations in temperature, food supply and salinity on adult oyster

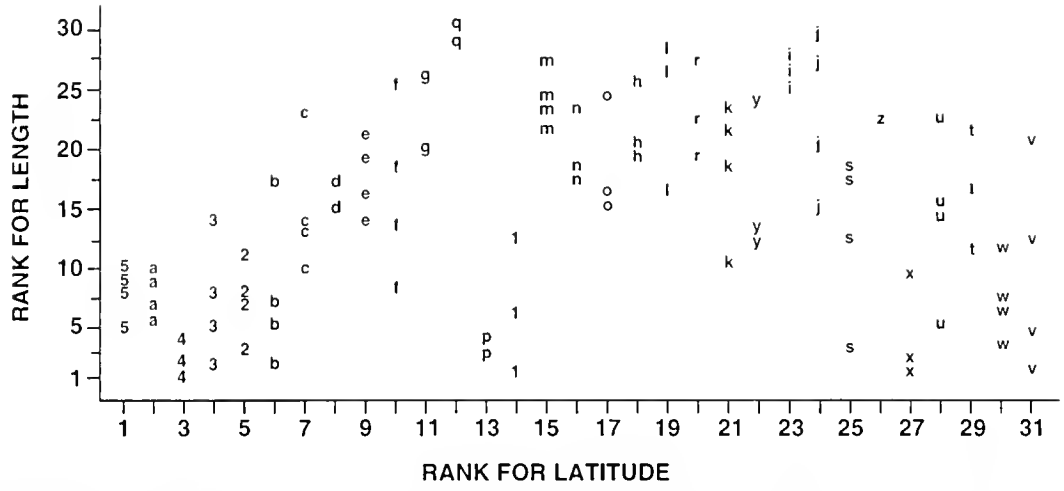

Figure 1. Mean adult oyster size (length) versus latitude plotted as the rank-order of latitude versus the rank-order of size [see Wilson et al. (1992) for details]. The four values for each size and latitude, referenced by letter (a–z) or number (1–5), are those given in Table 1 for 1986 to 1989. Bays with the characteristically smaller sizes are the more southerly bays on either side of the Gulf of Mexico (on the left), the bays in the Florida Panhandle (right), and Tiger Pass and the Mississippi Delta.

TABLE 1.

Oyster population mean length (cm) and fraction of the population in advanced reproductive state (spawning or ready to spawn) for thirty-one bay systems around the Gulf of Mexico that were sampled from 1986 to 1989 as part of the NOAA Status and Trends program. Details of the sampling sites are given in Wilson et al. (1992). Bays are listed beginning with the southern most bay in Texas and proceding clockwise around the Gulf of Mexico. The high fraction ready to spawn in the northern Gulf of Mexico in 1986 (bays l to s) resulted from sampling late in the year. Year and Julian Day were used in the statistical analysis of these data to control for this effect.

	Length				Fraction in Advanced Reproductive State			
Bay Systems	1986	1987	1988	1989	1986	1987	1988	1989
a Laguna Madre	8.16	6.95	6.04	6.03	0.14	0.86	0.27	0.15
b Corpus Christi Bay	7.41	5.67	5.52	7.04	0.13	0.00	0.14	0.23
c Aransas Bay	8.47	8.20	8.19	6.38	0.05	0.02	0.04	0.05
d San Antonio Bay	8.68	8.36	–	–	0.09	0.70	–	–
e Matagorda Bay	9.38	8.30	6.92	7.07	0.20	0.05	0.05	0.21
f East Matagorda Bay	10.13	8.37	6.72	6.29	0.10	0.00	0.14	0.23
g Brazos River	–	–	8.57	7.14	–	–	–	0.33
h Galveston Bay	9.03	8.56	8.55	8.33	0.14	0.09	0.04	0.10
i Sabine Lake	10.44	9.65	9.66	8.40	0.00	0.15	0.00	0.00
j Lake Calcasieu	11.48	8.27	7.99	9.32	0.00	0.00	–	0.00
k Joseph Harbor	8.36	8.79	8.19	7.06	0.67	0.00	–	0.14
l Vermillion Bay	8.72	9.66	9.91	9.06	0.93	0.00	0.25	0.00
m Caillou Lake	9.73	10.36	8.18	8.20	0.83	0.14	0.00	0.13
n Lake Barre/Felicity	8.96	9.22	7.17	7.49	0.97	0.04	0.00	0.21
o Barataria Bay	10.08	9.57	7.04	6.86	0.89	0.00	0.15	0.35
p Tiger Pass	–	–	5.80	5.72	–	–	–	0.27
q Pass a Loutre	–	–	11.23	10.57	–	–	0.00	0.00
r Breton Sound	9.66	8.50	7.71	8.47	0.93	0.07	0.04	0.04
s Lake Borgne	8.94	7.27	7.52	5.68	1.00	0.00	0.07	0.00
t Mississippi Sound	8.40	7.15	7.10	7.20	0.00	0.00	0.00	0.13
u Mobile Bay	8.62	9.03	6.03	6.66	0.13	0.00	0.00	0.13
v Pensacola Bay	9.09	4.55	6.02	6.46	0.08	0.00	0.05	0.09
w Choctawatchee Bay	7.74	4.95	6.67	5.97	0.09	0.00	0.00	0.03
x St. Andrew Bay	6.01	4.81	6.53	6.35	0.64	0.00	0.10	0.06
y Apalachicola Bay	8.43	7.35	8.29	6.64	0.13	0.07	–	0.04
z Apalachee Bay	–	–	–	7.29	–	–	–	0.00
1 Cedar Key	7.44	5.16	6.71	5.39	0.07	0.00	0.08	0.00
2 Tampa Bay	6.58	5.90	6.37	6.44	0.25	0.41	0.23	0.57
3 Charlotte Harbor	6.52	5.30	6.47	6.64	0.00	0.00	0.48	0.27
4 Rookery Bay	6.70	5.26	4.67	5.47	0.00	0.13	0.11	0.13
5 Everglades	8.06	6.56	6.56	5.84	0.08	0.20	0.10	0.00

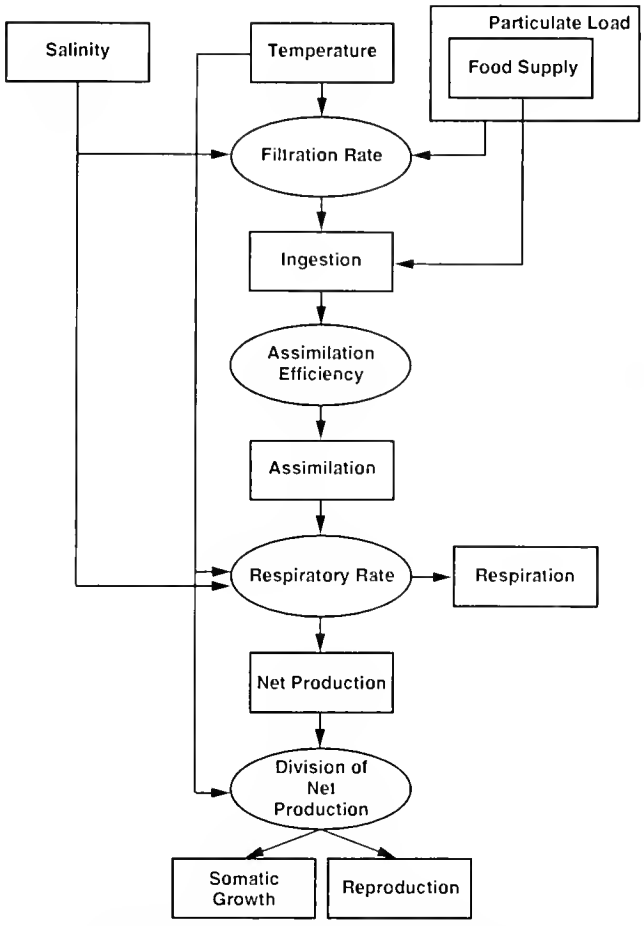

Figure 2. Schematic of the oyster population model.

size. Aside from reductions in oyster growth rate from diseases (Ray and Chandler 1955, Matthiessen et al. 1990) and perhaps genetic differences (Grady et al. 1989, Reeb and Avise 1990) these are likely to be the most important factors controlling size in oyster populations. The effect of latitudinal temperature effects is investigated with simulations that use environmental conditions appropriate for the Laguna Madre, Apalachicola Bay and Chesapeake Bay, as well as Galveston Bay.

THE MODEL

Basic Characteristics

The oyster population model (Fig. 2) 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 2), that 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. In Galveston Bay, for example, the largest oysters routinely collected are 7 to 8 g dry wt (Fig. 3), which corresponds to model size class 9. Thus, the largest size class, 10, is large enough to prevent boundary effects in the model solutions at the upper end of the size-frequency distribution. 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:

TABLE 2.

Biomass and length dimensions of the oyster size classes used in the model. Biomass is converted to size using the relationship given in White et al. (1988), denoted by WPR, and Paynter and DiMichele (1990), denoted by PD. The market-size/submarket-size boundary is about one size class smaller using the conversion from Paynter and DiMichele (1990). The upper size class length conversions obtained from the Paynter and DiMichele (1990) relationship are extrapolations and are, therefore, less accurate, as are the final two conversions obtained from the White et al. (1988) relationship. The range of length to biomass relationships in Galveston Bay, Texas is shown in Figure 3.

Model Size Class	Biomass (g ash free dry wt)	Length (WPR) (mm)	Length (PD) (mm)
1	1.3×10^{-7}–0.028	0.3–25	0.15–21.4
2	0.028–0.10	25–35	21.4–35.7
3	0.10–0.39	35–50	35.7–61.7
4	0.39–0.98	50–63	61.7–89.4
5	0.98–1.94	63–76	89.4–117.6
6	1.95–3.53	76–88	117.6–149.5
7	3.53–5.52	88–100	149.5–178.9
8	5.52–7.95	100–110	178.9–207.1
9	7.95–12.93	110–125	
10	12.93–25.91	125–150	

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.

The following 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), and the food available to the oysters was converted to caloric equivalents by using 5168 cal g dry wt^{-1}. The model calculations use biomass exclusively (and calories) and so are independent of oyster growth form and length-to-biomass relationships. To relate the biomass size classes, defined in Table 2, to lengths for comparison to the available measurements and the standard measures of fishery management, the length-to-biomass conversion given in White et al. (1988) was used. This conversion is only an approximation, however, given the variation in growth forms found in oysters within bays and throughout their latitudinal range. The model results are presented in terms of biomass, which can be converted to any local specific lengths by using an alternative length-to-biomass relation and the size class boundaries given in Table 2. One example, from Paynter and DiMichele (1990) is shown in Table 2 for comparison.

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. 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

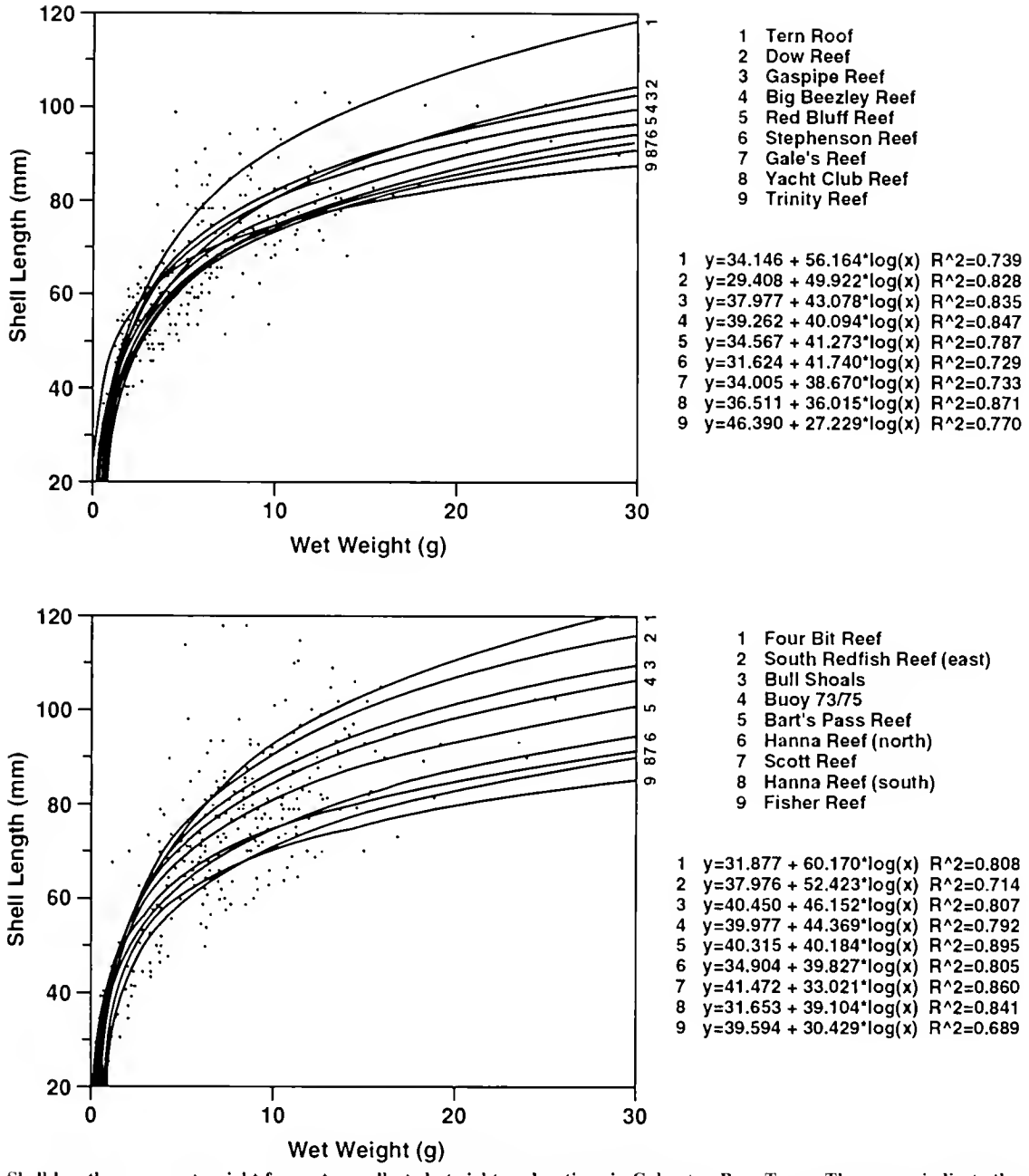

Figure 3. Shell length versus wet weight for oysters collected at eighteen locations in Galveston Bay, Texas. The curves indicate the empirical relationships obtained using the data from the different locations. The numbers on the curves correspond to those for the empirical relationships from each site.

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. Finally, each specific rate for each transfer between size classes was scaled to the relative size of the respective classes:

for transfers up: $W_j/(W_{j+1} - W_j)$
for transfers down: $W_j/(W_j - W_{j-1})$.

Governing Equation

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. 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):

$$NP_j = P_{gj} + P_{rj} = A_j - R_j. \quad (1)$$

Therefore, a governing equation for each oyster size class can be written as

$$\frac{dO_j}{dt} = P_{gj} + P_{rj} + (\text{gain from } j-1) - (\text{loss to } j+1) \qquad (2)$$

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) is modified as

$$\frac{dO_j}{dt} = P_{gj} + P_{rj} + (\text{gain from } j-1) - (\text{loss to } j+1) + (\text{gain from } j+1) - (\text{loss to } j-1). \qquad (3)$$

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. 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 Hilbert's (1977) biomass-length relationship to obtain filtration rate for each size class as a function of temperature (T) and biomass:

$$FR_j = \frac{K_j^{0.96} T^{0.95}}{2.95} \qquad (4)$$

and

$$K_j = W_j^{0.317} 10^{0.669} \qquad (5)$$

where filtration rate, FR_j, is given as ml filtered ind^{-1} min^{-1} and W_j is the ash-free dry weight in g for each size class. Powell et al. (1992b) 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 (1978), 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 ppt and ceases at 3.5 ppt. In mathematical terms:

$$\begin{aligned} S \geq 7.5 \text{ ppt} \quad & FR_{aj} = FR_j \\ 3.5 < S < 7.5 \text{ ppt} \quad & FR_{aj} = FR_j(S - 3.5)/4.0 \\ S \leq 3.5 \text{ ppt} \quad & FR_{aj} = 0 \end{aligned}$$

where S is the ambient salinity and FR_j is the rate obtained from equation (4). [Note that the second salinity relationship was misprinted in Powell et al. (1992b) and Hofmann et al. (1992).]

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

$$\tau = (4.17 \times 10^{-4}) 10^{0.418x} \qquad (7)$$

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:

$$FR_{\tau j} = FR_{aj}\left[1 - .01\left(\frac{\log_{10}\tau + 3.38}{0.0418}\right)\right]. \qquad (8)$$

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), Langefoss and Maurer (1975), and Valenti and Epifanio (1981).

Respiration

Oyster respiration, R_j, as a function of temperature and oyster weight in each size class was obtained from Dame (1972) as

$$R_j = (69.7 + 12.6T)W_j^{b-1} \qquad (9)$$

where b has the value 0.26. Equation (9) 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:

$$T < 20°\text{C} \qquad R_r = 0.007T + 2.099$$

and

$$T \geq 20°\text{C} \qquad R_r = 0.0915T + 1.324;$$

where R_r is the ratio of respiration at 10 ppt to respiration at 20 ppt:

$R_r = R_{10\ ppt}/R_{20\ ppt}$. Equations (9) and (10) were combined to obtain respiration over a range of salinities as:

$$\begin{aligned} S \geq 15 \text{ ppt} \quad & R_j = R_j, \\ 10 \text{ ppt} < S < 15 \text{ ppt} \quad & R_j = R_j(1 + [(R_r - 1)/5((15 - S))]) \\ S \leq 10 \text{ ppt} \quad & R_j = R_j R_r. \end{aligned}$$

Shumway and Koehn (1982) identified effects of salinity on respiration at 20 ppt; however, we used a 15 ppt 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

$$P_{rj} = R_{effj} NP_j, \text{ for } j = 4, 10, \tag{14}$$

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. Stauber 1950, Loosanoff 1965, Dupuy et al. 1977), including empirical temperature-dependent relationships (Loosanoff and Davis 1953, Kaufman 1979), 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 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. (1993).

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. 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 years 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. (1992b) and Hofmann et al. (1992), 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. $\pm 10\%$) 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 the effect of variations in environmental conditions on characteristic adult oyster size and fecundity.

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. 1992b) 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 ppt to remove the effect of low salinity on oyster respiration and filtration rates and to emphasize temperature effects. For some Galveston Bay simulations, a low salinity (7 ppt) event was imposed and one Chesapeake Bay simulation used the salinity time series given in Galtsoff et al. (1947). Food and turbidity values were specified as described for each simulation. A summary of the environmental conditions used for the simulations is given in Table 3.

RESULTS

Basic Simulation

The time evolution of an oyster population that resulted from the settlement of a cohort of ten individuals in mid-May (day 140) that were subsequently exposed to the monthly-averaged temperatures from Galveston Bay, a constant salinity (24 ppt) and a constant food supply of 0.5 mg l^{-1} was simulated. No recruitment or mortality was allowed so that the same individuals were tracked from settlement onwards, about 5.5 years. This simulation provided a basic case to which other simulations could be compared. Following settlement, the oyster population increases in biomass during the first 1.5 years of the simulation (Fig. 4a) after which it reaches a steady population distribution that is in equilibrium with the imposed environmental conditions. The majority of the population at the end of the simulation is in size classes 5 and 6 (63 to 88 mm). In the first two years of the simulation, gonadal tissue is present in size classes 4 to 6. However, as the population stabilizes, gonadal tissue is confined to size classes five and larger. Gonadal tissue development occurs in the adult size classes throughout the summer and into the fall, with the maximum development as a fraction of body weight occurring in late July of each year.

A fall larval set, exposed to the same environmental conditions, results in a similar population distribution (Fig. 4b). The oyster population stabilizes with the same size-frequency distribu-

TABLE 3.

Summary of the environmental conditions used for the oyster population simulations. Inclusion of a time varying monthly-averaged temperature, salinity, food concentration or turbidity time series is indicated by V. For simulations that used constant salinity or food conditions the values are given in ppt or mg l^{-1}, respectively. Some simulations used an idealized (I) food time series that included increased concentrations in the spring and fall to simulate blooms. Exclusion of an environmental variable is denoted by N.

Area	Temperature	Salinity	Food	Turbidity	Figure
Galveston Bay	V	24	0.5	N	4a, b
Galveston Bay	V	24	1.0	N	5a
Galveston Bay	V	24	1.5	N	5b
Galveston Bay	V	V	V	N	6a
Galveston Bay	V	V	V	V	6b
Galveston Bay	V	7	0.5	N	7a
Galveston Bay	V	7	1.0	N	7b
Galveston Bay	V	7	1.5	N	7c
Chesapeake Bay	V	V	V	N	9a
Laguna Madre	V	24	V	N	9b
Laguna Madre	V	24	0.5	N	10a
Apalachicola Bay	V	24	0.5	N	10b
Chesapeake Bay	V	24	0.5	N	10c
Laguna Madre	V	24	1.0	N	11a
Apalachicola Bay	V	24	1.0	N	11b
Chesapeake Bay	V	24	1.0	N	11c
Laguna Madre	V	24	I	N	13a
Galveston Bay	V	24	I	N	13b
Chesapeake Bay	V	24	I	N	13c

tion and gonadal tissue development is nearly identical. Consequently, a spring settlement is used to initialize the simulations described in the following sections.

Overall, the growth rates, gonadal tissue production and adult size of the simulated oyster populations shown in Figure 4 are in agreement with measurements from Galveston Bay. Some oysters reach size class 5 (63 mm) in about 45 days and size class 6 (76 mm) in about 72 days after settlement. These growth rates are similar to those found for oysters in Galveston Bay and around the Gulf coast in general (Powell et al. 1992a, Ingle and Dawson 1952, Hayes and Menzel 1981). Gonadal tissue production and spawning in oyster populations in the northern Gulf of Mexico is normally restricted to the summer months (Wilson et al. 1990). Consequently, reproductively-advanced oysters make up the majority of the population only from April to October. This same pattern is seen in the simulated population. In Galveston Bay the upper limit on oyster size is 80 to 100 mm and the mean oyster length is about 85 mm (Table 1; Wilson et al. 1992). Adult oyster size at the end of the simulation approaches this value.

Local Controls on Adult Size

Food Supply

Food supply is an important factor governing the growth and development of post-settlement oyster populations. Within any one bay, local conditions can result in large variations in the food concentrations experienced by these populations. To investigate this effect on oyster adult size, constant food supplies that bracketed the range of typical food variations measured in Galveston Bay (Soniat et al. 1984) were tested. The pattern of development for an oyster population exposed to a food supply double that used in the basic simulation (Fig. 5a) is not substantially different. A stable size-frequency distribution develops in about 1.5 years. However, the details of the population do differ. The final size-frequency distribution shows that most of the individuals are in size classes 8 and 9, 100–125 mm. Gonadal tissue development occurs throughout the year, but reaches maximum development in the larger animals in the fall. A further increase in food supply by 50% results in a simulated population that rapidly increases in size (Fig. 5b) and has the majority of the individuals in size class 8 and larger. Development of gonadal tissue occurs in the larger individuals throughout the year. Overall, these simulations demonstrate that oyster size increases with increasing food concentration.

Food supply does not remain constant throughout the year in Galveston Bay at the levels used in the previous simulations. Rather, in many years, food supply shows maximum values in the spring and fall that are associated with the spring and fall plankton blooms and reduced food values in the winter. Hence, a monthly-averaged food time series from Galveston Bay (Soniat et al. 1984) was used with the model. This simulation also used observed salinity values for Galveston Bay. The time varying food supply results in the simulated oyster population shown in Figure 6a. The final adult size for this population is intermediate between that obtained for the constant low and medium food simulations. The majority of the adults are found in size classes 7 and 8 (88–110 mm). Maximum gonadal tissue production is also associated with these size classes and occurs in the late summer and fall. A constant salinity of 24 ppt results in a simulated population (not shown) that is almost identical to that shown in Figure 6a.

Turbidity

In estuarine systems, like Galveston Bay, total seston includes inorganic particles that can interfer with filtration and reduce ingestion rates at high enough concentrations. Hence, the overall food supply is effectively reduced. When monthly-averaged turbidity values (Soniat et al. 1984) from Galveston Bay are included as part of the food supply, the effect is to reduce the overall size of the oyster population and gonadal tissue development (Fig. 6b). The final adult size is reduced to 63 to 88 mm (size classes 5 and 6) and is similar to that obtained at the low constant food supply of 0.5 mg l^{-1}. Gonadal tissue development is confined to a smaller portion of the year.

Salinity

Estuarine systems are frequently characterized by extended periods of low salinity. As many laboratory and field studies have shown, the filtration and respiration rates of oysters are adversely affected at salinities below 7.5 ppt and 15 ppt, respectively. Consequently, episodes of low salinity could result in reduced size and reduced gonadal tissue development. To test the effect of this environmental variable, the development of oyster populations during extended periods of low salinity (7 ppt) over a range of food concentrations was simulated (Fig. 7).

The effect of low salinity is to reduce the overall size of the adult population and to hinder the development of gonadal tissue at a given food concentration. The effect of low salinity is most pronounced at low food concentration (Fig. 7a) where the scope for growth is most reduced. The final adult size is reduced relative to the equivalent high salinity case (cf. Fig. 4a) and gonadal tissue

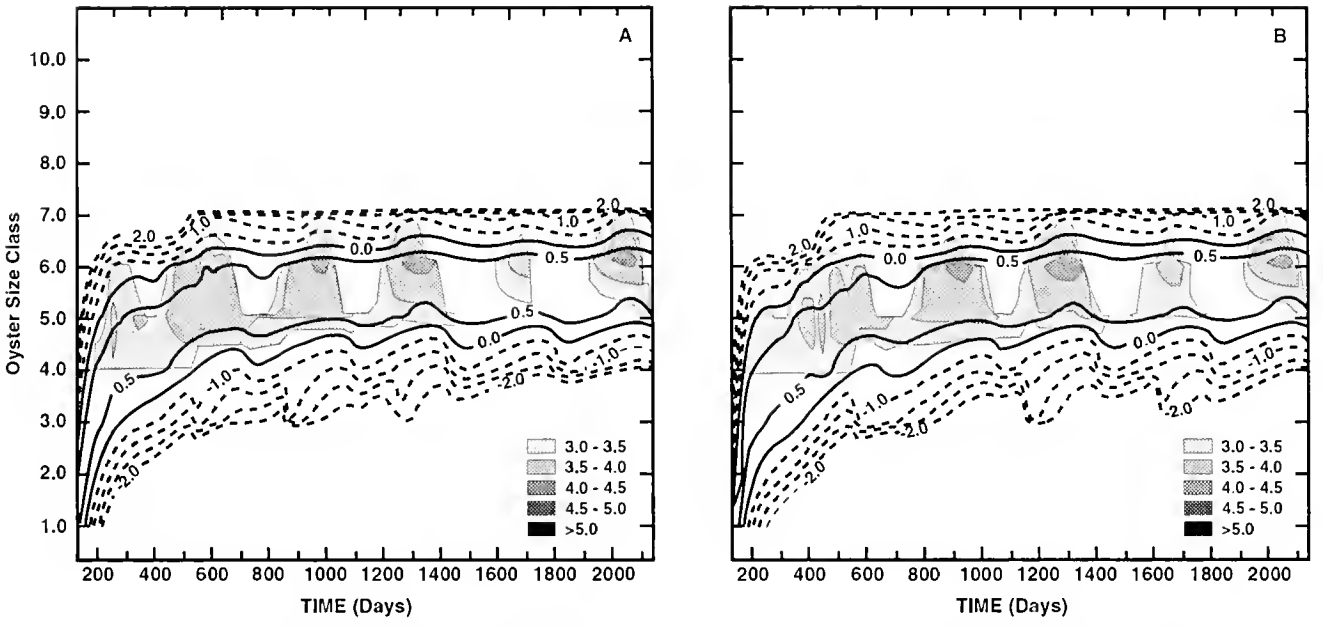

Figure 4. Comparison of the time evolution of oyster populations and gonadal tissue development produced by recruitment of a cohort of ten individuals into size class 1 on A) Julian Day 140 (mid-May) and B) Julian Day 240 (early August). Isolines represent the number of individuals which are given in terms of the logarithm of the number of oysters ($\log_{10} N$). Size class boundaries are defined in terms of biomass (ash free dry weight) as shown in Table 2. Hence, the zero contour corresponds to one individual. Population values less than this are indicated by the dashed lines; solid lines are population values greater than one individual. Shading for the amount of gonadal tissue development represents the logarithm of calories ($\log_{10}$ 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 gonadal tissue 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. The caloric values can be expressed as Joules by using a conversion of 4.18 Joules cal^{-1}.

production is less. Similar trends are observed for low salinity conditions at the higher food concentrations (Fig. 7b, c). However, higher food concentrations offset the deleterious effects of low salinity somewhat by providing more energy for growth. Comparison of the simulated populations at low (Fig. 7) and high salinity conditions (Figs. 4 and 5) shows that the effect of reduced salinity is minor relative to that of reduced food. Therefore, the detrimental effects of low salinity on oyster populations can be reduced by high, but not unusually high food supplies.

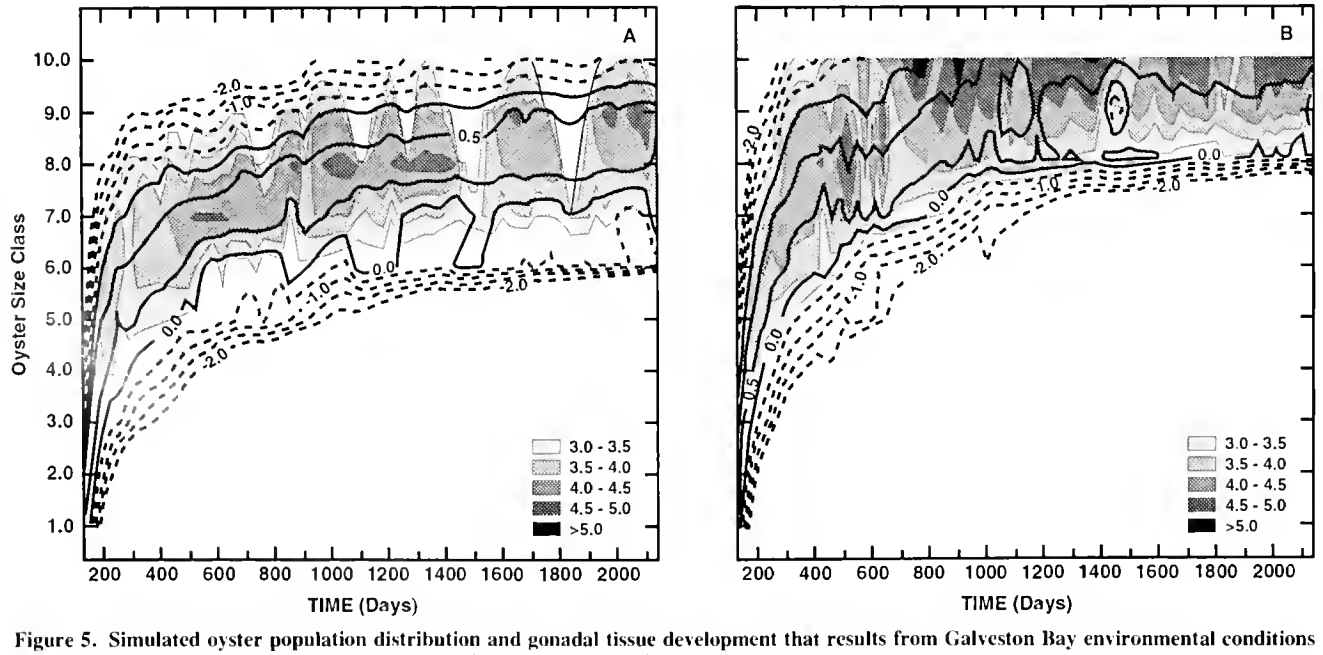

Figure 5. Simulated oyster population distribution and gonadal tissue development that results from Galveston Bay environmental conditions and constant food concentrations of A) 1.0 mg l^{-1} and B) 1.5 mg l^{-1}. Otherwise same as Figure 4.

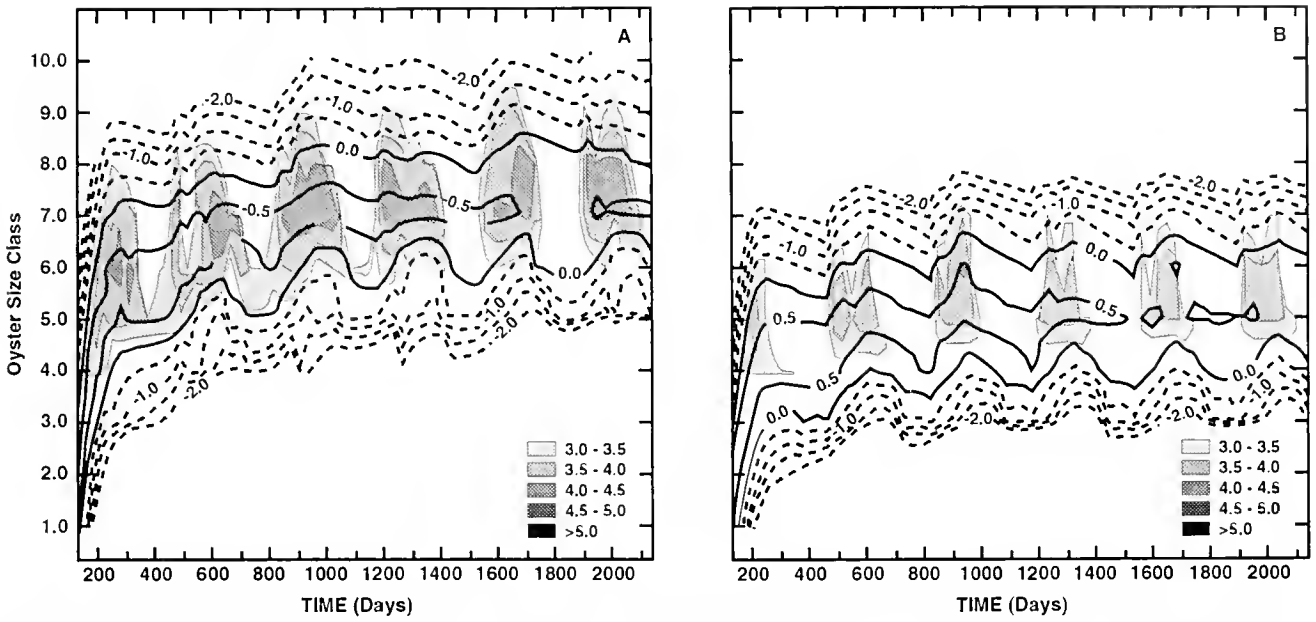

Figure 6. Simulated oyster population distribution and gonadal tissue development that results from Galveston Bay environmental conditions and food conditions A) without, and B) with turbidity. Otherwise same as Figure 4.

Latitudinal Controls on Adult Size

Temperature

The monthly temperature distributions that are characteristic of Laguna Madre, Texas (26°N), Galveston Bay, Texas (29°N), Apalachicola Bay, Florida (30°N) and Chesapeake Bay, Virginia (38°N) show that all three bays reach about the same temperature (28°C) in the summer (Dekshenieks et al. 1993). The primary difference over this latitudinal range is in the winter temperatures and duration of cold conditions. To test the effect of temperature on oyster size and gonadal tissue development over such a latitudinal range, a series of simulations that used idealized temperature time series were done. All simulations used six months of warm (28°C) temperature. The remaining six months were set at 25°C, 20°C, 15°C and 10°C to represent winter conditions in the four bays, respectively.

For all the temperature conditions, the mode of the oyster population, after 5.5 years of simulation, was found in size class 7, 88–100 mm (Fig. 8). However, the population distribution about this mode varied considerably from bay to bay. The small temperature difference between winter and summer conditions in Laguna Madre, resulted in the oyster population being dominated by essentially a single size class. Adult size increased between Laguna Madre and Galveston Bay, with about 40% of the population found in size class 8. This model result agrees with observations of increased adult oyster size in Galveston Bay relative to Laguna Madre. However, the simulated size distributions suggest that adult size decreases between Galveston Bay and Chesapeake Bay, which is opposite of the trend seen in the measurements. This difference in simulated and observed adult size arises from the similar time periods used for the warm and cool temperatures.

As a check on the above results, realistic temperature distributions for Chesapeake Bay and Laguna Madre were used with the model (Fig. 9). The simulated population size-frequency distribution for Chesapeake Bay shows that oysters of size classes 6 and 7 (70–100 mm) are produced by the summer of the second year. The juvenile growth rates and adult size obtained from the model agree with those reported for Chesapeake Bay oyster populations by Butler (1953b) and Beaven (1952). Yearly fluctuations in biomass are higher in Chesapeake Bay because scope for growth is negative for longer periods during the winter.

Adult size in Chesapeake Bay (size class 8) is larger relative to that in the Laguna Madre (size class 7). This difference arises despite the shorter growing season in Chesapeake Bay (Butler 1953b). The Chesapeake Bay simulation (Fig. 9a) allows more time at intermediate temperatures where somatic, but not reproductive, tissue is developed. The practical result is a larger adult population. Thus, the temperature range as well as the length of time exposed to a temperature are important determinants of adult size.

Food Supply

A low (0.5 mg l^{-1}) constant supply of food alters the size distribution of adult oysters from Laguna Madre to Chesapeake Bay (Fig. 10). The simulated adult size is essentially the same throughout the Gulf of Mexico. Adult oysters in Laguna Madre (Fig. 10a), Galveston Bay (Fig. 4a) and Apalachicola Bay (Fig. 10b) are found in size class 6. Gonadal tissue production is about the same in the three bays, with that in Laguna Madre being somewhat higher and extending over more of the year. Chesapeake Bay oysters (Fig. 10c) are slightly smaller (size class 5) which results from decreased filtration rate and hence reduced net production in response to the colder winter temperatures in this bay. Winter temperatures in Laguna Madre allow a higher rate of filtration which results in this bay having the largest oysters at the low food levels.

Doubling the available food supply to 1.0 mg l^{-1}, results in the largest oysters being produced at the mid-latitude sites, Galveston Bay (Fig. 5a) and Apalachicola Bay (Fig. 11b). The smaller adult size occurs in Laguna Madre (Fig. 11a) because more of the available food supply is used to produce reproductive rather than somatic tissue. Adult size in Chesapeake Bay (Fig. 11c) is also smaller than that in the mid-latitude bays. However, this arises

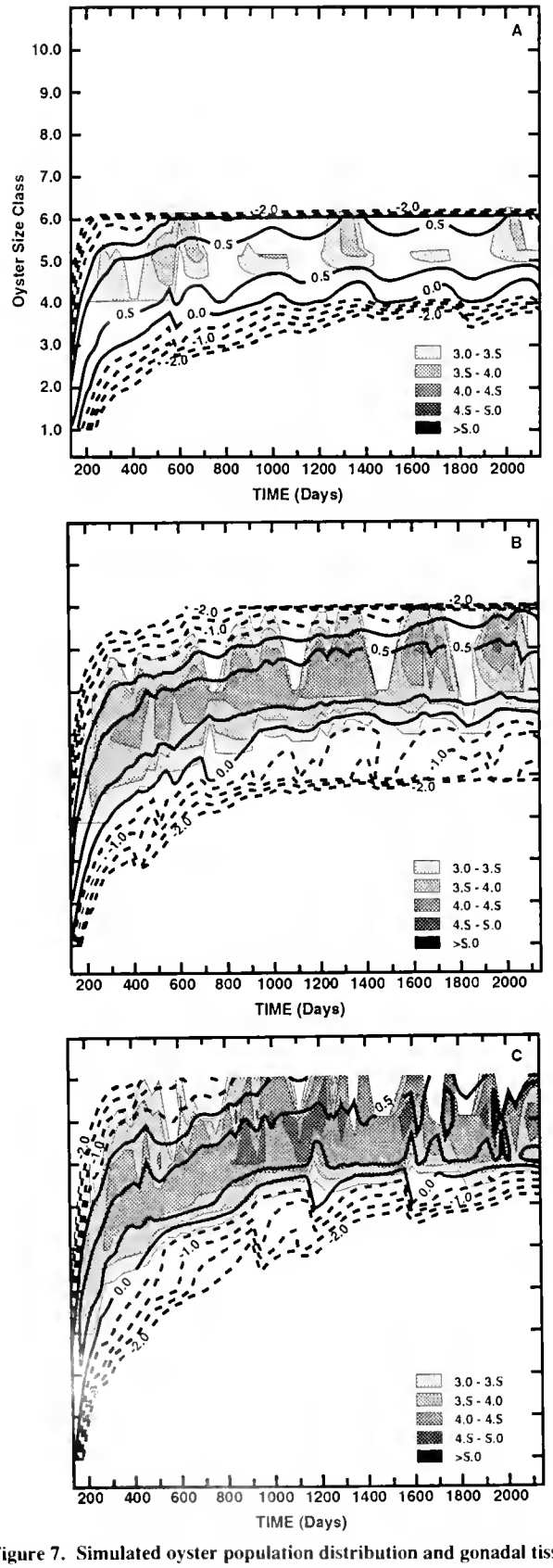

Figure 7. Simulated oyster population distribution and gonadal tissue development that results from Galveston Bay temperatures, low salinity (7 ppt) conditions and food concentrations of A) 0.5 mg l^{-1}, B) 1.0 mg l^{-1}, and C) 1.5 mg l^{-1}. Otherwise same as Figure 4.

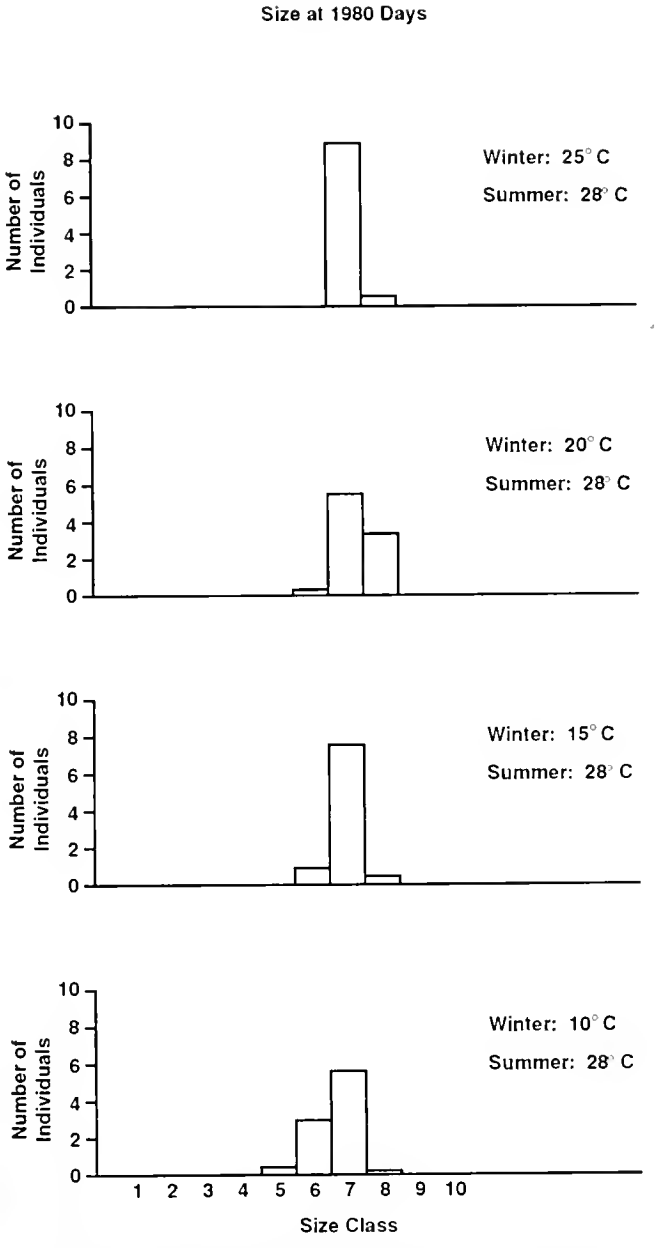

Figure 8. Simulated size frequency distribution from year six for four idealized temperature time series. Other environmental conditions were constant salinity (24 ppt), Galveston Bay food conditions and no turbidity.

due to the colder temperatures which limit winter net production rather than the production of reproductive tissue.

Environmental Controls on Reproductive Potential

The simulations presented in Figures 4–11 show that gonadal tissue development changes for a given set of environmental conditions. This in turn determines the reproductive potential (spawning) of an oyster population. The ability to check the accuracy of the reproductive portion of the population model is limited due to the paucity of observations that provide measurements of oyster reproductive state, oyster size, and environmental conditions concurrently. However, there are some general trends that should appear in the simulated populations.

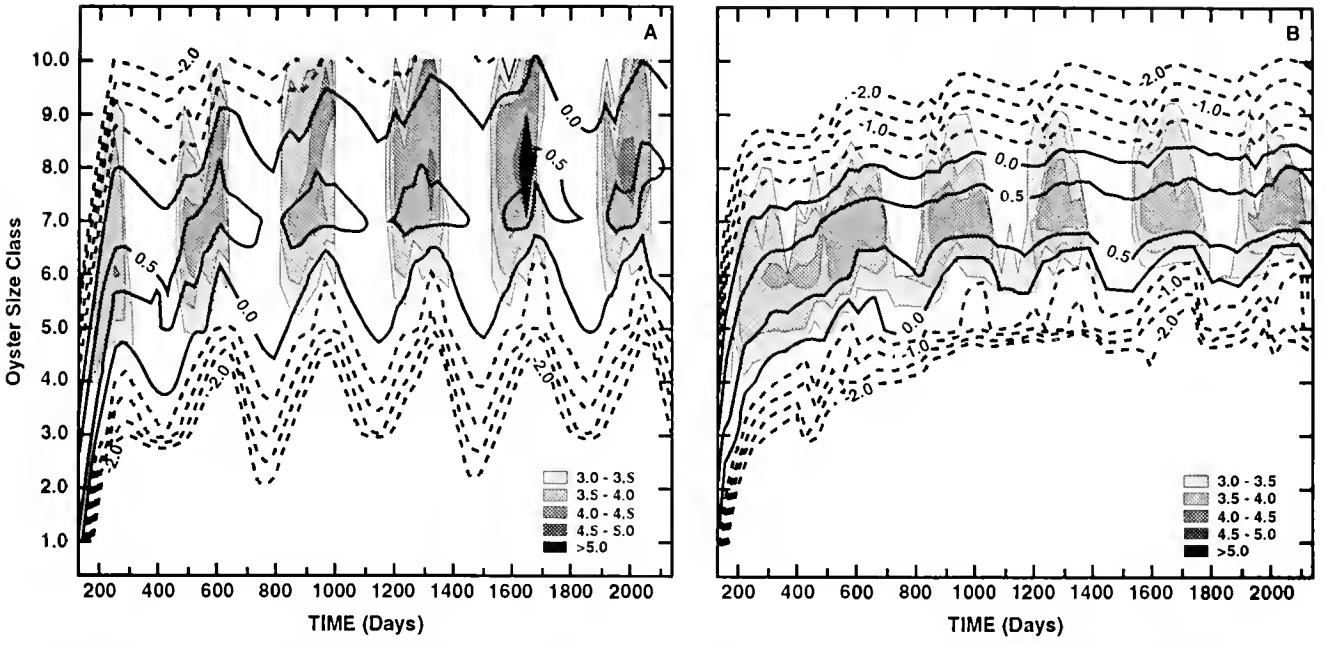

Figure 9. Simulated oyster population distribution and gonadal tissue development that results from temperature, salinity and food conditions characteristic of A) Chesapeake Bay and B) Laguna Madre. Observations on food distributions are lacking for Laguna Madre. Hence, the Galveston Bay food time series was used in this simulation. Otherwise same as Figure 4.

The spawning frequency and pattern associated with the simulated populations from Laguna Madre, Galveston Bay and Chesapeake Bay is shown in Figure 12. In general spawning is associated with the larger size classes and the spawning season tends to be longer at lower latitudes. Also, the most southerly bays tend to have continuous spawning; whereas, that in Chesapeake Bay tends to be confined to discrete pulses. This same trend is observed in the observations from the NOAA Status and Trends program (Table 2). More oysters were found in late reproductive phase, ready to spawn or spawning at lower latitudes.

Spawning season is usually defined by the period of time during which mature eggs are present or by the period of actual spawning. The simulated spawning season, as defined by significant spawning events, is about 100 days in Laguna Madre (Fig. 12a), somewhat shorter in Galveston Bay (Fig. 12b) and even shorter in Chesapeake Bay (Fig. 12c). A tendency towards a spring and fall spawning peak occurs in Galveston Bay (last two years of simulation) and an even stronger tendency towards this occurs in Chesapeake Bay. Significant gonadal material is present for about 200 days (7 months) in Galveston Bay, 160 days (5 months) in Chesapeake Bay, and nearly all year in Laguna Madre. These features of the stimulated spawning season are within the range of values reported for oyster populations and fit the trend toward shorter spawning seasons at higher latitudes (e.g. Hopkins 1935, Stauber 1950, Ingle 1951, Heffernan et al. 1989, and previous references). The development of reproductive material in the simulated oyster populations, from initiation to first spawning, takes about 40 days in Galveston Bay and 60 days in Chesapeake Bay. This is somewhat slower than the 20 to 40 days suggested by Kaufman (1979) and Loosanoff and Davis (1953). However, these time intervals were based on results from constant temperature incubations, which will result in shorter times. Hayes and Menzel (1981) recorded mature gametes in oysters that were 40 to 50 days old, which is similar to what is observed in the simulated populations from Galveston Bay. Egg production, over a two month period, recorded for Delaware Bay oysters held in the laboratory was 3×10^7 to 4×10^7 eggs per female (Davis and Chanley 1955). This study did not report food levels. Egg number, estimated from the simulation results for Chesapeake Bay and Galveston Bay, using the approach described in Klinck et al. (1992), is 1.7×10^8 and 3×10^8 eggs per female, respectively, for a spawning period of about 100 days.

The extent to which these differences and similarities in spawning frequency and pattern result from variations in environmental conditions is discussed in Hofmann et al. (1992). For this study, the interest is in the extent to which these differences and similarities result from variations in adult size. Oyster populations in Laguna Madre (Fig. 13a), Galveston Bay (Fig. 13b) and Chesapeake Bay (Fig. 13c) show a restriction in the period of reproductive effort, as measured by spawn production, over the course of the six-year simulation. This is a consequence of the increased size of the population rather than of increased age. Smaller oysters are more likely to have a positive energy balance and cal allocate a larger fraction of their total assimilated energy to reproduction. As a result, they can spawn more frequently. This trend is independent of the pattern or frequency of spawning and is observed for all ranges of environmental conditions.

A summary of reproductive effort, derived from the simulations, as it relates to average adult size, food supply and latitude is given in Table 4. These results show the strong relationship that exists between reproductive effort, temperature and food supply. Overall reproductive effort is more variable than adult size. For example, in Galveston Bay a reduction in food supply, produced by increased turbidity, gives a 67% reduction in average adult size, but an 85% decrease in reproductive effort (Fig. 6a vs. Fig. 6b). Similarly, the change in temperature that occurs between Galveston Bay and Laguna Madre reduces adult size by 6%, but increases reproductive effort by 23%. Higher temperatures produce higher filtration rates which give increased net production.

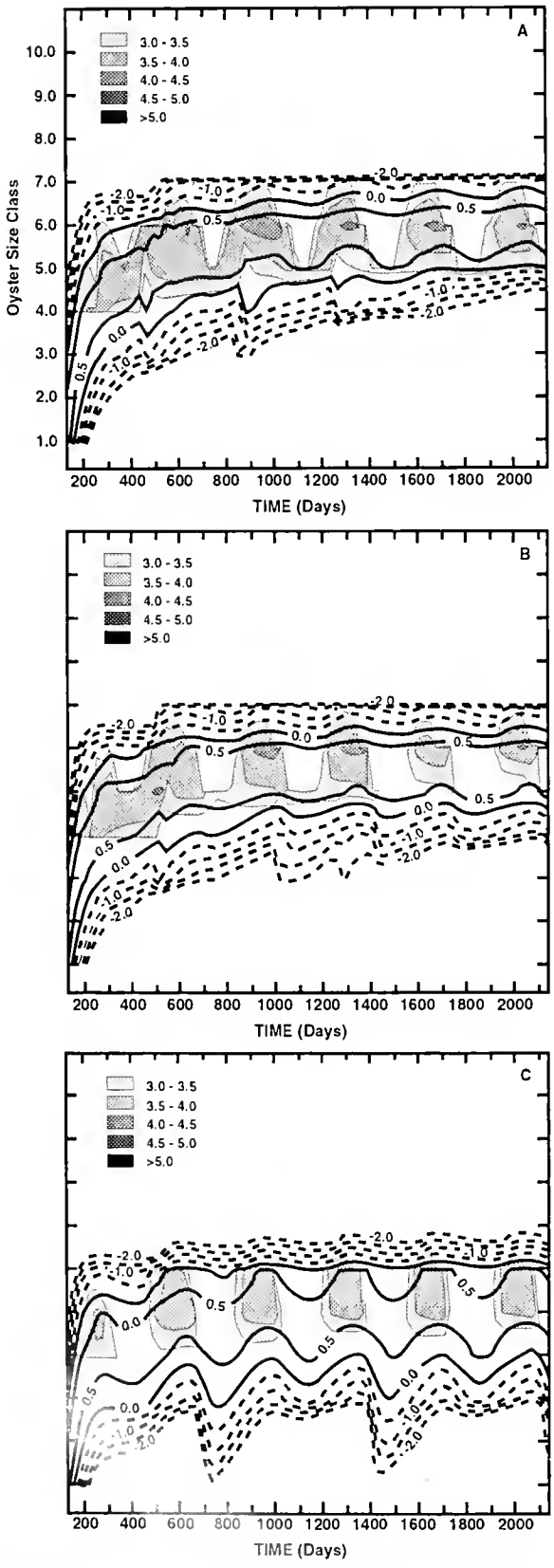

Figure 10. Simulated oyster population distribution and gonadal tissue development that results from constant low food (0.5 mg l^{-1}) supply and environmental conditions characteristic of A) Laguna Madre, B) Apalachicola Bay and C) Chesapeake Bay. Otherwise same as Figure 4.

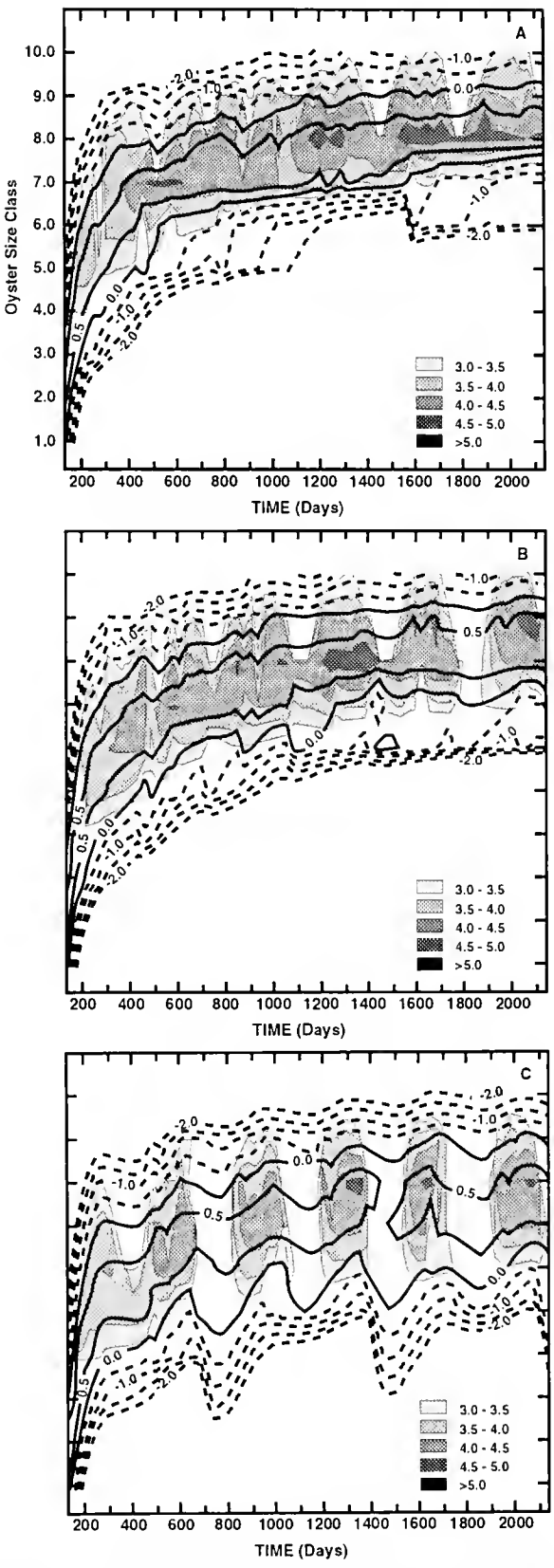

Figure 11. Simulated oyster population distribution and gonadal tissue development that results from medium food (1.0 mg l^{-1}) supply and environmental conditions characteristic of A) Laguna Madre, B) Apalachicola Bay and C) Chesapeake Bay. Otherwise same as Figure 4.

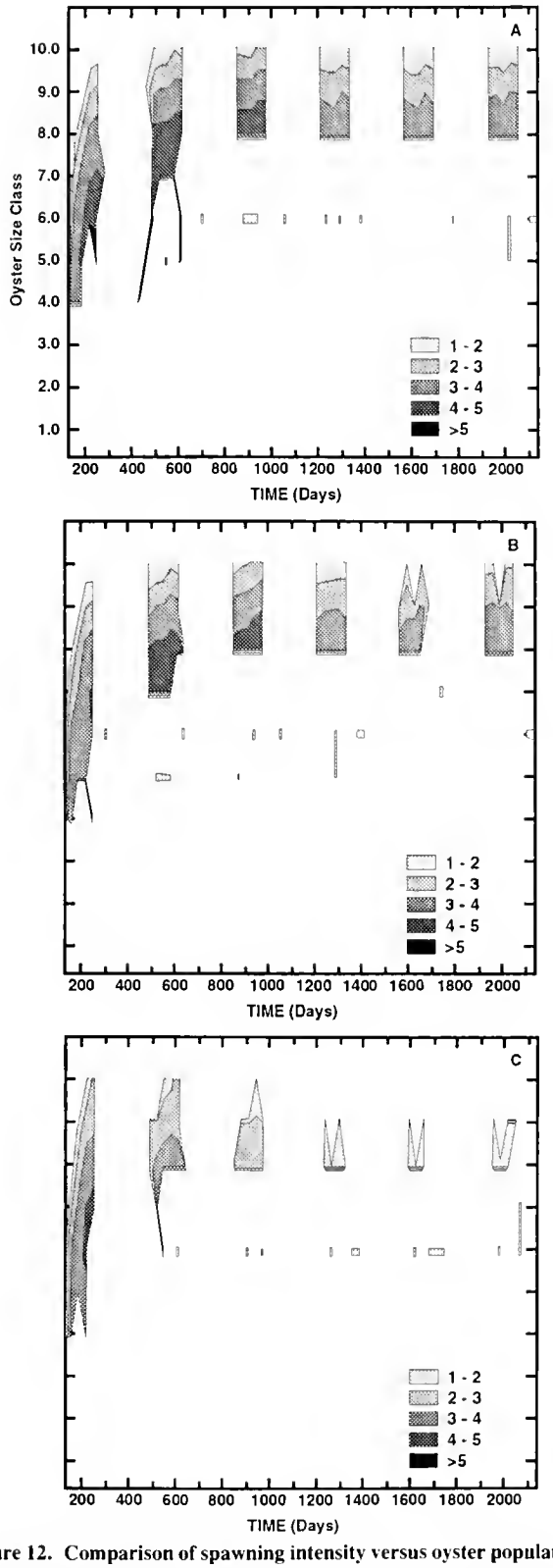

Figure 12. Comparison of spawning intensity versus oyster population size in A) Laguna Madre, B) Galveston Bay and C) Chesapeake Bay. Spawning intensity is shown as $\log_{10}$ calories spawned with a contour interval of 1. Spawning intensity for Laguna Madre and Chesapeake Bay was obtained from the simulated oyster populations shown in Figures 9b and 9a, respectively. The Galveston Bay spawning intensity was obtained from the constant salinity simulation that was essentially identical to the simulation results shown in Figure 6a.

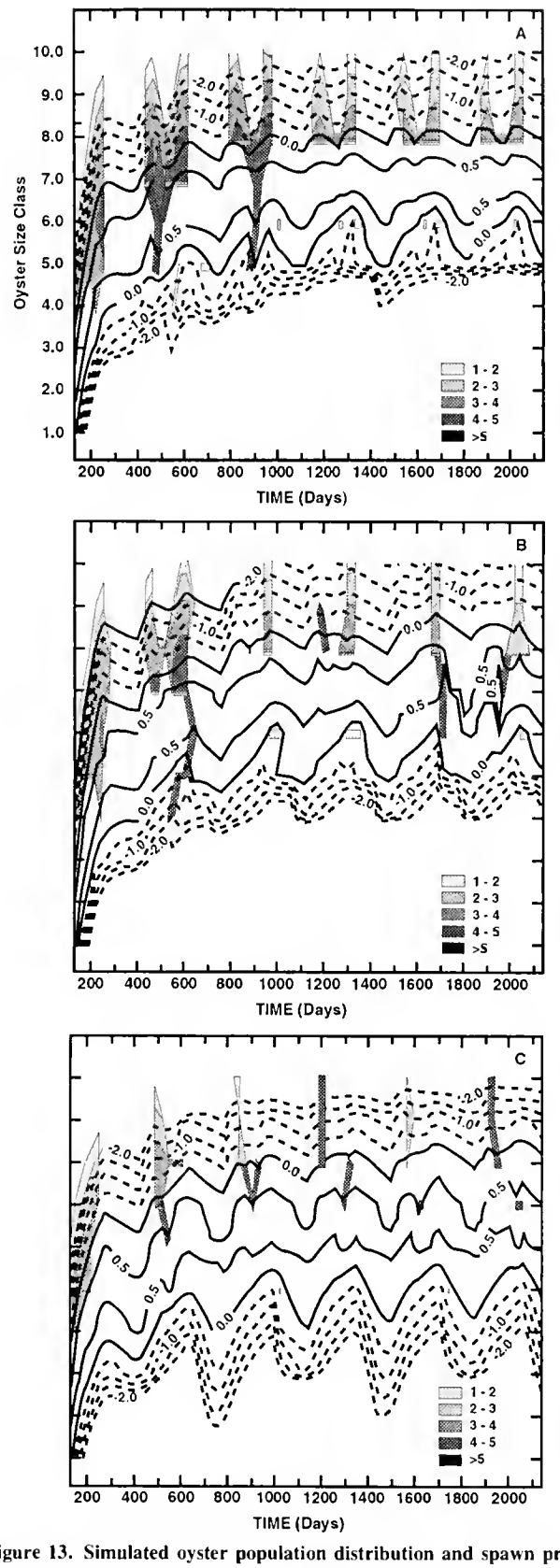

Figure 13. Simulated oyster population distribution and spawn production for A) Laguna Madre, B) Galveston Bay and C) Chesapeake Bay obtained using an idealized food time series. Spawning intensity is shown as $\log_{10}$ calories spawned with a contour interval of 1. Otherwise same as Figure 4.

TABLE 4.

Reproductive effort, average adult size and the ratio of the two calculated from year six of the simulated populations shown in the indicated figures. One simulation used is not shown (NS). This simulation used monthly-averaged temperature and food conditions from Galveston Bay, Texas, a constant salinity of 24 ppt and no turbidity. The results of this simulation were similar to those shown in Figure 6a. Size and reproductive effort are based on simulations that used the environmental time series defined in Table 3. Lower food supply, higher turbidity, or the inclusion of disease (e.g. *Perkinsus marinus*) could be expected to reduce thses values.

Location	Reproductive Effort (kcal)	Average Size (g dry wt)	Ratio (kcal:g dry wt $^{-1}$)	Figure Number
Laguna Madre vs.	266.71	4.87	54.77	11a
Galveston Bay	260.92	5.12	50.96	NS
Laguna Madre vs.	218.79	4.62	47.36	13a
Galveston Bay	179.03	4.89	36.61	13b
Galveston Bay vs.	129.77	4.73	27.44	13a
Chesapeake Bay	47.47	4.24	11.19	13c
Galveston Bay vs.	156.49	5.18	30.21	6a
Galveston Bay	24.21	1.81	13.36	6b

However, most of the net production is allocated to reproductive rather that somatic tissue development.

DISCUSSION AND SUMMARY

General Characteristics

Adult size and reproductive effort in oyster populations are determined by the temperature- and season-dependent allocation of net production to somatic and reproductive tissue development which in turn depends upon the temperature regulation of filtration rate. Salinity and turbidity affect oyster physiology through a reduction in the rate of food acquisition and cannot be distinguished from a simple reduction in food supply. Although respiration rate varies non-linearly with body mass and is affected by salinity, the overall effect of environmental conditions on respiration rate is small and can be ignored, in most situations.

A summary of simulated adult oyster size that results from variations in local and latitudinal controls on growth is given in Figure 14. These simulations considered only environmental control on oyster biomass. Oyster growth form is extremely plastic, although Kent (1988) argues for some predictable influences of local habitat. Nevertheless, the shell length achieved in the various simulated populations may vary over a wide range (Table 2). Unfortunately, much of the available oyster measurements are in terms of shell length or condition index rather than biomass. In this discussion, except where noted, oyster size is considered strictly in terms of biomass, and where needed, conversions to length are done as shown in Table 2.

The simulations indicate that adult oysters in Chesapeake Bay tend to be about the same size in terms of biomass as those in Galveston Bay (Fig. 14a), when presented with equivalent food supplies, salinities and levels of turbidity, despite the difference in temperature regimes. Water temperatures in Chesapeake Bay tend to be colder for longer periods than in Galveston Bay. Thus, the temperature-dependent control on the allocation of net production results in more going to somatic rather than reproductive tissue development.

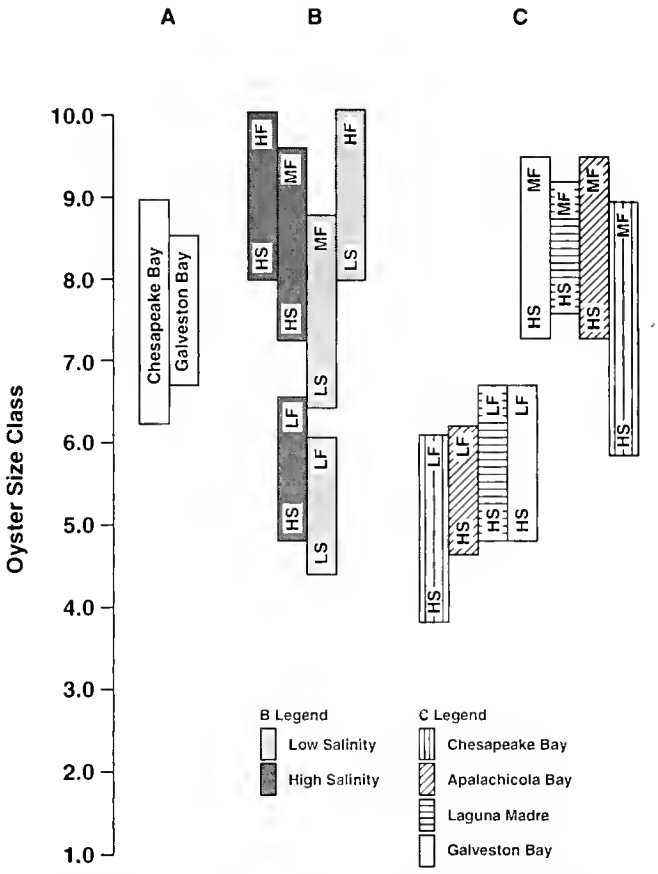

Figure 14. Comparison of adult size from year six of the simulations from A) Galveston and Chesapeake Bays (Figs. 6a and 9a), B) Galveston Bay for high and low salinity at a range of food concentrations (Figs. 4a, 5 and 7) and C) four bays and a range of food concentrations. High and low salinity values are 24 ppt and 7 ppt and are designated by HS and LS, respectively. Designations for high (1.5 mg l^{-1}), medium (1.0 mg l^{-1}), and low (0.5 mg l^{-1}) food concentrations are HF, MF and LF, respectively.

Variations in local environmental conditions also affect adult oyster biomass. Low salinity conditions in an environment such as Galveston Bay can result in reduced adult size (Fig. 14b). However, the effect of low salinity can be compensated for by increases in food supply. Low salinity conditions combined with high food conditions can result in adult biomass that is similar to that obtained during high salinity conditions. The largest reduction in adult oyster size occurs when low salinity is combined with a restricted food supply.

The importance of food in determining adult biomass over a latitudinal range is illustrated in Figure 14c. For all bays, low food conditions produced adult oysters that were about the same size, size classes 5 to 6. The only exception is Chesapeake Bay where somewhat smaller, size class 4, adult oysters are produced by low food conditions. Medium food conditions result in larger adult oysters for all bays with minimal overlap with the size produced by low food conditions. Galveston and Apalachicola Bays have similar sized adult oyster populations. Individuals in Laguna Madre tend to be a bit smaller. The warmer temperatures in Laguna Madre result in more of net production going to form reproductive tissue, thereby producing more spawn and smaller individuals. Chesapeake Bay populations show a wider range of adult size, but

many individuals reach adult size typical of the lower latitude sites despite the cooler temperatures and more restricted growing season (e.g. Butler 1953b).

Adult Size (Biomass)

The shape of the growth curve for bivalves—whether size continuously increases at some declining rate or asymptotes to some maximum size (e.g. Levinton and Bambach 1970)—is probably more a function of environment than genetics. It is significant that the simulated oyster populations reached sizes characteristic of populations throughout the latitudinal range from Laguna Madre to Chesapeake Bay solely on the basis of physiology and environment. No upper limit for oyster growth or adult size was included in any of the formulations used to describe oyster physiology. Limitations on size in the simulated populations come from the balance between winter and summer somatic production less the energy expended in reproduction:

$$P_{gJ_{summer}} - P_{gJ_{winter}} = A_J - P_{rJ}. \quad (15)$$

In adult oysters, net production is normally negative in the winter and for the most part is balanced by somatic growth in the spring and fall. Cessation or slowing of growth in the summer (e.g. Beaven 1950) in disease-free oyster populations is normally due to reproduction and spawning which accounts for most of the net production in older animals. Hence, the relationship given above should result in a stable, but seasonally-oscillating, variation in adult oyster size. In the simulated population distributions, the balance between winter loss in net production and spring-summer-fall gain begins in the second or third year depending on the ambient temperature and food supply. Exceptions to this occur only when food supply is very high.

Growth rate in the hard clam, *Mercenaria mercenaria*, has a concave parabolic relationship with temperature (Ansell 1968). Growth rates are lowest at low and high seasonal temperatures and maximum at intermediate temperatures. Multiplying equations 4 and 12, and assuming a food supply adequate to minimize the effect of respiration on the energy budget and ignoring the dependence of filtration rate on length, yields a parabolic dependence for oyster growth rate on temperature of the same form

$$G \alpha bT - aT^2 \quad (16)$$

where a and b are the constants in equation 12 and T is temperature. If equation (16) is applied over the latitudinal range from Laguna Madre to Chesapeake Bay, then oyster growth rate and hence size should decrease at the southern and northern ends of the distribution. Maximum growth rate and largest adult size would be found near the center of this range. However, both the oyster and the hard clam (Ansell 1968) deviate from this expected distribution in that adult size remains constant over a wide latitudinal range that includes habitats from the northern Gulf of Mexico to north of Delaware Bay.

The observed rather than expected [as suggested by equation (16)] latitudinal distribution in size is also reproduced in the simulated oyster population distributions. This relationship between size and latitude arises through temperature effects on the allocation of net production to somatic and reproductive tissue growth and on filtration rate which determines the rate of food acquisition. The longer periods of low temperature in the spring and fall found at higher latitudes result in more time in which food is plentiful occurring at temperatures that favor somatic growth. As a result, decreased filtration rates at lower temperatures are balanced by an increase in food apportioned to somatic growth and size remains stable. Reproductive potential, however, declines in these populations.

Reduced size at lower latitudes is common in bivalves (e.g. Bauer 1992). Such a gradient in animal size can result from variations in temperature in one of two ways. First, an environment characterized by low food supplies and warm temperatures can produce large adult oysters despite increased reproduction because the total gain in energy from higher winter filtration rates results in a net accumulation of somatic tissue. The decline in size at low latitudes in the Gulf of Mexico suggests that this is not the normal condition. Alternatively, an environment characterized by moderate-to-high food supply and warm temperatures can produce smaller adult oysters because the greater allocation of net production to reproduction balances the positive effect of temperature on the rate of food acquisition. This is the more usual case.

Stunting, the presence of a relatively small adult size in a population, is generally considered to result from restricted food supply. The results of this modeling study suggest that, at least for oysters, temperature and reproductive effort are also important in restricting animal size. Hence, stunted populations can occur at the edge of the species' range where physiology directly limits size as well as in populations that fail to reach the size expected for their position within the latitudinal range.

The observed oyster sizes from around the Gulf of Mexico (Fig. 1) show two exceptions to the general trend of decreasing size at lower latitudes. It should be noted that the data presented in Figure 1 are in terms of length, rather than biomass, and so are subject to the aforementioned caveats concerning the plasticity of oyster growth form. First, the adult length observed at lower latitudes on both sides of the Gulf of Mexico is about 1 to 2 cm less than the average length observed in the northern Gulf. Such a length decrease is not easily produced in the simulated populations with a simple reduction in temperature and one biomass-length relationship. A 0.5 to 1 cm reduction in length is typical of the simulated populations. A temperature-dependent change in growth form modifying the size-to-biomass relationship may also be involved. Second, oysters from Moblie Bay through the Florida Panhandle area and in Tiger Pass on the Mississippi Delta are unusually small. This region characteristically has the coldest winter temperatures in the Gulf of Mexico (Collier 1954). However, the possibility that the colder temperatures reduce the growing season and thus limit adult size is not supported by the simulated populations. Even colder temperatures in Chesapeake Bay fail to reduce adult biomass. Either food supply is unusually meager in these two areas or mortality rates are unusually high. Thus, stunting may be of local (Tiger Pass) or regional (Florida Panhandle) extent. The effect of a change in growth form can be discounted in this case because the length-biomass relationship given in White et al. (1988) is adequate for at least some of these populations.

Butler (1953b) showed that oysters in Chesapeake Bay and the northern Gulf of Mexico reached about the same size in terms of length. The simulations summarized in Figure 14 generally show that Gulf of Mexico oysters slightly exceed Chesapeake Bay oysters in length when biomass is converted using a single length-biomass relationship. A latitudinal difference in growth form would explain this differential. Kent (1988) describes a wide range in growth forms from Chesapeake Bay, so that within-bay variations cannot be discounted. However, the relationship given in Paynter and DiMichele (1990) for a Chesapeake Bay population from Tolley Point Bar predicts oysters much longer for a given

weight and this prediction agrees with a biomass-length relationship obtained by Newell (University of Maryland, pers. comm.) from the Choptank River subestuary of the Chesapeake Bay. Lunz (1938) suggested that a primary influence of anthropogenic activities on oyster growth form was to decrease width and length, but with more of an effect on width. If true, this would explain a perceived variation between oyster size reported by Butler (1953b) and the more recent measurements reported by Paynter and DiMichele (1990) and Newell (University of Maryland, pers. comm.). Unfortunately, the observations reported in Butler (1953b) are not in terms of biomass. The same trend might explain the tendency in the simulated oyster populations from Chesapeake Bay to be slightly lower in weight and, therefore, length, than the northern Gulf of Mexico oysters (e.g. Fig. 11). The weight obtained from the simulated populations would result in a longer oyster in Chesapeake Bay using the conversions of Paynter and DiMichele (1990) and Newell (University of Maryland, pers. comm.).

The simulated oyster populations suggest an explanation for the concordance in year-to-year oscillations in oyster size throughout the Gulf of Mexico (Wilson et al. 1992). Climatic cycles, such as El Niño, change the Gulf-wide temperature and rainfall regime (Powell et al. 1992a). Size, through the direct effect of temperature on the allocation of net production to somatic and reproductive tissue or indirectly through variations in food supply, could be affected by climatic variations in temperature and rainfall. Furthermore, such climatic effects are likely introduced through variations in temperature during the colder part of the year. For example, the difference between a warm and cold winter could be sufficient to significantly alter adult size.

Reproduction

The reproductive processes included in the oyster population model are based upon simple empirical relationships; however, the simulated population distributions show trends typical of oyster populations throughout the east coast of the U.S. and the Gulf of Mexico. This suggests that reproductive effort in oysters is primarily a function of a genetically-determined temperature-dependent allocation of net production into somatic and reproductive tissue development and an environmentally determined scope for growth. This temperature dependency may be described by simple linear relationships such as those given by equations (12) and (13) which may reflect temperature-dependent reaction rates in protein synthesis or hormonal control. The mechanism underlying the temperature-dependent allocation of net production would appear to be an important unknown in the reproductive physiology of oysters.

Reproductive potential is the result of the same physiological and environmental conditions that govern adult size, i.e. the temperature- and season-dependent rate of food acquisition and the temperature-dependent allocation of net production into somatic growth and reproduction. However, small changes in either result in more pronounced changes in reproductive effort than in adult size. For example, the rate of food acquisition is higher in warmer months when most net production is allocated to reproduction. Hence, small changes in available food are magnified during this period. The effect of small variations in environmental conditions on oyster reproduction and spawning is discussed in detail by Hofmann et al. (1992).

The wide range of reproductive efforts produced from small changes in temperature or food supply suggests that comparisons of reproductive effort between oyster populations can only be made within the context of a complete environmental analysis of food supply, environmental conditions and a total energy budget for the animal. The wide range of reproductive efforts reported for bivalves in general (see Powell and Stanton 1985 for a review) probably results from these interactions. Thus, correlations between size and reproductive effort will be location and time specific, and general conclusions based upon such correlations may not be valid. For example, the relationship between temperature and reproduction given by Kaufman (1979) requires similar rates of food acquisition among populations to provide valid comparisons.

The assumption that populations of larger individuals should reproduce more is not always correct. For many situations, populations of smaller individuals may have a greater reproductive effort per unit of biomass. The simulated population distributions suggest that decreases in reproductive effort are related to increased size rather than to age. The apparent reproductive senility in these populations results from the differential scaling of filtration and respiration rate with body size, which reduces scope for growth at a given food supply in larger animals.

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THE EFFECT OF MANUAL HANDLING ON OYSTER GROWTH IN LAND-BASED CULTIVATION

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ABSTRACT Growth trials carried out previously at the University of Hawaii have shown that 0.1-gm *Crassostrea virginica* spat can be grown to 55-gm market-sized animals in as little as 198 days. During those earlier growth trials, oysters were weighed at two-week intervals in order to obtain accurate growth data. The process resulted in shell breakage and possible oyster stress. There was speculation that this kind of excessive handling of oysters may reduce oyster growth.

In an effort to determine the effect of cleaning and biweekly handling on oyster growth rate, two identical grow-out tanks were stocked with *Crassostrea virginica* spat and operated simultaneously. The oysters in one tank were weighed biweekly and the oysters in the other tank were weighed only at the initiation and completion of the 214-day experiment. Except for the biweekly weighing of one tank's oysters, both tanks were treated identically. It was found that oyster weights from both tanks followed a normal distribution. However, oysters that were weighed biweekly had a final average weight of 34.8 gm, with a standard deviation of 2.2, while the oysters in the unhandled tank had a final average weight of only 28.7 gm, with a standard deviation of 2.7. It is our conclusion that the frequent handling of the oysters increased the oyster growth rate.

KEY WORDS: oyster growth, aquacultural engineering, land-based

INTRODUCTION

The production of oysters in land-based culture systems is becoming more and more attractive as supplies of wild stock decline. In land-based production facilities the oysters' environment can be carefully regulated and monitored, thus eliminating uncertainties that are inherent in natural production.

Previous growth trials carried out in land-based oyster flumes at the University of Delaware were unimpressive. In that system, *Crassostrea virginica* spat had grown to only 20 gm in 379 days. One explanation attributed the slowed growth to excessive handling of the oysters. In those trials, oysters were removed from the system, air dried, and weighed at three-week intervals. Researchers felt that the handling period may have caused mechanical damage to the oysters resulting in the slow growth (Bolton 1982).

The Aquacultural Engineering Laboratory at the University of Hawaii has used two different land-based culture methods for the grow out of *Crassostrea virginica*—suspended culture (Jakob et al. 1988) and tray culture (Jakob et al. 1989). As can be seen from Fig. 1, both methods have proven effective at producing 55-gm animals from spat in as little as 198 days. While these growth rates are, to our knowledge, the fastest ever reported, there was speculation regarding the effect that periodic handling and cleaning of the oysters may have on their growth rate.

This experiment was designed to test the effect that manual handling—cleaning and weighing—may have on oyster growth.

MATERIALS AND METHODS

Tank Design

Two identical tanks utilizing a suspended culture grow-out system were constructed for this experiment. Figure 2 shows a cross section of the tank used in this study. The tanks were made of plywood and reinforced and waterproofed with fiberglass and polyurethane resin. Tank dimensions were 1.8 m long by 1.2 m wide by 1.5 m tall. The total water volume of each tank was 1,400 liters. Both tanks were equipped with overhanging covers to reduce light intrusion.

Water entered each tank through a distribution pipe which ran the length of the incoming trough. Holes along the distribution pipe jet aerated the water into the trough. The water ran out over an inlet weir, down through the oysters, under the dividing wall and then out of the system through an exit weir. A settling zone allowed for the removal of oyster feces before the water was discharged. The bottom of the tank was sloped 30 degrees to aid tank cleaning.

Suspended Culture Design

The oysters were attached to 16 mm wide by 1.5 mm thick by 61 cm long flexible PVC strips with a quick bonding cyanoacrylate glue. The oysters were spaced 9 cm apart along the strip and each strip held 6 individual oysters. The frames holding the oyster strips within the tank were constructed of 1.9 cm PVC pipes and fittings; overall dimensions of the frames were 61 cm wide by 56 cm deep. The oyster strips were attached to the frames with Velcro which allowed easy removal and reattachment for weighing purposes.

The strips of oysters were spaced 7 cm apart along both sides of the frame. Hence, each frame held a total of 16 strips, or 96 oysters. The oyster frames were spaced 9.5 cm apart within the tank for a total of 18 frames, or 1,728 oysters per tank.

Pond water to the tanks was supplied by a 2 hp submersible pump which was selectively placed in one of five available 0.5 ha earthen marine shrimp ponds. The five ponds were operated under a semi-intensive management regime by Amorient Aquafarm Inc. of Kahuku, Hawaii.

Tank Operation

On March 11, 1988 *Crassostrea virginica* spat with an average weight of 0.2 gm were placed into the tanks. Previous growth trials carried out by the Aquacultural Engineering Laboratory have suggested that dissolved oxygen concentrations tend to be the most growth limiting factor for oysters (Jakob et al. 1989). Therefore, the amount of flow to the tanks was set so that exiting water from the tanks contained dissolved oxygen levels above the critical low limit of 3.6 mg/liter (Galtsoff 1964). The tanks received identical

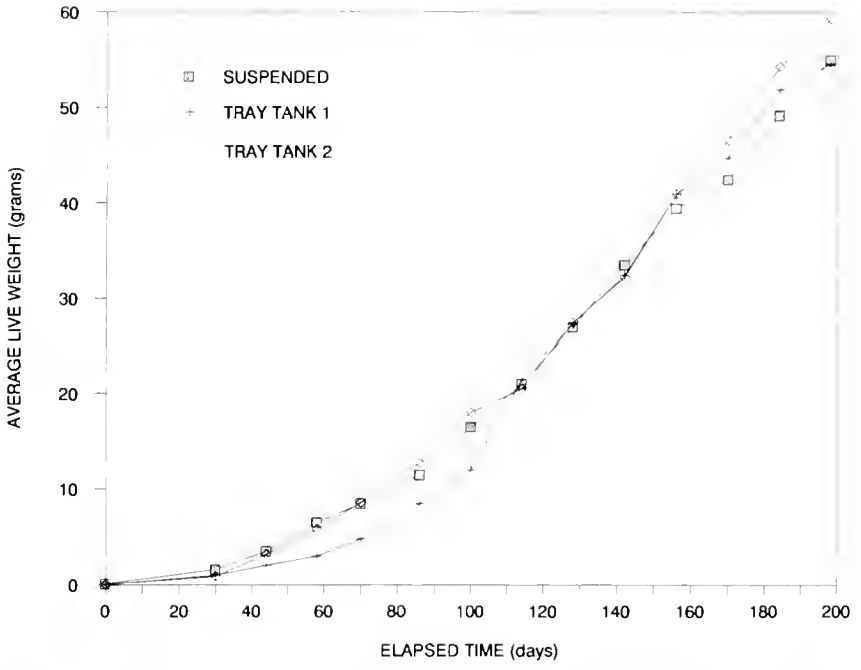

Figure 1. Average growth rate of *Crassostrea virginica* using two different culture methods.

flow rates which were set by the tank which had the lowest exiting dissolved oxygen concentration. Water flowing into the tanks was monitored with a cumulative water meter. The flow rates were adjusted on a weekly basis.

The oysters in both tanks were washed on a weekly basis. The tanks were drained and the tanks and oysters hosed down with fresh water. The accumulation of sediment was easily removed from the oysters using this procedure. Cleaning time was approximately ten minutes per tank. Every other week the oysters from the designated handled tank were washed and then removed from the tank. The strips of oysters were removed from the frames one at a time. The oysters on each strip were counted and the strip was weighed. After weighing, the strip was replaced on the frame in its original position. This data was used to obtain the average growth rate for the handled tank. Final oyster weights were obtained by removing the oysters from the strips and taking individual weights.

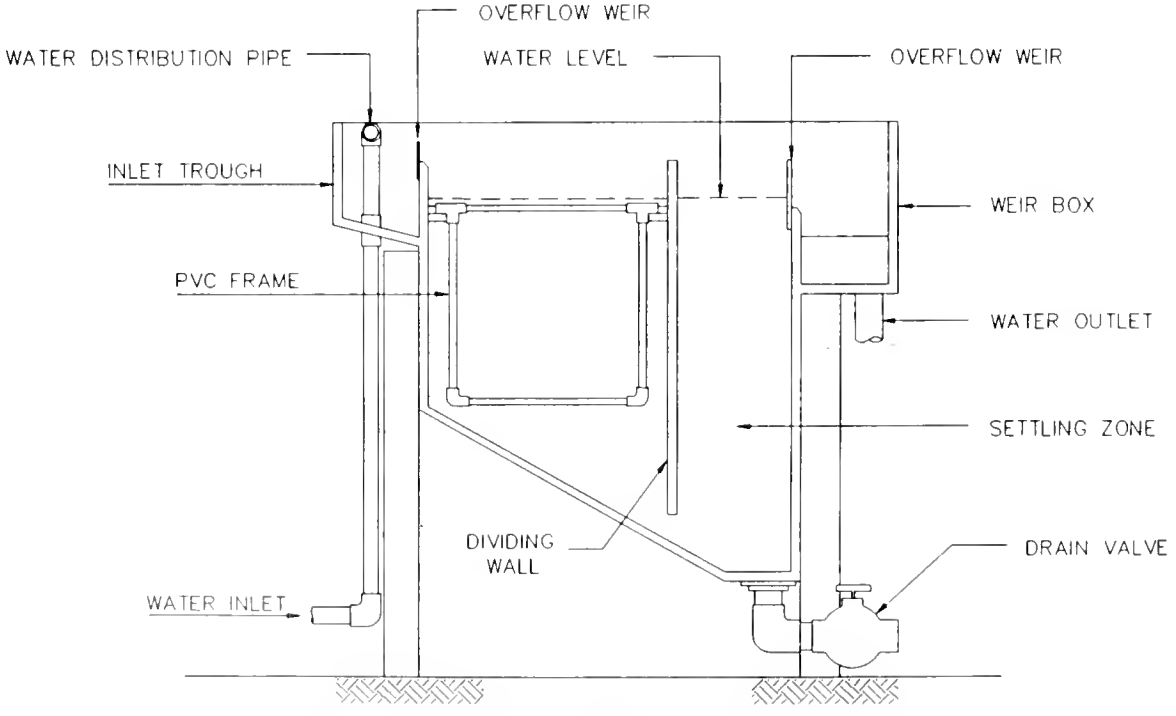

Figure 2. Cross section of the growth tank used for the experiment (for clarity, the oyster strips are not shown).

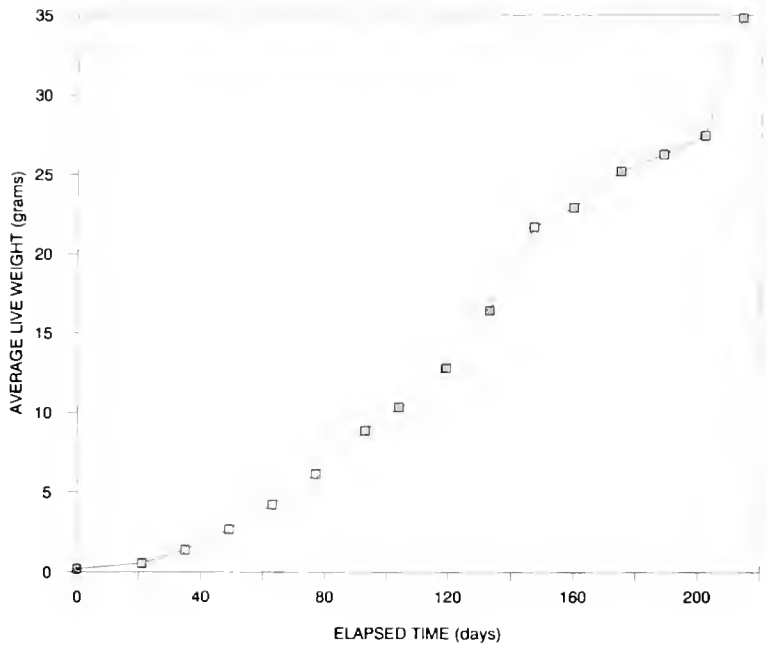

Figure 3. Average growth rate of the oysters in the handled tank over the 214-day trial.

RESULTS

Figure 3 shows the average growth rate for the oysters that were weighed on a weekly basis. It was found that after 214 days, the oysters from the handled tank had reached an average weight of 34.8 gm, with a standard deviation of 2.2. Oysters from the unhandled tank had an average weight of 28.7 gm, with a standard deviation of 2.7. Mortality for both tanks was fairly high at 22 percent for the oysters that were handled and 30 percent for the unhandled oysters.

Figures 4 and 5 show the weight distributions of the oysters in the handled and unhandled tanks, respectively.

DISCUSSION

While some mechanical damage to the oysters did occur when they were weighed, it was apparent that this handling procedure did not have a negative effect on oyster growth when compared to oyster growth for the unhandled tank. Both tanks showed relatively normal weight distributions (Figs. 4 and 5). Previous growth trials carried out by the Aquacultural Engineering Laboratory have shown that this type of normal distribution is to be expected. The difference in average growth is statistically significant ($P < .01$).

The oysters in this system grew at approximately one-half the rate of those grown in previous trials. Because the tanks operated

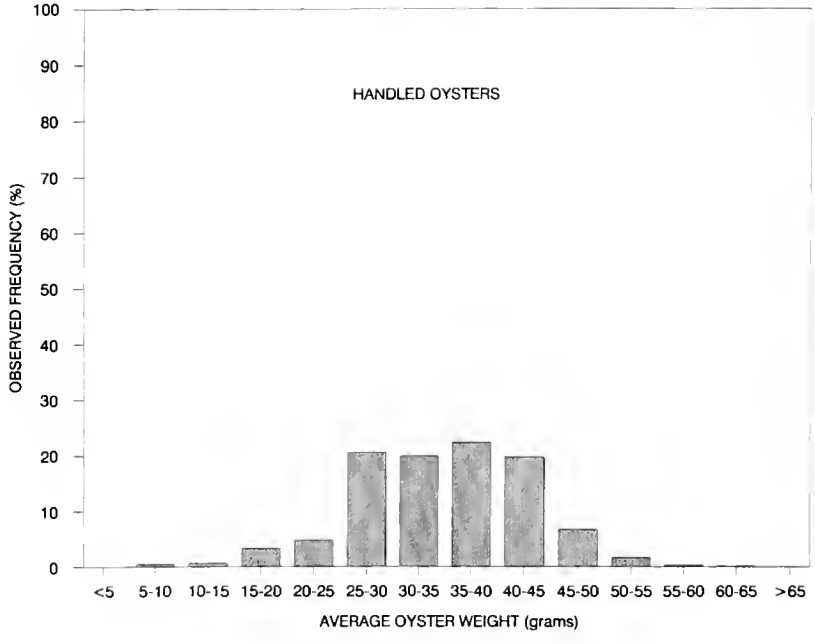

Figure 4. Final weight distribution of the oysters in the handled tank.

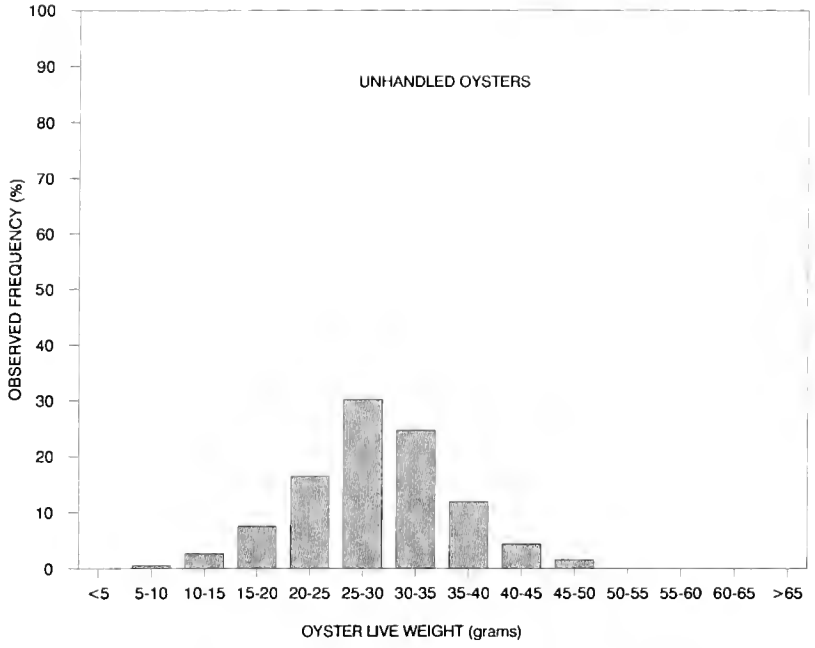

Figure 5. Final weight distribution of the oysters in the unhandled tank.

well, it is assumed that the oyster spat used was inferior. This conclusion is supported by the fact that oysters from the same seed stock placed in a fluidized bed system also had growth rates which were slightly less than those experienced in the suspended culture tanks.

The average size of the oyster spat obtained was approximately 5 mm. Because of the small size, some of the oysters were glued shut when they were attached to the strips. These oysters were counted as mortalities. The glued shut oysters accounted for 31 percent of the total mortality rate in the handled tank and 22 percent of the total mortality in the unhandled tank.

ACKNOWLEDGMENT

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THE COMBINED EFFECTS OF SALINITY, TEMPERATURE, ANTIBIOTIC AND AERATION ON LARVAL GROWTH AND SURVIVAL OF THE MANGROVE OYSTER, *CRASSOSTREA RHIZOPHORAE*

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ABSTRACT The combined effects of temperature and salinity on individual shell length, dry weight, survivorship and total biomass of *Crassostrea rhizophorae* veligers, were studied within the natural range of variation found in tropical mangrove swamps. Gametes from sexually mature adults collected in the Jacuruna estuary, Todos os Santos Bay, Brazil (13°00′S, 38°30′W), were fertilized in the laboratory and the larvae cultured at 20, 25 and 30°C and at salinities of 15, 20, 25, 30, 35 and 40 ppt. Nine day studies, both with and without antibiotic (1 mg/L erithromycin), accompanied or not by aeration, revealed that in combination with antibiotic, aeration increased survival significantly at higher temperatures, but reduced individual growth and total biomass. Larvae grew most rapidly at 30°C and intermediate salinities (25 to 35 ppt). Larval survival was lowest at 30°C at extreme salinities (15 and 40 ppt). Both shell length and mean dry weight increased more rapidly between the 5th and 7th days (63 to 100%) than between the 7th and 9th days (3% to 15.3%). The use of antibiotic, at 30°C and lower salinities (15 and 20 ppt), improved survival and total biomass gain until the 7th day. Thereafter, the gain in biomass dropped because of high mortality at this temperature. Total biomass was always highest at the lowest temperature (20°C) under moderate salinities (20–30 ppt).

INTRODUCTION

The mangrove oyster, *C. rhizophorae* (Guilding 1828), is an important species for commercial exploitation along the Brazilian coast. Extensive oyster beds, especially near urban areas, are being over fished, and productivity has decreased significantly. Oyster culture offers a solution for the decline of marketable oysters, but the reliability of seed supply for stocking purposes can only be assured by hatchery reared spat, due to the unpredictable environment in the estuarine areas.

The improvement of rearing techniques, beginning with the work of Loosanoff and Davis (1963) has allowed studies of the effects of various environmental factors on the growth and survival of oyster larvae (Walne 1966, Calabrese and Davis 1970, Helm and Spencer 1972, Breese and Malouf 1977, Helm and Millican 1977, Helm 1977, Newkirk et al. 1977, Nascimento 1980, Dos Santos and Nascimento 1985, Enright et al. 1986, Robert et al. 1988, His et al. 1989). These studies have, in general, been conducted in the laboratory by varying one factor at a time. The importance of combined temperature and salinity effects, and the need to consider these factors jointly, has been emphasized by Kinne (1963, 1970). He concluded that observations on the effects of several factors in combination make it possible to better understand the interactions between the environment and the tested organism.

The combined effects of environmental parameters have recently been investigated for several bivalve species (Brenko and Calabrese 1969, Calabrese 1969, Lough and Gonor 1973, Kennedy et al. 1974, Lough 1974, Gruffydd 1975, Pechenik et al. 1990) but little is known about the combined effects of two or more environmental factors on oyster larvae. Davis and Calabrese (1964) were the first investigators to look at the combined effects of factors such as food and salinity on the temperature tolerance of *C. virginica* larvae, by using factorial experiments. Helm and Millican (1977) studied the effects of salinity and temperature on *C. gigas* larvae. For *C. rhizophorae*, the effects of environmental factors are known only for embryos (dos Santos and Nascimento 1985). The present study was undertaken to determine the joint influence of salinity and temperature on the growth, development and survival of the mangrove oyster, from D-stage to umbonned larvae.

The mortality of bivalve larvae during cultivation is a problem for most hatcheries, where this difficulty is generally overcome by increasing the input of early larval stages. The use of antibiotics is often considered, but should be avoided as a routine practice (Calabrese 1969). Even so, it is important to know what alterations an antibiotic, such as erythromycin, may cause in the normal pattern of response of young oyster larvae to the combined effects of temperature and salinity. Similarly, it is important to know how aeration may modify the same response patterns, even though it has been previously identified as detrimental to young larvae (Helm and Spencer 1972, dos Santos and Nascimento 1985).

MATERIALS AND METHODS

Adult *C. rhizophorae* were obtained from natural stock in Salinas da Margarida, Bahia, Brazil (12°52′S, 38°44′N). Immediately prior to each experiment, gametes were collected from mature individuals of each sex by stripping the gonads into filtered sea water (0,1 μm glass fiber membrane), at 25°C and 28 ppt salinity. Eggs were fertilized by procedures previously described by dos Santos and Nascimento (1985). Within 24 h of fertilization, most embryos had developed into the straight-hinged or prodissococh-1 larval stage, and all experiments where initiated with theselarvae. Rearing conditions were held at temperatures of 20, 25, and 30°C with salinities of 15, 20, 25, 30, 35 and 40 ppt.

Larvae were fed a diet consisting of a mixture of 50 cells μl^{-1} of Prymnesiophyte T-ISO (*Isochrysis* aff *galbana*) and 50 cells μl^{-1} of *Chaetoceros gracilis*. These species were grown in semi-continuous unialgal cultures in Conway medium, prepared from autoclaved sea water (Walne 1974). All algal cultures were harvested in the exponential growth phase.

Three series of experiments were carried out in duplicated 1 L beakers: (1) no antibiotic or aeration, (2) Erithromycin (1 mg/L) without aeration and (3) Erythromycin (1 mg/L) with aeration. Water soluble Erithromycin (1 mg/L) was added to the filtered sea water of experiments 2 and 3 before introducing larvae.

Sea water was exchanged every 48 h, at which time the larvae were collected on an appropriate mesh sieve. The larvae were then washed into filtered sea water, resuspended and evenly mixed in clean sea water in a graduated cylinder. At each water exchange, small, known volumes containing approximately 50 larvae were sampled with an autopipette, for measuring with the ocular micrometer of a monocular microscope. A count of these larvae served to estimate the total number of larvae surviving. Another sample of approximately 200 larvae was removed for the estimation of dry weight. Two drops of neutralized formalin (4.0%) were added to this sample, to kill the larvae, and the supernatant seawater was removed with a pipette. The larvae were then washed twice with 3% (w/v) ammonium formate solution, to remove the inorganic salts. After the second wash, larvae were retained on previously dried and weighed glass fiber filters. The filters were then dried at 60°C for 48 h, cooled in a dessicator and re-weighed to the nearest 0.1 mg on a Mettler precision balance. Mean larval weight was calculated by dividing the total weight by the number of larvae in the sample.

After subsampling, the volume of fresh seawater in the cultures was adjusted to give a larval density of 10/mL and the remaining larvae were returned to the rearing vessels with fresh food.

Survival was calculated every 48 h, as a percentage of the initial population remaining in each replicate. Increments of growth in shell length and dry weight were calculated as the increase over the values determined for the initial samples.

All statistical analyses were performed utilizing SPSS/PC+, Statistical Package for the Social Sciences and a Leading Edge 386SL Notebook computer. Factorial Analyses of Variance and Multiple Classification Analyses were utilized to evaluate the effects of temperature, salinity, antibiotic and aeration on larval shell length, dry weight, survivorship and total biomass. Because the experimental design was not orthogonal (i.e. aeration was not utilized in the absence of antibiotic), the effects of antibiotic and aeration were evaluated in separate analyses.

Length, weight and total biomass were log-transformed prior to ANOVA. The angular transformation, with Bennett's correction for 0.0 ($p = \frac{1}{4N}$) and 1.00 ($p = 1.0 - \frac{1}{4N}$) values, was applied to proportional survival, relative to initial larval densities.

Multivariate, third degree polynomial regressions were calculated for each dependent variable upon salinity, temperature and interaction (Temp × Sal) values. The resultant equations were utilized to calculate response surfaces and topographic projections of the effects of the independent variables on larval length, weight, survivorship and total biomass for the fifth day of the study. The calculation of generalized response equations for these surfaces smoothed the data. Consequently, mean values cited in the subsequent results and discussion, derived from the original data and analyses of variance, do not correspond exactly with values plotted on the response surface figures.

RESULTS

Day 3

Without aeration nor antibiotic, temperature effects on all four dependent variables (length, dry weight, survival and total biomass) were significant ($p < .0005$) by the third day. All four variables assumed maximum values at the lowest temperature tested (20°C). Salinity effects were not significant on length ($p = .170$) nor weight ($p = .786$), but were reflected in survivorship ($p = .015$) and in total biomass ($p = .036$). Both survivorship and biomass were maximum at salinity 35 ppt. Temperature × salinity interaction was significant for survival ($p = .029$) and marginally so for biomass ($p = .058$), but temperature effects were dominant, yielding superior survivorship and biomass at 20°C, irrespective of salinity. The absolute maxima for survivorship and total biomass occurred at temperature 20°C and salinity 35 ppt.

The presence of antibiotic, without aeration, revealed a significant interaction with temperature on all four dependent variables ($p < .0005$), reducing their values at 20°C and increasing their values at 30°C. Antibiotic × salinity interaction was not significant.

In the presence of antibiotic, aeration revealed highly significant interaction with temperature ($p < .001$) for all four parameters. The effects were most notable in relation to survivorship and total biomass, which were greatly enhanced at 20 and 30°C, but only moderately so at 25°C. Aeration effects were independent of salinity.

Day 5

Larval growth and survival trends for the fifth day, without antibiotic nor aeration, are presented graphically in Figures 1 to 4. In the absence of antibiotic and aeration, temperature × salinity interaction was significant on length of larvae ($p = .001$, Fig. 1) and survivorship ($p = .036$, Fig. 3), marginally so on weight ($p = .068$, Fig. 2), but not on total biomass ($p = .138$, Fig. 4). Temperature effects were dominant and highly significant ($p < .0005$) on all four parameters, with maximum values at 20°C and minima at 30°C. Salinity had significant effects on length ($p < .0005$), weight ($p = .045$) and survivorship ($p = .020$), with maximum values in the range from 25 to 35 ppt, but not on total biomass ($p = .233$). A general tendency was observed for all parameters to be maximum at 20°C under higher (30–40 ppt) salinity conditions.

In the absence of aeration, antibiotic × temperature interaction was highly significant ($p < .0005$) for all parameters. Interactions with salinity were not significant. The presence of antibiotic slightly reduced growth and survivorship at lower temperatures but enhanced them at 30°C. Overall maximum biomass production was at 20°C and 35 ppt salinity, in the absence of antibiotic. With the addition of antibiotic, maximum biomass production shifted to higher temperatures (25 and 30°C) and slightly lower salinities (25–30 ppt), but maximum values were 5% below those observed with the absence of antibiotic.

In the presence of antibiotic, aeration × temperature interaction was significant ($p < .001$) on all parameters. No interactions with salinity were significant. Aeration effects were most notable on survivorship and, consequently, on total biomass production. Although survivorship and biomass production were enhanced at 30°C, maximum values were still observed at 20°C under salinities of 30–35 ppt. Under these conditions, survivorship was approximately five times that observed at 30°C.

Day 7

In the absence of antibiotic and aeration, larval mortality was 100% at 30°C. At 20 and 25°C, temperature effects were highly

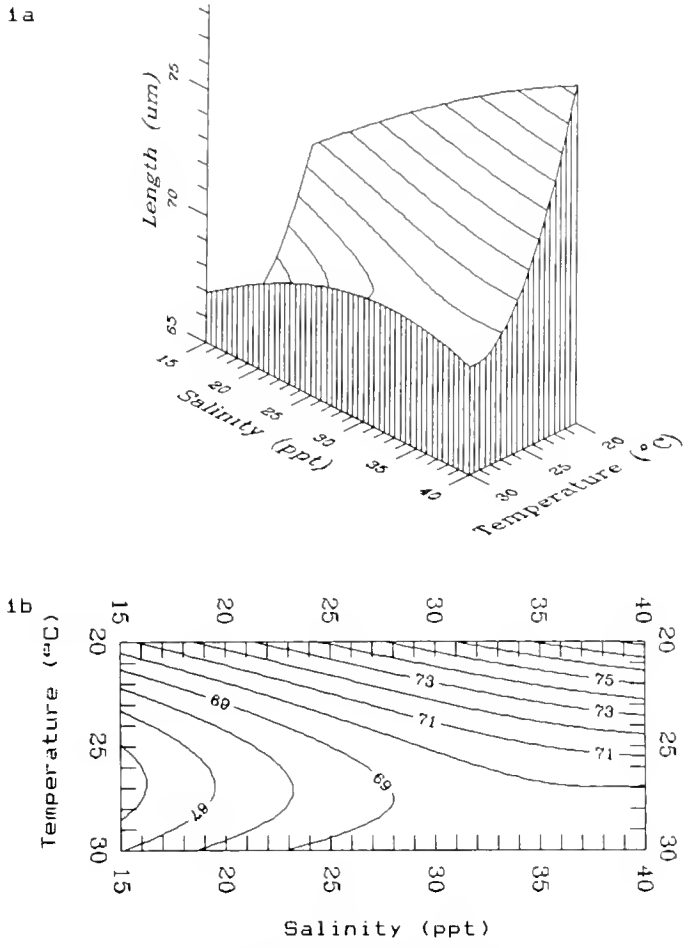

Figure 1. Response surface (a) and topographic projection (b) of the mean shell length of *Crassostrea rhizophorae* larvae, as a function of temperature and salinity, on the fifth day of cultivation without the use of antibiotic and aeration.

significant ($p < .0005$) on all parameters. Salinity had no significant effect on length nor weight, but showed significant interaction with temperature ($p < .0005$) for survivorship and total biomass production. Both survivorship and biomass were maximum at 20°C and salinity 30 ppt.

Antibiotic, without aeration, had a highly significant effect ($p < .0005$), enhancing larval survival at 30°C, but survivorship was still extremely low (mean 1.7%). Because of 100% mortality at 30°C, in the absence of antibiotic, interaction terms were not analysed. At lower temperatures, antibiotic had a negative effect on survival. Antibiotic did not significantly effect length, but had effect on larval weight ($p = .004$), reducing weight at 20°C and augmenting weight at 25°C. Maximum individual weight was at 30°C under salinities of 25–30 ppt, but survival was extremely low. There was no significant effect of antibiotic on total biomass production ($p = .611$).

In the presence of antibiotic, aeration had highly significant interaction with temperature ($p < .0005$) for length, weight and survivorship, and moderately significant interaction for total biomass production ($p = .027$). No significant interaction was observed with salinity, but salinity effect were significant for length ($p = .003$), weight ($p = .006$) and survivorship ($p = .019$). Aeration slightly increased average length and weight at 20°C, but reduced these parameters slightly at 30°C. Aeration increased survivorship at all three temperatures, but survivorship was still maximum at 20°C and salinity of 35–40 ppt. The effects of aeration on total biomass production followed the same pattern as that for survivorship, but maximum biomass was observed at slightly lower salinities (25–35 ppt).

Day 9

Only temperatures of 20 and 25°C were completely analysed on the ninth day, due to 100% mortality at 30°C in the absence of antibiotic. Without antibiotic nor aeration, temperature × salinity interaction was significant on length ($p = .014$), survivorship ($p = .014$) and biomass ($p < .0005$), but only marginally so on weight ($p = .052$). All parameters were maximum at 20°C and salinities of 25–35 ppt.

The presence of antibiotic increased survivorship at 30°C, but it was still extremely low (approx. 1%). Survival was reduced by antibiotic at the lower temperatures. Antibiotic also significantly increased average length, weight and biomass at 25 and 30°C.

Aeration, in the presence of antibiotic, revealed significant

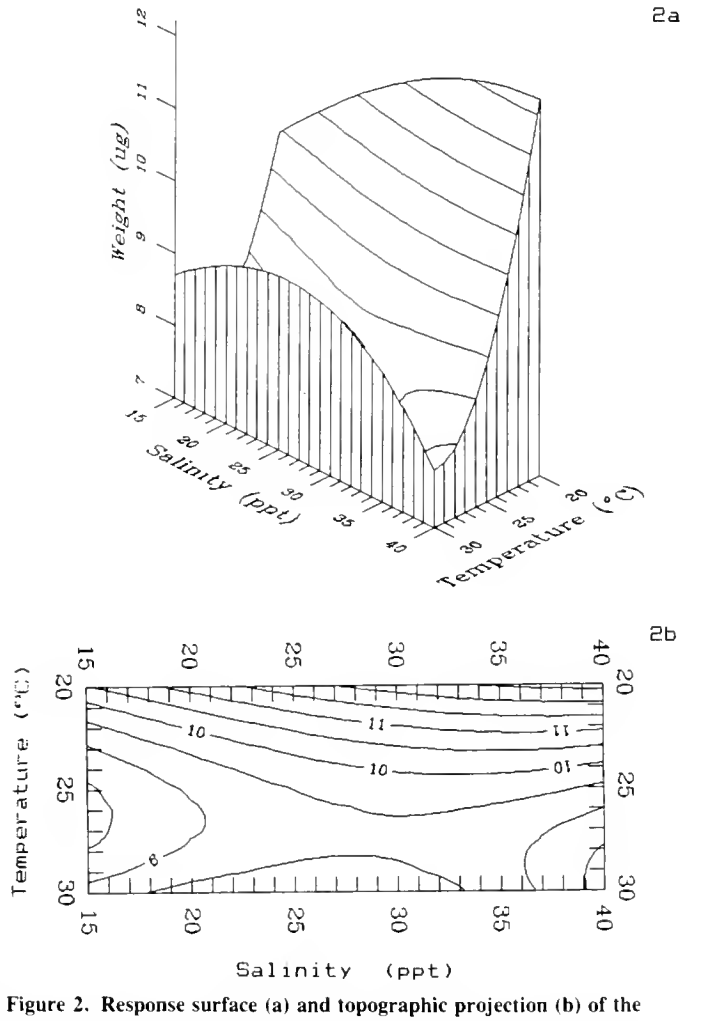

Figure 2. Response surface (a) and topographic projection (b) of the mean dry weight of *Crassostrea rhizophorae* larvae, as a function of temperature and salinity, on the fifth day of cultivation without the use of antibiotic and aeration.

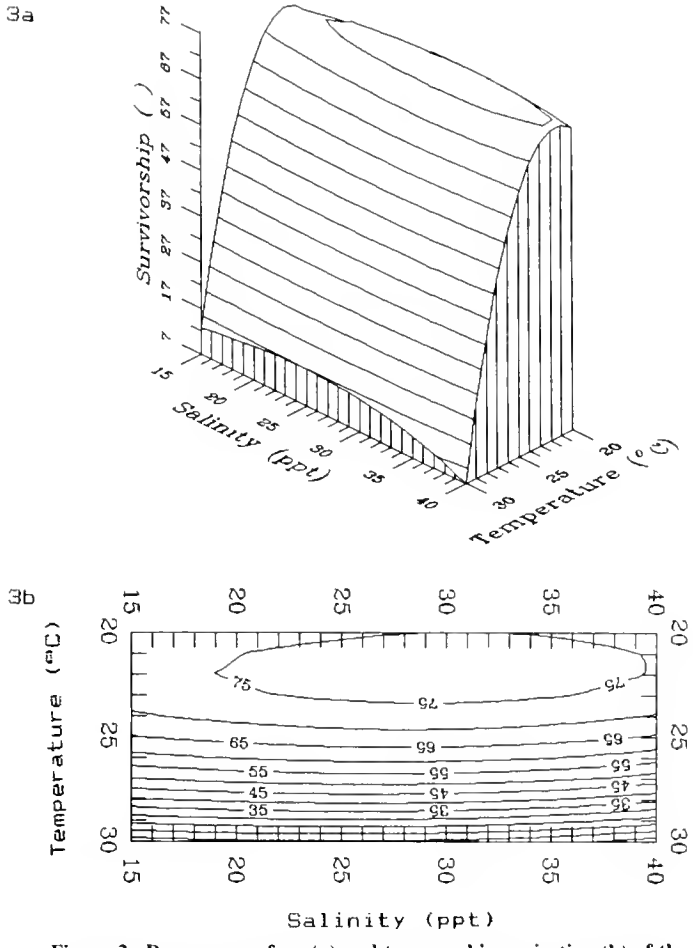

Figure 3. Response surface (a) and topographic projection (b) of the survivorship of *Crassostrea rhizophorae* larvae, as a function of temperature and salinity, on the fifth day of cultivation without the use of antibiotic and aeration.

interactions with temperature and salinity ($p < .0005$). Aeration reduced length and weight slightly at 25 and 30°C and increased survivorship at 20°C. Maximum biomass values were still observed at 20°C, with aeration.

DISCUSSION AND CONCLUSIONS

The results observed in this study confirm that, in the absence of antibiotic and aeration and with constant food availability, the survival of *C. rhizophorae* veligers is determined primarily by temperature. Similar conclusions have been reported for several other species of bivalve larvae (Lough 1974, Lough and Gonor 1973). As previously reported for *C. rhizophorae* embryos (dos Santos and Nascimento 1985), young larvae have euryhaline characteristics, in spite of a tendency to survive and grow better at higher salinities (25–40 ppt). Although temperature × salinity interaction was generally significant for larval survival and total biomass production, temperature effects were always dominant, yielding superior survivorship and biomass at 20°C, irrespective of salinity, during the period studied. In this respect, *C. rhizophorae* veligers differ from *Mytylus edulis* veligers, which require a narrow range of salinity (near oceanic), despite the fact that later larvae (16–17 days) and adults are euryhaline. For *M. edulis* larvae, no true temperature × salinity interaction was observed (Lough 1974).

The present study shows that temperature × salinity interaction is at times significant for growth parameters of *C. rhizophorae* larvae, but temperature effects were always predominant. The analysis of growth for other species of bivalve larvae has also shown a pronounced interaction effect between temperature and salinity (Davis and Calabrese 1964). Within a suitable range of salinity, growth of *C. rhizophorae* larvae depends primarily on temperature. This finding is in agreement with Robert et al. (1988), who concluded that, discounting nutrition, temperature was clearly the dominant factor influencing the development of *Ostrea edulis* larvae. *O. edulis* larvae developed normally within a wide salinity range, growth being dependent upon temperature. Even so, the optimal salinity for their growth was estimated to be 25 to 35 ppt, with reduction of larval growth rate occurring at lower salinities, as previously reported by Davis and Ansell (1962).

Davis and Calabrese (1964) reported that clam (*M. mercenaria*) and oyster (*C. virginica*) larvae also differed in their growth response to varying salinities. While oyster larvae were more sensitive than calm larvae to temperature differences, the latter appeared more sensitive to salinity variations. Within the salinity range of 15 to 27.5 ppt, growth of *C. virginica* larvae was ap-

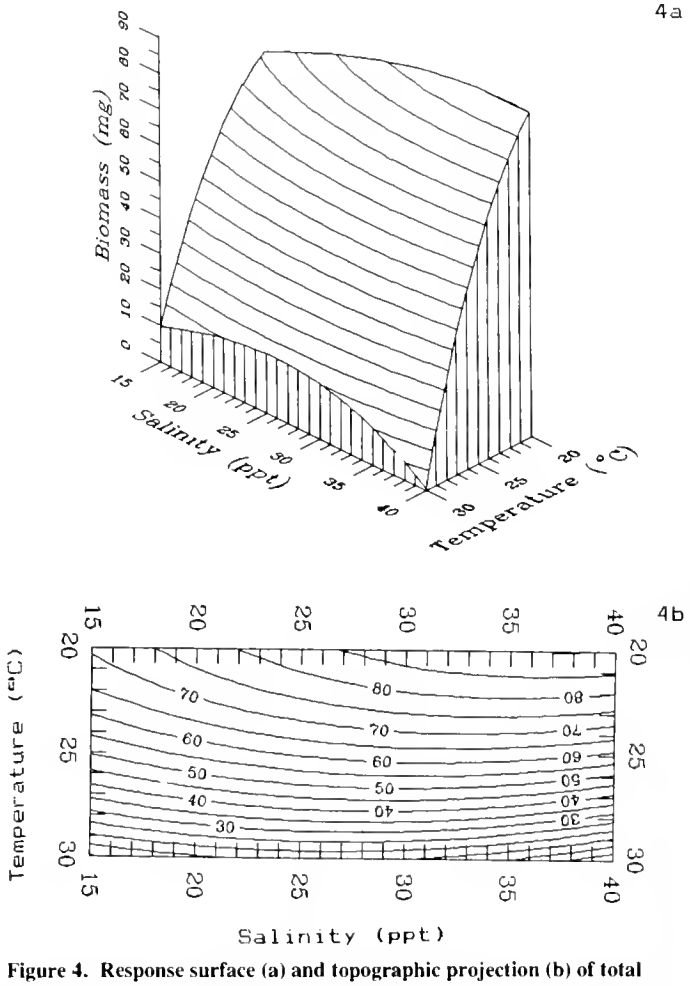

Figure 4. Response surface (a) and topographic projection (b) of total biomass of *Crassostrea rhizophorae* larvae, as a function of temperature and salinity, on the fifth day of cultivation without the use of antibiotic and aeration.

proximately constant within each temperature tested, but revealed an optimum in the range of 30 to 32.5°C.

In the present study, length and weight increments of *C. rhizophorae* veligers were maximum at the highest temperature tested (30°C), but survival was lowest at this temperature. The presence of antibiotic and of aeration revealed significant interactions with temperature for all four parameters, but were especially notable in relation to survivorship and total biomass. Length was enhanced at 30°C. This probably resulted from increased activity of enzyme systems at the higher temperatures (Calabrese 1969).

In general, individual dry weight followed the same pattern as length increment. It appears, however, that the extreme salinities tested were detrimental to growth in length, since some of the lowest values for this parameter were observed at 30°C under salinities of 15 and 40 ppt. Robert et al. (1988) found that *O. edulis* veligers grow best at 30°C. Their data is in contrast with those of Walne (1966) and Davis and Calabrese (1969), who observed optimum growth at 26 and 27°C, respectively. The differences were attributed to the geographical range of the species. However, maximum growth of *C. gigas* larvae also occurred at 30°C, with a substantial reduction at salinities of 20 and 30 ppt (His et al. 1989).

The present study demonstrated that patterns of larval shell length, weight and survivorship differed under the same combinations of temperature and salinity. Consequently, it may be advisable to consider biomass production as the most significant parameter for cultivation purposes. Intuitively, biomass seems the best parameter to maximize because it integrates individual weight and survivorship, indicating a reasonable number of larvae of moderate size. If larger larvae tend to survive better and grow well during later phases of production, biomass may indeed be the best parameter to maximize. Further studies, during subsequent stages of oyster development, are needed to confirm this supposition.

Throughout the period of cultivation, larval biomass was greatest at 20°C. It has been noted that the temperature of the water is relatively stable and seldom reaches 30°C in the estuarine regions where *C. rhizophorae* reproduce well (Nascimento et al. 1980). The salinity, however, is extremely variable. This explains why, of the two environmental variables studied, temperature variations were clearly the dominant factor influencing larval growth. Natural populations of *C. rhizophorae* are well adapted to salinity variations but not to extremes of temperature. Several studies have suggested the possibility that the salinity and temperature at which the parent adults are maintained during maturation may influence the tolerance and developmental response of their larvae to these environmental factors (Stickney 1964, Helm and Millican 1977). Based on this assumption, and taking maximum biomass production as the best end point for cultivation purposes, it is advisable to cultivate *C. rhizophorae* veligers near 20°C at salinities between 25 and 35 ppt, without the use of antibiotics.

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TETRAPLOID INDUCTION WITH MITOSIS I INHIBITION AND CELL FUSION IN THE PACIFIC OYSTER (*CRASSOSTREA GIGAS* THUNBERG)

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ABSTRACT Tetraploid induction was attempted in the Pacific oyster, *Crassostrea gigas* (Thunberg), with mitosis I inhibition and cell fusion. Mitosis I was blocked with heat shocks (35–40°C, for 2–23 min), producing up to 45% tetraploid embryos. Zygote-zygote fusion was successfully obtained with polyethylene glycol (PEG), without producing significant levels of tetraploidy. Sperm fusion was also tested, but the PEG treatment used in this study didn't lead to the fusion of sperm cells. On the other hand, PEG-induced blastomere fusion produced significant levels of tetraploids (up to 30%). There was no evidence that the tetraploid embryos produced in this study survived beyond D-stage. The poor viability of tetraploids produced in this study was probably caused by disruptions of the polar lobe formation by heat shock and cell fusion treatments.

KEY WORDS: tetraploidy, triploidy, heat shock, cell fusion, *Crassostrea gigas*, aquaculture.

INTRODUCTION

Tetraploidy refers to the condition of a cell or organism possessing four sets of chromosomes instead of two sets found in diploidy. Tetraploidy provides an opportunity to study the effects of genome duplication on development and gene expression. In the Pacific oyster (*Crassostrea gigas* Thunberg), tetraploidy is also important for commercial reasons. It can be potentially used to cross with diploids and produce triploids which have become an important part of the oyster culture industry. Currently, triploid oysters are produced by blocking the release of the second polar body, primarily with cytochalasin B (CB) (Allen et al. 1989). There are several disadvantages with the use of CB: 1) CB is highly toxic, and health concerns may limit CB's use in food production; 2) induction is rarely 100% effective, which complicates hatchery management; and 3) blocking polar body II may have deleterious effects on the survival and growth of induced triploids (Chourrout et al. 1986, Guo et al. 1990). On the other hand, the use of tetraploids may eliminate all these problems. For cultured mollusks in general, tetraploids can also be useful in hybridization and various breeding schemes.

Tetraploidy is commonly induced by inhibiting the first mitosis in fertilized eggs with heat, pressure, colchicine, or CB treatments. Viable tetraploids have been produced with mitosis I inhibition by heat and pressure in a number of fish (Chourrout 1982, 1984, Myers et al. 1986) and amphibians (Fischberg 1958, Jaylet 1972, Reinschmidt et al. 1979). In mammals tetraploidy is probably lethal, but tetraploid embryos have been constructed by fusing two diploid cells (Eglitis 1980, Spindle 1981). In the Pacific oyster, tetraploid induction with CB and pressure induced mitosis I inhibition have been tested without success (K. Cooper unpublished, S. K. Allen personal comm.). Also, meiosis I inhibition (Stephens 1989, Guo et al. 1992a, b) and gynogenesis (Guo et al. 1993) failed to produce viable tetraploids. Recently Guo and Allen (1994) succeeded in producing viable tetraploid Pacific oysters by manipulating chromosome sets in eggs from triploids. Their success proves tetraploid Pacific oyster is viable and will likely renew interests in tetraploid induction in mollusks. Therefore, it becomes necessary to report our failed attempts on tetraploid induction with heat-induced mitosis I inhibition and cell fusion.

MATERIALS AND METHODS

The experiments described here were conducted in summer of 1990. Broodstocks were provided by the Coast Oyster Company (Washington, USA). Gametes of the Pacific oyster were obtained by dissecting gonads of fully matured animals. Eggs were passed through a 100 μm nytex screen to remove the large tissue debris and rinsed on a 20 μm screen. Sperm suspension were filtered through a 20 μm screen. Fertilization was conducted at 25°C. After treatments, embryos were cultured in natural sea water at 25°C at a density of 100/ml (Breese and Malouf 1975). The natural sea water used in this study had a salinity of 28–30 ppt.

Mitosis I Inhibition

Mitosis I in fertilized zygotes was inhibited with heat shocks. Heat treatments were applied when a majority of the fertilized zygotes released polar body II, which is usually around 55 min post-fertilization (PF) at 25°C. For treatments, zygotes were transferred to 35, 38, and 40°C sea water for 2, 5, 10, 15, and 20 min. The temperature range of 35–40°C was selected based on results on mitosis I blocking in other animals (Thorgaard et al. 1981, Bidwell et al. 1985), and this range was also effective in blocking the release of polar bodies in several mollusks, including the Pacific oyster (Quillet and Paneley 1986, Arai et al. 1986, Yamamoto and Sugawara 1988). One million eggs were used in each group. Two replicates were made for each treatment with different pairs of parents.

Cell Fusion

Tetraploid induction was tested with zygote-zygote fusion, blastomere fusion and sperm fusion followed with inhibition of

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polar body II. Artificial sea water (ASW) was used for all solutions and treatments, which is consisted of 423 mM NaCl, 9 mM KCl, 22.94 mM $MgCl_2$, 25.50 mM $MgSO_4$, 9.27 mM $CaCl_2$, and 2.15 mM $NaHCO_3$ (Meijer et al. 1984).

For zygote-zygote fusion, fertilized zygotes (200,000) were first treated with trypsin (0.5%) for 30 min and exposed to a area solution (1 M with 1 mM $CaCl_2$) for 30 seconds. The trypsin treatments were applied at 10 min post-fertilization. The zygotes were then treated with 50% PEG (mol. wt. 8000) for 4 min. This procedure was based on studies of Bennet and Mazia (1981a, b) and Sekirina et al. (1983), and optimized for the Pacific oyster zygotes by testing different trypsin durations (0–40 min), PEG molecular weights (1,000, 3,450, 8,000) and durations (1–8 min). Two replicates were made using a different pair of parents.

Sperm fusion was attempted by treating sperm with 50% PEG (mol. wt. 8000) for 4 min. The treated sperm were used for fertilization. Three replicates were made. Blocking polar body II was not attempted because of the lack of evidence of successful sperm fusion. Blastomere fusion was tested by treating 2-cell embryos directly with 50% PEG (mol. wt. 1,000, 3,450, 8,000) for 1, 2, and 4 min. Two replicates were made for each group. For both sperm fusion and blastomere fusion, half a million eggs were used for each group.

Data Collection

Survival of treated zygotes was determined as percentage of cleavage at 90–120 min PF. The induction of tetraploidy was determined by chromosome counts at 6–8 hrs PF according to methods described by Guo et al. (1992a). The Pacific oyster has a diploid chromosome number of 20 (Ahmed 1973). Ploidies were classified as the following: 9–10 chromosomes, haploid; 18–20, diploid; 28–30, triploid; 35–40 tetraploid. Other chromosome numbers were considered aneuploids. Because chromosome analysis is difficult in later stages, ploidy of 24-hour old larvae were determined by flow cytometry (Allen 1983, Guo et al. 1993).

RESULTS

Heat Shock

Heat shocks were applied to the fertilized eggs of the Pacific oyster at 55 post-fertilization (at 25°C). At this time, the second polar body was released in over two-thirds of the fertilized eggs.

The majority (>75%) of the treated eggs tolerated heat shocks up to 5 min at 40°C, 10 min at 38°C, and 23 min at 35°C (Table 1). At 40°C, heat shocks longer than 5 min resulted in mortality of the majority of the treated eggs. Only 7.5% of the treated eggs survived the 10 min shock, and all the treated eggs were killed in the 15 and 20 min shocks.

At 38°C, the 10 min heat shock resulted in a 25% reduction in egg survival, and more than half of the eggs treated for 15 min died (Table 1). The 20 min heat shock at 38°C caused mortality in the majority of the eggs, and the 12% that divided were mostly abnormal.

At 35°C, heat shocks were 1–3 min longer than the scheduled duration due to unexpected delays in releasing the heat shocks. The treated eggs tolerated all durations (up to 23 min) well (Table 1), and all divisions appeared to be normal.

Compared with the untreated controls, the development of eggs treated with heat shocks was delayed and less synchronized. When the untreated eggs entered the 8-cell stage, the treated eggs in most groups were either at 1-cell, 2-cell or 4-cell stages. Abnormalities in the first mitotic division were obvious in the eggs treated for longer than 5 min at 40°C, and 10 min at 38°C.

TABLE 1.

Percentage (s.d.) of eggs survived and tetraploids induced from heat shock treatments. The heat shocks were applied to the fertilized eggs at 55 min post-fertilization at 25°C. A minimum of 20 metaphases were analyzed in each of the two replicates.

Heat Treatment/ Durations	Percent Eggs Survived	Percent Tetraploids at 8 Hours
40°C		
2 min	100.0 (0.0)	20.4 (0.6)
5 min	87.0 (9.9)	15.0 (4.9)
10 min	7.5 (3.5)	12.5 (n.a.)*
15 min	0 (0.0)	–
20 min	0 (0.0)	–
38°C		
2 min	97.5 (3.5)	5.0 (n.a.)
5 min	95.0 (7.1)	6.7 (9.4)
10 min	75.0 (7.1)	10.6 (0.8)
15 min	48.0 (2.8)	25.0 (n.a.)
20 min	12.0 (3.5)	30.0 (n.a.)
35°C		
3 min	100.0 (0.0)	0.0 (0.0)
7 min	97.0 (4.2)	2.8 (4.0)
12 min	97.5 (3.5)	13.2 (9.7)
17 min	89.0 (1.4)	21.7 (n.a.)
23 min	88.0 (4.2)	45.0 (7.1)
25°C: control	100.0 (0.0)	0.0 (0.0)

* n.a. designates the group where the chromosome count is not available in one of the two replicates.

Induction of tetraploidy was evident. Karyological analysis of 8-hour old embryos revealed that tetraploids were produced in almost all heat-shocked groups (Table 1). The only heat treatment which didn't yield detectable levels of tetraploids was the 3 min heat shock at 35°C. Less than 10% tetraploids were found in three groups, 7 min at 35°C, 2 min at 38°C and 5 min at 38°C. The highest levels of tetraploidy, 45%, was produced by the 23 min shock at 35°C. It appeared that heat shock at a lower temperature (35 or 38°C) for a longer duration (20 or 23 min) was more effective in inducing tetraploids than a higher temperature (40°C) for a shorter duration (2 or 5 min). An example of the tetraploid metaphase plates observed is presented in Figure 1.

At 24 hours post-fertilization, D-stage larvae were found in all

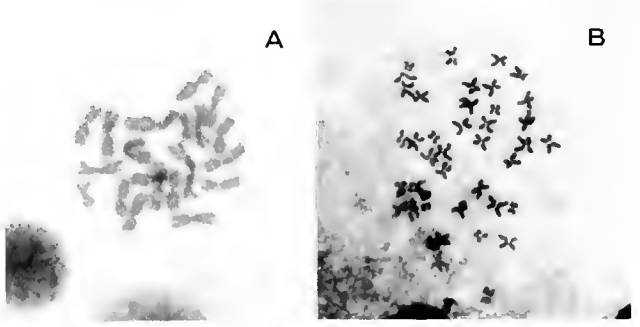

Figure 1. Metaphase of diploid (A) and tetraploid (B) embryos from control and heat treated groups. The embryos were 8 hours old.

groups where the treated eggs did not suffer complete mortality. Survival of the tetraploid embryos was poor. Even in the group where 45% of the 8-hour old embryos were tetraploids, flow cytometric analysis showed virtually no detectable tetraploids among the D-stage larvae (Fig. 2).

Cell Fusion

Zygote-Zygote Fusion

After trypsin treatment, zygotes appeared to be adhesive and formed clusters. The urea treatment further enhanced the formation of zygote clusters. Application of PEG induced fusion of 20% of the treated zygotes. The majority of the fusions occurred between two zygotes (Fig. 3). In a few instances, fusion of three and four zygotes were also observed. Ploidy of embryos was determined by karyological analysis at 8-hours post-fertilization. Only 2.2% tetraploid embryos was recorded (1 in 45) (Table 2). On the other hand, an average of 15.6% aneuploid embryos were found, compared with only 5% aneuploids in the control group.

Sperm Fusion

Direct observation of the sperm under the microscope didn't reveal any difference between the treated and untreated sperm. Even in the untreated group, sperm under a cover glass had a tendency to form clusters, and the clustered sperm might appear to be fused.

There was no evidence that the treatment of sperm with 50% PEG 8,000 induced significant levels of sperm fusion. Fertilization with the treated sperm did not lead to the production of significant levels of triploids or aneuploids (Table 3). In the three treated groups, only an average of 1.7% triploids and 6.7% aneuploids were found, which were about the same as in the control group.

Blastomere Fusion

In the presence of PEG, the treated embryos were dehydrated with a shrunken appearance. The shrunken embryos came back to a normal shape within 3 minutes after the removal of PEG treatment. After the completion of PEG treatment, the majority of the treated embryos had regained their normal appearance. Because the PEG treatment was brief and shrunk the embryos, the fusion of blastomeres could not be directly observed. The production of larger numbers of tetraploid embryos in PEG treated groups suggested the PEG treatments were effective in inducing blastomere fusions (Table 4). At the first mitotic division, abnormalities were common among the recovered embryos, and the deformed embryos often appeared to have daughter cells and polar lobes of abnormal proportions.

Treatments with PEG of the three molecular weights (1,000, 3,450 and 8,000) had similar effects on blastomere fusion and viability of treated embryos. While more tetraploids were induced by longer PEG treatments, prolonged PEG treatment (4 min) caused heavy mortalities in the treated embryos (40–55%) (Table 4). Compared with other molecular weights, PEG 1,000 produced the highest level of blastomere fusions (at least 30%). No tetraploids were detected among D-stage larvae, as analyzed by flow cytometry.

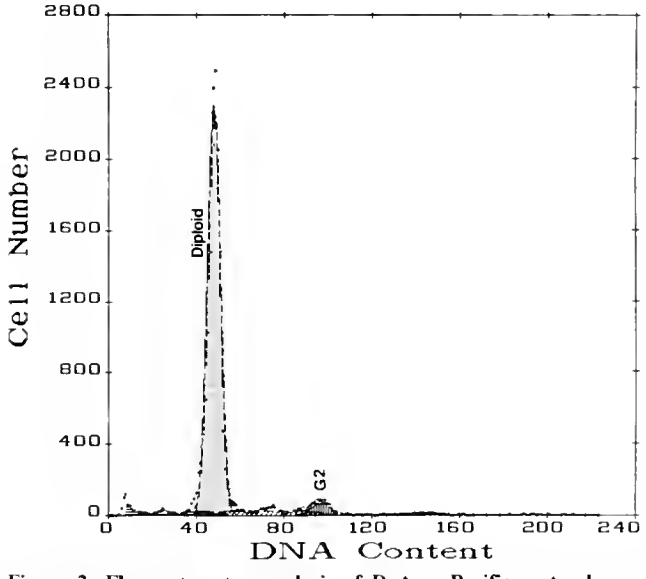

Figure 2. Flow cytometry analysis of D-stage Pacific oyster larvae from a heat-treated group, where 45% of the 8-hour old embryos were tetraploids.

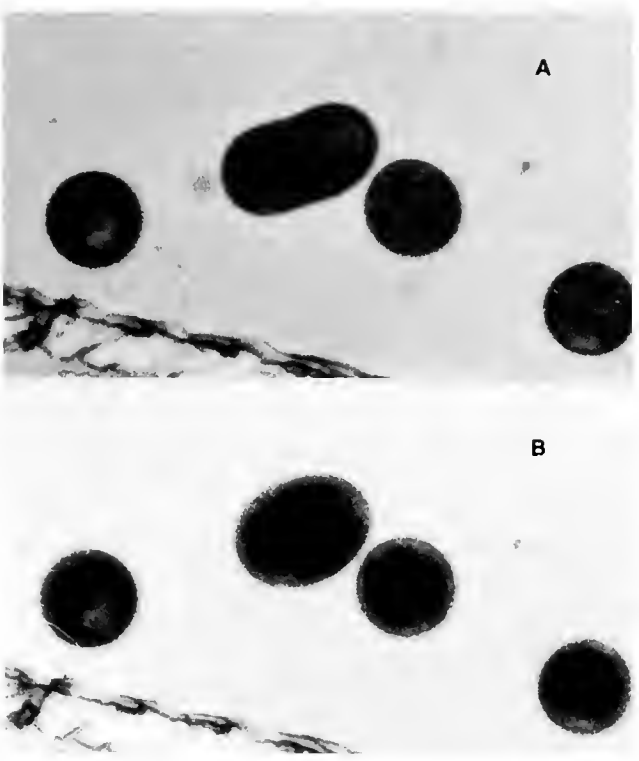

Figure 3. The fusion of two zygotes of the Pacific oyster at one (A) and three (B) minutes after treatments. The zygotes were trypsinized for 30 min and then treated with 50% polyethylene glycol (mol. wt. 8000).

DISCUSSION

Induction of Tetraploids

Significant levels of tetraploids were produced by mitosis I blocking with heat shocks. The highest percentage of tetraploids produced was 45%, which is lower than that produced in newts (94%) (Fischberg 1958) and channel catfish (62%) (Bidwell et al. 1985), but higher than that produced in rainbow trout (16%) (Thorgaard et al 1981).

TABLE 2.

Ploidy levels of embryos resulting from zygote-zygote fusion

Groups	Fused (%)	n	Haploid (%)	Diploid (%)	Triploid (%)	Tetraploid (%)	Aneuploid (%)
Replicate 1	24	25	0	76	0	4	20
Replicate 2	16	20	0	90	0	0	10
Pooled	**20**	**45**	**0**	**82.2**	**0**	**2.2**	**15.6**
Control	0	20	0	95	0	0	5

The failure to produce higher levels of tetraploidy in heat shocked eggs may be due to two factors. First, the development of the treated eggs was not synchronized. Synchronization of egg development in the Pacific oyster is greatly affected by female condition and other environmental factors. Unsynchronized egg development was used to explain the difficulty in producing a high percentage of triploids (Lu 1986, Downing 1987, Downing and Allen 1987). This may also explain why a longer heat shock at a lower temperature (23 min at 35°C) produced more tetraploids than shorter shocks at higher temperatures (2 min at 40°C) in this study. The longer treatment probably encompasses more eggs in mitosis I than the short treatment. The second factor was that the heat shock conditions used in this study may not be optimally effective in blocking the first mitosis in the Pacific oyster. Detailed studies necessary to define optimum heat shock conditions have not been conducted with this species as they have with other animals (Fischberg 1958, Thorgaard et al 1981, Chourrout 1982, Bidwell et al. 1985).

Zygote-Zygote Fusion

Zygote-zygote fusion was successfully produced with PEG and other treatments. The levels of zygote-zygote fusion (20%) obtained in this study were higher than that obtained in starfish oocytes (up to 6%) (Sekirina et al. 1983). Using a different procedure in sea urchins, Bennett and Mazia (1981a) obtained a level of fusion as high as 40%.

In many cases, the fused zygotes produced three or four daughter cells at the first mitosis. Similar abnormalities were observed in the fused eggs of sea urchins (Bennet and Mazia 1981a). Despite the abnormal cleavage in some of the fused zygotes, the early cleavage of the fused zygotes observed in this study suggests, as in other organisms, that the fusion treatments are not lethal to eggs (Vassetzky et al. 1983, Vassetzky and Sekirina 1985, Vassetzky et al. 1986).

The chromosome behavior of the fused zygotes, however, is not clearly understood. Results in this study showed no significant production of tetraploid embryos in the groups where a significant proportion of the zygotes were fused, while tetraploid embryos have been produced by zygote-zygote fusion in mice (Dyban et al. 1981). Instead, results of this study seem to indicate that the fusion of zygotes in the Pacific oyster results in the formation of aneuploids. Bennett and Mazia (1981a) observed that the fused zygotes of sea urchins formed either a tetrapolar spindle or two centrally located bipolar spindles. The formation of tetrapolar spindles in the fused zygotes of the Pacific oyster would explain the production of aneuploid embryos.

The major problem with zygote-zygote fusion was that the fused zygotes could not be easily separated from the unfused zygotes. Because of the low levels of fusion and the potentially poor viability of the fused zygotes, development of fused zygotes can be easily overwhelmed by unfused zygotes. In echinoderms, the large oocytes (100–120 μM) made it possible to separate the fused from unfused zygotes manually by micropipeting under a dissecting microscope (Vassetzky et al. 1986). The small egg size of the Pacific oyster (about 45 μM) made the separation by micropipeting difficult. Until a good separation technique is developed, zygote-zygote fusion may not be of much practical use for the production of tetraploid Pacific oysters.

Sperm Fusion

The PEG treatments used in this study didn't lead to significant levels of sperm-sperm fusion. Using a treatment of 50% PEG 4,000 for 1 min, Ueda et al. (1986) successfully fused sperm and obtained 33% triploid rainbow trout from fertilization with fused sperm. By the evidence that the triploids contained two Y chromosomes, they confirmed that the triploids were produced by fused sperm, rather than residual effects on polar body II. In this study, fertilization with the treated sperm produced only 1.7%

TABLE 3.

Ploidy levels of resultant embryos from sperm fusion.

Groups	Fertilized (%)	n	Haploid (%)	Diploid (%)	Triploid (%)	Tetraploid (%)	Aneuploid (%)
Treated 1	90	20	0	95	0	0	5
Treated 2	95	20	5	85	5	0	5
Treated 3	90	20	0	90	0	0	10
Pooled	**91.7**	**60**	**1.7**	**90**	**1.7**	**0**	**6.7**
Control	100	20	0	95	0	0	5

Before fertilization, sperm were treated with 50% PEG 8,000 for 4 min.

TABLE 4.

Tetraploid induction with blastomere fusion produced by different durations of treatment with PEG of different molecular weights: percentage (s.d.) of eggs killed and tetraploids induced.

PEG Treatments	Eggs Killed	Tetraploids Induced
PEG 1,000		
1 min	12.5 (10.6)	5.0 (7.1)
2 min	29.0 (8.5)	15.0 (7.1)
4 min	40.0 (9.9)	30.0 (14.1)
PEG 3,450		
1 min	11.5 (9.2)	5.0 (7.1)
2 min	32.5 (24.7)	0.0 (0.0)
4 min	49.0 (22.6)	10.0 (0.0)
PEG 8,000		
1 min	22.5 (10.6)	5.0 (7.1)
2 min	30.0 (:7.1)	10.0 (0.0)
4 min	55.0 (21.2)	20.0 (n.a.)*

Ten metaphases were analyzed in each of the two replicates.
* Designates the groups where chromosome count of one of the replicate was not avaialble.

triploids and 6.7% aneuploids, which could as well be formed spontaneously. Spontaneous triploids and aneuploids may occur in the Pacific oyster at frequencies of 1.3% and 8.7%, respectively (Guo et al. 1992a), which are very close to the values observed in this part of the study.

Blastomere Fusion

Although the fusion of blastomeres was not directly observed, the occurrence of significant levels of tetraploids (up to 30%) in the PEG treated groups suggests that blastomere fusion occurred at a considerable frequency. Working with mouse embryos, Spindle (1981) observed the fusion of blastomeres in 82% of the 2-cell embryos which were exposed to 40% PEG 1,000 for 1 min, and the fused embryos developed uniformly as tetraploids.

Results of this study suggest at least some of the fused blastomeres may develop abnormally, which differs from the observation in mice where all the fused blastomeres had normal cleavages (Spindle 1981, Ozil and Modlinski 1986). The difference was probably due to the fact that the first two cleavages in eggs of the Pacific oyster normally form polar lobes, while the mouse eggs cleave into two equal-sized blastomeres.

Survival of Tetraploids

The failure to detect tetraploids among D-stage larvae suggested that the viability of the tetraploids induced in this study was very low, if not zero. There were several potential causes for the poor survival of tetraploids. Both mitosis 1 blocking and blastomere fusion affect the first cleavage. In many mollusks including the Pacific oyster, the first cleavage is characterized by the formation of a polar lobe. The polar lobe contained important morphogenic determinants for mesodermal derivatives, including the shell gland, which are distributed through the D blastomere (Wilson 1904). Both heat shocks and blastomere fusion affected the first cleavage and the polar lobe formation. Therefore, it is possible that the poor viability of the tetraploids induced in this studies was caused by disruptions to the polar lobe formation. Probably due to the same reason, CB and pressure treatments applied to mitosis 1 also didn't produced viable tetraploids (K. Cooper unpublished). It seems that inhibition of mitosis 1 is not a viable approach to tetraploid induction in the Pacific oyster due to the unequal division and formation of the polar lobe. In fish and amphibians where viable tetraploids were produced by mitosis 1 blocking (Fischberg 1958, Jaylet 1972, Reinschmidt et al. 1979, Chourrout 1982, Myers et al. 1986), the first cleavage were equal divisions.

However, the disruption of polar lobe formation was probably not the only cause for the poor viability of tetraploid Pacific oysters. Blocking PB1 and gynogenesis don't involve mitosis 1 and the polar lobe, but neither methods produced viable tetraploid in the Pacific oyster (Stephens 1989, Guo et al. 1992a, Guo et al. 1993). On the other hand, triploids produced by blocking PB1 and diploids produced by gynogenesis were viable. Guo (1991) hypothesized that the poor viability of the induced tetraploids was caused by a cell-number deficiency due to the cleavage of a normal eggs by a large, tetraploid nucleus. Unlike fish and amphibians, most mollusks follow a "mosaic" type of development where a cell's fate is programmed by the number of divisions and distribution of morphogenic determinants (Gilbert 1988). A reduction in cell number at the end of cleavage may stop development by retention different morphogenic determinants in one cell, or simply by a lack of sufficient number of cells needed for further morphogenesis. The cell-number deficiency can be corrected by an increase in the zygote volume. Tetraploids produced by zygote-zygote fusion don't suffer from the cell-number deficiency. Unfortunately, zygote-zygote fusion in this study didn't produce a satisfactory number of tetraploids to test this hypothesis. The production of viable tetraploids with eggs from triploids which are significantly larger than eggs from diploids (Guo and Allen 1994), seems to support the cell-number deficiency hypothesis. On the other hand, a small number of tetraploids produced by polar body inhibition in normal eggs survived metamorphosis in the mussel, *Mytilus galloprovincialis* (Scarpa et al. 1993), suggesting the cell-number deficiency may be tolerable to some embryos or species.

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SMALL SCALE VARIABILITY OF BIOCHEMICAL INDICES OF GROWTH IN *MYA ARENARIA* (L.)

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ABSTRACT The small-scale variability of biochemical indices of growth (RNA:DNA, protein:DNA, and protein:RNA) was investigated in the gills, adductor muscle, and digestive gland of the intertidal bivalve *Mya arenaria* (L.). Field and tank experiments were conducted in order to determine the effect of age and body size on the indices, and to assess the spatial variability of the indices and their rapidity of response to emersion. The protein:DNA and protein:RNA ratios of the gills and the digestive gland varied positively with the animal's age or size while the biochemical indices of the adductor muscle were not affected by these parameters. An important spatial variability was observed. The indices of the adductor muscle were significantly different among animals collected within a sampling surface of 12.5 × 12.5 m^2 but remained homogeneous within 2.5 × 2.5 m^2 quadrates. The protein:DNA ratio of the gills was even more variable, being significantly different among animals collected within a sampling surface of 2.5 × 2.5 m^2. The indices were only slightly affected by emersion: they did not significantly vary until the clams had been exposed for at least 14 hours. This emersion independence probably reflects the adaptation of intertidal animals to their habitat, where they are regularly subjected to hypoxia.

KEY WORDS: bivalve, *Mya arenaria*, growth indices, spatio-temporal, protein, RNA, DNA

INTRODUCTION

RNA:DNA ratio and related biochemical indices are increasingly used in growth studies. The short-term index of growth RNA:DNA represents the organism's potential growth rate (Pease 1976) while the protein:DNA ratio is an indicator of cell size (Barron and Adelman 1984), which is representative of the net protein synthesis. The protein:RNA ratio is interpreted as the synthesis activity of ribosomal RNA, as 85–94% of the cellular RNA is ribosomal (McMillan and Houlihan 1988). These indices provide early information about the impact of ecological changes on the animal's growth (Bulow 1970, Buckley 1984, Wright and Hetzel 1985, Buckley and Lough 1987). The biochemical indices are also sensitive to relatively small modifications of a variety of physico-chemical factors (Bulow 1970, Kearns and Atchison 1979, Buckley 1982, Buckley et al. 1984, Barron and Adelman 1984).

Despite the increasing interest in biochemical indices of growth, many aspects still have to be investigated in order to use these indices properly, especially in studies concerned with bivalves. For example, the individual variability induced by the animal's age or size is not well known (see Haines 1973, on fishes; Regnault and Luquet 1974, on *Crangon vulgaris;* Pease 1976, on *Crassostrea virginica;* McKee and Knowles 1987, on *Daphnia magna*). Nevertheless, this factor must be taken into account to maximize the homogeneity of the indices within a sample. Neither the small-scale spatial variability of the biochemical indices of growth nor their rapidity of response to emersion have yet been investigated. Such information is needed in order to plan accurate sampling designs and to avoid methodological bias that could be introduced by spatial heterogeneity of the indices or by differences in the duration of emersion experienced by the animals before being collected. Previous studies concerned with the spatial variation of bivalves growth used shell length increment as a measure of growth. They were generally done at 1 to 100 km scale (e.g. Brousseau 1979, Beukema and Meehan 1985, Essink and Bos 1985, Jones et al. 1990, Roseberry et al. 1991). In addition to its practical interest for the improvement of sampling designs, the study of individual and small-scale variability allows a better understanding of the factors that can affect growth in bivalves.

The intertidal bivalve *M. arenaria* (L.) is particularly convenient for the study of individual and spatio-temporal variability. The adult stage of this infaunal species is widely distributed, abundant, and sedentary. Surprisingly, bivalves have seldom been selected for studies on biochemical indices of growth. To our knowledge, there have been only six studies using this group: Pease (1976) and Wright and Hetzel (1985) worked on *Crassostrea virginica*, Robbins et al. (1990) on *Pecten maximus*, Whyte et al. (1991) on *Patinopecten yessoensis* eggs, Frantzis et al. (1993) on *Abra ovata*, and Robinson (pers. comm.) on the seasonal variations in *Mya arenaria*.

The aim of our study was to improve protocols for the use of short-term biochemical indices of growth (RNA:DNA, protein:DNA, and protein:RNA) in natural environments for *M. arenaria*. More specifically, we wanted to estimate the following variables acting on the biochemical indices in different tissues:

1. the individual variability, according to the animal's age and size;
2. the spatial variability, at a 150 m^2 scale; and
3. the response delay to emersion.

MATERIALS AND METHODS

The study was conducted at the Parc du Bic, in the Saint-Lawrence Estuary (48°21′31″N and 68°47′43″W), Québec, Canada. In this area, the semi-diurnal tides have a maximal amplitude of 4 m. The bivalve *M. arenaria* was collected from late summer to autumn 1989, a period during which 70 to 95% of the animals are sexually inactive (Roseberry 1988). The clams were dug at the mid-intertidal level, where the immersion period accounts for about 65% of the tidal cycle. The animals were kept in cool sea water during transport to the laboratory.

Age was estimated by counting the annual growth rings, which are well marked in the clams from the studied area and allow an accurate age estimation (Roseberry 1988). The length and height of the shells were measured with a Vernier caliper. The total wet flesh and the tissue samples were weighed after being briefly dried with a paper towel. As the total flesh wet weight of *M. arenaria* has been shown to be closely correlated to the dry weight (Roseberry 1988), only the wet weight was measured.

Tissue Sample Treatment

Three tissues were selected for the biochemical analyses: the gills, which are associated with respiration and food transport; the digestive gland, which is an important site for digestive enzymes production and an energy storage organ (Bayne et al. 1976); and the adductor muscle, which is representative of the somatic growth. Tissues were kept on ice during dissection and were immediately frozen at −80°C. Prior to biochemical analysis, the tissues were homogenized in 13 ml of distilled water (<4°C).

Biochemical Analysis

Duplicate biochemical analysis were performed on each tissue sample. The extraction of RNA and DNA was done with the Schmidt-Thannhauser procedure of Munro and Fleck (1966), as modified by Wright and Hetzel (1985), with some modifications which will be specified later. We used Bakers Yeast RNA type XI and Calf Thymus DNA Type I as standards (Sigma Chemical Co.).

For each tissue, the optimal time for the 0.3 N KOH and the 0.6 N percloric acid (PCA) hydrolysis was determined using internal standards.

A 50-minute hydrolysis in 0.3 N KOH released a maximal amount of RNA from the adductor muscle, while 60 minutes were needed for the gills and the digestive gland. A 5-minute hydrolysis at 80°C in 0.6 N PCA was shown to be optimal for the extraction of DNA from the three tissues, rather than the 15 minutes suggested by Wright and Hetzel (1985), which caused a significant protein hydrolysis. These proteins interfered with the DNA absorbancy. The adductor muscle had to be centrifuged at 14,000 g instead of 6,000 g in order to get a clear supernatant. Proteins were measured with the Bradford (1976) method; bovine albumin (fraction V, Sigma) was used as a standard.

The percentage of recovery for RNA was 74.2 ± 6.3 (N = 7) in the gills, 73.3 ± 7.1 (N = 6) in the adductor muscle, and 98.2 ± 14.9 (N = 8) in the digestive gland. The percentage of recovery for DNA was 98.3 ± 5.1 in the gills (N = 3), 83.6 ± 11.8 (N = 5) in the adductor muscle, and 95.5 ± 13.5 (N = 4) in the digestive gland. Proteins were recovered with an efficiency of 111.2% ± 6.0 (N = 20) for the three tissues. This suggests that some molecules other than proteins reacted with the dye.

Individual Variability

The individual variability of the biochemical indices in relation to the animal's age and size was studied. For this purpose, 23 *M. arenaria* were collected within a 3 × 3 m^2 quadrat in early August 1989. Their ages ranged from 7 to 18 years, total wet flesh weight varied from 0.84 to 14.86 g, shell length from 2.8 to 7.3 cm, and shell height from 1.7 to 4.4 cm. Linear regressions were used to describe the relationships between each parameter (age and animal size) and each biochemical index (RNA:DNA, protein:DNA, and protein:RNA). F-tests were conducted to detect significant differences of the regression coefficients from zero. Adductor muscle aliquots with DNA concentrations smaller than 1.5 μg/ml were excluded from statistical tests because of the extremely low position of these values on the DNA standard curve: the associated biochemical ratio values were strongly biased without any evident biological meaning. This was caused by the diminished precision of this part of the standard curve due to Beer's law.

Spatial Variability

In mid-October 1989, the spatial variability was investigated at a 150 m^2 scale by a hierarchical sampling design. Quadrates were delimited on a relatively homogeneous surface without macrophytes or important variations in the sediment quality. A quadrat of 12.5 × 12.5 m^2 enclosed 3 quadrates of 2.5 × 2.5 m^2, each of them containing 3 quadrates of 0.5 × 0.5 m^2. Their locations had been randomly selected. Three *M. arenaria* were collected in each 0.5 × 0.5 m^2 quadrat and animal density was estimated by counting the siphon holes at the sediment surface (Roseberry 1988). The animal's age varied from 12 to 18 years, total wet flesh weight from 4.1 to 9.5 g, shell length from 4.3 to 6.0 cm, and shell height from 2.8 to 3.8 cm. A nested ANOVA was performed to detect significant differences among the groups (quadrates of 2.5 × 2.5 m^2) and among the subgroups (quadrates of 0.5 × 0.5 m^2), and to separate the variance between the different spatial levels. The Bartlett's test was used to check the homogeneity of variances and graphical analyses showed that the residuals were normally distributed and independent. In this experiment involving spatial variability, only the gills and the adductor muscle were analysed. A simple ANOVA was used to evaluate the effect of animal density on the biochemical indices.

Delay of Response to Emersion

Thirty-eight clams were exposed to hypoxia in order to study the response time of the biochemical indices to emersion. Their age ranged from 12 to 20 years, total fresh flesh weight from 3.0 to 10.6 g, shell length from 4.4 to 6.7 cm, and shell height from 3.0 to 4.2 cm. Prior to the experiment, the animals were kept in running seawater and fed with *Dunaliella tertiolecta* (phytoplankton concentration varying from 2,000 to 4,000 cells/ml). Water temperature fluctuated between 3.0 and 6.6°C. The animals were maintained under these conditions for 21 days to ensure that they were in a similar physiological condition before emersion. The clams were then removed from the water and placed on trays in a refrigerated room (6°C). Ten control animals were immediately dissected (time of emersion = 0 h). Subsequent analyses were performed on four animals collected at logarithmic time intervals, i.e. 1.75, 4.75, 8.5, 13.75, 21, 31 and 45 hours of emersion. Since this study is the first to investigate the effect of emersion on biochemical indices of growth, the emersion period was prolonged to 45 hours in order to evaluate the sensitivity of the biochemical indices to this stress, in *M. arenaria*. ANOVAs were performed to detect significant differences among the biochemical ratios observed after the various periods of emersion. When significant differences were found, data were further analyzed with the Tukey multiple comparisons test.

RESULTS

Variability Induced by Tissue Sample Weight, Age, and Animal Size

According to the F-tests (Table 1) the animal's age and size positively affected the protein:DNA and protein:RNA ratios of the gills and the digestive gland. In other words, the net protein syn-

TABLE 1.

Effects of morphological parameters on biochemical ratios.

Tissue and Biochemical Ratios	Morphological Parameters: Age	Shell length	Shell Height	Flesh Weight
Gills (N = 22)				
R:D	0.74	1.94	1.23	1.86
	(0.04)	(0.09)	(0.06)	(0.09)
P:D	30.91	32.51	36.40	29.80
	(0.61)	(0.62)	(0.65)	(0.60)
	***	***	***	***
P:R	11.26	7.79	10.63	7.71
	(0.36)	(0.28)	(0.35)	(0.28)
	**	*	**	*
Digestive gland (N = 23)				
R:D	0.21	0.10	0.12	0.05
	(0.01)	(0.01)	(0.01)	(0.00)
P:D	4.65	3.94	4.37	2.88
	(0.18)	(0.16)	(0.17)	(0.12)
	*		*	
P:R	13.07	12.83	14.20	8.78
	(0.38)	(0.38)	(0.40)	(0.29)
	**	*	***	**
Adductor muscle (N = 20)				
R:D	3.28	3.57	3.02	2.85
	(0.15)	(0.17)	(0.14)	(0.14)
P:D	1.50	1.04	1.24	0.56
	(0.08)	(0.05)	(0.06)	(0.03)
P:R	3.82	2.95	3.12	1.73
	(0.18)	(0.14)	(0.15)	(0.09)

Linear regression analysis: the first value is the F-ratio, the value in parentheses is the R^2; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. P:D = protein:DNA, P:R = protein:RNA, R:D = RNA:DNA.

TABLE 2.

Spatial variability of the biochemical indices.

Tissue and Biochemical Ratios	N	Source of Variation: Among Quadr. 2.5 m side	Among Quadr. 0.5 m side	Within Quadr. 0.5 m side (error term)
Gills				
P:D	26	10.40***	3.14*	
		(32.1)	(28.3)	(39.5)
P:R	27	3.52	2.55	
		(6.7)	(31.8)	(61.6)
R:D	27	0.27	0.66	
		(0)	(0)	(100.0)
Adductor Muscle				
P:D	26	24.98***	1.43	
		(69.6)	(3.8)	(26.6)
P:R	27	5.09*	2.58	
		(15.5)	(29.1)	(55.4)
R:D	27	22.79***	0.71	
		(71.0)	(0)	(29.0)

The F ratios from the nested ANOVAS are given. The values in parentheses are the percentages of variance explained at each level. P:D = protein:DNA, P:R = protein:RNA, R:D = RNA:DNA, A.M. = adductor muscle. * = $p < 0.05$, *** = $p < 0.001$.

thesis and the activity of the RNA molecules in these tissues were more important in old and large animals than in young and small ones, while the quantity of RNA per cell remained constant. Nevertheless, the animal's age and size had no effect on the biochemical indices of the adductor muscle.

In the gills, a maximum of 65% of the biochemical ratios variability was explained by the morphological parameters, as indicated by the R^2 values (Table 1). The R^2 values fell to 12–40% for the protein:DNA and protein:RNA ratios of the digestive gland. This reflects the wide distribution of the dependent variables along the regression line.

Spatial Variability

The nested ANOVAs (Table 2) indicated a significant spatial variability for biochemical indices of both gills and adductor muscle despite the important percentage of variance explained by the error term (within the 0.5 × 0.5 m^2 quadrates). In the gills, the protein:DNA ratios were significantly different among the 2.5 × 2.5 m^2 quadrates included within the 12.5 × 12.5 m^2 quadrate and among the 0.5 × 0.5 m^2 quadrates included within each of the three 2.5 × 2.5 m^2 quadrate. In the adductor muscle, the protein:DNA, protein:RNA and RNA:DNA ratios were significantly different among the 2.5 × 2.5 m^2 quadrates but remained homogeneous among the three 0.5 × 0.5 m^2 quadrates enclosed in each 2.5 × 2.5 m^2 quadrate. The animal's age and size did not significantly vary among the 2.5 × 2.5 m^2 quadrates nor among the 0.5 × 0.5 m^2 quadrates (nested ANOVAs, $p > 0.05$), so the preceeding results were not biased by an uneven distribution of age and size among the quadrates.

The biochemical indices were plotted against animal density for the gills and the adductor muscle (Fig. 1). According to the ANOVAs, the biochemical indices of the gills were unaffected by the animal density ($F = 2.40$, 1.64 and 0.58 for protein:DNA, protein:RNA and RNA:DNA, respectively, $p > 0.05$, df = 5). However, *t*-tests showed that the mean value of protein:DNA and protein:RNA ratios were significantly higher at a density of 3 clams/0.25 m^2 than at densities ≥4 clams/0.25 m^2 ($t = 2.06$ and 2.73 for protein:DNA and protein:RNA, respectively, $p < 0.05$). No significant differences of age or size were detected between the two groups (*t*-test, $p > 0.05$). In the adductor muscle, the protein:DNA and RNA:DNA ratios significantly differed according to animal density ($F = 16.67$ and 3.30, respectively, $p < 0.05$, df = 5). The protein:DNA values were greater at a density of 4 animals/0.25 m^2 than at any other density. The RNA:DNA values were higher at a density of 4 than at densities of 3, 8, and 14 animals/0.25 m^2 (Tukey multiple comparison test, $p < 0.05$). No significant effect of density on the protein:RNA ratio was observed ($F = 2.03$, $p > 0.05$).

Delay of Response to Emersion

The temporal pattern of response to emersion revealed by the biochemical indices of growth is presented for the gills (Fig. 2) and the adductor muscle (Fig. 3).

In the gills, only the protein:DNA ratio was found to vary significantly during the course of the experiment. The protein:DNA and protein:RNA ratios of the adductor muscle were significantly different among the various periods of emersion (Table 3). According to the Tukey test ($p < 0.05$), the protein:DNA ratio of the gills was significantly lower after a 21 h period of emersion than after 4.75 h, and subsequently rose to values comparable to those observed during the first 15 h of the experiment.

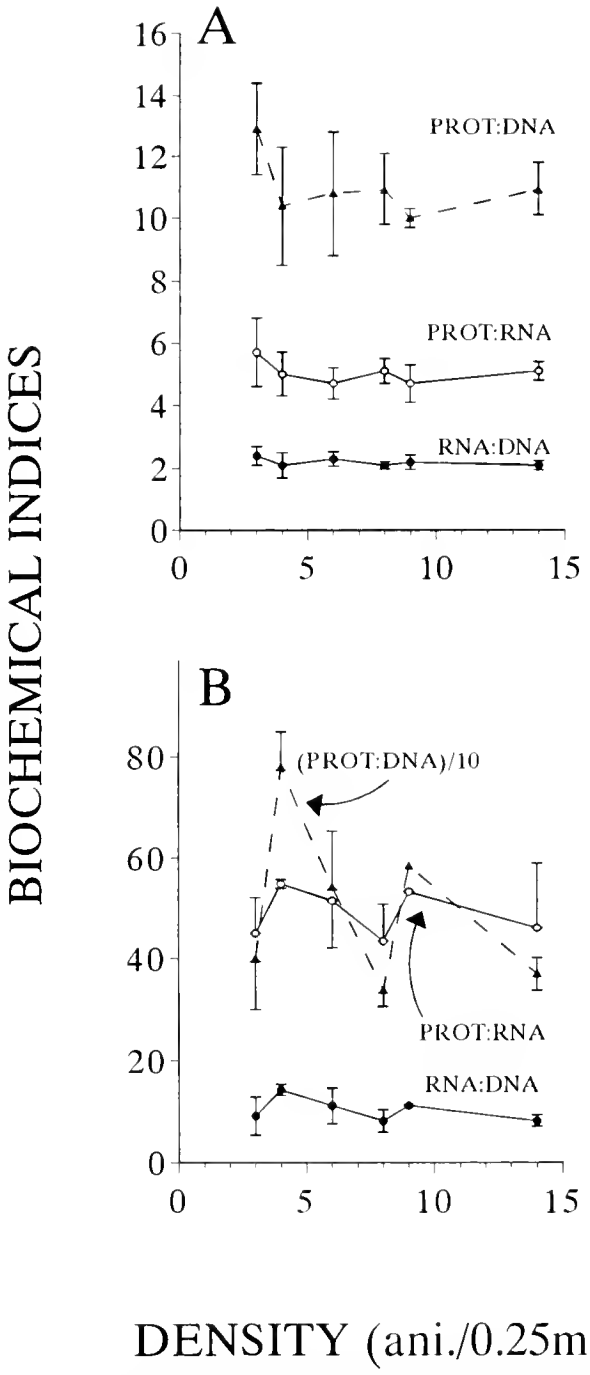

Figure 1. Variations of the biochemical indices in the gills (A) and adductor muscle (B) with respect to the animal density (mean ± s.d.). N = 3 to 9 for each point. prot:DNA = protein:DNA, prot:RNA = protein:RNA. (Protein:DNA)/10 is given for the adductor muscle.

In the adductor muscle, the protein:DNA ratio noted after 31 h of emersion was significantly higher than those observed after 1.75 and 4.75 h. The maximal protein:RNA value was attained at 13.75 h and it was significantly higher than at all the other times except 31 h. The protein:RNA ratio was also higher at 31 h than at 4.75 h. There was no statistical differences among the age and size of the animals collected at the various times (ANOVA tests, $p > 0.05$).

DISCUSSION AND CONCLUSION

Variability Induced by Age and Size

Since the animal's age and size influenced the protein:DNA and protein:RNA ratios of the gills and the digestive gland (Table 1) it would be advisable to restrict the range of these parameters when collecting clams for future research works. An alternative solution for comparative studies purposes (e.g. inter-sites comparisons) would be to select groups of clams with similar frequency distribution of age and size. Doing so would minimize unwanted effects of these parameters that could interfere with other factors one wishes to study.

The positive relationships between the net protein synthesis and the activity of RNA molecules in the gills, on one hand, and the size of the animals, on the other hand, is consistent with the observations of Widdows et al. (1979). These authors reported a positive relationship between maximum filtration rates and shell length in *Mytilus edulis*. The more important protein synthesis observed in the gills of large clams was possibly associated to the larger amount of mucus needed to trap and convey the higher quantity of particles filtered by these individuals. Higher fitration rate in large animals may in turn explain the positive relationship between biochemical ratios in the digestive gland and animal's age and size. The digestive gland is a major site of digestive enzymes production and serves as a storage organ for energetical reserves, including proteins (Bayne et al. 1976, Sastry 1979). In the adductor muscle, the biochemical ratios were independent of morpho-

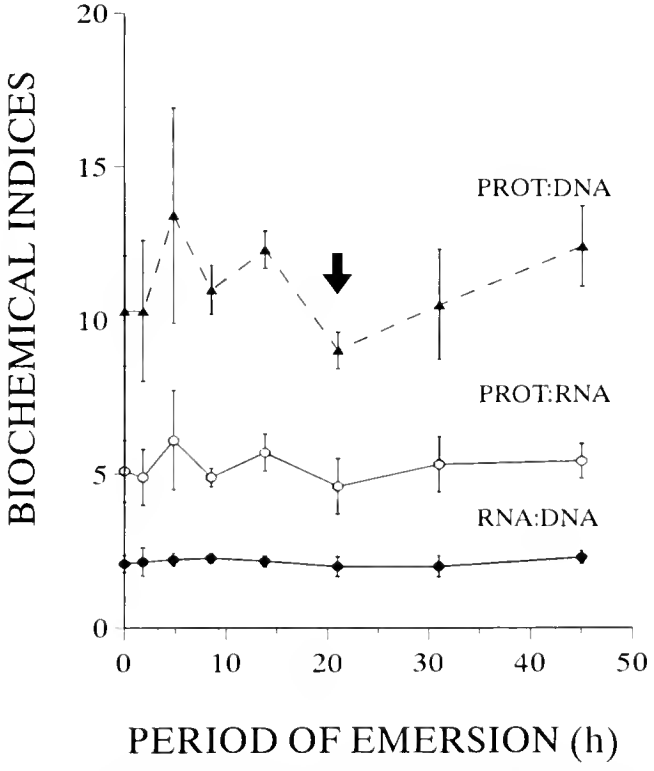

Figure 2. Temporal variation of the biochemical indices in the gills, with respect to the period of emersion (mean ± s.d.). The arrows indicate the points which are significantly different from one or more of the preceding points (Tukey test, see text for details). Abbreviations are as in Fig. 1.

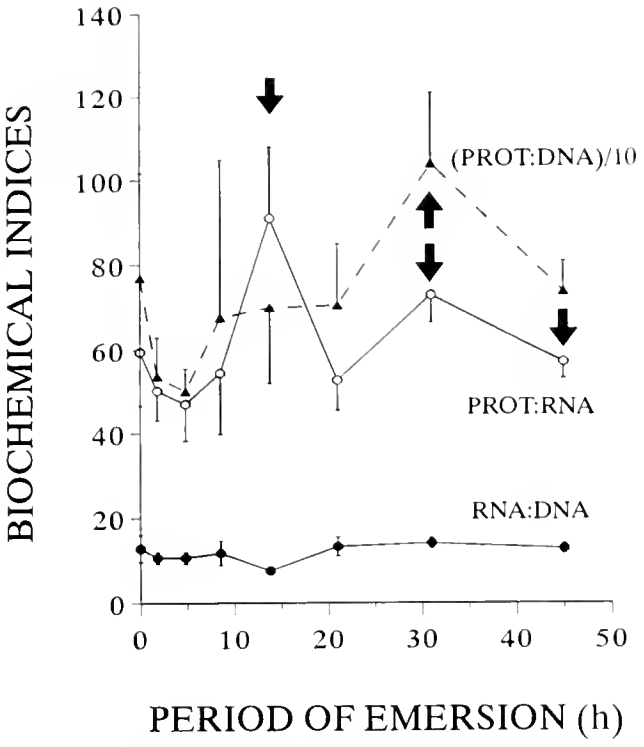

Figure 3. Temporal variation of the biochemical indices in the adductor muscle with respect to the period of emersion (mean ± s.d.). Abbreviations and symbols are as in Figure 2. (Protein:DNA)/10 is given.

logical parameters as it was also noted by Pease (1976) for *C. virginica*. Since the digestive gland acts as a buffer organ, storing and distributing metabolites to other tissues, the biochemical indices of muscular tissues do not necessarily reflect the effect of animal's size on the quantity of ingested food particles.

Spatial Variability

A non-negligible small-scale spatial variability was detected for biochemical indices of the gills and the adductor muscle (Table 2). Because of this sensitivity, the biochemical indices of growth are well suited for studies concerned with the variability of growth rate within a population. Nevertheless, this sensitivity can bias the results of studies conducted at a larger spatial scale unless an accurate sampling design is used. For example, in the context of an inter-site comparison of *M. arenaria* growth rate, the animals should be collected over areas larger than 12.5 × 12.5 m^2. This would avoid the unintentional selection of a group of animals whose biochemical indices could reach particularly low or high values. Those values would not be representative of the study area.

Numerous factors can give rise to spatial heterogeneity of bivalve growth, including animal density (e.g. Stiven and Kuenzler 1979, Peterson and Black 1987, Vincent et al. 1989), current speed (Wildish et al. 1987; Grizzle and Morin 1989), sediment disturbance (Emerson 1990), sediment resuspension rate (Grant et al. 1990, Utting 1988) and food availability (Fréchette and Bourget 1985). These factors are, in fact, closely interrelated. This experiment was not designed to take into account all these parameters, but we did know the animal density in each of the selected 0.5 × 0.5 m^2 quadrates. Although the protein:DNA and protein:RNA ratios of the gills were significantly greater at a density of 3 clams/0.25 m^2 than at densities ≥4 clams/0.25 m^2 (Fig. 1) no linear relationships could be detected between the biochemical indices of the studied tissues and the number of animals per quadrat. Animal density thus appears to have little effect on protein synthesis in the gills and the adductor muscle at a 150 m^2 scale. Nevertheless the range of densities was narrow and the biochemical indices may have responded to this factor, had the range of densities been more important. Indeed, previous studies have reported a decrease of shell or flesh growth rate at high animal density in various bivalve species (Peterson and Black 1987, Peterson and Beal 1989, Vincent et al. 1989). In the present study, environmental factors other than density may have been the cause of the spatial heterogeneity of the biochemical indices. There are good indications that small-scale variations in the substrate can have an impact on growth. Turner and Miller (1991) noticed higher shell growth rates and lower pseudofaeces production in *Mercenaria mercenaria* living at the crest of ripples compared to those occupying the trough. In the same species, Grizzle and Morin (1989) noted a tendency towards higher growth rates in sand compared with mud within a 12 m transect. More detailed studies which would take into account the micro-variations of the environment are needed to get a better understanding of the small-scale spatial heterogeneity of the biochemical indices.

TABLE 3.

ANOVAS with the biochemical indices of the gills and the adductor muscle as dependent variables and the period of emersion as independent variable.

Tissues	Biochemical Indices	F
Gills (N = 38)	Protein:DNA	2.63*
	Protein:RNA	1.12
	RNA:DNA	0.73
Adductor muscle (N = 35)	Protein:DNA	2.61*
	Protein:RNA	5.17***
	RNA:DNA	2.36*

df = 7. Symbols are as in Table 1.

Time of Response of the Biochemical Indices to Emersion

The biochemical ratios of the gills and the adductor muscle appeared to be little affected by emersion, as shown by the kinetics of their response to emersion (Figs. 2 and 3). They did not significantly differ from the values observed during the first hours of the experimentation until the animals were exposed for at least 13.75 h. This long response delay suggests that the length of emersion before collection in the field should not affect the biochemical ratios of the gills or the adductor muscle. This relative emersion independence of the biochemical indices would reflect the adaptation of intertidal bivalves to their highly variable environment, where they are regularly exposed to hypoxia at low tide. Similarly, metabolic rate of intertidal bivalves has been shown to be unaffected by short-term changes in temperature within the normal temperature range (Anderson 1978, Hoffmann 1983, Wilbur and Hilbish 1989).

Pedersen (1992) has demonstrated the existence of an endog-

enously controlled alternance of high and low metabolic activity for *M. arenaria*. In the present study, it is unlikely that such an endogeneous rythm could have caused the temporal variations of the biochemical indices during the course of emersion. Indeed, the heat dissipation and O_2 uptake rythms that were reported by Pedersen (1992) for intertidal clams showed marked inter-individual variability: the interval between the onset of two successive activity periods ranged from 7 to 21 h. Furthermore, patterns of activity tended to weaken with decreasing O_2 tension in the water. The endogeneous rythms amplitude should then have been attenuated in the hypoxic conditions of the present experiment. The emersion stress by itself could have been sufficient to produce the observed temporal variations.

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GENETIC CHARACTERISTICS AND RELATIONSHIPS OF FIVE RAZOR CLAM (*SILIQUA PATULA* DIXON) POPULATIONS ALONG THE PACIFIC COAST OF NORTH AMERICA

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ABSTRACT We electrophoretically examined razor clams (*Siliqua patula* Dixon) from five intertidal locations along the Pacific coasts of Alaska, British Columbia, Washington, and Oregon to determine the level of genetic variation within the species and among regions. All five locations exhibited high levels of within population genetic diversity. The average heterozygosity ranged from 0.25 to 0.27 and the mean number of alleles per locus ranged from 3.4 to 4.6, based on 24 loci. We found no reduction of genetic diversity within the Washington populations compared to populations that had not experienced large mortalities due to the pathogen NIX. The genetic diversity among regions was low, and alleles that occurred at a frequency of 0.05 or greater in one collection, were generally observed in all other collections. However, we found significant differences in allele frequencies among collections. The most genetically similar populations were the two from Washington, while the greatest genetic differences occurred when the two Washington collections were combined and compared to the Alaska sample. Differences in the susceptibility of razor clams to NIX among locations may have a genetic basis.

KEY WORDS: razor clam, *Siliqua*, genetic, electrophoresis, enzymes, nuclear inclusion X (NIX)

INTRODUCTION

The genetic characterization of naturally occurring populations is playing an increasingly important role in the management of fisheries resources and in conservation measures taken to protect animals that are threatened or endangered. Such characterizations achieve their greatest utility in management practice only when significant levels of genetic variability can be detected within and among populations.

Historically, several approaches have been used to identify inter and intraspecific variability including serological, immunological, and DNA studies (Utter 1991). Currently, one of the most widely applied methods for determining the genetic structure of a species is the analysis of enzyme polymorphisms as revealed by starch-gel electrophoresis. Allele frequency data gathered in this way from different regions throughout a species' range may be used to examine the historical patterns of gene flow among populations. However, prior to making comparisons among populations it must be determined that an adequate amount of electrophoretically detectable genetic variation exists within the species.

This paper describes the results of an electrophoretic examination of 49 enzyme encoding loci from the Pacific razor clam (*Siliqua patula* Dixon). In addition to quantifying genetic variation within the species, we compared the average heterozygosity and allelic diversity found in Washington State populations to populations from Oregon, Alaska, and British Columbia. Washington populations have experienced large mortalities due to outbreaks of a pathogen belonging to the eubacterial class *Proteobacteria* (Kerk et al. 1992) and given the operational name Nuclear Inclusion X (NIX) by Elston (1986). Outbreaks of NIX are not known to occur or occur only at very low levels in the other three regions.

MATERIALS AND METHODS

Razor clams were collected between fall of 1990 and spring of 1991 from five intertidal locations along the Pacific coasts of Alaska, British Columbia, Washington, and Oregon (Fig. 1). Collection sites and sample sizes were as follows: Clam Gulch (Kenai Peninsula), AK (N = 102); McIntyre Bay, Graham Island (Queen Charlotte Islands), B.C. (N = 96); Copalis Beach, WA (N = 100); Long Beach, WA (N = 100); Seaside, OR (N = 43). The clams were delivered live to the laboratory where they were immediately dissected and isolated tissues were stored at −80°C for subsequent electrophoretic analysis. Enzyme extracts were prepared by homogenizing tissues from each clam in equal volumes of TC-1 gel buffer (Shaw and Prasad 1970) containing 10 mg pyridoxal 5′-phosphate per 100 ml buffer. Homogenates were centrifuged at 1000 × g for 5 minutes and the supernatants used for electrophoresis. Extensive descriptions of the general starch-gel electrophoretic methodology we employed exist elsewhere (see Aebersold et al. 1987, Utter et al. 1974, Shaklee and Keenan 1986) and will not be discussed here.

Gels consisted of 11.5% hydrolyzed potato starch in buffer. A variety of electrophoretic buffer systems were used in the initial screening protocol. These were CAME6.8 and CAME6.3 modified from Clayton and Tretiak (1972) with 0.001 M EDTA 2 Na dihydrate, CAM6.1 and CAM6.05 (Clayton and Tretiak 1972), TC-4 (buffer "a" of Schaal and Anderson 1974), TRIS-GLY (Holmes and Masters, 1970), LiOH-RW (Ridgeway et al., 1970), EBT (Boyer et al. 1963), and TECB (Shaklee and Tamura 1981).

Gel slices were stained using 2% agar overlays following the recipes of Harris and Hopkinson (1976), Shaw and Prasad (1970), and Grant and Cherry (1985). When an enzyme exhibited multiple isozymes the loci were numbered sequentially from number one beginning at the least anodal locus. Three enzymes (AAT, IDH, and MDH) exhibited multiple isozymes that did not form interlocus heteropolymers. Enzyme and gene nomenclature follow the guidelines of Shaklee et al. (1990).

Preliminary enzyme survey protocols included siphon muscle, anterior adductor muscle, mantle fold, foot muscle, gill, gonad, and style. Based on this survey, we determined that adductor muscle yielded the largest number of resolvable loci of any single tissue. Therefore, we used this tissue throughout the rest of the analysis except for the Oregon clams where siphon muscle was used as this was the only tissue available from this collection.

Variant alleles were identified in terms of the mobility of the isozymes they code for, relative to the mobility of the most common isozyme which was designated 100 (e.g., *GPI*100*, *GPI*112*, *GPI*86*). Alleles that coded for isozymes that migrated

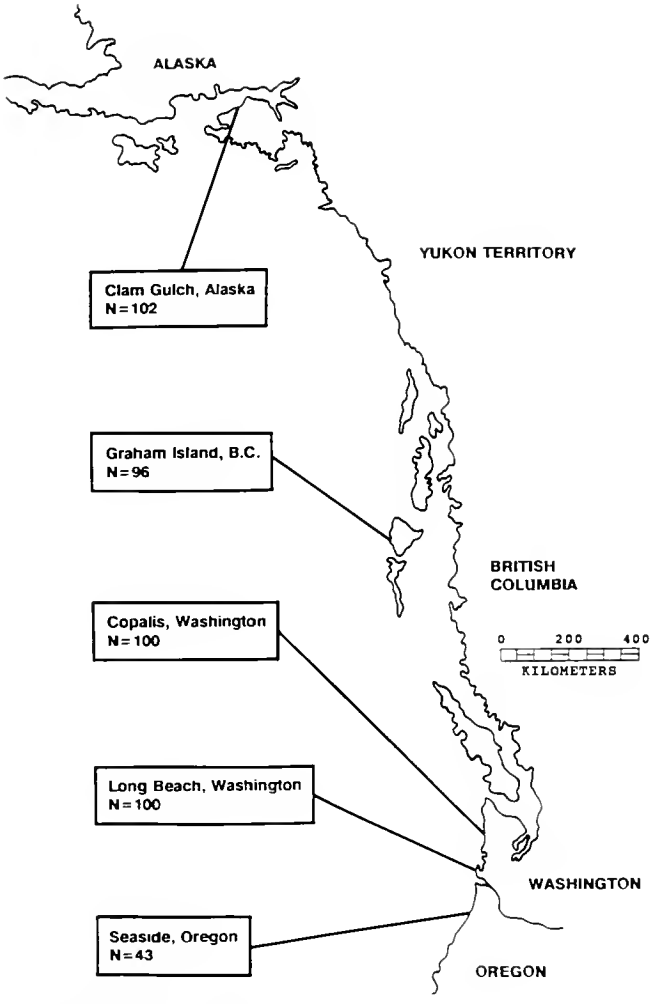

Figure 1. Sampling locations and sample sizes of Pacific razor clam (*Siliqua patula*).

towards the cathode are identified by a minus sign in front of the mobility. Alleles with similar mobilities were confirmed by running them in close proximity to previously identified mobility standards. A locus was considered polymorphic if more than one allele was detected. Statistical computer analyses of electrophoretic data were conducted using BIOSYS-1 (Swofford and Selander 1981).

RESULTS

Relative rankings of buffer/enzyme combinations are summarized in Table 1. Relative staining activities and tissue distribution of enzymes are listed in Table 2. Of the enzymes assayed, ten did not exhibit sufficient activity to determine whether or not genetic variation existed. These were adenosine deaminase (ADA), alcohol dehydrogenase (ADH), adenylate kinase (AK), creatine kinase (CK), fructose-bisphosphate aldolase (FBALD), fumarate hydratase (FH), glutamate dehydrogenase (GLUDH), glycerol-3-phosphate dehydrogenase (G3PDH), L-iditol dehydrogenase (IDDH), and phosphoglycerate mutase (PGAM). Sixteen enzymes demonstrated at least one locus exhibiting evidence of variation but locus/loci were unscorable due to lack of resolution, activity, or a genetic model to account for observed banding patterns. These were, acid phosphatase (ACP), aconitate hydratase (AH), alanine aminotransferase (ALAT), esterase-D (ESTD), *N*-acetyl-beta-glucosaminidase (bGLUA), beta-*N*-actylgalactosaminidase (bGALA), glutathione reductase (GR), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), guanine deaminase (GDA), lactoylglutathione lyase (LDL), alpha-mannosidase (aMAN), peptidase-3 (PEPS), proline dipeptidase (PEPD), peptidase (leu-tyr) (PEP-LT), tripeptide aminopeptidase (PTPB), and purine-nucleoside phosphorylase (PNP). One enzyme, aspartate aminotransferase (AAT) exhibited one locus (*AAT-3**) that appeared to be monomorphic but had insufficient activity to be scored reliably in all collections.

Of 49 enzyme encoding loci screened, 24 loci were resolved adequately for reliable scoring and were examined in all five collections for subsequent comparative analyses. Only one of these enzymes, strombine dehydrogenase, was monomorphic in each of the five areas. Allele frequencies and sample sizes for the 23 variable loci are presented in Table 3.

Because of the high number of alleles encoding isozymes with only slightly different mobilities at the GPI* locus, we combined some of these alleles into allele classes. The following mobilities were grouped into a single allele class and scored as *GPI*112: GPI*103, GPI*112, GPI*113, GPI*114, GPI*115,* and *GPI*116.* Similarly, *GPI*86, GPI*87, GPI*88, GPI*89,* and *GPI*91* were grouped and scored as *GPI*86; GPI*70, GPI*72, GPI*73, GPI*74, GPI*76, GPI*77,* and *GPI*78* were grouped and scored as *GPI*73;* and *GPI*123, GPI*124,* and *GPI*127* were grouped and scored as *GPI*127.* Allele classes were assigned the mobility of the allele occurring most frequently within that class.

Genetic Diversity Within Populations

We examined the genotype counts at each locus in each population for agreement with Hardy-Weinberg equilibrium expectations by chi-square analysis. Because the chi-square test is suspect where the expected frequencies of some classes are low (Sokal and Rohlf 1969) and many loci in the razor clam had a large number of alleles which resulted in many expected genotype classes, we pooled the genotypes into three classes. The classes were: 1) homozygotes for the most common allele; 2) heterozygotes for the most common allele and one of the other alleles; and 3) all other genotypes. One of the 22 tests in the Long Beach collection was significant; *GPI** had an excess of heterozygotes ($P = 0.022$). In the Copalis collection, *MPI** had a deficit of heterozygotes ($P = 0.010$) (1 of 17 tests). The *PGM** locus (1 of 13 tests) had an excess of heterozygotes in the Oregon collection ($P = 0.003$). Two of eighteen comparisons in the Alaska collection disagreed with expectations. The *AAT-1** locus had an excess of heterozygotes ($P = 0.044$) and *PEPC** had a deficit of heterozygotes ($P = 0.004$). Two loci (of 17) in the McIntyre Bay collection had deficits of heterozygotes (LDH* $P = 0.026$, *MPI** $P = 0.024$).

The number of loci out of Hardy-Weinberg equilibrium was slightly higher than expected by chance at the 0.05 level, but the deviations were about evenly split between excesses and deficits of heterozygotes and *MPI** was the only locus to exhibit more than one deviation from Hardy-Weinberg equilibrium expectations.

We also examined the variable loci in each collection to determine if there was a consistent excess or deficit of heterozygotes from Hardy-Weinberg expectations. A consistent deficit could indicate that more than one gene pool was present in a collection, whereas a consistent excess of heterozygotes could indicate a recent mixing of two distinct gene pools in the previous generation or natural selection for heterozygous individuals. No significant patterns of within population heterozygosity were revealed.

TABLE 1.

Relative ranking of buffer/enzyme combinations used in razor clam (*Siliqua patula*) genetics study.

	Buffer								
Enzyme	A	B	C	D	E	F	G	H	I
AAT-1	+ + +	n.a.	n.a.	n.a.	+ + +	+ + +	n.a.	n.a.	n.a.
AAT-2	+ + +	n.a.	n.a.	n.a.	+	+	n.a.	n.a.	n.a.
AAT-3	+ + +	n.a.	n.a.	n.a.	+	+	n.a.	n.a.	n.a.
ACP	+	n.a.	n.a.	n.a.	n.a.	n.a.	+	n.a.	n.a.
AH	+	n.a.	n.a.	n.a.	+	n.a.	n.a.	+	n.a.
ADA	n.a.	n.a.	n.a.	n.a.	n.a.	+	n.a.	n.a.	n.a.
AK	n.a.	n.a.	n.a.	n.a.	n.a.	+	n.a.	+	n.a.
ADH	n.a.	n.a.	n.a.	n.a.	n.a.	+	n.a.	n.a.	n.a.
ALAT	+	n.a.	n.a.	n.a.	n.a.	+	n.a.	+ + +	+ + +
ARK	+ +	n.a.	n.a.	n.a.	n.a.	+ + +	n.a.	+ + +	n.a.
CK	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	+	n.a.
ENO	+ + +	+ + +	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
ESTD	+	n.a.	n.a.	n.a.	n.a.	+ + +	n.a.	+ + +	n.a.
FBALD	+	n.a.	n.a.	n.a.	n.a.	+	n.a.	n.a.	n.a.
FDHG	n.a.	n.a.	n.a.	n.a.	n.a.	+ + +	n.a.	+	+ +
FH	+	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
bGLUA	+	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	+
bGALA	n.a.	n.a.	n.a.	n.a.	n.a.	+	n.a.	n.a.	n.a.
GPI	n.a.	n.a.	n.a.	n.a.	n.a.	+ + +	+ + +	n.a.	n.a.
GLUDH	n.a.	n.a.	n.a.	n.a.	+	n.a.	n.a.	n.a.	n.a.
GR	+ +	n.a.	n.a.	n.a.	+	+ +	+ +	+ +	+ +
GAPDH	+ +	n.a.	n.a.	n.a.	+	n.a.	n.a.	+ +	n.a.
G3PDH	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	+	n.a.
GDA	+	n.a.	n.a.	n.a.	+ +	+	n.a.	n.a.	n.a.
IDDH	+	n.a.	n.a.	n.a.	+	n.a.	n.a.	n.a.	n.a.
IDHP-1	+ + +	+ + +	+ +	n.a.	+ + +	n.a.	n.a.	n.a.	n.a.
IDHP-2	+ + +	+ + +	+ +	n.a.	+ + +	n.a.	n.a.	n.a.	n.a.
LDH	+	n.a.	+	n.a.	+	+ + +	n.a.	+	n.a.
LGL	+	+ +	n.a.	n.a.	n.a.	+	n.a.	+ +	+ +
MDH-1	+ + +	+ + +	n.a.	n.a.	+ +	n.a.	n.a.	n.a.	n.a.
MDH-2	+ + +	+ + +	n.a.	n.a.	+ +	n.a.	n.a.	n.a.	n.a.
MEP	+ + +	n.a.	n.a.	n.a.	+ + +	n.a.	n.a.	n.a.	n.a.
MPI	n.a.	n.a.	+	n.a.	n.a.	+ + +	n.a.	+ +	n.a.
aMAN	+	n.a.	n.a.	n.a.	n.a.	+	n.a.	+	+
OCDH	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	+ + +	n.a.
PEPA	n.a.	n.a.	n.a.	+	n.a.	+ +	+ +	n.a.	+
PEPB	n.a.	n.a.	+	+ +	+	+ +	+	n.a.	n.a.
PEPC	+ +	n.a.	+ + +	+ +	+	+ +	n.a.	+	n.a.
PEPD	n.a.	n.a.	n.a.	+	+ +	+	n.a.	n.a.	n.a.
PEP-LT	n.a.	n.a.	n.a.	+	n.a.	+ +	+	n.a.	n.a.
PEPS	n.a.	n.a.	n.a.	+	n.a.	+ +	+	n.a.	+
PGAM	+	n.a.	n.a.	n.a.	+	n.a.	n.a.	n.a.	n.a.
PGDH	+ + +	n.a.	n.a.	n.a.	+ + +	n.a.	n.a.	n.a.	n.a.
PGK	+ + +	+	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PGM	+	n.a.	n.a.	n.a.	+	+ + +	+	n.a.	n.a.
PNP	+	n.a.	n.a.	n.a.	+	+	+	n.a.	n.a.
SOD	n.a.	n.a.	n.a.	n.a.	n.a.	+	+ +	+	n.a.
STDH	n.a.	n.a.	n.a.	n.a.	n.a.	+ +	+ +	n.a.	n.a.
TPI	n.a.	n.a.	n.a.	n.a.	n.a.	+ + +	+ + +	n.a.	n.a.

Ranking symbols are as follows: n.a. = not assayed, + + + = good, + + = fair, + = poor. Buffer symbols are as follows: A = CAME6.8, B = CAME6.3, C = CAM6.1, D = CAM6.05, E = TC-4, F = TRIS-GLY, G = LiOH-RW, H = EBT, I = TECB. Relative rankings are based on each buffers' capacity to resolve variability into interpretable banding patterns. Rankings for enzymes encoded by monomorphic loci are based on activity only.

Genetic Diversity Among Populations

We used a contingency chi-square test summed over all loci (where the frequency of the most common allele was less than 0.95 in any one collection) to test for significant differences in allelic counts between collections. Two loci, *MDH-2** and *MPI** had significantly different allele counts between the two Washington collections, but the probability summed over all loci was not significant ($P = 0.057$). The two Washington collections were tested together against each of the other three collections. The

TABLE 2.

Tissue distribution and relative staining activity of 49 enzymes in the razor clam (*Siliqua patula*).

Enzyme	Tissue					
	Anterior Adductor Muscle	Mantle Fold Muscle	Siphon Muscle	Gonad	Gill	Style
AAT-1	+ + + +	+ + +	+ + + +	+ + +	+ +	n.d.
AAT-2	+ + +	+ +	+ + +	+	n.d.	n.d.
AAT-3	+ + +	+ +	+ + +	+	n.d.	n.d.
ACP	n.a.	n.d.	+	+ +	+ +	n.d.
AH	n.a.	+	+ +	+	n.d.	n.d.
ADA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AK	n.a.	n.a.	n.d.	n.a.	n.a.	n.a.
ADH	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ALAT	+ + + +	+ +	+ + +	+ + +	n.d.	n.d.
ARK	+ + + +	+ + +	+ + + +	+	n.a.	n.d.
CK	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ENO	+ + + +	+ +	+ + + +	+ +	n.a.	n.d.
ESTD	+ + +	+	+ + +	+ +	n.d.	n.d.
FBALD	n.a.	+	+ + +	n.a.	n.d.	n.d.
FDHG	+ + +	+	+ + +	+ +	n.a.	n.d.
FH	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.
bGLUA	n.a.	+	+	+	n.a.	n.a.
bGALA	n.a.	n.a.	+	n.a.	n.a.	n.a.
GPI	+ + + +	+ + + +	+ + + +	+ + + +	+ +	n.d.
GLUDH	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
GR	+ + +	+ + +	+ + +	+ + +	+ + +	n.d.
GAPDH	+ + + +	+ + +	+ + + +	+	n.a.	n.d.
G3PDH	n.a.	n.a.	n.d.	n.a.	n.a.	n.a.
GDA	n.a.	n.a.	n.a.	+ +	+ +	n.a.
IDDH	n.a.	n.a.	n.d	n.d.	n.d.	n.d.
IDHP-1	+ + + +	+ + +	+ + + +	+ + + +	+ +	n.d.
IDHP-2	+ + + +	+ + +	+ + + +	+ + + +	+ +	n.d.
LDH	+ + +	n.a.	+ + +	+ +	n.d.	+
LGL	+	n.a.	+	n.a.	n.a.	+
MDH-1	+ + + +	+ + + +	+ + +	+ + + +	n.a.	n.a.
MDH-2	+ + + +	+ + + +	+ + +	+ + + +	n.a.	n.a.
MEP	+ + + +	+ + + +	+ + + +	+ + + +	n.a.	n.a.
MPI	+ + +	n.a.	+ + + +	+ + +	+ + +	n.d.
aMAN	n.a.	n.a.	+ + +	+ + + +	+ + + +	n.a.
OCDH	+ + + +	+ + +	+ + + +	+	n.d.	n.d.
PEPA	+ + + +	+ + +	+ + + +	+ + +	+	n.d.
PEPB	+ + + +	+	+ + + +	+ + + +	n.d.	n.d.
PEPC	+ + + +	+	+ + + +	+ + +	n.d.	n.d.
PEPD	n.a.	+ + +	+ + + +	+ + + +	n.d.	n.a.
PEP-LT	n.a.	+	+	+ +	n.d.	n.d.
PEPS	n.a.	+	+ + +	+ + + +	n.d.	n.d.
PGAM	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.
PGDH	+ + + +	n.a.	+ + + +	n.d	n.a.	n.a.
PGK	+ + + +	+ + +	+ + + +	n.a.	n.a.	n.a.
PGM	+ + + +	+ + + +	+ + + +	+ + + +	n.d.	n.d.
PNP	n.a.	+	+ + +	+ + +	n.a.	n.a.
SOD	+ + + +	+	+ + + +	+ + + +	+ +	n.a.
STDH	+ + + +	+ + +	+ + + +	n.a.	n.a.	+ +
TPI	+ + + +	+ + + +	+ + + +	+ + + +	+ + +	+ + +

Relative staining activity was not necessarily associated with interpretability of observed banding patterns. Symbols are as follows: n.a. = not assayed, + + + + = very strong, + + + = strong, + + = weak, + = trace, n.d. = not detected.

probability summed over all loci was significant at the 0.05 level for each of these three tests. The heterogeneity test between McIntyre Bay and Clam Gulch was also significant.

We used the genetic chord distance measure of Cavalli-Sforza and Edwards (1967) to calculate the genetic distances among the five collections (Table 4). Cluster analysis using the unweighted pair-group method with arithmetic averaging (UPGMA) described by Sneath and Sokal (1973) was used to visualize the genetic distance relationships (Fig. 2).

The average heterozygosity and mean number of alleles per locus were similar in all five collections (Table 5). Often, large population mortalities result in genetic bottlenecks that reduce the

TABLE 3.

Population allele frequencies of variable loci from the razor clam (*Siliqua patula*).

	Population				
Locus	**Clam Gulch, AK**	**McIntyre Bay, B.C.**	**Copalis Beach, WA**	**Long Beach, WA**	**Seaside, OR**
*AAT-1**					
(N)	102	96	100	100	41
**-100*	0.618	0.714	0.660	0.690	0.671
**-180*	0.299	0.250	0.325	0.305	0.293
**-230*	0.029	0.010	0.005	0.000	0.012
**120*	0.039	0.010	0.005	0.005	0.012
**-375*	0.000	0.010	0.000	0.000	0.000
**-75*	0.005	0.005	0.005	0.000	0.000
**340*	0.005	0.000	0.000	0.000	0.000
**-370*	0.005	0.000	0.000	0.000	0.000
**-300*	0.000	0.000	0.000	0.000	0.012
*AAT-2**					
(N)	101	94	100	100	42
**100*	0.896	0.920	0 915	0.900	.905
**140*	0.050	0.059	0.060	0.070	0.095
**150*	0.010	0.000	0 015	0.010	0.000
**71*	0.000	0.005	0.005	0.005	0.000
**52*	0.030	0.005	0.005	0.005	0.000
**86*	0.010	0.000	0.000	0.000	0.000
**1*	0.005	0.011	0.000	0.010	0.000
*ALAT-1**					
(N)	101	96	100	100	41
**100*	0.663	0.568	0.525	0.570	0.622
**126*	0.079	0.229	0.240	0.215	0.207
**67*	0.243	0.182	0.195	0.185	0.159
**50*	0.005	0.000	0.025	0.020	0.012
**150*	0.010	0.021	0.015	0.010	0.000
*ARK**					
(N)	102	96	100	100	42
**100*	0.975	0.995	0.990	0.985	0.988
**150*	0.000	0.000	0 005	0.000	0.012
**60*	0.005	0.000	0.000	0.000	0.000
**130*	0.000	0.000	0.005	0.005	0.000
**125*	0.020	0.000	0.000	0.010	0.000
**120*	0.000	0.005	0.000	0.000	0.000
*ENO**					
(N)	102	93	100	99	41
**100*	0.858	0.876	0.795	0.803	0.890
**77*	0.127	0.124	0.205	0.192	0.110
**113*	0.015	0.000	0.000	0.005	0.000
*ESTD-2**					
(N)	102	96	100	100	42
**100*	0.740	0.812	0.810	0.830	0.798
**89*	0.225	0.161	0.170	0.150	0.179
**81*	0.010	0.010	0.005	0.005	0.012
**105*	0.000	0.010	0.015	0.000	0.000
**102*	0.010	0.000	0.000	0.000	0.012
**91*	0.010	0.000	0.000	0.015	0.000
**77*	0.000	0.005	0.000	0.000	0.000
**68*	0.005	0.000	0.000	0.000	0.000
*FDHG**					
(N)	102	96	100	100	42

TABLE 3.

continued

Locus	Population				
	Clam Gulch, AK	McIntyre Bay, B.C.	Copalis Beach, WA	Long Beach, WA	Seaside, OR
**100*	0.975	0.943	0.925	0.930	0.929
**67*	0.000	0.000	0.015	0.010	0.000
**74*	0.015	0.016	0.020	0.005	0.000
**128*	0.000	0.031	0.015	0.010	0.012
**83*	0.005	0.010	0.025	0.030	0.060
**154*	0.005	0.000	0.000	0.000	0.000
**120*	0.000	0.000	0.000	0.010	0.000
**137*	0.000	0.000	0.000	0.005	0.000
*GPI**					
(N)	102	96	100	100	41
**100*	0.289	0.344	0.295	0.335	0.329
**112*[a]	0.407	0.354	0.365	0.335	0.305
**86*[a]	0.127	0.161	0.200	0.195	0.183
**73*[a]	0.098	0.115	0.090	0.105	0.134
**127*[a]	0.078	0.005	0.025	0.015	0.037
**133*	0.000	0.000	0.010	0.000	0.012
**59*	0.000	0.021	0.015	0.010	0.000
**40*	0.000	0.000	0.000	0.005	0.000
*IDHP-1**					
(N)	102	96	100	100	42
**100*	0.912	0.911	0.945	0.910	0.964
**70*	0.005	0.031	0.035	0.045	0.000
**109*	0.083	0.047	0.020	0.035	0.036
**135*	0.000	0.000	0.000	0.005	0.000
**125*	0.000	0.000	0.000	0.005	0.000
**90*	0.000	0.005	0.000	0.000	0.000
**118*	0.000	0.005	0.000	0.000	0.000
*IDHP-2**					
(N)	102	96	100	100	42
**100*	0.495	0.604	0.550	0.585	0.595
**93*	0.436	0.380	0.395	0.380	0.357
**89*	0.054	0.005	0.010	0.000	0.024
**113*	0.000	0.010	0.030	0.005	0.024
**81*	0.000	0.000	0.000	0.005	0.000
**91*	0.010	0.000	0.000	0.000	0.000
**108*	0.000	0.000	0.010	0.015	0.000
**67*	0.005	0.000	0.000	0.000	0.000
**104*	0.000	0.000	0.005	0.010	0.000
*LDH**					
(N)	102	96	100	100	42
**100*	0.907	0.896	0.905	0.890	0.917
**90*	0.000	0.000	0.005	0.000	0.000
**93*	0.000	0.010	0.005	0.020	0.000
**91*	0.020	0.083	0.075	0.060	0.060
**101*	0.074	0.010	0.010	0.030	0.024
*MDH-1**					
(N)	102	96	100	100	42
**100*	1.000	1.000	1.000	0.995	1.000
**37*	0.000	0.000	0.000	0.005	0.000
*MDH-2**					
(N)	102	96	100	100	42
**100*	0.887	0.891	0.945	0.990	0.976
**135*	0.113	0.089	0.055	0.005	0.024

TABLE 3.

continued

Locus	Population				
	Clam Gulch, AK	McIntyre Bay, B.C.	Copalis Beach, WA	Long Beach, WA	Seaside, OR
*128	0.000	0.000	0.000	0.005	0.000
*61	0.000	0.005	0.000	0.000	0.000
*147	0.000	0.005	0.000	0.000	0.000
*78	0.000	0.005	0.000	0.000	0.000
*86	0.000	0.005	0.000	0.000	0.000
*MEP**					
(N)	102	96	100	100	42
*100	0.931	0.927	0.945	0.950	0.917
*86	0.069	0.068	0.055	0.045	0.083
*80	0.000	0.005	0.000	0.005	0.000
*MPI**					
(N)	102	96	100	100	42
*100	0.559	0.536	0.625	0.450	0.548
*94	0.314	0.292	0.220	0.350	0.167
*85	0.074	0.068	0.065	0.085	0.095
*104	0.049	0.094	0.090	0.105	0.167
*108	0.000	0.005	0.000	0.010	0.024
*77	0.000	0.005	0.000	0.000	0.000
*74	0.005	0.000	0.000	0.000	0.000
*OCDH**					
(N)	102	34	100	100	42
*100	1.000	0.971	1.000	0.990	1.000
*124	0.000	0.029	0.000	0.005	0.000
*91	0.000	0.000	0.000	0.005	0.000
*PEPA**					
(N)	102	96	100	100	41
*100	0.828	0.797	0.765	0.790	0.780
*102	0.044	0.120	0.100	0.105	0.122
*104	0.029	0.000	0.045	0.010	0.073
*95	0.020	0.062	0.045	0.060	0.000
*90	0.064	0.010	0.035	0.010	0.024
*74	0.015	0.010	0.010	0.025	0.000
*PEPC**					
(N)	102	96	100	100	42
*100	0.868	0.927	0.945	0.870	0.881
*83	0.025	0.005	0.010	0.010	0.000
*76	0.005	0.010	0.010	0.020	0.000
*88	0.039	0.010	0.010	0.025	0.036
*107	0.015	0.005	0.025	0.015	0.060
*92	0.044	0.010	0.000	0.020	0.012
*68	0.000	0.010	0.000	0.005	0.012
*61	0.000	0.005	0.000	0.015	0.000
*113	0.005	0.016	0.000	0.020	0.000
*PGDH**					
(N)	102	96	100	100	42
*-100	0.843	0.870	0.865	0.860	0.810
*300	0.074	0.078	0.095	0.085	0.095
*-260	0.000	0.031	0.010	0.005	0.012
*590	0.039	0.000	0.005	0.000	0.000
*-200	0.000	0.005	0.005	0.000	0.012
*445	0.010	0.000	0.000	0.010	0.012
*735	0.010	0.000	0.000	0.000	0.000
*275	0.015	0.010	0.020	0.025	0.048

TABLE 3.

continued

Locus	Population				
	Clam Gulch, AK	McIntyre Bay, B.C.	Copalis Beach, WA	Long Beach, WA	Seaside, OR
*-283	0.010	0.005	0.000	0.010	0.000
*270	0.000	0.000	0.000	0.005	0.012
*PGK**					
(N)	100	96	100	100	42
*100	0.700	0.714	0.735	0.725	0.690
*124	0.210	0.172	0.170	0.200	0.214
*61	0.050	0.052	0.050	0.040	0.048
*86	0.000	0.000	0.005	0.000	0.012
*38	0.020	0.000	0.000	0.005	0.000
*122	0.000	0.026	0.030	0.020	0.012
*152	0.000	0.005	0.005	0.005	0.000
*76	0.005	0.031	0.005	0.005	0.012
*110	0.015	0.000	0.000	0.000	0.000
*49	0.000	0.000	0.000	0.000	0.012
*PGM**					
(N)	101	95	100	100	41
*100	0.366	0.474	0.455	0.510	0.293
*110	0.406	0.263	0.350	0.345	0.402
*91	0.045	0.068	0.105	0.035	0.134
*118	0.094	0.132	0.050	0.075	0.122
*84	0.035	0.021	0.005	0.020	0.012
*79	0.000	0.000	0.015	0.005	0.000
*125	0.054	0.042	0.010	0.010	0.037
*71	0.000	0.000	0.005	0.000	0.000
*60	0.000	0.000	0.05	0.000	0.000
*SOD**					
(N)	102	96	55	100	42
*100	0.995	0.995	1.000	0.995	1.000
*80	0.005	0.005	0.000	0.000	0.000
*124	0.000	0.000	0.000	0.005	0.000
*TPI**					
(N)	102	32	100	100	42
*100	0.975	0.984	1.000	0.990	0.988
*68	0.020	0.016	0.000	0.005	0.000
*157	0.000	0.000	0.000	0.005	0.000
*136	0.005	0.000	0.000	0.000	0.012

N = the number of razor clams successfully scored in each collection.

[a] Allele designation represents multiple alleles of similar mobilities.

amount of genetic variation within populations. However, there appears to be no marked decrease of heterozygosity or average number of alleles per locus in the Washington collections due to the large mortality experienced with the NIX outbreaks.

DISCUSSION

The mean heterozygosity in razor clam is high (0.261 averaged over all five collections) compared to other molluscs. Fujio et al. (1983) conducted an electrophoretic survey of 25 species of marine molluscs. They reported mean heterozygosities ranging from 0.059 in the oyster *Ostrea circumpicta* to 0.216 in the oyster *Tiostreal lutaria*. Grant and Cherry (1985) reported mean heterozygosities in the mussels *Mytilus galloprovicialis* and *Mytilus edulis* of 0.24 and 0.18 respectively. The proportion of polymorphic loci when averaged over all five collections also was high (0.875 based on 24 loci) when compared to the multi-species study of Fujio et al. where the highest proportion of polymorphic loci was 0.667 based on 24 loci screened in *Tiostreal lutaria*.

Although significant allele frequency differences at some loci existed among all collections, we identified no abundant (frequency >5%) private alleles—those alleles found only in one population. In general, most alleles occurring at frequencies greater than 2% were found throughout all five surveyed areas. Absence of some of the low frequency alleles in certain populations is likely due to chance sampling error as a result of sample sizes. This finding indicates that there is some gene flow among razor clam populations.

The amount of gene flow among razor clam populations is difficult to estimate. The effective population sizes of razor clam populations are likely to be extremely large compared to those of

TABLE 4.

Matrix of genetic distances (Cavalli-Sforza and Edwards 1967 chord distance).

Population	1	2	3	4	5
Long Beach, WA	*****				
Seaside, OR	0.118	*****			
Clam Gulch, AK	0.122	0.125	*****		
Copalis Beach, WA	0.089	0.100	0.122	*****	
McIntyre Bay, B.C.	0.092	0.117	0.124	0.092	*****

most vertebrate species studied and the expected rate of genetic drift would therefore be very small. Thus, allele frequencies should be very stable over long periods of time if the juvenile recruitment for a beach is coming from the present parental population. A small amount of gene flow per generation would keep many of the same alleles in populations, but greater gene flow levels would be needed to keep the allele frequencies the same. On the other hand, even small amounts of genetic drift over many generations would cause genetic differentiation.

While a larval life span of from 5–16 weeks (Lassuy et al. 1989) suggests a potential for genetic exchange over wide geographic distances, it is not clear how oceanographic conditions along the Eastern Pacific Coast might limit the potential for far-ranging larval distribution. For example, larval distribution across major fresh water plumes such as those that occur at the mouth of the Columbia River and the entrances to Grays Harbor and Willipa Bay may be restricted. Bourne (1979) discussed some of the significant oceanographic features of the waters near Masset (Graham Is.), British Columbia and speculated that changes in oceanographic conditions in that region may have impacted larval settlement resulting in steadily declining razor clam recruitment on Masset beaches since 1971.

While evidence for non-panmixia exists, describing the razor clam populations as discrete stocks may not be appropriate. Perhaps there is a continuum of gradual genetic change in populations along the Eastern Pacific Coast. Analyzing widely separated populations and finding significant differences may be an artifact of the sampling design. Although, the sampling design was intended this way because we were unsure if any genetic differences could be identified. Further sampling from areas in between our existing collections would be necessary to determine if razor clams exist as discrete stocks or if a gradual continuum exists and to determine what, if any, relationship may be revealed between observed allele frequencies and oceanographic conditions. Repeat sampling at these same locations would allow the comparison of genetic change within areas to the diversity among locations.

The large number of alleles per locus and high average heterozygosity in razor clams suggests a great potential for genetic adaptation to environmental change. Even after large mortalities experienced by the Washington razor clam populations, there is no significant decrease in these measures of genetic diversity compared to the other populations. The large genetic diversity within razor clams may allow the Washington populations to adapt resistance to the NIX pathogen quickly provided the brood stock is derived from the same population or from populations subject to the same disease pressure.

CONCLUSIONS

Pacific razor clams have a large amount of genetic variation within populations and a small amount of diversity among populations. Significant differences in allelic composition exist among all five populations sampled. Although the Washington beaches were the most genetically similar of the areas studied, these razor clam populations have restricted gene flow. We found no reduction of genetic variation in the Washington populations compared

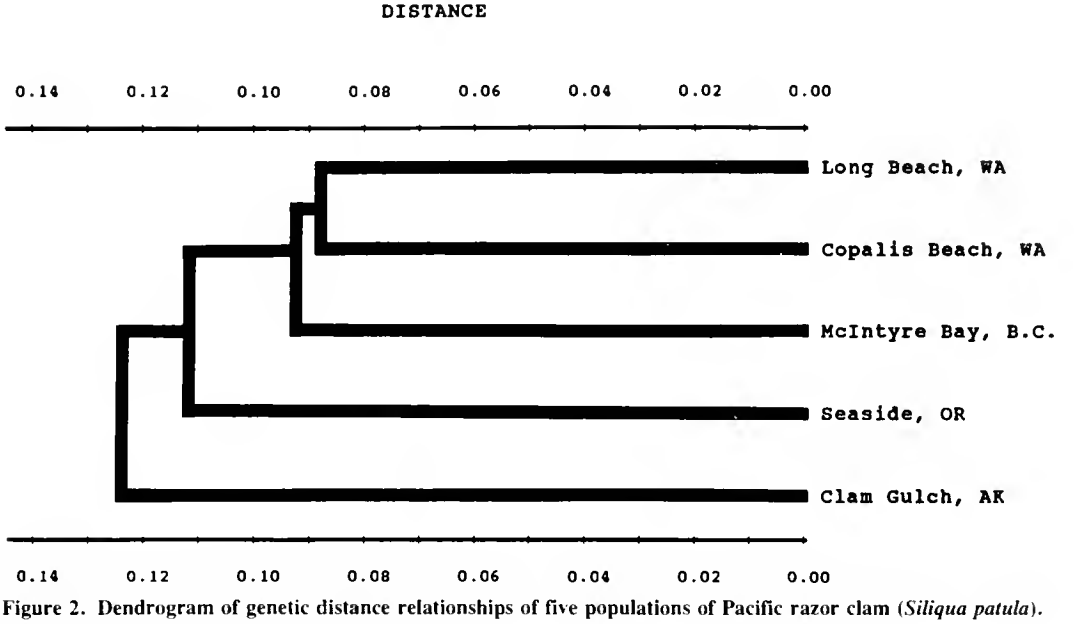

Figure 2. Dendrogram of genetic distance relationships of five populations of Pacific razor clam (*Siliqua patula*).

TABLE 5.

Genetic variability measures based on 24 loci from razor clam populations (standard errors in parentheses).

Population	Mean No. of Alleles per Locus	Percentage of Loci Polymorphic[a]	Mean Heterozygosity	
			Direct-Count	Hardy-Weinberg Expected[b]
1. Long Beach, Washington	4.6 (0.4)	95.8 (0.049)	0.270 (0.047)	0.265
2. Seaside, Oregon	3.4 (0.4)	83.3 (0.050)	0.262 (0.050)	0.267
3. Clam Gulch, Alaska	4.1 (0.4)	87.5 (0.046)	0.268 (0.047)	0.273
4. Copalis Beach, Washington	4.0 (0.5)	79.2 (0.047)	0.254 (0.049)	0.259
5. McIntyre Bay, British Columbia	4.2 (0.4)	91.7 (0.043)	0.250 (0.047)	0.264

[a] A locus was considered polymorphic if more than one allele was detected.
[b] Unbiased estimate (see Nei 1978).

to the other populations that had not experienced large mortalities due to NIX.

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EVALUATION OF GRADING, DEPURATION, AND STORAGE TIME ON CRAWFISH MORTALITY DURING COLD STORAGE[1]

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ABSTRACT Red swamp crawfish (*Procambarus clarkii* Girard) were subjected to three postharvest treatments (grading, grading and purging, or no additional treatment) and three cold storage (4 ± 0.2°C) periods (2, 4, or 6 days) in a factorial arrangement to determine the effects of these factors on crawfish mortality during cold storage. Significant differences were observed for both main effects. Mean mortalities during cold storage were 10.5, 10.3, and 19.5% for the control, graded, and graded/purged groups, respectively. Mean percentage loss of 7.8, 10.8, and 21.7% were observed for the 2-, 4-, and 6-day periods of cold storage, respectively. Crawfish that were graded and then purged for 24 hr and crawfish held for 6 days in a cooler had significantly higher mean mortalities during cold storage. An additional mean mortality of 11.6% was observed during the 24-hr purge period. Overall, mortality losses were generally highest early in the harvest season and declined over time. Results from this research demonstrate that substantial loss can occur both during and after the purging process and present evidence to rebut the idea that purging may be beneficial in increasing the shelf life of crawfish during cold storage.

KEY WORDS: Crawfish, depuration, grading, shelf life, cold storage.

INTRODUCTION

Louisiana is the international leader in freshwater crawfish production. Total annual harvest for the 1991/92 season was estimated at 40.5 million kg (live weight, *Procambarus* spp.), with 27 million kg produced from Louisiana's 46,600 ha of culture ponds (LCES, 1992). Sixty percent of the production is sold live, and the majority of live crawfish are marketed locally through private household and restaurants (Roberts and Dellenbarger 1989). Live crawfish are sold packaged 18–23 kg in mesh bags or sacks (similar to those used to package onions in U.S. markets). These allow live crawfish to be tightly packed, thus restricting their movements and their ability to inflict damage to each other with their chelae. Sacked crawfish are stored in coolers until sold. The storage time may be only a few hours or as long as one week. Environmental conditions in the cooler are maintained at temperatures of 1.6–4°C with high relative humidity to insure maximum shelf life of live crawfish (Moody 1989).

Crawfish are sometimes held in depuration or "purging" systems (where feed is withheld) for 24–48 hr prior to being placed in coolers. This procedure eliminates or greatly reduces the contents of the gut, which is exposed when the abdomen is "peeled" for consumption. The process also "washes" the outer surface and gill area. Purging is intended to render a cleaner, more attractive product that exhibits a fresher odor and taste when cooked (Lawson and Drapcho 1989). Improved quality, resulting from purging, is a major contributor to repeat sales and historic loyalty to certain producers (Haby and Younger 1989). Purging has been surmised to increase the shelf life of crawfish held in a cooler (Haby and Younger 1989; Huner and Barr 1991). It has been theorized that since the gill area is likely to be freer of mud and debris, oxygen transfer might be enhanced; and second, by largely eliminating the gut contents, the potential for stress and acute disease should be reduced. It has been reported (Treece 1984) that bacterial counts were reduced in peeled tail meats of purged crawfish.

Grading of crawfish by size has become the most recent development in the Louisiana crawfish industry. The gain of lucrative export markets, largely in Sweden, that demand only the largest crawfish was the impetus for grading (Moody 1989). Since domestic crawfish buyers also pay premium prices for larger crawfish, grading has become a standard industry practice. Currently, crawfish are graded into three or four sizes. The largest are used in the export market as whole, cooked product. The medium sizes are used in the domestic restaurant and live market trades as whole, spicy boiled crawfish. The smaller size is processed for the abdominal (tail) meat.

The shelf life of live crawfish during refrigerated storage is a key factor for merchants. These dealers must know how long they can safely store crawfish in cold storage without impacting survival and quality. Little is known of the relationship between grading or purging and their effect on crawfish shelf life. This study was conducted to document the effects of grading, purging, and storage time on crawfish mortality during cold storage.

MATERIALS AND METHODS

Red swamp crawfish (*Procambarus clarkii* Girard) produced at the Louisiana State University Agricultural Center's Rice Research Station, Crowley, were used for this project. The crawfish were trapped, using conventional baited traps, from typical rice-forage (*Oryza sativa* L.) crawfish ponds and were assumed to be in excellent condition. The day's catch was equally divided among the treatments. Postharvest treatment of crawfish after harvest, but prior to cold storage, were evaluated for their effect on crawfish mortality during storage in a cooler. The treatments were : (1) crawfish exposed to no additional handling after harvest (control); (2) crawfish exposed to a grader soon after harvest (graded); and, (3) crawfish held for 24 hr in a flow-through purge system after being graded (graded/purged). Crawfish mortality during cold storage was evaluated after 2, 4, and 6 days in a cooler.

[1]Approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript number 93-86-7339.

Equipment

The grader used in this study was a shop-built prototype developed by faculty of the Louisiana Agricultural Experimental Station, Louisiana State University Agricultural Center (McClain et al. 1993). This machine utilizes a passive grading system whereby crawfish grade themselves underwater. The grader includes a rectangular tank that holds water and a grader section, with three stacks of horizonal grates, that fits inside the tank. The grates are made up of parallel bars where the spacing is successively reduced from layer to layer. As a group of mixed crawfish is placed in the water above the top grate, they instinctively migrate down through the grates. They are impeded only when the spacing between bars is small enough to halt their descent. The grader section is raised out of the water, and graded crawfish are delivered (via chutes) to a platform. Immediately after the grading process, the three size grades were combined, a portion of the random mixture was sacked and placed in cold storage, and an equal portion was placed into the purging system.

The purge system was a flow-through type composed of a water-filled (770 L) rectangular tank with five suspended baskets that held crawfish in the water column. The basket frames were semicircularly shaped of aluminum and covered with plastic mesh (6.35 mm) wire. They sat crosswise inside the tank. Crawfish clung to the sides and bottom and equally occupied the entire submerged surface area (0.62 m^2). Loading rates ranged from 7 to 21.5 kg/m^2 and were within the 10–24 kg/m^2 range of those commonly used in commercial purging systems (Lawson and Drapcho 1989). Profuse supplemental aeration (0.17 m^3/min) was supplied via a regenerative blower and diffused through two 15.2-cm "airstones" per basket placed directly beneath the baskets. Water was continuously added to the end of the tank opposite the drain at the rate of 2.12 L/min, and the turn-over time for complete water replacement was 6.05 hr. Water used in both the grader and purge system was well water filtered through a commercial water softening system (Table 1). Water analysis was determined using the appropriate test in a commercially available test kit (Hach DRELL/5, Hach Chemical Co., Loveland, Colorado).

TABLE 1

Water quality characteristics of well water and purge system effluent[1] during the study period.

Parameter	Well Water		Purge System Effluent	
pH	7.6	(7.4–7.8)	8.2	(8.0–8.6)
Total Alkalinity (mg/L as $CaCO_3$)	297	(271–353)	272	(248–332)
Total Hardness (mg/L as $CaCO_3$)	4	(1–11)	22	(4–68)
Total Ammonia (mg N/L)	0.14	(0.09–0.38)	1.15	(1.02–1.50)
Nitrite (mg N/L)	0.01	(0–0.02)	0.28	(0.04–0.73)
Nitrate (mg N/L)	0.75	(0.04–1.20)	1.90	(0.07–4.53)
Total Iron (mg/L)	0.04	(0.02–0.05)	0.10	(0–0.14)
Dissolved Oxygen (mg/L)	–		7.8	(7.3–9.5)
Temperature (C)	25.0		24.5	(24–25)

[1] Effluent measured at full loading capacity and after 24 hours of purging. Values are expressed as means and ranges (in parentheses) unless no variability was observed.

Procedure

Crawfish were harvested approximately 8:00–9:00 a.m. and packaged in traditional mesh sacks immediately after harvest (control group) or after the grading process (graded group) and placed in a walk-in cooler. Crawfish removed from the purging system after 24 hr (graded/purged group) were first assessed for mortality and sexual maturity, and then sacked and placed in the cooler. Crawfish were placed under refrigeration on shelving in single layers with wet burlap sacks loosely placed over the crawfish and rewet daily in order to maintain adequate humidity. The temperature was maintained at 4 ± 0.2°C, a random sack from each group was removed after 2, 4, and 6 days in the cooler. Crawfish were individually examined for movement and classified as alive or dead and mature or immature. Sexual maturity was determined by the presence of ischial hooks at the base of the third and fourth pereopods in males (Form I), and subjectively for females by examining the relative size of the cephlothorax and chelae, color and hardness of exoskeleton, length of pleopods, and cornification of the annulus ventralis (Holdich and Lowery 1988).

Treatment comparisons were performed eight times from January to May during the 1993 harvest season (1/18, 2/1, 2/15, 3/1, 3/15, 4/12, 4/26, and 5/17) to cover a wide array of environmental and biological conditions. Tests at each period were conducted on crawfish harvested the same day under similar conditions. Mortality and maturity data were analyzed with analysis of variance (ANOVA) using the Micro-SAS Statistical Software System (SAS version 6.04, SAS Institute, Cary, N.C.). Significant ($P \leq 0.05$) differences between means were compared using Duncan's multiple range test. The relationship between the proportion of mature animals and mortality of crawfish after purging and cold storage was determined by simple linear correlations.

RESULTS

Approximately 835 kg of crawfish (36,828 individuals) were tested and individually examined during the study. Significant differences were observed for both main effects ($P = 0.0054$ and $P = 0.0001$ for postharvest treatment and length of cold storage, respectively) but not for their interaction. Results of mortality computations for both main effects are depicted in Table 2. Crawfish that were graded and then purged for 24 hr had the highest mean death rate during cold storage, and crawfish held for 6 days in a cooler had significantly higher mean mortalities. When postharvest treatments were examined and compared at each storage period, the graded/purged group exhibited significantly higher mortalities at both 2- and 4-day storage times. Although not significantly different, the graded/purged group exhibited a similar trend at the 6-day storage time. No significant difference was observed between the control and graded groups. An additional mortality of 11.6% was observed during the 24-hr purge period, bringing the mean death loss to 39.5% for crawfish in the graded/purged group that was held for 6 days in a cooler compared with a mean of approximately 18.5% for the other two groups.

Examination of mortalities for each postharvest treatment reveals a trend of increased death rate with increased storage time (Table 2). However, mean mortality values for crawfish held 2 and 4 days in cold storage were not significantly different at 7.8 and 10.8%, respectively. The 21.7% mortality for those held 6 days was significantly higher. Mean mortalities and differences among storage period varied with test dates and generally declined over the season (Fig. 1).

No significant differences, due to either postharvest treatment

TABLE 2.

Mean percent mortality of crawfish exposed to increasing periods of cold storage (4 ± 0.2°C) after different post-harvest treatments.

Post-Harvest Treatment	24-hr Purge	Period of Cold Storage 2-day	4-day	6-day	Mean
Control	–	4.0^{B2}	8.7^{B2}	18.8^{A1}	10.5^{B}
Graded	–	5.5^{B2}	7.2^{B2}	18.3^{A1}	10.3^{B}
Graded/Purged	11.6	14.1^{A1}	16.5^{A1}	27.9^{A1}	19.5^{A}
Mean		7.8^{2}	10.8^{2}	21.7^{1}	

Significant differences ($P \leq 0.05$) among post-harvested treatments (within columns) and storage times (within rows) are indicated with alphabetical and numerical superscripts, respectively.

or cold storage time, were detected in the percentage of dead crawfish that were estimated to be physiologically mature. The percentage of dead mature crawfish was lower at each test date than the percentage of mature crawfish in the daily catch (Fig. 2). The percentage of mature crawfish in both the dead group and daily catch greatly increased after 15 March, but the increase was usually much greater in the original catch. The mean mortality for all treatments was substantially lower after 15 March. Mean percent mortality at each test date was correlated with the percentage of mature crawfish in the daily catch ($R^2 = 0.4650$) but was not significant ($P = 0.0915$). The influence of basket-loading capacity on percent mortality during the purge period was also computed but was negatively correlated ($R^2 = 0.4526$) and not significant ($P = 0.0675$).

DISCUSSION

There is much evidence from this study to refute the idea that purging may be beneficial in increasing the shelf life of crawfish during cold storage. The opposite appears to be likely. Although crawfish in this study were graded prior to being purged, it was initially assumed that the grading component would have little impact compared with the purging component of this treatment. However, there could be an additive or confounding effect involved. Purging crawfish prior to cold storage may exert increased stress, thus increasing the likelihood of higher losses and decreased shelf life. Purging prior to cold storage usually insures that crawfish have been handled and subjected to stress for an additional day (or longer) compared with other crawfish held for the same length of time in cold storage.

Results of this study were very similar to trends obtained from a previous preliminary study conducted in the same laboratory during 1992 (McClain et al. in press). That study subjected 24,761 crawfish from six test dates (3/11/92–4/22/92) to the same three postharvest treatments and surveyed the mortalities after 6 days in cold storage. The mortality during cold storage was 6.88, 11.07, and 19.94% for the control, graded, and graded/purged groups, respectively. The deaths during cold storage were considerably less during the 1992 study; however, that study lacked data from January, February, and the beginning of March. Data collected during this period in 1993 had the highest mortalities (Fig. 1).

It is not clear why the mortalities were higher during the January–March period. Although the correlation between mortality and maturity was not significant in this study, the profile of mortality and maturity over time (Fig. 2) suggests that when a high percentage of the catch was mature (4/12–5/17), the overall mortality during handling and storage was substantially decreased. This was corroborated by the data that showed a disproportionate share of immature crawfish in the dead group (Fig. 2), demonstrating that immature crawfish were more susceptible to the stress of purging and cold storage.

Losses incurred during the purging process in this study (11.6%) were much higher than those incurred in the 1992 preliminary study (3.33%; range, 2.40%-4.11%). Lawson and Baskin (1985) experienced mortalities exceeding 5% and attributed those to low dissolved oxygen concentrations in the system, and later, partially attributed those losses to disease problems (Lawson and Drapcho 1989). Dissolved oxygen concentration in the present purge system was maintained above 7 mg/L, and other critical water quality parameters are not suspected (Table 1). No assay for disease was performed. Huner and Barr (1991) suggested that mortality should be less than 10% when crawfish of good quality are stocked into purge systems at water temperatures in the 22–26°C range. It is apparent that the higher mean mortality associated with the purging process in 1993 was caused by high mortalities during the early part of the harvest season (Fig. 1). Mortalities through 1 March averaged 18.6% while mean mortality for the latter half of the harvest season was only 4.5%. Although

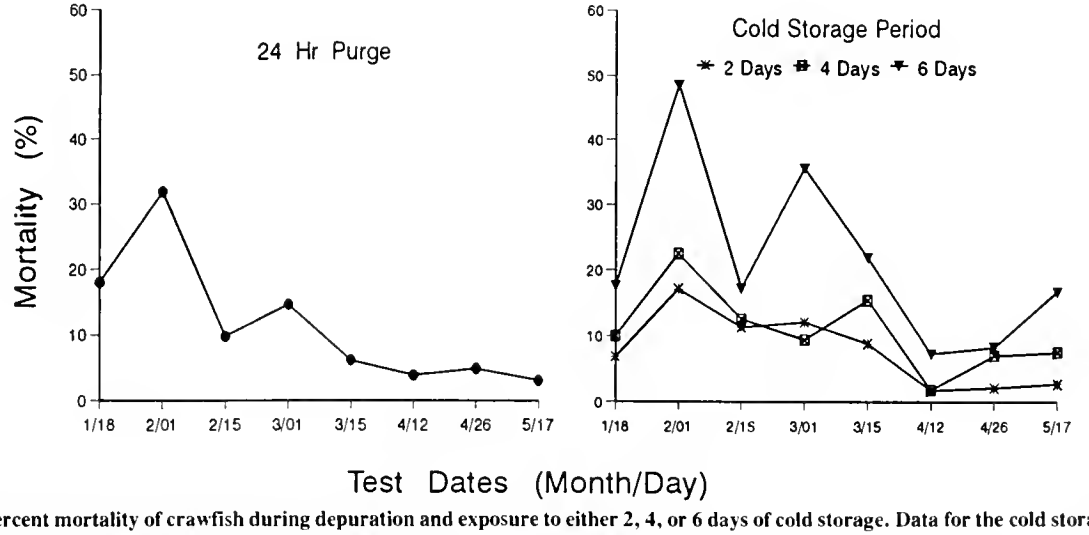

Figure 1. Percent mortality of crawfish during depuration and exposure to either 2, 4, or 6 days of cold storage. Data for the cold storage periods represent means across postharvest treatments at each test date of the study period.

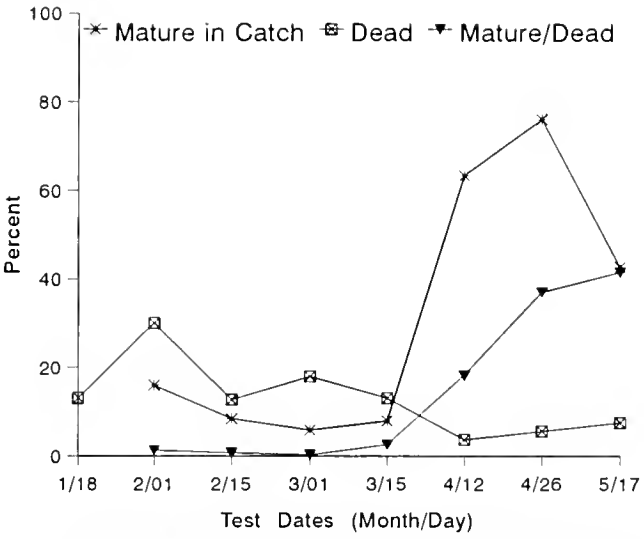

Figure 2. Mean percent of mature crawfish in the original catch (mature in catch), mortality (dead), and maturity of the dead group (mature/dead) for each test date of the study period.

water temperature was not measured the day of harvest, high mortalities during this early period may have been associated with thermal shock. Purged crawfish were exposed to increased water temperature in the purge system; they sometimes experienced a temperature gradient of approximately 15–20°C. Also, a higher percentage of crawfish during this period were immature, which may have contributed to higher mortalities. Minimizing the potential for thermal shock and segregating crawfish according to size grade during the purging process may aid to decrease the mortality during purging. This warrants further investigation.

Utilizing the passive grading technique of allowing crawfish to sort themselves underwater appeared to have had little effect on shelf life of crawfish during cold storage. Individual differences did sometimes occur at various test dates, but they were inconsistent with storage time and no trend was obvious. Time during cold storage was a significant factor affecting live crawfish shelf life, regardless of postharvest treatment. The expected trend of increased mortality with storage time was apparent with a significant increase at the 6-day period. Huner and Barr (1991) stated that crawfish can be held under refrigeration for up to 5 days with less than 10% mortality. This was only achieved in the graded and control groups during the latter half of the season in the present study.

In conclusion, findings from this study demonstrate that substantial loss can occur both during and after the purging process and that purging to improve the product does exact a cost by increasing mortality. This should be taken into consideration in commercial enterprises. Further research is needed to better define the causes affecting survival and shelf life of live crawfish and means to improve conditions, especially in regards to purging. While the impact of a water-based grader on crawfish shelf life during cold storage appears negligible, little is known of the effects from other types of graders used in the industry. Since grading is fast becoming an industry standard, further investigations are needed.

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UPTAKE AND RETENTION OF *SALMONELLA* BY BIVALVE SHELLFISH

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ABSTRACT The sensitivity of detecting *Salmonella* in seawater by the means of bivalve molluscs has been tested. Mussels were artificially contaminated by seawater inoculated by a *Salmonella* suspension at five different concentrations. Mussel contamination by *Salmonella* was effective and rapid. The presence of *Salmonella* was detected in mussel tissue (25 g) from *Salmonella* concentrations in seawater as low as 3.5 CFU per litre by both a conventional method and the ORGANON ELISA technique. The ELISA rapid method was in agreement with the conventional technique and its sensibility and selectivity were respectively 94.3% and 88.8%.

A study of *Salmonella* retention in oysters, artificially contaminated at a low level and stored in air at 12–15°C has shown that these bacteria were still detectable after a five day-period.

The survival of *Salmonella* in mussels and oysters, air-stored at 10°C, was followed by a direct *Salmonella* enumeration from individual bivalves. A storage period as long as twenty days did not produce appreciable changes in *Salmonella* counts in these shellfish.

KEY WORDS: shellfish, bivalve, mussel, *Mytilus edulis*, oyster, *Crassostrea gigas*, bacteria, *Salmonella*, bacterial uptake, bacterial retention

Shellfish are often considered to be responsible for gastroenteric outbreaks (Fleet 1978). The causative agents of these diseases may be bacteria, viruses, or dinoflagellates. Most epidimiologic data indicate that gastroenteritis of viral etiology or due to toxic dinoflagellates have a greater incidence than that of bacterial origin (Bean and Griffin 1990, Bryan 1988).

The reported outbreaks related to *Salmonella* associated to shellfish are concerning generally typhoid fever and a past period (1900–1950). However salmonellosis of shellfish origin remains a reality (Rippey, 1991).

More generally, salmonellosis is often considered as a major risk among foodborne bacterial hazards, and its incidence is increasing dramatically (Notermans et al. 1992). Transmission from domestic animals is prevailing (Oosterom 1991) and the increasing distribution of *Salmonella* in the environment increases the potential risk of illness from consuming bivalves.

The published data dealing with *Salmonella* in shellfish mainly concern the geographic distribution of *Salmonella*-contaminated shellfish, and their relationship with seawater contamination and with usual indicators (and especially fecal coliforms or *Escherichia coli*).

In contrast, few studies deal with the processes of accumulation, retention and release of *Salmonella* in shellfish (Prieur et al. 1990). More work has been carried out on the behaviour of *Salmonella* in shellfish depuration (Rowse and Fleet 1984, Timoney and Abston 1984).

Therefore, there is a lack of data to elucidate the relationship between *Salmonella* and bivalve shellfish. This lack may probably be due to the relative difficulty in detecting *Salmonella*, the lack of a convenient technique for enumerating these bacteria, and hazards associated with *Salmonella* experiments. Furthermore, these difficulties are adding to those encountered in experiments conducted with living shellfish.

The present study has been performed with the purpose of providing information on two points:

- the sensitivity of *Salmonella* uptake by bivalve shellfish,
- the behaviour of *Salmonella* in shellfish stored in air.

To this end, artificial contamination of shellfish by *Salmonella* has been achieved in controlled conditions, followed by *Salmonella* detection both by a conventional method and a rapid ELISA technique. Main advantages to using ELISA technique are:

- a shortened response time
- an easy to use procedure
- a result independent of technician interpretation
- the possibility of grouping samples to obtain a simultaneous and similar detection for large series.

These last two points were important in the present comparative study.

A quantitative study on *Salmonella* evolution in air-stored shellfish has been conducted to complete the detection results.

MATERIAL AND METHODS

Bivalves

Two bivalves were chosen for this study: the mussel *Mytilus edulis* and the Pacific oyster *Crassostrea gigas*. In France, mussels and oysters are considered to be the most frequent vehicles in shellfish-borne diarrhoeal diseases. Before undertaking the experiments, the molluscs were placed under clean running seawater for five to seven days, and controlled for the absence of *Salmonella* in 25 ml of shellfish tissue.

Bacterial Strain

Artificial contamination was obtained by a suspension of *Salmonella worthington* which had been recently isolated from naturally polluted mussels. Another strain, obtained from the same source, was used in the quantitative study: this second strain belonged to the serovar *Salmonella agona*.

EXPERIMENTAL PROCEDURES

Salmonella *Uptake by Mussels*

Contamination of Mussels by *Salmonella*

Five sample-runs of thirty mussels were placed respectively in five similar ten-litre empty aquaria (these five tanks were labelled A, B, C, D, E). The contamination of the mussels was achieved by connecting these tanks to five 30 litre flasks containing seawater contaminated by different concentrations of *Salmonella*. The same supply in every aquarium was obtained by delivering seawater through a multichannel peristaltic pump. The flow rate was fixed at 10 litres per hour. Consequently after one hour the tanks were full and overflowing seawater was collected in a container (and decontaminated with hypochlorite). After three hours, the flasks delivering seawater were empty, and no further seawater was supplied. Mussels were kept in the contaminated tanks for 24 hours with a light aeration supplied.

Contamination of the 30 litre flasks was achieved with a suspension of *Salmonella worthington* in sterile seawater prepared 24 hours before. This procedure was applied according to the conclusions of Munro et al. (1987) showing that bacteria which has been adapted for one day to seawater conditions presented a subsequent reduction in death.

The *Salmonella* concentration in this suspension, just before inoculation in the tanks, was obtained by pour plate enumeration in nutrient agar. The concentrations in tanks A to E were inferred from this enumeration and from the respective dilution factors. They are given in Table 1.

Sampling

One seawater sample and one mussel sample were taken before contamination to verify the absence of *Salmonella*.

During the experiment, mussels were sampled after 40 minutes, 3 hours, and 24 hours. Mussels filtration activity was visually controlled during the contamination period, and only active mussels were sampled for analysis.

Seawater samples were taken from the supply flasks just after contamination by *Salmonella* and in the tanks with mussels after 2 hours 30 minutes and 24 hours.

A seawater sample consisted of 100 ml of seawater. A mussel sample was obtained from ten mussels (approximately 40 grams).

Sample Preparation

100 ml of seawater were filtered through a cellulose membrane (pore size: 0.45 μm). This membrane was introduced to a stomacher polyethylene bag containing 100 ml of Buffered Peptone Water (BPW Merck 7228). After homogenization for one minute in the "stomacher" system, the content of the bag (BPW plus membrane) was transferred into a sterile flask and incubated overnight at 37°C. This constituted the pre-enrichment in *Salmonella* detection.

Mussels were briefly flamed and asepticaly opened. A previous study had shown that mussel liquid content gave inconsistent bacteriological results (Plusquellec et al. 1990) and it was therefore eliminated for the present study. The mussel tissue was collected and diluted by BPW (one part of mussel flesh for 4 parts of BPW). After homogenization one minute in the "stomacher" system (which had been shown to be appropriate to this use, Trollope 1984) 125 ml of this suspension was transferred into a sterile flask and incubated at 37°C. This pre-enrichment corresponded therefore to 25 ml of mussel tissue, in agreement with French standards.

Salmonella Detection

Conventional technique. The pre-enrichment in BPW was incubated overnight at 37°C and then transferred into two enrichment media: Rappaport-Vassiliadis (RV) medium (Merck 10236). 10 ml of medium was inoculated by 0.1 ml of pre-enrichment; Muller-Kaufman Tetrathionate (MKTT) medium (Merck 10863). 20 ml of medium was inoculated by 2 ml of pre-enrichment. The two enrichments were incubated for 24 hours at 42°C in a water bath.

Isolation from these media was performed on two selective agars: Brillant Green Agar (DIFCO 0285); Hektoen Agar (Biomerieux 51531).

These two media were incubated at 37°C for 24 to 48 hours. Typical colonies were sampled and confirmed by Api Rapidec Z procedure (Biomerieux).

ELISA technique. This technique was applied after the enrichment stage of the conventional method and therefore performed from the same initial sample.

0.5 ml of the RV enrichment and 0.5 ml of the MKTT enrichment were inoculated in 5 ml of M Broth (DIFCO), which was incubated at 41°C for 24 hours, and constituted the post-enrichment for the ELISA method.

The ELISA detection was achieved b;y using the Organan *Salmonella* Tek (Transia) according to instructions.

The results were obtained by measuring optical density in a microtitration plate photometer (450 nm).

Salmonella *Persistence in Shellfish*

Preliminary Study

Oysters were placed in 20 litres of seawater in an aerated aquarium. Contaminated was achieved by addition of a *Salmonella worhington* suspension in this tank. The expected *Salmonella* concentration was 10 to 100 *Salmonella* per litre and the actual concentration obtained was 45 *Salmonella* per litre.

After a three hour contamination period, oysters were taken and dried on paper and then placed in dishes and stored for 7 days in a ventilated room, temperature of which varied between 12°C to 15°C.

Oysters were analysed after 40 minutes and 3 hours during the contamination period and after 72 hours, 82 hours, 96 hours, 120 hours, and 168 hours during storage. A sample consisted of two oysters. The whole content of the oyster (flesh and liquid) was analysed according to the procedure described for mussel analysis. The results were therefore relative to the whole content of two oysters.

Quantitative Study

Oysters and mussels were placed in a 40 litre aerated aquarium, and after 24 hours were contaminated by addition of a *Salmonella agona* suspension.

Salmonella concentration in the water was measured after 5 minutes, 150 minutes, and 180 minutes. *Salmonella* counts in seawater were obtained by direct enumeration on a new selective medium: the Rambach Agar (Merck 7500) which has been described as adapted for this use (Monfort et al. 1994).

After this three hours contamination period, shellfish noted as active during the contamination step, were taken and distributed in 140 mm Petri dishes into sample runs of five bivalves.

One oyster sample and one mussel sample were analysed immediately, the others were stored at 10°C and analysed in terms of time.

For this quantitative study, five shellfish were analysed individually (a, b, c, d, e). The whole content of the bivalve (flesh plus liquid) was suspended 1/3 in saline Peptone Water (Merck 15421) before homogenization and *Salmonella* direct enumeration on Rambach agar according to the procedure presented by Monfort et al.

EXPERIMENTAL RESULTS

Salmonella *Uptake by Mussels*

The results obtained from the detection of *Salmonella* in seawater and mussel samples are respectively presented in Table 1. The conventional method (CM) produced either a positive or a negative response (obtained either after isolation or after confirmation step).

The ELISA response is given by an optical density measurement. Measurements are related to a positive and a negative control. In the present case the threshold for a positive response was 0.52 OD unit.

Just after contamination, positive responses were obtained in 100 ml of seawater by the conventional technique, even at the lowest *Salmonella* concentrations (corresponding to less than one in 100 ml). These positive results may therefore be considered as unexpected in terms of probability.

The results obtained by the ELISA method were more inconsistent, and the response in tank B for the earliest samples did not follow the positive results given by the conventional method.

As in seawater, *Salmonella* were detected in mussel samples for each contamination level, as early as the first sampling time (40 minutes).

In contrast with the results obtained from seawater, a positive response is obtained both by the conventional and the ELISA method after a 40 minute contamination period.

Salmonella *Retention in Shellfish*

Preliminary Study

The presence of *Salmonella* in oysters was followed for seven days after emersion. The results of this study are presented in Table 2, the positivity threshold for ELISA detection was here 0.36 OD units.

The *Salmonella* concentration in the contaminating seawater was 45 CFU per litre, and as in the case of mussels, oysters exhibited contamination by *Salmonella* after an immersion period as short as 40 minutes.

Quantitative Study

Salmonella evolution in shellfish in the course of time is reported in Table 3 and has been represented in Figure 1a and Figure 1b by the average (m) and the standard deviation (s) of *Salmonella* counts on Rambach agar.

It appeared from these representations that *Salmonella* concentrations in shellfish did not present a clear evolution in the duration of the experiment (20 days). This observation concerned the two bivalves observed here and was corroborated by a statistical analysis. An analysis of variance (ANOVA) indicated no significant difference related to the time ($F < 1$, nonsignificant). This result was obtained for mussels as well as for oysters and in addition no significant difference between these two bivalves could be noted.

After six days some mussels presented spoilage signs (shell half-opened, liquid excreted). These spoiled mussels were identified and a Student t test indicated that *Salmonella* counts in these bivalves did not differ significantly from non-perceptibly spoiled mussels ($t = 1.23$ NS $p = 0.20$).

The liquid excreted by spoiled mussels was analysed. *Salmonella* concentrations in this liquid was in the same range as in mussel tissue but with greater fluctuations.

After a twenty-day survey, all the mussels were badly spoiled (with a strong ammonia odor and flesh beginning to dry up). However, no significant changes in *Salmonella* counts could be detected.

Similar observations could be inferred from oysters, the major difference was that spoilage appeared later and less markedly in *Crassostrea gigas* than in *Mytilus edulis*.

Individual variability among bivalves appears more clearly in Figure 2a and 2b. Inter-bivalve variability could be assessed through the coefficient of variation in each series of five bivalves (CV). These coefficients were varying from 4.3% to 22.4%. Differences in variability relevant to storage time could not be noted.

Additionally, variability in oyster did not differ from variability in mussel, although the average weight of these two bivalve con-

TABLE 1.

Compared *Salmonella* detection from mussels and contaminating seawater by ELISA and a conventional method (CM).

		Seawater							Mussels				
		A*	B	C	D	E			A	B	C	D	E
Time‡	Detection	3.6†	9.2	36	360	1800	Time§	Detection	3.6	9.2	36	360	1800
5 min	ELISA	2.71 (+)	0.26 (−)	2.94 (+)	3.06 (+)	3.13 (+)	40 min	ELISA	2.67 (+)	2.5 (+)	2.92 (+)	2.60 (+)	2.82 (+)
	CM	+	+	+	+	+		CM	+	+	+	+	+
150 min	ELISA	2.81 (+)	0.29 (−)	2.94 (+)	2.79 (+)	2.82 (+)	3 h	ELISA	2.46 (+)	2.67 (+)	2.58 (+)	2.61 (+)	2.77 (+)
	CM	+	+	+	+	+		CM	+	+	+	+	+
24 h	ELISA	0.67 (+)	0.50 (−)	0.12 (−)	2.83 (+)	2.97 (+)	24 h	ELISA	2.98 (+)	2.73 (+)	0.07 (−)	2.86 (+)	2.89 (+)
	CM	−	−	−	+	+		CM	+	+	−	+	+

* Letters on the first row of boxheads are Tank designations.

† Values on second row of boxheads are *Salmonella* (CFU/l).

‡ Water control $T = 0$: CM: negative; ELISA: 0.03 (−).

§ Mussel control $T = 0$: CM: negative; ELISA: 0.12 (−).

TABLE 2.

***Salmonella* detection in oysters after contamination and air-storage.**

Time	Detection	Seawater	Oysters
0 min*	ELISA	0.03 (−)	0.31 (−)
	CM	−	−
40 min*	ELISA	2.87 (+)	
	CM	+	
90 min*	ELISA		2.69 (+)
	CM		+
3 h*	ELISA	2.83 (+)	2.88 (+)
	CM	+	+
24 h	ELISA		2.47 (+)
	CM		+
72 h	ELISA		2.16 (+)
	CM		+
82 h	ELISA		0.57 (+)
	CM		+
96 h	ELISA		0.22 (−)
	CM		−
144 h	ELISA		2.80 (+)
	CM		+
168 h	ELISA		0.21 (−)
	CM		−

* Immersion period.

tents were quite different (4.2 g ± 2.31 for mussels, 8.09 g ± 3.48 for oysters).

Comparison Between ELISA and Conventional Techniques

Forty-four of the *Salmonella* detections already presented were carried out in parallel by the conventional technique and the ELISA method, allowing a limited comparison between these two methods. The distribution of these paired results is shown in Table 4.

ELISA and conventional detection methods are in agreement in 41 cases (93.1%). Inconsistent responses have been obtained essentially for seawater in tank B for which two negative results by ELISA were associated with positive responses by the conventional method. In tank A, after 24 hours, a negative result by conventional technique is related to a slightly positive ELISA response (Table 1). A further conventional detection obtained from the post-enrichment medium has confirmed this negative result.

Agreement between the two methods is confirmed by the Mac Nemar test (recommended by Flowers et al. 1987) which indicates no significant difference between the two methods ($p < 0.05$). The sensitivity and the specificity of the ELISA technique in reference to the conventional technique can be assessed from these paired detections according to the formulations recommended by Beumer et al. (1991). The sensitivity of the ELISA method obtained like this is 94.3% and its specificity is 88.8%.

DISCUSSION

The first study, related to *Salmonella* uptake in mussels, was performed with the aim to assess the sensitivity of mussel flesh for indirect detection of *Salmonella* from seawater. The high sensitivity of this bivalve material as an indicator of *Salmonella* in seawater appears clearly here: the presence of *Salmonella* in mussel tissue is detectable after a short time and for low *Salmonella* concentrations in contaminating seawater.

Thus observation confirms the rapid uptake of enteric bacteria by bivalves which has been previously noted in laboratory or in situ experiments (Timoney and Abston 1984, Trollope and Al Salihi 1984). The maximal concentration in mussels exposed to contaminated water was reported to be reached in as short a period as 30 minutes (Plusquellec et al. 1990).

For short contamination times, and low *Salmonella* concentrations, it can be considered that the *Salmonella* presence is established more clearly in mussel tissue than in the contaminating seawater. However, the results observed here for the *Salmonella* presence in 100 ml of seawater are rather surprising, because positive responses were obtained for samples containing theorically less than one *Salmonella* unit. In absence of negative detections the actual sensitivity thresholds for *Salmonella* detection from 25 g of mussel tissue and 100 ml of seawater cannot be concluded from the present study.

After 24 hours in static seawater, *Salmonella* was still detected in mussels in most cases (with an unexplained exception in tank C) whereas it had disappeared in seawater with concentration levels under 360 CFU per litre (Table 1). Thus, mussels seem to be able to retain *Salmonella* in their tissue for as long as 24 hours. However, this observation is obtained here in a closed system. In contrast, experimental data from studies on depuration of mussels and other bivalve species in pure running seawater have shown a rather rapid elimination of *Salmonella* and related bacteria (Nishio et al. 1981, Timoney and Abston 1984, Martinez Manzanares et al. 1991, Plusquellec et al. 1990).

Mussels have often been described as a possible sampling material in monitoring contamination of seawater. The value of mussels in this indirect monitoring is related to their ability to concentrate pollutants and especially bacterial pathogens, and to retain them in their tissue for some period. These properties have been pointed out in the contamination by indicator bacteria (Trollope and Al Salihi 1984, Plusquellec et al. 1983, Turick et al 1988).

Two points in the present study tend to corroborate this ability in the case of *Salmonella:* Firstly, mussels appear to be very sensitive organisms in the detection of *Salmonella* at low contamination levels in seawater. Secondly, immersed mussels retain bacteria for a while and therefore provide us with some information which is not the instantaneous contamination in the surrounding seawater but which is relative to some previous time period.

These two points can be considered as positive with regard to the *Salmonella* survey of seawater contamination through mussel analysis.

The second experiment was performed with the oyster *Crassostrea gigas* contaminated in static seawater, and confirmed that rapid contamination also occurs in this bivalve. Within less than one hour the contamination by *Salmonella* appears as effective (Table 2).

The preliminary study of *Salmonella* persistence in oysters as a function of time indicates that *Salmonella* can still be recovered from oysters six days after emersion. This observation is obtained here with oysters contaminated at a low level (45 *Salmonella* per litre).

In the quantitative study which followed this preliminary approach, bivalve were contaminated at an upper level to allow a quantitative survey (9.3×10^2 *Salmonella* per ml of contaminating seawater).

The quantitative data confirm entirely the previous observations: the main feature of this quantitative study is the absence of "evolution" in *Salmonella* counts for a 20-day storage.

TABLE 3.

***Salmonella* quantitative evolution in mussels and oysters during air storage.**

	Mussels (days)										
	0	**1**	**2**	**3**	**6**	**7**	**8**	**9**	**10**	**15**	**20**
a	2,39	3,22	3,41	2,77	2,6	3,44	4,12	3,16	3,41	3,04	3,86
b	3,47	3,71	3,43	3,67	3,6	3,38	3,01	3,11	3,73	3,36	3,44
c	3,44	3,25	3,76	3,74	4,74	3,45	3,28	2,47	3,31	3,98	3,42
d	3,39	3,69	3,37	3,43	3,31	3,74	3,41	3,67	3,53	4,51	2,3
e	3,14	4,24	3,37	3,74	3,23	3,38	2,97	3,4	2,81	3,89	3,25
m	3,166	3,622	3,468	3,47	3,496	3,478	3,358	3,162	3,358	3,756	3,254
s	0,453	0,417	0,165	0,412	0,785	0,15	0,464	0,446	0,344	0,571	0,579
CV (%)	14,31	11,5	4,766	11,86	22,46	4,315	13,82	14,11	10,24	15,21	17,78
						excreted liquid					
							3,28	3,62	3,56	2,32	2,39

	Oysters (days)					
	0	**2**	**6**		**10**	**20**
a	3,15	3,87	3,65		3,13	3,75
b	3,67	3,95	3,39		2,92	3,4
c	3,46	3,73	3,59		3,73	3,18
d	3,34	3,67	3,4		2,51	2,47
e	2,65	2,99	3,85		2,51	3,1
m	3,254	3,642	3,576		2,96	3,18
s	0,387	0,381	0,191		0,507	0,47
CV (%)	11,89	10,46	5,348		17,13	14,78
			excreted liquid			
			3,13		3,58	1,69

The behaviour of *Salmonella* during the storage at 10°C in living shellfish, is quite similar in *Crassostrea gigas* and in *Mytilus edulis*, showing a survival rate next to 100% in both bivalves.

The apparent longer persistence observed in the quantitative study versus the preliminary study may be due to difference in storage temperature, in season or in the contamination level (which was in the preliminary study close to the detection threshold). This seems more likely than difference between *Salmonella agona* and *Salmonella worthington* behaviours.

An absence of evolution in *Salmonella* counts average may have resulted from opposite evolutions in individual bivalves. *Salmonella* enumeration on individual bivalves and a subsequent analysis on inter-bivalves variability allows to affirm the opposite: *Salmonella* survival during air-storage is general and similar for all bivalves. Thus, the individual variability in shellfish which had been pointed out by Heffernan and Cabelli (1971) during contamination and depuration, does not appear as a determining factor during air storage.

Spoilage on shellfish no more appears as a source of variation in *Salmonella* concentrations in the bivalve. After twenty days at 10°C, shellfish (especially mussels) were highly spoiled, whereas *Salmonella* counts remained close to their initial values.

These conclusions are consistent with most of the data dealing with the fate of bacteria in air-stored bivalves, which generally concluded to a very slow evolution of *Salmonella* in air-stored shellfish. Kelly and Arcisz (1954) had followed the behaviour of *Salmonella shottmuelleri* in *Crassostrea virginica* and *Mya arenaria* stored in air at 5°C and 9°C. These authors observed a low decrease of *Salmonella* counts which is slightly influenced by the temperature and a little more rapid in *Mya arenaria*. *Salmonella* evolution paralleled *E. coli* behaviour in the same experiment. Similarly, Fraiser and Koburger (1984) reported a non-significant evolution in *Salmonella* contamination of oysters air-stored for ten days at 5°C. In contrast, Son and Fleet (1980) indicated a more rapid decrease in *Salmonella* concentrations, but their study was related to heavily contaminated oysters stored at high temperature (20–25°C).

This conclusion does not seem dependent on the living state of bivalves. Similar experiments conducted on shucked oysters artificially contaminated with *Salmonella typhi* (Nishio et al. 1981) did not show any change in *Salmonella* counts during the first ten days.

It will be important to understand the reasons of the long persistence of *Salmonella* in air-stored shellfish and, firstly, to simultaneously compare it with *Salmonella* persistence in seawater, in shuckled shellfish, and in miscellaneous seafoods stored in similar conditios.

Salmonella behaviour in stored shellfish may be compared to other bacterial groups. Arumugaswamy et al. (1988) have studied the response of *Campylobacter jejuni* and *Campylobacter coli* during storage. Survival of these pathogens in the oysters differs markedly from *Salmonella* behaviour. After 3 to 10 days (according to the storage temperature) viable *Campylobacter* were no longer detected. On the other hand, Hood and Ness (1984) reported that during air-storage of oysters at low temperature the Vibrio group increases significantly.

This long persistence in air-stored shellfish is also an argument for the use of shellfish as material for *Salmonella* detection in seawater. Additionally to advantage previously presented, shell-

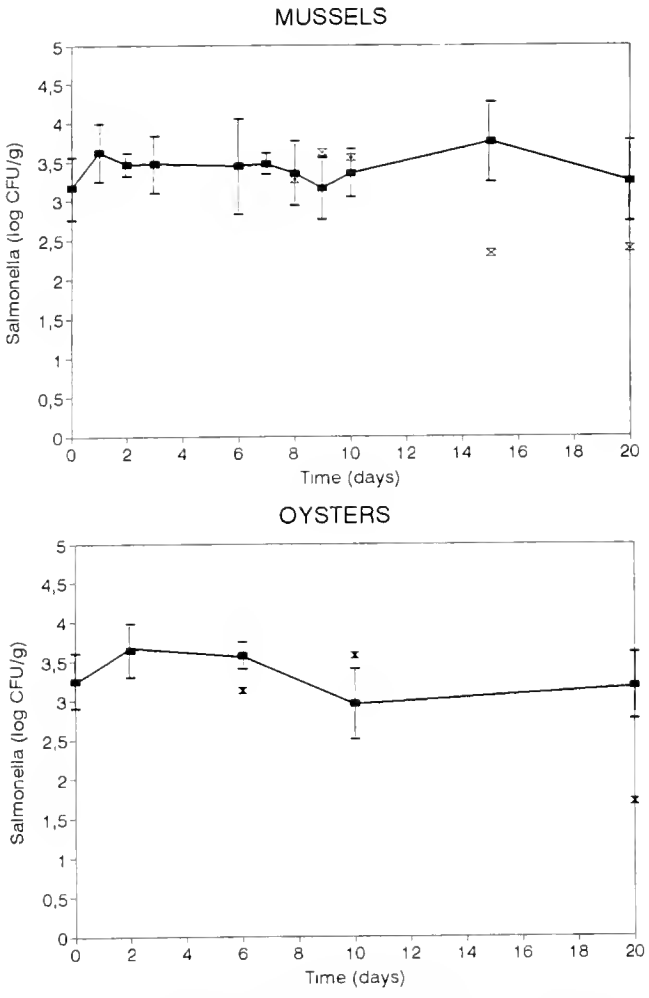

Figure 1. a: *Salmonella* evolution in mussels during air-storage. (x indicates counts from excreted liquid). b: *Salmonella* evolution in oysters during air-storage. (x indicates counts from excreted liquid).

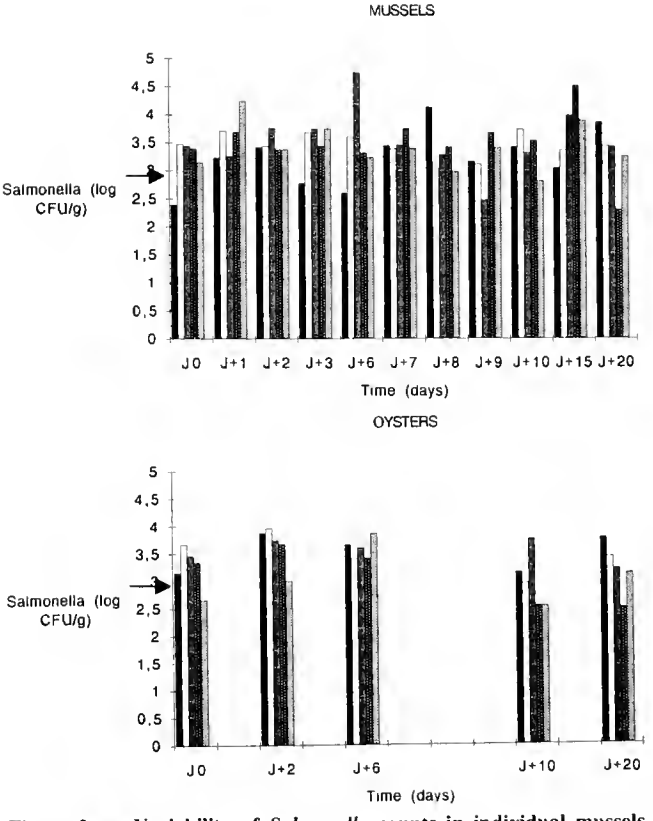

Figure 2. a: Variability of *Salmonella* counts in individual mussels during air storage. → indicates *Salmonella* concentration per ml in contaminating waer. Columns represent counts for each of individual mussels at each sample time. b: Variability of *Salmonella* counts in oysters during air storage. → indicates *Salmonella* concentration per ml in contaminating water. Columns represent counts for each of individual mussels at each sample time.

fish may allow a differed *Salmonella* detection from a marine site when instantaneous analysis is not possible.

The experiments outlined here allow, in addition, a comparison between the ELISA technique and the conventional *Salmonella* detection method, as applied to shellfish. Nevertheless, this comparison remains limited to the present conditions of experimental contamination.

The general conclusion is that the ELISA results are in good agreement with the usual technique. Sensibility and specificity are satisfactory but are slightly lower than the values quoted by Beumer et al. in their comparison between *Salmonella* detection by ELISA and other methods. Their comparative studies were general, and (although no specific validity studies were applied to shellfish) West (1989) mentioned a study related to crustaceans which indicates a high false positive rate by ELISA in the case of heavily contaminated products. This did not appear to be a problem in this study. On the other hand, negative responses from ELISA technique paired with positive results by the conventional technique, are related to low contamination levels. This observation states the question of the respective sensitivity thresholds of these two techniques. Monfort et al. (1994) have assessed this threshold for the conventional method (10^4 to 10^5). It appeared lower than the values generally quoted for rapid indirect methods (10^5 to 10^6) (D'Aoust 1992).

CONCLUSION

The ability of bivalves to accumulate and retain enteric pathogens such as *Salmonella*, with a high sensitivity is pointed out by the present study. The detection through mussel tissue appears as a quite consistent and reliable method to reveal low contamination levels in seawater.

As a consequence, the present study emphasizes the value of mussels as a sampling material in the monitoring of *Salmonella* in seawater.

Regarding to the fate of *Salmonella* in air-stored shellfish, it appears from our study that these pathogens can persist in shellfish for a long time without appreciable changes. Therefore, *Salmonella* evolution in edible shellfish is sufficiently slow to allow

TABLE 4.

Distribution of paired detections by ELISA and the conventional method.

		ELISA Technique	
		Positive	negative
Conventional Method	Positive	33	2
	Negative	1	8

survival of these pathogens beyond the time generally requested for the marketing of living shellfish. *Salmonella* behaviour in shellfish is quite different from their evolution in seawater (Morinigo et al. 1990), and it would be interesting to elucidate the reasons of this particular behaviour.

The use of new methods such as ELISA techniques (which appeared from the present study adapted for *Salmonella* detection in shellfish) would allow a more intensive sampling, necessary to study more thoroughly relationships between *Salmonella* and shellfish.

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A QUALITATIVE APPROACH TO MANAGING SHELLFISH POPULATIONS: ASSESSING THE RELATIVE IMPORTANCE OF TROPHIC RELATIONSHIPS BETWEEN SPECIES

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ABSTRACT Simple qualitative models are applied to investigate aspects of how ecological interactions between a variety of predators and competitors of shellfish can be used to assist with the development of resource management policies. The modeling approach illustrates how typically complex interspecific interactions which vary with species and shellfish life stage can influence shellfish populations. Model results show the importance of managing shellfish predators, as well as selected groups of fouling species that may function as larval predators or competitors or both with juvenile shellfish life stages. While the modeling approach is limited by the lack of estimating intensities of interspecific interaction, it allows first-order insight into the relative importance of different types of interactions on shellfish populations and which variables are likely to be the most important in affecting shellfish abundance. Coupled with rigorous experimental hypothesis testing, the qualitative modeling approach provides assistance regarding which life stages of a shellfish species or interacting species needs to be managed in order to maximize shellfish productivity. Examples using the eastern oyster (*Crassostrea virginica*) are given.

KEY WORDS: loop analysis, resource management, qualitative interactions

> . . . fisheries regulations, which represent to some often large degree an educated guess, should be formulated using modern principles of experimental design to allow rigorous testing of their influences on key population and system variables.
>
> C. H. Peterson (1990)

INTRODUCTION

Shellfish resource management practices range from harvesting regulations which place limits on the minimum size and quantity of adults to re-seeding programs designed to supplement natural populations with hatchery-reared juveniles. Other management policies include shellfish predator control, enhancing the suitability of larval and juvenile habitats, planting adult shellfish for spawning purposes and maintaining natural "seed beds" to enhance juvenile shellfish abundance for subsequent transplant to managed shellfish beds (e.g. MacKenzie 1970, Kassner and Malouf 1982, Kennedy 1989). While the ultimate goal of all the various resource management practices is to enhance shellfish abundance, they differ both conceptually and pragmatically by concentrating on the management or manipulation of different phases of the shellfish's life history. The importance of this was recently illustrated by Malinowski and Whitlatch (1988) who examined the theoretical relationship between harvestability and life history features of three commercially important bivalves: *Crassostrea virginica* (Gmelin) (eastern oyster), *Mya arenaria* (L.) (softshell clam) and *Mercenaria mercenaria* (L.) (northern quahog). They demonstrated that for all three species population growth rates were 2–3 orders of magnitude more sensitive to changes in survivorship of larval and juvenile stages of the life cycle than proportional changes in either survivorship or fecundity of adult size classes. Ulanowicz et al. (1980) also illustrated the importance of reducing the mortality of newly recruited juvenile oysters. Their computer model was designed to forecast changes in the oyster harvest based on spat produced three to seven years earlier. The model was successful (explaining 56% of the variation in oysters harvested in the Chesapeake Bay) only after spat production was weighted by the management effort four, five, six and nine years prior to the harvest year. Collectively, these results indicated the greatest return would be realized in shellfish production if management efforts were directed to increasing juvenile survivorship and the quality or quantity of juvenile shellfish habitat.

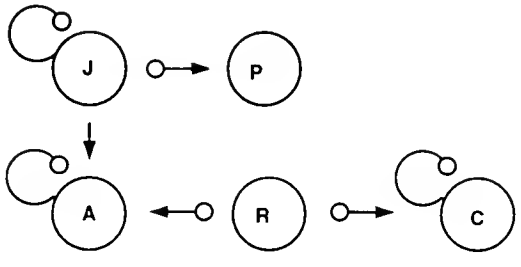
The success of any shellfish resource management program will largely depend on the life history attributes of the exploited species and how the species interacts with its abiotic and biotic environment. For instance, Peterson (1990) recently documented how knowledge of predation activities can be used to evaluate aspects of the bay scallop and northern quahog resource management along the southeastern Atlantic coast of the United States. Information on predator foraging ecology (e.g. prey size selectivity and preferences) and habitat selection were paramount in understanding how predators could be directly incorporated into resource management programs. Others have noted the importance of understanding competitive interactions between shellfish and other groups of species (e.g. Cole and Knight-Jones 1949, MacKenzie 1983) and how these species may be managed to enhance the shellfish resource.

For the past several years we have documented the role a variety of sessile invertebrates (e.g. barnacles, tunicates, bryozoans) have on settlement and postsettlement survivorship and growth of *C. virginica* (Osman et al. 1989, 1990, Zajac et al. 1989). Our studies found that many interspecific interactions between the sessile species and oysters could be pronounced and often changed with oyster life stage. For example, two species of solitary tunicates (*Ciona intestinalis* [L.], *Styela clava* Herdman) were found to prey on oyster larvae and function as competitors with juvenile oysters. Other species (e.g. colonical tunicates, *Botryllus schlosseri* (Pallas) and *Botrylloides* sp.) competed only with juvenile oysters and had little or no influence on larvae or larger oysters. Other studies have also noted how the activities of predators can change with oyster size. For example, the flatworm *Stylochus*

ellipticus, blue crab (*Callinectes sapidus* Rathbun) and mud crabs (*Neopanope sayi* Smith) can be important predators of oyster spat and juveniles, but not necessarily of adult oysters (e.g. Loosanoff 1956, Webster and Medford 1959, Kranz and Chamberlin 1979, MacKenzie 1981, Elner and Lovoie 1983, Bisker and Castagna 1987, Eggleston 1990).

Because of the observed complexity of these interspecifc interactions, it is necessary to examine the relation between specific life stages and particular species in order to fully understand how best to increase the productivity of the species being managed. However, while it is often relatively easy to identify a specific ecological interaction it is commonly more difficult to assess how best to direct shellfish management practices in order to enhance the harvestable shellfish population given a particular interaction. In this paper we present a simple qualitative modeling approach ("loop analysis" *sensu* Levins 1973, 1975a, b), that uses the knowledge of interspecific ecological interactions between a shellfish species with potential competitors and predators to assist with the development and testing of shellfish resource management practices. An advantage of this modeling approach is that it requires only qualitative information on ecological interactions. Specific models can be based on interactions that are known to occur, but without the detailed quantitative data that are usually absent. It

MODEL 1



INTERACTION MATRIX

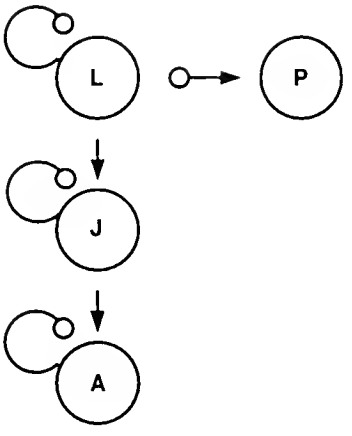
	J	A	P	R	C
J	-	+	+	0	0
A	0	-	0	-	0
P	-	0	0	0	0
R	0	+	0	0	+
C	0	0	0	-	-

"PREDICTION MATRIX"

	J	A	P	R	C
J	0	0	+	0	0
A	0	+	0	-	-
P	-	-	+	+	+
R	0	+	0	+	+
C	0	-	0	-	+

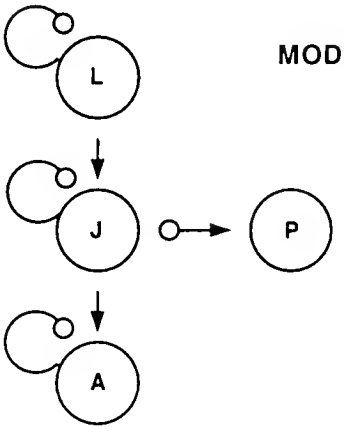
Figure 1. A loop model illustrating an example of the effects of a predator (P) which consumes juvenile shellfish (J) and a competitor (C) which competes with adult shellfish (A) for a common resource (R). Lines connecting variables show causal effects on one another. Lines ending in circle heads show negative effects while lines ending in arrows represent positive effects. Negative intra-variable links (links starting and ending with the same variable) indicate self-damping or density-dependent effects. The interaction matrix summarizes qualitative relationships (+ = positive effect; − = negative effect; 0 = no effect) between all variables in the model. The "prediction matrix" summarizes the effects on equilibrium abundance of variables (horizontal) when increases in the growth rate of another variable (vertical) occurs (see text for further explanation). Qualitative changes are expressed as positive (+), negative (−) or no change (0).

MODEL 2A



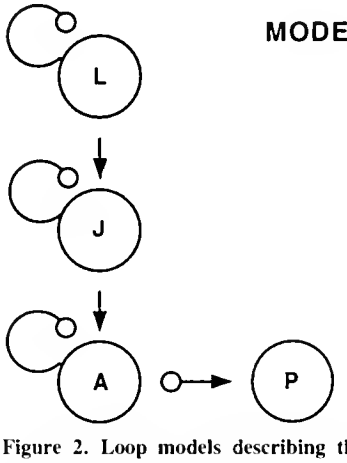
	L	J	A	P
L	0	0	0	+
J	0	+	+	0
A	0	0	+	0
P	-	-	-	+

MODEL 2B



	L	J	A	P
L	+	0	0	+
J	0	0	0	+
A	0	0	+	0
P	0	-	-	+

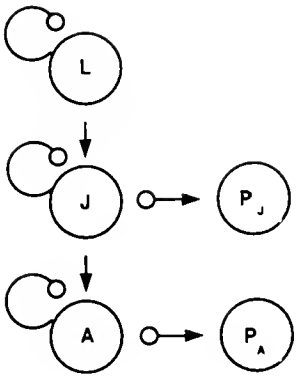
MODEL 2C



	L	J	A	P
L	+	+	0	+
J	0	+	0	+
A	0	0	0	+
P	0	0	-	+

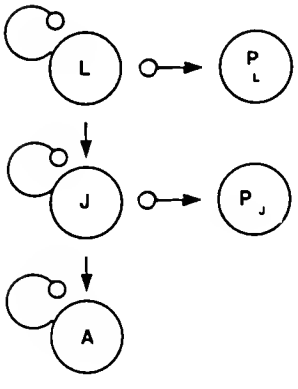
Figure 2. Loop models describing the effects of different types of predators (P) on three stages of oysters (L = larvae; J = juveniles; A = adults). Model 2A describes the effects of a predator preying only on the larval stage of the oyster population, while Model 2B and Model 2C illustrate the effects of predators exclusively on juvenile and adult oysters, respectively. Models 2D-2F describe the effects of two different types of predators, each specializing on a different oyster life stage (P_L = predators of oyster larvae; P_J = predators of juvenile oysters; P_A = predators of adult oysters) while Model 2G illustrates the influence of three predators, each feeding on a single oyster life stage. Each model is associated with its own "prediction matrix" (see Fig. 1 and text for details).

MODEL 2D



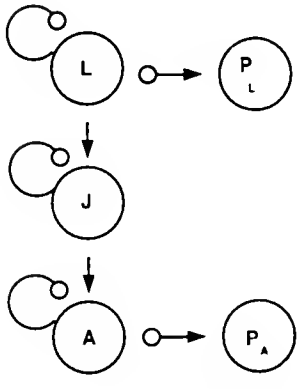
	L	J	A	P_J	P_A
L	+	0	0	+	0
J	0	0	0	+	0
A	0	0	0	0	+
P_J	0	-	0	+	-
P_A	0	0	-	0	+

MODEL 2E



	L	J	A	P_L	P_J
L	0	0	0	+	0
J	0	0	0	0	+
A	0	0	+	0	0
P_L	-	0	0	+	-
P_J	0	-	-	0	+

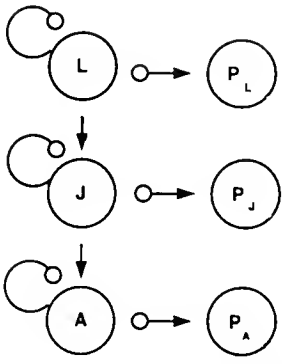
MODEL 2F



	L	J	A	P_L	P_A
L	0	0	0	+	0
J	0	+	0	0	+
A	0	0	0	0	+
P_L	-	-	0	+	-
P_A	0	0	-	0	+

Figure 2. Continued.

MODEL 2G



	L	J	A	P_L	P_J	P_A
L	0	0	0	+	0	0
J	0	0	0	0	+	0
A	0	0	0	0	0	+
P_L	-	0	0	+	-	0
P_J	0	-	0	0	+	-
P_A	0	0	-	0	0	+

Figure 2. Continued.

should be emphasized, however, that the chief utility of loop models is to develop insights as to the outcome of complex biological interactions and the relative potential of a particular management strategy. Because the approach makes no distinctions between large and small quantitative changes in a particular ecological interaction, model results must be tested rigorously before adopting any management practice.

Our principal goal in this study is not to present a detailed resource modelling overview using loop analysis methodology. Rather, it is to illustrate how various types of ecological interactions influence different life stages of a shellfish species and how knowledge of the nature of these interactions can be used to provide insights as to which life stage(s) of a shellfish species or the interacting species needs to be managed in order to enhance the shellfish resource. More sophisticated resource management uses of loop analysis are to (a) analyze the stability properties of a system and (b) determine the effects of how changes in one variable influence other variables in the system. Given this ability, it is then possible to identify which variables may have the greatest influence on the system.

The examples presented below illustrate how different types of ecological interactions potentially can influence the abundance of discrete life stages of a shellfish species. While we frequently illustrate ecological interactions of eastern oysters with other types of benthic species, we feel the approach is general enough to be applied to a broad array of management considerations and other shellfish species.

METHODS

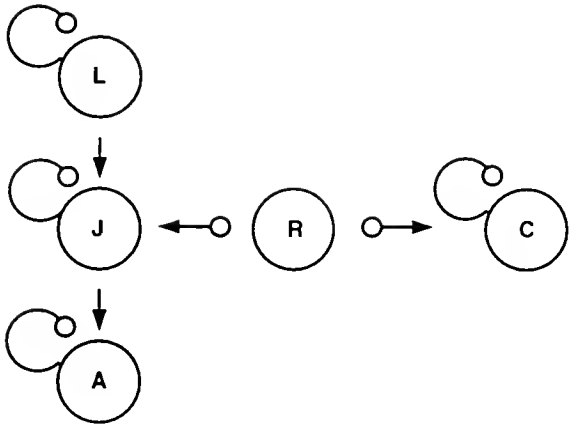
Loop Analysis

Loop models use network analysis to study biological systems. The models provide a framework for formulating qualitative relationships between variables within a particular biological system, permit analysis of the stability properties of that system, and determine the effects of changes in one variable on the remainder of the system (e.g. Lane and Levins 1977, Puccia and Levins 1985, Giavelli and Bodini 1990). For studies related to resource management, loop analysis has previously been used to examine multispecies fisheries (Saila and Parrish 1972, Cobb and Caddy 1989), species introductions (Li and Moyle 1981), and "undesirable" species (Briand and McCauley 1978).

Loop analysis is based upon the correspondence between systems of differential equations, matrices and graphs and mathematical descriptions can be found in Puccia and Levins (1985), Edelstein-Keshet (1988) and Jeffries (1989). Ecological relationships are expressed graphically between different components of a system. The relationships are expressed as signs, indicating the type of feedback or influence each variable has upon another: positive, negative or zero. For instance (+, −) denotes a predator-prey or parasite-host interaction, while (−, −) represents competition between two species.

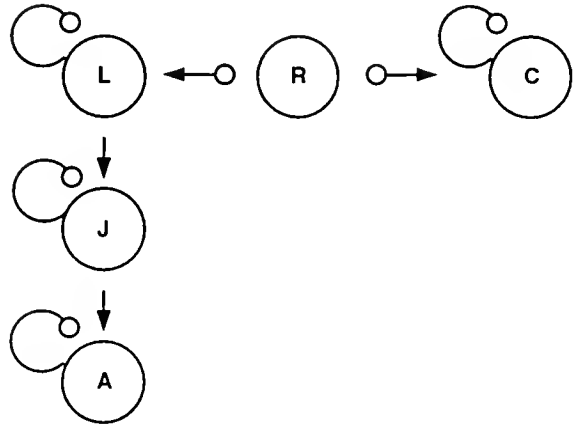
An ecological system is displayed graphically as a network of vertices (circles) and edges (arrows). Each variable is represented by a circle and arrows represent direction of interactions. A loop is one or more interactive links that connect one or more variables in a closed circuit. For example, Figure 1 depicts two different life

MODEL 3A



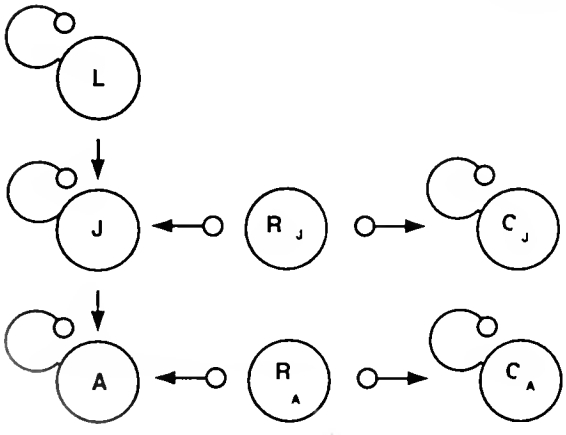
	L	J	A	C	R
L	+	+	+	-	-
J	0	+	+	-	-
A	0	0	+	0	0
C	0	-	-	+	-
R	0	+	+	+	+

MODEL 3B



	L	J	A	C	R
L	+	+	+	-	-
J	0	+	+	0	0
A	0	0	+	0	0
C	-	-	-	+	-
R	+	+	+	+	+

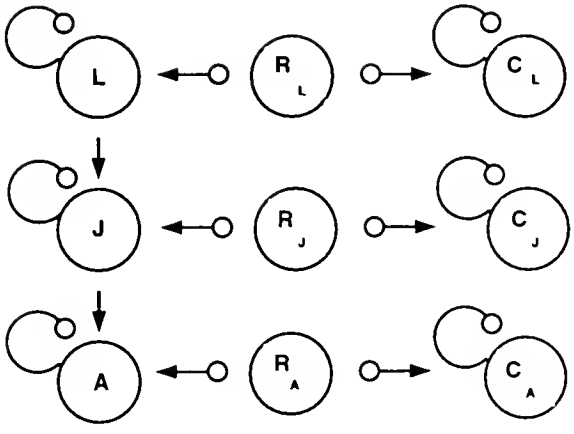
MODEL 3C



	L	J	A	C_J	C_A	R_J	R_A
L	+	+	+	-	-	-	-
J	0	+	+	-	-	-	-
A	0	0	+	0	-	0	-
C_J	0	-	-	+	+	-	+
C_A	0	0	-	0	+	0	-
R_J	0	+	+	+	-	+	-
R_A	0	0	+	0	+	0	+

Figure 3. Loop models describing the effects of different types of competitors (C) on three stages of oysters (L = larvae; J = juveniles; A = adults) when competing for a shared resource (R). Models 3A and 3B show the effects of a competitor of juvenile and larval oysters, respectively. Model 3C examines the effects of two competitors; one competing with juvenile oysters (C_J) for a unique resource (J_R), while the other is competing with adult oysters (C_A) over a shared resource (A_R). Model 3D illustrates the influence of three different types of competitors on three oyster life stages. Each model is associated with its own "prediction matrix" (see Fig. 1 and text for details).

MODEL 3D



	L	J	A	C_L	C_J	C_A	R_L	R_J	R_A
L	+	+	+	-	-	-	-	-	-
J	0	+	+	0	-	-	0	-	-
A	0	0	+	0	0	-	0	0	-
C_L	-	-	-	+	+	+	-	+	+
C_J	0	-	-	0	+	+	0	-	+
C_A	0	0	-	0	0	+	0	0	-
R_L	+	+	+	+	-	-	+	-	-
R_J	0	+	+	0	+	-	0	+	-
R_A	0	0	+	0	0	+	0	0	+

Figure 3. Continued.

stages of a shellfish species (J = juveniles and A = adults), and the arrow connecting juveniles to adults represents growth between successive stages. Each variable has a self-regulating or self-damping loop indicating they exploit depletable resources and density-dependent processes (e.g. space or food limitation) influence their growth. The model contains a predator (P) which consumes only juveniles. The qualitative predator-prey interaction is depicted by a negative (circle head) effect on juveniles and a positive (arrow) effect for the predators. Also included is a competitor (C) of adult oysters. Because of stability criteria associated with loop analysis (see below), the competitive interaction is displayed as two variables (C, A) competing for a shared resource (R). Qualitative interactions among all the variables in the example are summarized as an interaction matrix (see Fig. 1).

Each loop model must meet several stability criteria (Quirk and Ruppert 1965 for mathematical proof; May 1973 for biological importance): (a) no positive feedback on any single variable can exist, (b) at least one self-regulating loop must occur, (c) only predator-prey, self loops and single + and − effects can be included, (d) there can be no loops containing three or more variables, and (e) no node can be devoid of an input arrow. Given these conditions, each "predation community" (subgraphs composed of all interconnected predation links in a loop) must *fail* "color tests" developed by Jeffries (1974) such that it is not possible to color each node in the subgraph black or white using the following scheme: (a) each self-regulating node is black, (b) the subgraph contains at least one white point, (c) each white point is connected by a predation link to at least one other white point, and (d) each black point connected by a predation link to one white node is also connected by a predation link to one other white node.

If a loop model is sign-stable it is then possible to examine the qualitative relationships between variables and examine how each component affects others in the model. A "prediction matrix" (PM) is calculated using the equation:

$$PM = -\text{inv}[A]$$

where $[A]$ represents the interaction matrix. The "prediction matrix" in Figure 1 shows how an increase in the rate of change of a specific variable (row) influences the equilibrium abundance of any other variable (column). For example, increasing the growth of predators leads to decreases of juvenile and adult life stages and increased growth of predators, competitors and the resource. In contrast, increasing the growth of competitors leads to decreases of adults and the resource it is consuming, and has no effect on juveniles or the predators.

Assumptions

In addition to criteria required for model stability, several assumptions were made in constructing the loop models presented in this study. Self-damping loops were included for all the shellfish life stages and competitors, but not for mobile predators or resources (e.g. food, space). This assumes that competitors are regulated by density-dependent processes while predators and resources are not. To maintain stability requirements or reflect ontogenetic changes in trophic relationships between species or both, we sometimes separated oyster competitors and predators into two separate life stages (denoted by numeric subscripts [see Figs. 4–6]). A linkage between oyster adults and larvae was not included. We assumed that shellfish management policies are primarily directed to local, rather than system-wide, populations (e.g. a shellfish bed rather than shellfish in an entire estuary). Lastly, in the construction of our models we assumed that the goal of management was to enhance the abundance of adult oysters.

The Data Base

Much of the information used in the construction of the loop models was derived from our previous work examining the effect(s) of a variety of common sessile invertebrates on *C. virginica*. Primary objectives of this work were to examine (1) whether post-settlement interactions among sessile invertebrates influenced oyster recruitment patterns, and (2) how these interactions and

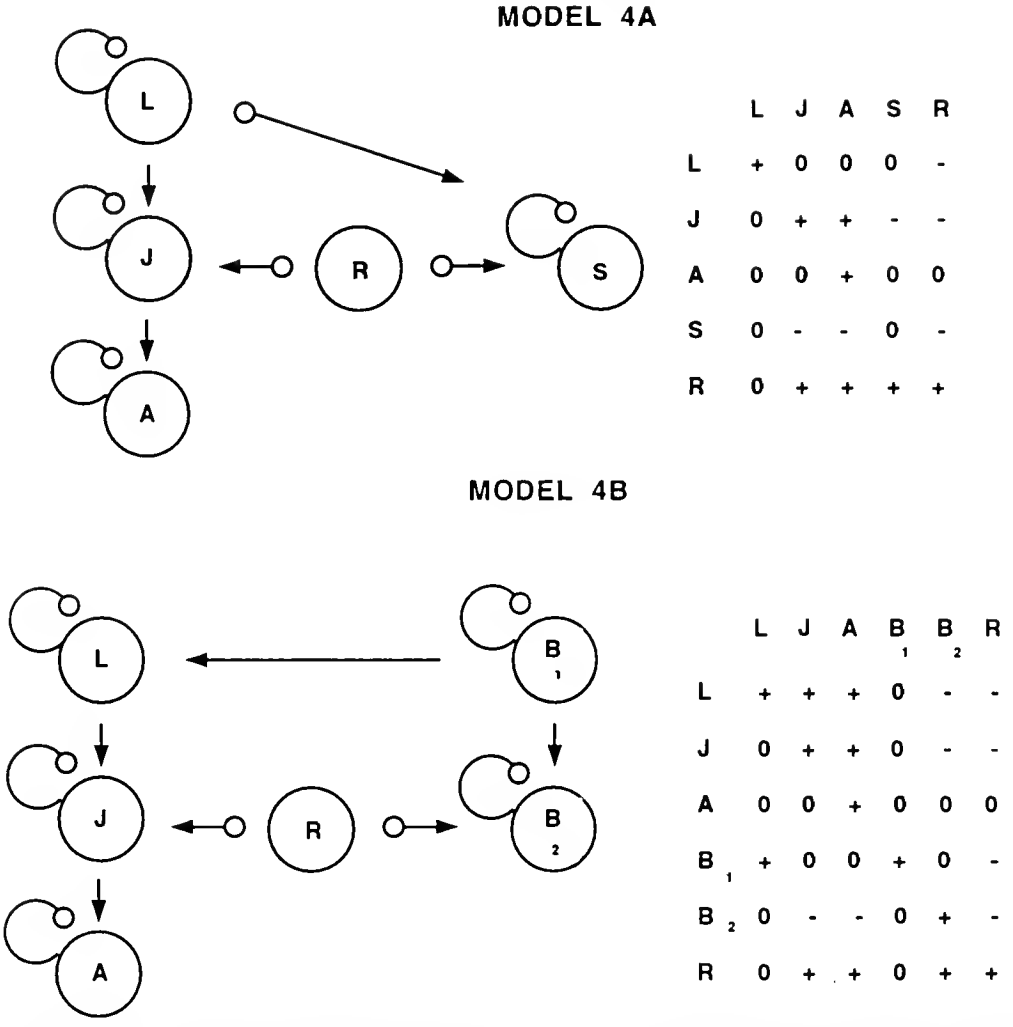


Figure 4. Loop models describing the effects of ontogenetic changes in the trophic relationships between two different sessile species on three oyster life stages. Model 4A illustrates the effects of a solitary ascidian (S) in which the ascidian is a predator of larval oysters and acts as a competitor with juvenile oysters. Model 4B examines the influence of encrusting bryozoans on the oyster population. The bryozoan acts to enhance the settlement of larval oysters (B_1) while it functions as a competitor (B_2) with the juvenile oyster life stage. Each model is associated with its own "prediction matrix" (see Fig. 1 and text for details).

their effects changed with oyster life-history stage. In most experiments, treatments consisted of 100 cm^2 panels which contained known densities of a single taxon of particular sessile invertebrate. Panels were exposed to competent oyster larvae in the laboratory in order to assess the effect of a particular species on larval settlement and post-settlement juvenile oyster growth and mortality (see Osman et al. 1989 for details). Other data on the qualitative nature of ecological interactions between oysters and other species that were incorporated into our models were obtained from observations or from the literature. Table 1 summarizes information on the qualitative ecological associations between three life stages of oysters and a variety of benthic species commonly found to influence eastern oyster survivorship.

RESULTS

Predator Effects

Figure 2 (Models 2A-2G) depicts how various types of predators specializing on different oyster life stages influence adult oysters. For example, Model 2A shows how predators (solitary ascidians [*S. clava, C. intestinalis*], sea anemones [*Diadumene leucolena*]) feeding only on oyster larvae can have a negative effect on all three oyster life stages. Model 2B, in comparison, illustrates how a predator which consumes only juvenile oysters (e.g. flatworms [*S. ellipticus*]) can negatively influence juvenile and adult oyster life stages.

Models containing predators that are limited to the consumption of a single oyster life stage (Models 2A-2C) show that regardless of the specific oyster life stage being consumed, predators can negatively influence adult oysters. Alternative models assessing the effects of multiple numbers of specialized predators consuming different oyster life stages generally show that their effects on oysters remain pronounced (Models 2D-2G). The models show that management efforts directed to reducing adult oyster mortality should first be directed to those predators consuming the oldest oyster life stage. For example, predators of juvenile oysters can also negatively affect adult oysters (Model 2E).

All the predator models, except Model 2A, suggest that enhancing the number of oyster larvae or juveniles in the presence of a predator will have no positive effect on adult oysters. Therefore, if shellfish predation is the primary management problem, the loop

TABLE 1.

Examples of the qualitative nature of ecological interactions of a variety of species commonly associated with three different life stages of the eastern oyster, *Crassostrea virginica*.

Interacting Species	Effect on Oyster Larvae	Effect on 1 to 3 Month Old Oysters	Effect on 3–12 Month Old Oysters
Barnacles *Balanus* spp.	Positive (enhanced settlement)	No effect	No effect
Solitary ascidians *Ciona intestinalis* *Styela clava*	Negative (predation)	Negative (competition)	No effect
Foliose bryozoan *Bugula turrita*	Positive (enhanced settlement)	Negative (competition)	Positive? (camouflage from predators)
Colonial ascidians *Botryllus schlosseri* *Botrylloides* sp.	No effect	Negative (competition)	No effect
Encrusting bryozoans *Crytosula pallasiana* *Schizoporella errata*	Positive (enhanced settlement)	Negative (competition)	Positive? (camouflage from predators)
Flatworms *Stylocus ellipticus*[1]		Negative (predation)	
Starfish *Asterias forbsii*[2]		Negative (predation)	Negative (predation)
Slipper shells *Cepidula fornicata*[3] *Cepidula plana*		Negative (competition)	
Jingle shells *Anomia simplex*[4]		Negative (competition)	
Sea anemones *Diadumene leucolena*[5]	Negative (predation)		
Crabs[6]		Negative (predation)	Negative (predation)

Unless otherwise noted information is summarized from Osman et al. (1989, 1990) and Zajac et al. (1989). Empty cells indicate no information available on the nature of the interspecific interaction.

[1] Loosanoff 1956, Webster and Medford 1959.

[2] Various sources (e.g. Galtsoff and Loosanoff 1939, Galtsoff 1964).

[3] MacKenzie 1981.

[4] Loosanoff 1965.

[5] MacKenzie 1977, Steinberg and Kennedy 1979.

[6] Various species (e.g. Lunz 1947, Menzel and Nichy 1958, Kranz and Chamberlin 1978).

models indicate that predator control measures appear to be a viable means of managing the oyster resource. Alternative management approaches such as efforts aimed at increasing larval and juvenile oyster abundance through seeding programs or enhancing the quantity of juvenile habitat appear to have little positive effect in increasing the number of harvestable oysters.

Competitor Effects

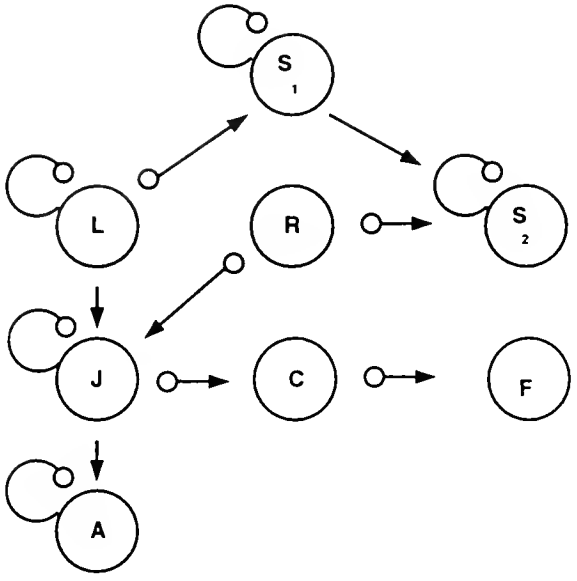
Loop models presented in Figure 3 illustrate several examples of how several types of competitors can influence different oyster life stages. Models 3A-3B examine the effects of competitors interacting with a single oyster life stage, while Models 3C and 3D illustrate the simultaneous influence of competitors on two or three oyster life stages, respectively. Results generally show that regardless of oyster life stage being affected, oyster competitors can negatively influence adult oysters. Competitor effects can cascade through the system similar to those demonstrated in the oyster-predator models (Fig. 2).

Unlike results generated by the predator models, all the examples containing oyster competitors show that management efforts directed to enhancing the numbers of oyster larvae and juveniles can lead to positive effects on adult oysters. Secondly, the loop models show in those cases where it is important to control oyster competitors, efforts should be directed to managing all stages of oyster development and competitors of those stages prior to and including the life stage of interest (e.g. Models 3C-3D).

Effects of Changing Trophic Relations Between Species

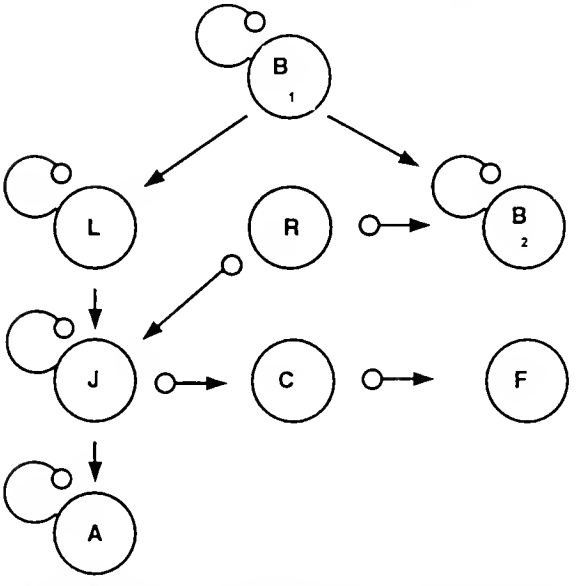
We have previously documented how changing trophic interactions among various sessile invertebrates species quantitatively influence different life stages of the eastern oyster (Osman et al. 1989, 1990, Zajac et al. 1989). Several types of models were constructed to depict how these ontogenetic changes in trophic

MODEL 5A



	L	J	A	S_1	S_2	C	F	R
L	+	0	0	+	0	0	0	-
J	0	+	+	0	-	0	+	-
A	0	0	+	0	0	0	0	0
S_1	-	-	-	+	+	0	0	0
S_2	0	-	-	0	+	0	-	-
C	0	0	+	0	0	0	+	0
F	0	+	+	0	-	-	+	-
R	0	+	+	0	+	0	+	+

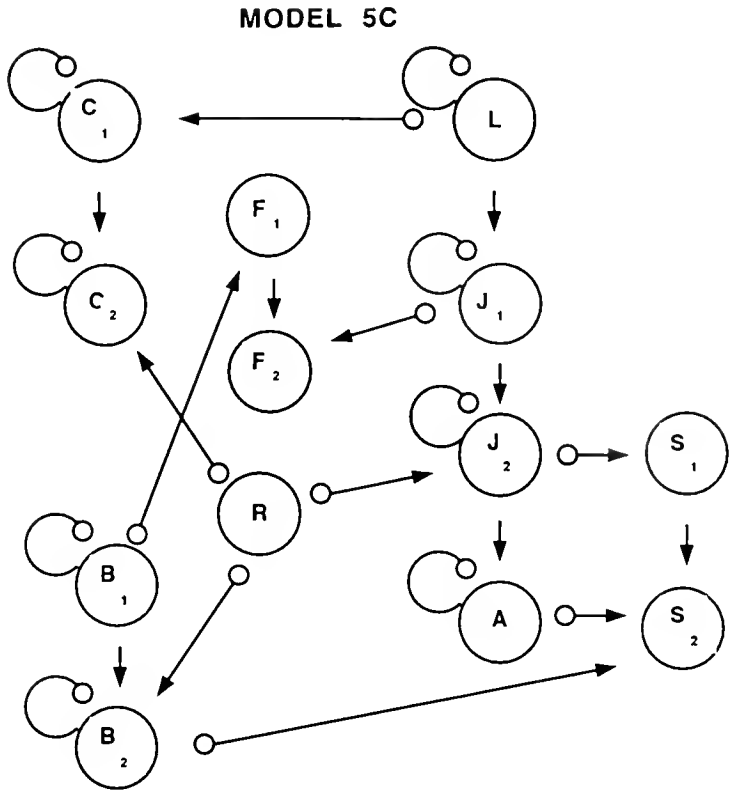
MODEL 5B



	L	J	A	B_1	B_2	C	F	R
L	+	+	+	0	-	0	+	-
J	0	+	+	0	-	0	+	-
A	0	0	+	0	0	0	0	0
B_1	+	0	0	+	0	0	0	-
B_2	0	-	-	0	+	0	-	-
C	0	0	+	0	0	0	+	0
F	0	+	+	0	-	-	+	-
R	0	+	+	0	+	0	+	+

Figure 5. Loops models describing the effects of complex trophic interactions on three oyster life stages. Model 5A includes the effects of an ascidian which preys on larval oysters (S_1) and acts as a competitor (S_2) with juvenile oysters. Also included in the model is the effects of crabs which prey on juvenile oysters while themselves are being preyed on by fish predators. Model 5B illustrates the effects of the crab and fish predators as well as incorporating the effects of bryozoans (see Fig. 4). Model 5C examines the effects of multiple groups of competitors and predators on different oyster life stages: C_1 and C_2 represent an ascidian species which preys on oyster larvae and competes with juvenile oysters; B_1 and B_2 illustrate the effects of barnacles which act as space competitors with juvenile oysters and are preyed upon by flatworms (F) and starfish (S); flatworms (F_1 and F_2) prey upon juvenile oysters and barnacles while starfish (S_1 and S_2) feed on juvenile and adult oysters as well as barnacles. Each model is associated with its own "prediction matrix" (see Fig. 1 and text for details).

relationships may affect oyster populations (Fig. 4). Model 4A illustrates the effect of the solitary ascidian, *S. clava*, on three oyster life stages. The ascidian is capable of ingesting oyster larvae, competing with juvenile oysters for space but has no apparent effect on adult oysters (Table 1). Model results show that the effects of the ascidian can be as pronounced as those of various types of predators depicted in Figure 2. In contrast, however, is the result that increasing juvenile oysters would be an effective management practice for enhancing adult oysters. Alternatively, increasing oyster larvae has no effect on adult oysters.

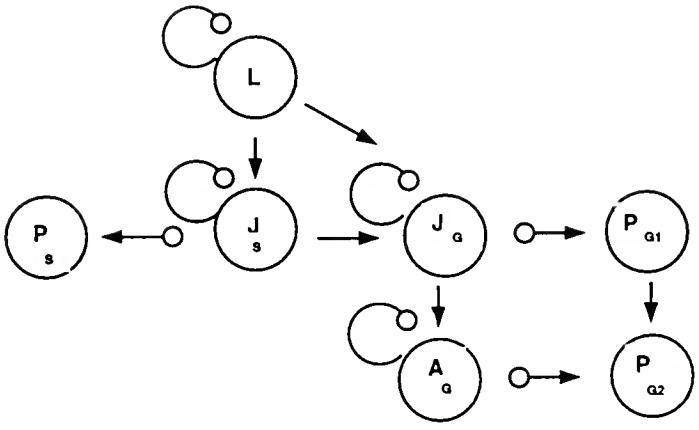


	L	J_1	J_2	A	C_1	C_2	B_1	B_2	F_1	F_2	S_1	S_2	R
L	+	0	0	+	+	0	0	0	0	+	-	-	-
J_1	0	0	0	0	0	0	0	0	0	+	0	0	0
J_2	0	0	0	-	0	+	0	-	0	0	+	+	+
A	0	0	0	0	0	+	0	-	0	0	+	+	+
C_1	-	0	0	+	+	0	0	0	0	-	-	-	-
C_2	0	0	0	+	0	0	0	0	0	0	-	-	-
B_1	0	-	0	+	0	-	0	+	+	+	-	-	-
B_2	0	0	0	0	0	-	0	+	0	0	-	0	-
F_1	0	-	0	+	0	0	-	0	+	+	-	-	0
F_2	0	-	0	+	0	-	0	+	0	+	-	-	-
S_1	0	0	-	-	0	+	0	0	0	0	+	+	+
S_2	0	0	0	-	0	+	0	-	0	0	+	+	+
R	0	0	0	-	0	+	0	0	0	0	+	+	+

Figure 5. Continued.

The effects of encrusting bryozoans (e.g. *Cryptosula pallasiana* (Moll), *Schizoporella errata* (Waters)) on the three oyster life stages are shown in Model 4B. These species were shown to enhance the settlement of oyster larvae, act as space competitors with juveniles and may enhance the survival of adult oysters by camouflaging them from certain types of epibenthic predators (Table 1). The model shows the competitive effects of the bryozoans can negatively influence juvenile and adult oysters and suggest that control measures of these organisms may be warranted. The model also shows that management efforts directed at enhancing both larvae and juvenile oysters could translate into an increased number of adult oysters. Lastly, both models suggest that man-

MODEL 6A



	L	J_S	J_G	A_G	P_S	P_{G1}	P_{G2}
L	+	0	0	-	+	+	+
J_S	0	0	0	0	+	0	0
J_G	0	0	0	-	0	+	+
A_G	0	0	0	0	0	0	+
P_S	0	-	0	+	+	-	-
P_{G1}	0	0	-	-	0	+	0
P_{G2}	0	0	0	-	0	0	+

Figure 6. Loops models illustrating the effects of different types of predators and competitors in oyster seed bed and grow-out areas. In Model 6A predators forage on juveniles in seed beds (denoted by subscript "s") and juveniles and adults in grow-out areas (denoted by subscript "g"). Note that juvenile oysters in seed beds (J_S) are transplanted to grow-out areas (J_G) and that oyster larvae (L) are contributing to oyster populations in both areas. Model 6B describes the effects of competitors in oyster seed bed (denoted by subscript "s") and grow-out areas (denoted by subscript "g"). Each model is associated with its own "prediction matrix" (see Fig. 1 and text for details).

agement policies of enhancing the resource (e.g., spreading more cultch) that juvenile oysters and fouling species are competing for should have a positive effect on juvenile and adult oysters.

Multiple Predator/Competitor Effects

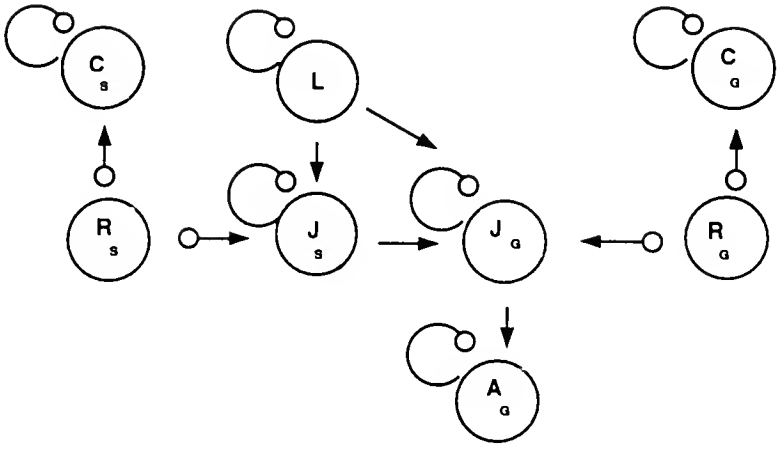
The loop models have thus far been limited to relatively simple relationships between shellfish and various types of competitors and predators. Most natural systems, however, are comprised of complex assemblages of interacting organisms, and several models were developed to illustrate the utility and limitations of the loop analysis modeling approach in assessing the multiple predator/competitor effects on oyster populations (Fig. 5).

Model 5A describes an ascidian species that preys on oyster larvae and is a space competitor with juvenile oysters. Mudcrabs, which typically feed on juvenile oysters, are also included in the model. These predators, however, are themselves prey for a demersal fish (e.g. toad fish). The model shows the predaceous fish negatively effects the mudcrabs and that its presence can lead to positive effects on juvenile and adult oysters. The ascidian also has a negative effect on all three oyster life stages, either directly or indirectly. While it is apparent that predator control measures would have a positive effect on harvestable oysters, information on the relative quantitative effects of the two types of predators on oyster mortality must be known before a specific management policy is adopted.

In Model 5B the ascidian is replaced by encrusting bryozoans which enhance oyster larval settlement and compete with juvenile oysters for space (Table 1). As in the previous example, increasing juvenile oysters and fish preying on the mudcrabs continues to have a positive effect on adult oysters. In addition, methods for enhancing the abundance of oyster larvae also lead to positive effects on adult oysters. As in Figure 1, both Models 5A and 5B show that the use of "biological control" procedures (e.g. Gibbons and Castagna [1985] for the northern quahog) to reduce of shellfish predation appears to be a viable oyster management option.

The models thus far show that controlling the abundance of the top-most predator is always results in a positive effect on adult

MODEL 6B



	L	J_S	J_G	A_G	C_S	C_G	R_S	R_G
L	+	+	+	+	-	-	-	-
J_S	0	+	+	+	-	-	-	-
J_G	0	0	+	+	0	-	0	-
A_G	0	0	0	+	0	0	0	0
C_S	0	-	-	-	+	+	-	+
C_G	0	0	-	-	0	+	0	-
R_S	0	+	+	+	+	-	+	-
R_G	0	0	+	+	0	+	0	+

Figure 6. Continued.

shellfish. Even when ecological complexity is increased to include multiple types of competitors and predators, as in Model 5C, this management strategy results in positive effects on harvestable oysters. Model 5C shows a system in which solitary ascidians prey on oyster larvae and compete with the juveniles. Barnacles also compete with both ascidian and juvenile oysters for available substrate space, and flatworms prey on juvenile oysters as well as barnacles. Starfish, which feed on juvenile and adult oysters, are also included in the model. As in the previous models, results of this model show that the control of the top predator, in this case starfish, can lead to a positive effect on adult oysters.

These models also illustrate some of the limitations of the qualitative modeling approach. Adding ecological complexity generates model results which are sometimes difficult to interpret. For example, Model 5A shows that increasing the juvenile oyster predator (C_1) leads to a positive effect on adult oysters, while Model 5C shows that enhancing the number of oyster larval and juvenile competitors results in a positive effect on adult oysters. In the first case, one must have information on whether competitive interactions are quantitatively more important than predator-prey interactions or vice versa. In the second instance, insight into the strengths of the predator-prey interaction between the crab and juvenile oyster relative to those of the crab and predaceous fish is needed. In both cases there is a requirement for fairly extensive knowledge of the relative strengths of interspecific interactions between oysters and their competitors and predators.

Seed Beds and Grow-out Area Models

A common oyster management practice in some regions in the U.S. (e.g. Long Island Sound) involves collecting natural oyster spatfall on artificially-planted shell cultch in "seed bed" areas (see MacKenzie 1970 for details). The seed beds are typically found in estuarine habitats and salt ponds where abundant oyster populations naturally occur. Several months after larvae have attached to the shells, seed oysters are then transplanted to managed beds for subsequent growth to harvestable size. Two loop models were developed to examine the relative importance of different types of biotic interactions within seed beds and what effect they may have on the management of oysters found in seed beds and grow-out areas (Fig. 6).

Model 6A illustrates the effects of benthic predators consuming

juvenile and adult oysters in seed beds and grow-out areas. The model suggests that seed bed predators can lead to reduction of juveniles in seed beds, as well as reduction in the number of spat transplanted to grow-out areas. In addition, enhanced seed bed oyster larvae and juvenile survivorship lead to reduced numbers of adult seed oysters. As with previous examples illustrating the effects of predators on oyster abundance, Model 6A points to the importance of first managing predators in the seed bed and then in the grow-out areas.

Model 6B compares the relative importance of the effects of competitors in seed bed and grow-out areas. In this example the competitor (e.g. barnacles, encrusting ascidians) competes only with juvenile oysters. The model shows that competitors inhabiting both areas can negatively effect adult oysters. Unlike the previous example, however, increasing larval or juvenile abundance or both in the seed bed areas can lead to a positive effect on adults. In cases where oyster competitors are a major management problem, the model shows two alternative solutions to management of the seed beds; reduce competitor abundance or develop methods for enhancing seed bed larval or juvenile survivorship.

DISCUSSION

Loop model results show the effects of predation on shellfish abundance can vary widely depending upon the specific type of predator and the oyster life stage consumed. Model results, however, generally reinforce the importance of the common management practice of controlling the top-most and generalized predators (e.g. starfish, crabs) to enhance the shellfish resource. Somewhat less intuitive are model results which show the effects of predators feeding exclusively on oyster larvae can cascade to older oyster life stages. Several studies have empirically or theoretically shown the importance of high rates of larval and juvenile mortality in influencing shellfish abundance (MacKenzie 1979, Blundon and Kennedy 1982, Malinowski and Whitlatch 1988, Osman et al. 1989, Peterson 1990, Osman and Abbe 1994). Obviously, it is important to quantitatively understand the relative intensity of predation and size-specific predator behavior of different suites of predators before decisions are made regarding specific methods of predator control. The point to be made, however, is that predators of shellfish larvae can be potentially as important as predators of juvenile and adult shellfish life stages and should be considered when establishing predator control programs.

Results generated by the various predator models can be broadened to assess the effects of disease or parasites on oyster populations. Model results show that increases in larval or juvenile oysters in the presence of a disease organism likely will have little effect on enhancing adult oysters. This result suggest that while developing disease resistant strains of shellfish (e.g. Ford and Haskin 1987) or supplementing natural beds with juveniles may have a positive effect on the short-term, such management practices may not necessarily translate into long-term consequences on shellfish populations. Like predator effects, developing methods of controlling the disease organism appear to always lead to the greatest payoff in an oyster management framework.

A variety of fouling species share hard substrate habitats with oysters and interact with them (Table 1). It is generally believed that these organisms compete with each other and oysters for the available substrate space or act as predators of settling oyster larvae or both. From collections and observations, MacKenzie (1970, 1981, 1983) showed that fouling species commonly associated with oyster seed beds in Long Island Sound were an important source of mortality of oyster spat. Recent experimental studies have generally confirmed those observations and quantified the effect of these organisms on shellfish larval and post-settlement mortality and growth (e.g. Bros 1987, Osman et al. 1989, 1990, Zajac et al. 1989, Dalby and Young 1993). It is important to note, however, that not all species influence oysters in a similar manner (Table 1) and their effects typically vary with oyster size. Loop models illustrating the complex nature of ontogenetic changes in trophic relationships, generally confirmed the importance of fouling species on the abundance of harvestable oysters. Given the nature of the complexity of these interactions, more detailed quantitative information on these relationships is needed before management programs designed to control or limit a specific species or group of species are implemented. Such programs could involve eliminating selective groups of competitors to enhancing the abundance of competitively inferior species or species which are beneficial to the survivorship and growth of juvenile oysters. MacKenzie (1970) also suggested that the effects of fouling organisms on oyster spat can potentially be reduced by delaying planting of cultch in seed beds until oyster larvae are competent to settle. Studies on comparing oyster recruitment and growth on cultch planted at different times of the year have shown mixed results (e.g. Morales-Alamo and Mann 1990, Dalby and Young 1993) and are likely dependent upon the fouling species involved and particular habitat conditions.

The continued need to develop appropriate management practices for commercially important shellfish has been illustrated by several workers (e.g. Caddy 1989a, Malinowski and Whitlatch 1989, Peterson 1990). While standard applications of traditional yield-per-unit effort or yield-per-recruit models have been applied to the management of shellfish populations (e.g. Caddy 1989b,c), these approaches can often be improved by substituting models based on direct tests of explicit hypotheses based on the specific characteristics of the system being studied. It is important, for example, to understand not only the population biology of the exploited shellfish species, but also the nature of the species' ecological interactions with potential competitors and predators. These interactions can be complex, vary spatially and temporally, and change over the course of an organism's life cycle. Qualitative models that examine how different types of ecological interactions effect oyster population abundance represent a first step in understanding the relative importance of complex ecological interactions and how they may be used to assess the type of management strategy needed to enhance the shellfish resource. The greatest uncertainties in applying loop analysis to any ecological system is the lack of incorporating intensities of interspecific interactions and the lack of a quantitative aspect to any model result. However, the approach does allow the resource manager to utilize available ecological knowledge in a formalized manner to gain reasonably constrained, first-order insight(s) which may not be intuitively obvious. For example, most loop models suggested that management policies directed to enhancing the amount of resource (e.g. cultch) for juvenile oysters would translate to a positive effect on adult oysters. However, Model 5C showed that when multiple competitors and predators were interacting with juvenile oysters, increasing the juvenile resource negatively influenced adult oysters. Knowledge of the relative strengths of the various interspecific interactions is needed before a specific management policy is adopted. The use of a qualitative modeling provides insight as to which variables in the system are most likely to effect the shellfish population and provides a focus for the design of *in situ* and/or laboratory studies to examine the specific nature of the

ecological interactions and develop means of more effective management of the resource.

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A BIBLIOGRAPHY OF "*DREISSENA POLYMORPHA* IN EUROPEAN AND RUSSIAN WATERS: 1964–1993"*

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ABSTRACT A bibliography of over 1000 papers on the biology, impacts, and control of the zebra mussel (*Dreissena polymorpha*) in European and Russian waters is compiled to aid scientists and managers in addressing this species of economic and ecological importance. The bibliography primarily includes publications between the early 1960s and early 1990s but does contain some earlier references not found in another extensive bibliography published in 1964. This bibliography will be a valuable tool, especially to water users and environmental scientists in North America where zebra mussels have recently invaded and become established.

KEY WORDS: zebra mussel, *Dreissena*, European waters, Russian waters

INTRODUCTION

The introduction, reproduction, recruitment, and dispersal of the zebra mussel, *Dreissena polymorpha*, in North America in the mid- and late-1980s has led to interest in studies on the biology, impacts, and control of this species in freshwater (Nalepa and Schloesser 1992). Zebra mussels are small (<5 cm long) freshwater bivalves that use byssal threads to attach to hard surfaces such as rocks, hard-bodied invertebrates, and water pipes (Fig. 1). Their discovery in the Laurentian Great Lakes raised immediate concern because mussels became abundant enough (ca. 700,000/m^2; Griffiths et al. 1989) to obstruct the flow of water through pipes, hoses, screens, and condensers in drinking water and power generating facilities in 1989. In addition, zebra mussels spread at an alarming rate throughout North America; by 1993 zebra mussels were found in major waterways extending from Quebec to Louisiana and from New York to Oklahoma and are expected to invade approximately ¾ of the surface waters in the United States (Nalepa and Schloesser 1992). In 1993, densities of mussels in the Illinois River were about 100,000/m^2—a density equal to the maximum recorded for this species in Europe (Nalepa and Schloesser 1992). The magnitude and speed at which zebra mussels became established in the Great Lakes resulted in a great demand for information about the biology, effects, and control of this species in European and Russian waters where it occurred during the past 200 years.

The search for information about zebra mussels in Europe and Russia by the Great Lakes scientific community was time-consuming and expensive. An important bibliography (1,180 references between the years 1771 and 1964) concerning zebra mussels was published by Limanova in 1964 and translated from Russian to English in 1968. This document was used extensively by scientists in North America to learn about zebra mussels. Another bibliography by Limanova (1978) was also published but is available only in Russian (translation in progress; New York Sea Grant Extension). Both bibliographies by Limanova focused heavily on the Russian literature, and much of the European literature is contained in many journals that are difficult to obtain.

Some European and Russian literature has been summarized from a North American perspective, including 1) Mackie et al. (1989), which lists 192 references, includes an annotated bibliography of 122 studies, and short summaries of the general biology, problems, benefits, and controls of zebra mussels, and 2) New York Sea Grant Extension, which lists references and brief summaries in *Dreissena polymorpha* Information Review (New York Sea Grant Extension, Brockport, New York). At present, information about zebra mussels in North American waters is primarily being documented in meetings, workshops, and large conferences and is only now beginning to be published in available literature (bibliography in preparation, Schloesser, National Biological Survey, Ann Arbor, Michigan).

We believe the need for information concerning zebra mussels is likely to increase as mussels invade, become abundant, and impact natural and human systems throughout North America. To address this need, we list 1000 references to zebra mussels in European and Russian waters. This document contains references to published studies, primarily between 1964 and 1993, and does not duplicate Limanova (1964). The document is a compilation of several literature searches of various computerized databases and extensive searching for references and verification of citations in papers about zebra mussels in European and Russian waters. Databases were compiled from several different perspectives, including the electric power industry, the potable water industry, and research and development organizations. In addition, the authors collected references from many European, Russian, and North American investigators of zebra mussel biology and control. We believe the present bibliography, when combined with that of Limanova (1964) will provide fast, efficient, and economical information about zebra mussels in European and Russian waters. This information will be a valuable tool to help water users and environmental scientists understand and mitigate impacts caused by invading zebra mussels in surface waters of North America.

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Figure 1. Zebra mussels, *Dreissena polymorpha*, covering an exposed shoreline of Lake Erie of the Laurentian Great Lakes 1989. (Photo courtesy of Detroit Edison Electric Power Company.)

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ABSTRACTS OF TECHNICAL PAPERS

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CONTENTS

BIVALVE AQUACULTURE

BIVALVE STOCK ENHANCEMENT AND RESTORATION

FEEDING AND NUTRITION OF BIVALVES

BIVALVE GENETICS

MORE LIGHT ON SEX DETERMINATION IN BIVALVES FROM ALL-FEMALE, GYNOGENETIC *MULINIA LATERALIS* SAY. Standish K. Allen, Jr.* and **Ximing Guo,** Haskin Shellfish Research Lab, Institute of Marine and Coastal Sciences, Rutgers University, Box B-8, Port Norris, NJ 08349.

Little is known about the sex determining mechanism of bivalves. Some models have been proposed for oysters (*Crassostrea* spp.), a protandric hermaphrodite; other inferences have been drawn from sex ratio of triploid soft shell clams (*Mya arenaria*), a dioecious species. We produced gynogenetic dwarf-surf clam (*Mulinia lateralis*) to test induction techniques prior to application to commercial species. Eggs were fertilized with ultraviolet light-irradiated sperm (one female and male per replicate) and subsequently treated with cytochalasin B to block the release of the second polar body. Diploid and triploidy controls were also produced, using normal sperm for diploids and by blocking the second polar body in normally fertilized eggs for triploids. The survival of gynogenetic diploids was low: 0.7% to eight days post-fertilization (PF) compared with 15.2% in the triploids and 27.5% in the normal diploids. Larvae in all groups metamorphosed at 8–10 days PF, and there was no significant post-larval mortality. At sexual maturation (2–3 months PF), all gynogenetic diploids were female. These results suggested that the dwarf-surf clam may have an XX, XY (like many vertebrates) or an XX:2A, XY:2A (like Drosophila) sex determinating mechanism. Sex ratio in both diploid and triploid controls was skewed in favor of males, triploids more so than diploids. The results taken together favor an XX, XY system although karyotypes have shown no evidence of heteromorphic sex chromosomes. NJAES publication no. K-32904-2-93.

MITOCHONDRIAL DNA VARIATION IN THE BAY SCALLOP, *ARGOPECTEN IRRADIANS*, AND THE CALICO SCALLOP, *ARGOPECTEN GIBBUS*. Sandra G. Blake,* School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

The subspecies of the bay scallop, *Argopecten irradians*, have long been distinguished on the basis of shell morphology, with diagnostic features including rib count, valve color and valve inflation. There is a significant degree of overlap in these characters, however, and there are no clear geographic boundaries delineating the ranges of the subspecies. Variation in the mitochondrial DNA of four geographically separate populations was examined in order to clarify current taxonomic designations and published geographic ranges. The hypothesis, that the sampled populations share a common gene pool, was tested. A concurrent study was conducted for two populations of the calico scallop, *Argopecten gibbus*.

A sample of the northern bay scallop, *Argopecten irradians irradians*, was obtained from Buzzards Bay, Massachusetts. The southern bay scallop, *A. i. concentricus*, was harvested from Harker's Island, North Carolina, Rabbit Key, Florida and Crystal River, Florida. For temporal comparison, a second sample was obtained from the Harker's Island population one year after the first collection. Calico scallops were collected from the Cape Canaveral region on the Florida Atlantic coast, and from Cape San Blas, in the Florida Gulf.

Mitochondrial DNA was purified from scallop gonad and mantle tissue by cesium-chloride density-gradient ultracentrifugation, then subjected to restriction fragment length polymorphism (RFLP) analysis with eight restriction enzymes. The measured nucleotide sequence divergence between the North Carolina and Massachusetts scallops was found to be less than that between either of these populations and the Crystal River bay scallops. These preliminary data suggest that genetic variation in *Argopecten irradians* may not coincide with variation in the morphological features used to assign subspecies designations and geographic ranges. The genotypic diversities and variation measured within and between all of the sampled populations will be discussed, with particular attention given to issues of taxonomy and range.

THE SUITABILITY OF LAND BASED EVALUATIONS OF *CRASSOSTREA GIGAS* AS AN INDICATOR OF PERFORMANCE IN THE FIELD. Gregory A. Debrosse* and **Standish K. Allen, Jr.,** Haskin Shellfish Research Laboratory, Institute of Marine and Coastal Sciences, Rutgers University, Port Norris, NJ 08349.

Besides disease resistance, there are a host of ecological questions regarding the suitability of *Crassostrea gigas* for introduction to the mid-Atlantic. Are tank based comparisons of survival, growth, disease, etc. suitable for estimating the performance of *C. gigas* in the field? In June 1991, equal numbers of spat from three crosses—WFLA (*Crassostrea virginica*), YWAA (*C. gigas* form Miyagi), and XJPNA (*C. gigas* form Hiroshima)—were split into two replicates and reared in upwellers for the first summer and in a land-based tank the second. After the first season, *C. virginica* had the highest mortality (65%, 36%, and 13% for WFLA, YWAA, and XJPNA, respectively) and average spat size was about 30% greater in both *C. gigas* groups. For the second year, the 3 crosses were transferred to a 4200 gallon tank; two replicates of WFLA were also placed in Delaware Bay. Cumulative mortality for the second season (through November, 1992) was WFLA—60%; YWAA—73%; XJPNA—93%; and WFLA (Delaware Bay)—37%. YWAA grew fastest followed by XJPNA and WFLA; however WFLA grown on the tidal flats were larger than all tank reared groups. All oysters in the tank were infested with *Polydora websteri*. *C. gigas* heavily and WFLA lightly; WFLA (Delaware Bay) were virtually free of infestation. These data indicate that tank-based comparisons are not likely to be a true measure of performance in the local environment. Publication No. K-32100-2-93 NJAES.

RAPID MORPHOMETRIC DISCRIMINATION BETWEEN THE HARD CLAMS *MERCENARIA MERCENARIA*, *MERCENARIA CAMPECHIENSIS*, AND THEIR HYBRIDS. Robert T. Dillon, Jr.,* Department of Biology, College of Charleston, Charleston, SC 29424; **Nancy H. Hadley,** Marine Resources Research Institute, P.O. Box 12559, Charleston, SC 29412.

Previous efforts to distinguish between *M. mercenaria* and *M. campechiensis* have relied upon simultaneous multivariate analysis of six morphometric variables, in addition to shell ridging and internal shell color. Here we use nonparametric techniques to screen 12 simple ratios of easily-measured shell variables for their ability to distinguish 100 adult (50–75 mm standard length) *M. mercenaria* collected from Charleston and 100 *M. campechiensis* of similar size collected from Cedar Key, Florida. Entirely accurate discrimination was achieved using the ratio of shell weight (both valves) to the product of standard (maximum) shell length and lunule height (in the plane of the valves). All adult *M. mercenaria* showed values less than 9.6 g/cm · cm (median 7.6), while all *M. campechiensis* showed values greater (median 13.3). This ratio did not, however, effectively discriminate between 30-individual samples of smaller, two year old *M. mercenaria* and *M. campechiensis* (standard length 30–50 mm). A sample of 60 first-generation hybrids was examined after 8 years of culture (60–85 mm standard length). Their median morphometric ratio was 8.8 g/cm · cm, intermediate between pure lines but closer to the *M. mercenaria* value. 82% of hybrid values were less than the 9.6 cut-point. This indicates that the *M. mercenaria* genome is 58% dominant with respect to this character.

A UNIQUE PROCESS FOR PRODUCING TETRAPLOIDS ALSO DEMONSTRATES VIABILITY OF ANEUPLOID *CRASSOSTREA GIGAS* THUNBERG. Ximing Guo* and **Standish K. Allen, Jr.,** Haskin Shellfish Research Lab, Institute of Marine and Coastal Sciences, Rutgers University, Box B-8, Port Norris, NJ 08349.

We have developed a unique process for the production of tetraploid *Crassostrea gigas*, probably useful in shellfish generally: inhibition of polar body I in eggs from triploids fertilized with haploid sperm. The likely mechanism for tetraploid induction involves a "united bipolar" segregation, consisting of thirty dyads aligned in a single metaphase for meiosis II. After segregation, thirty chromosomes are released as polar body II and thirty chromosomes remain in the egg which, when united with the ten chromosomes from the sperm, forms a tetraploid. In this study, low survival in this cross suggests that the frequency of united bipolar segregation in triploid eggs was low. Other segregations led to aneuploids, most of which are undoubtedly inviable. However, 7 of 30 "tetraploid" progeny examined by karyology were viable aneuploids consisting of 21, 31, 32, 33, 38 (n = 2), and 41 chromosomes, demonstrating an apparent tolerance of this condition. While tetraploids themselves have enormous potential in shellfish breeding, incidental aneuploids could be quite useful too. For example, production of a trisomic series (lines of 2n + 1 shellfish, each with a unique extra chromosome) could lead to gene mapping of loci to particular chromosomes. Breeding aneuploids might be useful for gene transfer, especially in hybrids. These possibilities emphasize the need for improved chromosome banding and other techniques for obtaining complete karyotypes among shellfish species. NJAES publication no. K-32904-3-93.

HYBRID VIGOR IN PACIFIC OYSTERS: AN EXPERIMENTAL APPROACH USING CROSSES AMONG INBRED LINES. Dennis Hedgecock,* University of California, Davis, Bodega Marine Laboratory, Bodega Bay, CA 94923.

The genetic and physiological bases of hybrid vigor (heterosis) are poorly understood even for major agricultural crops. Two alternative genetic explanations for heterosis have co-existed for nearly 60 years: dominance and overdominance. In the 1980s, this debate re-kindled over reports that, for several bivalve molluscs, individual heterozygosity at allozyme-coding loci was positively correlated with fitness-related traits, primarily growth rate. Because this debate is not likely to be resolved without experimental manipulations of the genome, we have taken a classical approach to the study of heterosis in the Pacific oyster *Crassostrea gigas*, controlled crosses among inbred lines. In such mating experiments, heterosis (or potence, h_p) for one or more traits can be defined and quantified as $Q/L > 1.0$, where L is the difference between the trait values of the two parental inbred lines and Q is twice the deviation of the hybrid from the mid-parent value (Griffing 1990 *Genetics* 126:753).

Inbred lines of the Pacific oyster were initiated in 1989 by selfing of simultaneous hermaphrodites, resulting in families with an expected inbreeding coefficient of $f = 0.5$; allozyme analyses of the F_1 confirmed this expectation. Two sets of 2×2 crosses were carried out in May and June, 1993, with four of the inbred lines. Observations of larval survival, growth, and oxygen consumption were made in collaboration with Dr. Donal Manahan, University of Southern California. Limited data from the first cross suggest that the two parental inbred lines did not differ significantly from each other in survival of larvae but that hybrids grew faster than one of the parents. In the second cross, significant heterosis was found for larval survival, in one direction (h_p = 2.13) but not the other, and for growth, in both directions (h_p = 3.36). These are the first direct measurements of heterosis for a bivalve mollusc. Mass-specific, routine metabolic rates of larvae from the best parental inbred line were significantly greater than those in hybrid larvae (Appelmans and Manahan, unpubl. obs.), as expected from the growth data and from the literature on juvenile growth and metabolism.

A controlled growth study with juveniles from the first cross was initiated in December, 1993. In January, 1994, after 7 weeks of ad libidum feeding, the energy and protein metabolism and growth of juveniles from this experimental cross will be studied by Dr. Brian Bayne, Plymouth Marine Laboratory. Results from

these analyses will allow us, by comparison to larval data, to address the issue of marginal overdominance of traits that may differ between the larval and benthic phases of the oyster life cycle.

GROWTH OF TRIPLOID *CRASSOSTREA GIGAS* UNDER NATURAL CONDITIONS IN THE LOWER CHESAPEAKE BAY. Roger Mann* and **Eugene M. Burreson,** School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Pt. VA 23062; **Standish K. Allen, Jr.,** Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349.

To determine disease susceptibility, growth and survival of the Pacific oyster, *Crassostrea gigas*, under natural conditions in the lower Chesapeake Bay, 200 individually-typed triploid individuals from Rutgers University were placed in mesh bags in four replicate trays in the lower York River at VIMS. Control oysters consisted of 200 diploid *C. virginica* from Ross Rock in the upper Rappahannock River and 200 diploid *C. virginica* from Worlds End Aquaculture, Queenstown, MD, placed in separate mesh bags in the same trays as the *C. gigas*. All oyster were deployed on 29 June 1993. Salinity averaged 20 ± 2 ppt. during the summer and fall. Initial mean shell height of the *C. gigas* was 45 mm with a range from 34 to 70 mm. Initial mean total weight was 13 g with a range from 5 to 31 g. All live *C. gigas* were measured and weighed on 10 August, 8 September, 14 October and 7 December 1993 and in late winter and early spring 1994. Growth data will be discussed and compared with growth in *C. virginica*.

THE RELATIONSHIP BETWEEN HABITAT AND GENE FLOW AMONG INTERTIDAL BIVALVE DEMES. E. Whitfield McMillan,* Graduate Program in Marine Biology, University of Charleston, Charleston, SC 29424.

Three bivalve species common in the Charleston, SC, area typically inhabit different tidal heights. Hard clams (*Mercenaria mercenaria*) are found around mean low water, oysters (*Crassostrea virginica*) are mid-tidal, and ribbed mussels (*Geukensia demissa*) are dewatered for most of a normal tidal cycle. Thus one might expect clams to show the greatest gametic and larval dispersal capabilities, mussels the lowest, and oysters intermediate. I collected 50 small and 50 large individuals of each of these three species from two demes approximately 13 km apart and examined gene frequencies at 5–8 polymorphic enzyme loci using starch gel electrophoresis. Clam and oyster genotype frequencies appeared to conform to Hardy-Weinberg expected values, while mussels deviated significantly at two loci. Interdemal divergence was negligible in clams, while significant differences were detected at two loci in both oysters and mussels. I tested for a relationship between relative dispersal capability (as predicted by the tidal height inhabited) and interdemal divergence by calculating Nei's genetic distances between both large and small cohorts of the three bivalves. Genetic distances among large bivalves matched expectation (M > O > C) and were just slightly off prediction among the small cohorts. These results are not quite significant, but suggestive of a relationship between habitat and interdemal divergence in bivalve populations.

BROODSTOCK MANAGEMENT

DEVELOPMENT OF BREEDING PROGRAMS—PAST, PRESENT AND FUTURE. Standish K. Allen, Jr., Haskin Shellfish Research Laboratory, Institute of Marine and Coastal Sciences, Rutgers University, Box B-8, Port Norris, NJ 08349.

As practiced for centuries, agricultural genetics was a practical exercise in crop improvement, as the wild relative was domesticated and selected for desirable traits. Later, classic techniques of quantitative genetics, pedigreed lines, hybridization, polyploidy and gene transfer created the varieties and strains we know now. The state of shellfish breeding now seems analogous to the practical stage of early agriculture, with the "classic" techniques just emerging. While examples of the potential for genetic improvement have been demonstrated in research, very few have found their way to commercial use. For example, MSX-disease resistance was obtained rapidly in *Crassostrea virginica* but its commercial use is restricted by another pathogen. Growth rate shows high realized heritabilities in *Mercenaria mercenaria* but environment-genotype interaction may limit the use of fast growing lines. Triploid *C. gigas* have been adopted by the industry but there seems to be variation in the value added. At present, the wisest course for broodstock development seems to be controlled domestication, whereby brood stock are carefully managed to maintain genetic variation while selected for traits of local commercial importance. These managed populations will produce strains which may be outcrossed within or among commercial operations in the future. Research on classic and modern techniques (especially molecular) presents the possibility of accelerating genetic improvement. This combinatorial approach requires coordination on a national level and an infrastructure that will support these activities. NJAES contribution no. K-32100-12-93.

PLANS FOR A NATIONAL BROODSTOCK LABORATORY FOR MOLLUSCAN SHELLFISH. Christopher J. Langdon,* Coastal Oregon Marine Experiment Station, Hatfield Marine Science Center, Oregon State University, Newport, Oregon 97365.

Despite the long history of shellfish aquaculture in the United States, there have been few attempts to develop comprehensive broodstock management strategies for the industry. As a consequence, the industry is essentially farming animals without the benefits of domestication.

Hatchery programs need to be established before broodstock management strategies can have significant beneficial effects on production. The Pacific coast oyster industry has depended on hatcheries for seed production for about 20 years, while the At-

lantic and Gulf industries are only beginning to seriously consider hatcheries, partly as a result of dramatic declines in populations of naturally set oysters in the mid-Atlantic states over the last decade.

The industry's interest in hatcheries has resulted in plans to establish a National Broodstock Laboratory for Molluscan shellfish. The objectives of the Laboratory will be 1) to establish a repository for the conservation of genetic material, 2) to develop breeding programs for the commercial production of shellfish with desirable traits and 3) to establish a resource center for industry, researchers and other interested parties in the USA and abroad. A proposal to establish the National Broodstock Laboratory as a special research project will be submitted to the U.S. Department of Agriculture during winter 1994. It is anticipated that Congress will decide on funding the Laboratory by mid-summer 1994.

IDENTIFICATION AND ACQUISITION OF BROODSTOCK FOR THE AQUACULTURE OF MOLLUSCAN SHELLFISH. Roger Mann,* School of Marine Science, Virginia Institute of Marine Science, Gloucester Point, Virginia 23062.

Existing aquaculture industries are based on the culture of a limited number species that are suited for mass cultivation practices. In addition, cultivated populations are often derived from limited samples of wild populations that exhibit desirable characteristics. Alternative species or populations can sometimes be identified that possess characteristics that would allow expansion of aquaculture production. Introduction of non-indigenous species or populations to aquaculture sites should take into account economic, environmental, legal and socio-economic factors.

Animals chosen for the establishment of a broodstock population should be disease-free. International, federal and state regulations reduce the risks of the spread of undesirable organisms and diseases potentially associated with broodstock transferred between sites.

BROODSTOCK HUSBANDRY OF BIVALVE MOLLUSCS. Anja Robinson,* Coastal Oregon Marine Experiment Station, Hatfield Marine Science Center, Oregon State University, Newport, OR 97365.

The shellfish industry no longer depends solely on natural production and harvesting of species. A number of species are farmed in aquaculture facilities during some part of their life cycle. Since the shellfish industry has become increasingly dependent on hatchery production, we must increase our knowledge of good broodstock management practices.

Firstly, it is important to understand natural spawning cycles of broodstock species at different sites. The annual reproductive cycles of species vary from one location to another. Secondly, it is important to be able to manipulate the spawning time of broodstock animals for hatchery production. Broodstock can be held at hatcheries at elevated or lowered water temperatures to achieve a desired spawning time; however, holding broodstock animals at elevated temperatures for long periods of time can lead to reduced numbers of high quality eggs. To avoid loss of production, some hatcheries feed broodstock animals during conditioning for spawning.

Artificial spawning of conditioned broodstock animals is commonly carried out in hatcheries by temperature or chemical manipulations, sometimes by stripping. Larval survival and development greatly depend on the condition of broodstock animals.

BIVALVE RECRUITMENT

SPAWNER-RECRUIT DYNAMICS OF BAY SCALLOPS (*ARGOPECTEN IRRADIANS CONCENTRICUS*) IN FLORIDA. William S. Arnold,* Dan C. Marelli, and **Catherine P. Bray,** Florida Marine Research Institute, 100 8th Avenue S.E., St. Petersburg, Florida 33701-5095.

Bay scallops appear to be in a state of steady decline in Florida. Commercial landings, at one time significant throughout the Gulf coast of the state, are now practically nonexistent. Recreational landings are also on the decline; areas such as Tampa Bay and the Anclote estuary that once supported substantial recreational fisheries now support no recreational fishing at all. The decline appears to be progressing from south to north but no clear trend is evident.

We have initiated a study in the Homosassa Bay region of north central Florida to determine the relationship between adult abundance, timing of spawning, and subsequent settlement into this relatively isolated local population. We obtain information on spawning and settlement patterns by collecting 20 adult scallops for analysis of reproductive state and by retrieving and redeploying spat traps at a variety of sites within the study area. Twice each year (once before and once after the fishing season), we conduct transect surveys at each of 20 stations in the study region to estimate adult densities; those data are contoured to provide areal estimates of adult abundance.

We are currently integrating information from all three phases of the program to determine temporal and spatial relationships between the distribution of adult bay scallops and the settlement of the subsequent year class. Our results will be useful in determining the extent to which the local population is dependent upon allochthonous versus autochthonous input of recruits for the success of the following year class.

INTERTIDAL OYSTER REEFS AS A TOOL FOR ENHANCING SETTLEMENT, GROWTH, AND SURVIVAL OF THE OYSTER, *CRASSOSTREA VIRGINICA*. Ian K. Bartol,* School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

The construction of intertidal oyster reefs resembling those present during early colonial times is one frequently proposed but critically unevaluated solution for rejuvenating the declining Virginia oyster fishery. An intertidal reef environment may be more

advantageous than current subtidal habitats for the American oyster, *Crassostrea virginica,* because aerial exposure provides both temporal relief from predation pressure and less hospitable conditions for disease. To determine whether aerial exposure has an effect on settlement intensity, post-settlement survival, and growth of *C. virginica* on oyster reefs, a 30 × 300 m intertidal structure composed of cleaned fossil shells was constructed.

Settlement monitoring of the reef was performed on a weekly basis from June through September 1993, and involved destructive and non-destructive sampling at 4 tidal heights on upstream and downstream orientations of both exposed and sheltered reef locations. Growth and survivorship assessment, initiated in October 1993, includes monthly destructive sampling of naturally-set reef oysters and monthly photosampling of hatchery-reared oyster larvae (hatchery larvae were set on clean oyster shell and confined in plastic Vexar mesh bags). The examination of both naturally-set and hatchery-reared oysters continues at tidal heights and sites similar to those used in settlement monitoring. Digital image processing is being used to record mortality and growth of photosamples.

Preliminary inspection of settlement data reveals that settlement intensity is greatest subtidally. However, microscale spatial and temporal variation is prevalent. Possible explanations for this variation and early growth and mortality findings will be discussed.

FACTORS DETERMINING RECRUITMENT SUCCESS OF *CRASSOSTREA VIRGINICA* IN A TEMPERATE LATITUDE ESTUARY: A MODELING STUDY. Margaret M. Dekshenieks,* Eileen E. Hofmann, and **John M. Klinck,** Center for Coastal Physical Oceanography, Old Dominion University, Norfolk, VA 23529; **Eric N. Powell,** Department of Oceanography, Texas A&M University, College Station, TX 77843.

A size-structured, time-dependent population model has been developed to investigate growth and mortality of larvae and adults of *Crassostrea virginica* in a physically energetic system. This population model is coupled to a three-dimensional hydrodynamic model for the Galveston Bay system. In the coupled model, adult oysters spawn eggs into the water column, larvae develop from eggs to spat, and the spat are recruited to the benthic population as juveniles. Growth and mortality rates for adults and larvae are regulated by the environmental conditions supplied by the hydrodynamic model. Simulations that were designed to investigate recruitment success show that recruitment depends not only upon adult oyster abundance and fecundity, but also upon larval survivorship. Survivorship is influenced by both larval growth and larval mortality. Larval growth rate is regulated by four environmental factors; temperature, salinity, food concentration and turbidity. Larval mortality is calculated as a cumulative loss based on known causes of larval mortality from both laboratory and field experiments. Comparison of the simulated larval recruitment in Galveston Bay for yearly mean-flow and low-flow conditions show that during periods of mean-flow, recruitment is extremely low in over 50% of Galveston's reef area. Low recruitment values are prevalent in, but not limited to, areas in the Galveston Bay system under the immediate influence of river run-off. The number of spat recruited increases significantly under periods of low-flow conditions, even in the upper reaches of Galveston Bay. Larval survivorship, expressed as the ratio of the number of spat to the number of eggs spawned, also differs between mean-flow and low-flow simulations. Survivorship is low under mean-flow conditions, with few larvae surviving in the upper reaches of the Bay. Larval survivorship increases dramatically over the entire Bay under low-flow conditions.

ESTUARY-SCALE DISPERSAL OF POST-LARVAL MUSSELS, *MYTILUS EDULIS*, AMONG EELGRASS (*ZOSTERA MARINA*) MEADOWS AND SUBSEQUENT RECRUITMENT TO PLANTED LIVE AND MUSSEL SHELL CULTCH. Carter R. Newell, Great Eastern Mussel Farms, Inc., Tenants Harbor, Maine 04860 U.S.A.

Studies performed in the Mt. Desert Narrows, Maine estuary from 1985–1991 examined the dynamics of the settlement and subsequent development of seed mussel (*Mytilus edulis*) beds near a large subtidal eelgrass (*Zostera marina*) meadow. Earlier studies have shown that the eelgrass meadows act as a larval trap for mussels which attach in mid-summer to the eelgrass shoots and attached epiphytes with concentrations often exceeding 1000 post-larval mussels per blade. The subsequent disappearance of many of the post-larval mussels from the eelgrass beds at about 1 month after settlement, and their redistribution throughout the estuary, was investigated through transects within the eelgrass meadow, zooplankton tows on flood and ebb tides, collectors put out and retrieved throughout the study area, and bags of live and shell mussel cultch. The results suggest that dispersal from the eelgrass meadows occurs primarily at two sizes: early post-set (250–400 micron shell length) and larger juveniles (0.8–2 mm). The eelgrass meadows provide a predator refuge in between these two sizes. Greatest concentrations of byssus-drifting juveniles were obtained on flood tides, resulting in a concentration of post-larval mussels inshore of the eelgrass meadows. A hydrographic model was used to predict optimal placement of cultch for seed collection. The concentration of early post-larval mussels on eelgrass blades in early summer may be used as an index of the cost-effectiveness of planting cultch on any given year.

FACTORS LIMITING SUBTIDAL OYSTER RECRUITMENT IN COASTAL GEORGIA. Francis X. O'Beirn,* P. B. Heffernan, and **R. L. Walker,** Shellfish Research Laboratory, Marine Extension Service, University of Georgia, P.O. Box 13687, Savannah, GA 31416-0687.

In coastal Georgia, reports have indicated that the paucity of subtidal oysters is a result of predation and disease upon the adult forms. This study was initiated to determine if predation upon spat

is a major factor in determining lack of subtidal oysters in the southeast. Plastic meshes (3 mm and 6 mm) were used to cover the spat sampling arrays, located subtidally (ST), at mean low water (LW) and intertidally (IT), thus limiting access of predatory organisms. These were compared to controls and sampled on a biweekly, monthly, and seasonal basis. The levels of oyster settlement were significantly lower than the previous two years ($p < 0.0001$). The pooled biweekly data for the controls and the two treatments revealed significantly higher settlement subtidally than at low water and intertidally. For the monthly data, there were no significant differences in settlement between the tidal heights for the controls and the 3 mm mesh treatment. The 6 mm treatment had significantly higher settlement at low water than intertidally. The seasonal controls had higher intertidal settlement ($p < 0.0001$), the 3 mm mesh had LW > IT > ST, ($p = 0.0134$) and 6 mm mesh had LW > ST = IT, ($p = 0.0103$). Despite the non-significance of many of the ANOVAs, it is apparent that the treatments do not mimic the patterns of oyster settlement exhibited by the controls. It appears that the subtidal values in the treatments remain high, relative to the other tidal heights. These preliminary data do seem to indicate that predation upon the newly settled spat does play a role in the scarcity of subtidal oysters in the region.

SETTLEMENT OF *CRASSOSTREA VIRGINICA* LARVAE: EFFECTS OF WATER FLOW AND A WATER-SOLUBLE CHEMICAL CUE. Elizabeth J. Turner* and **Margaret A. Palmer,** Dept. of Zoology, Univ. of MD, College Park, MD 20742; **Mark Luckenbach,** Virginia Institute of Marine Sciences, College of William & Mary, Wachapreague, VA 23480; **Richard K. Zimmer-Faust,** Dept. of Biological Sciences, Marine Sciences Program and Belle W. Baruch Institute for Coastal & Marine Research, Univ. of S. Carolina, Columbia, SC 29208.

The importance of waterborne chemical cues in mediating settlement of pelagic larvae has been questioned, due to the slow swimming speeds of many marine invertebrate larvae in relation to the near-bed flows experienced by these larvae during settlement. We present results of flume experiments demonstrating enhanced settlement of oyster larvae in response to dilute ($<10^{-8}$ M) concentrations of a water-soluble peptide. The peptide was released from the bottoms of small target wells (7 cm in diameter) filled with crushed oyster shell, and the number of larvae settled in these wells was compared to identical substrates without the cue in flows of 2 cm s^{-1} and 6 cm s^{-1} (free-stream velocity). The enhancement effect was similar in both the 2 cm s^{-1} and the 6 cm s^{-1} flow, resulting in approximately 50% more settlers in the presence of the peptide, compared to the untreated oyster shell. Concurrent still-water experiments confirmed enhanced settlement in solutions of the waterborne cue, compared to seawater controls. Rapid vertical swimming or sinking in response to the waterborne cue may concentrate larvae in near-bottom waters and enhance larval settlement out of flowing seawater. Investigations are underway to determine if a threshold flow velocity exists, above which larvae will be unable to respond to waterborne cues.

CHEMICAL IDENTITY OF OYSTER LARVAL SETTLEMENT CUES. Richard K. Zimmer-Faust* and **Mario N. Tamburri,** Department of Biology, University of South Carolina, Columbia, SC 29208.

Oyster larvae (*Crassostrea virginica*) settle in response to water-soluble compounds released by both adult conspecifics and bacteria biofilms. We performed molecular weight fractionations of seawater used to bathe adult oysters (with intact films). Bioassays of these fractions indicated the presence of waterborne settlement inducers between 500 and 1000 Da. The inducers were degraded by reaction with proteases, but not by carbohydrases or by lipase. Of several proteases we applied, only those cleaving basic amino acids (lysine and arginine) from the C-terminal, and arginase (an enzyme condensing arginine at the C-terminal to ornithine) eliminated settlement-inducing activity. A tri-peptide having arginine at the C-terminal, glycyl-glycyl-L-arginine (hereafter referred to as GGR), is identified as a powerful settlement cue. This compound significantly evoked settlement at a concentration as low as 10^{-10} *M*. Dose-response curves for GGR and for the active fraction (500–1000 Da) of oyster bath water were essentially identical. Finally, tests of 21 free amino acids were done but only two compounds, lysine and arginine, evoked settlement. Larval settlers were far more sensitive to arginine than lysine, although significantly less sensitive to arginine than to peptides with arginine at the C-terminal.

Our combined results are all consistent in identifying low molecular weight peptides with arginine at the C-terminal as potent, water-soluble inducers of settlement in oyster larvae.

STOCK ASSESSMENT

THE POTENTIAL USE OF SHELL GROWTH MARKS TO DETERMINE NATURAL GROWTH PATTERNS AND AGES OF LARVAL AND POST-LARVAL BIVALVES: A SPECIES SURVEY. Brad S. Baldwin, Institute of Marine and Coastal Sciences, Rutgers University, Cook Campus, New Brunswick, NJ 08903.

The growth of larval and early post-larval stages of bivalves may greatly affect recruitment patterns of natural populations, yet little is understood about either of these two processes. I am exploring the possibility of using shell growth marks to determine the age and growth history of these important early life history stages for a variety of species. Such information could greatly improve our ability to: (a) model larval dispersal and determine locations of parental stocks, (b) determine spawning and settling dates, and (c) determine whether the growth of these life stages is limited in nature.

Based on a scanning electron microscope survey of the valves of several cultured species (*Arctica islandica, Argopecten irradi-*

ans, Crassostrea virginica, Dreissena polymorpha, Mya arenaria, Rangia cuneata, and *Spisula solidissima*) I have observed external, commarginal ridges on the larval prodissoconch II and post-larval dissoconch shell with little or no corresponding internal structure evident in fractured shell sections. Most external ridges on the prodissoconch II are spaced only 0.5–2 um apart but in some species more prominent ridges are present 4–20 μm apart. Small dissoconch ridges are spaced 1–2 μm apart but prominent ridges are separated by as much as 30–100 μm. Known age specimens will be examined to see whether age can be determined based on ridge number. The effects of temperature and food level on ridge production will also be investigated.

In most species there is a clear demarcation between the larval prodissoconch II and post-larval dissoconch shell; thus, realistic growth history information of each life stage can potentially be retrieved from individual, field collected post-larvae. This will be examined using specimens of *C. virginica* collected from Chesapeake and Delaware bays and *D. polymorpha* from Lake Ontario.

EVALUATION OF *MERCENARIA* SPP. ABUNDANCE AND GROWTH IN NORTHERN GULF OF MEXICO HABITATS. Loren D. Coen,* Marine Resources Research Institute, Box 12559, Charleston, SC 29422; **Kenneth L. Heck, Jr.,** Marine Environmental Sciences Consortium, Box 369, Dauphin Island, AL 36528; **Michael L. Judge,** Department of Biology, Manhattan College, Riverdale, NY 10471.

Mercenaria population abundances in vegetated and adjacent unvegetated habitats in Alabama and northwest Florida were estimated and field experiments were conducted on clam survival and growth to evaluate the favorability of nearshore vegetated habitats for hard clams. Seasonal field surveys were made at four sites over two years. In each habitat, plots were censused for larger clams by "treading." Small clams were sampled in 0.18 m^2 plots in each habitat using suction sampling and a 0.7 mm retaining mesh. Clam field densities were extremely low and patchy in vegetation (generally <0.07 adults and <0.6 juveniles/m^2). Clams were absent in adjacent sand.

Seven seasonal two-month experimental field studies incorporating location (habitat and distance into grass beds), caging and simulated nipping (predation) as main effects were conducted in Perdido Pass, Alabama and Big Lagoon, Florida. Both sites are shallow water (<1 m) dominated by seagrasses (*Halodule* and/or *Thalassia*) with surrounding bare sand. All clams were hatchery-derived (FL), individually numbered and measured (initial mean length 25–39 mm). Growth rates varied among seasons, siphon treatments and habitats with growth ranging from 1.5–5.5 in sand, and from 2.5–4.35 mm/mo in seagrass, with highest growth rates in both habitats during fall/winter. Siphon nipping reduced growth, often significantly. We conclude that for northern Gulf of Mexico sites, habitat and season significantly influence growth rates, with clams overall growing fastest in cooler months within sand; (2) location within vegetation significantly influences growth; and (3) simulated siphon nipping and caging can significantly affect clam growth.

ESTIMATING POPULATION PARAMETERS OF DELAWARE BAY OYSTERS. S. R. Fegley,* J. N. Kraeuter, S. E. Ford, D. R. Jones, and **H. H. Haskin.** Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349 and *Corning School of Ocean Studies, Maine Maritime Academy, Castine, ME.

We present estimates of the numbers of samples required to achieve a 95% level of confidence with proscribed precision for oysters in Delaware Bay. These are derived from long-term data sets collected on several life-history stages of oysters (*Crassostrea virginica*) in Delaware Bay. The estimates: 1) provide appropriate levels of replication required in future sampling, 2) demonstrate whether number of replicates required vary from year to year, and 3) help direct the analysis of the data. Three historical data sets (eyed larval abundance, initial oyster spatfall, and adult oyster abundances) were used. The negative k binomial distribution provided the best fit of the data in all cases. We chose to estimate the number of samples necessary for a 95% probability of having a range of levels of precision about the annual mean. These data are compared to the results of 35+ years of data. Samples of adult oysters had good (<33%) precision in all years. Precision of the spat data was moderate (around 50% of the mean) and generally poor (>50% of the mean) for the larval data in most years. Surprisingly, over each of the data sets the required sample size for a given level of precision between years shows relatively little variation. We will discuss the consequences of these different levels of precision in the analysis and future sampling of the respective population parameters and suggest alternative practices for gathering and analyzing such data.

EASTERN OYSTER STOCK ASSESSMENT IN MARYLAND'S CHESAPEAKE BAY. M. L. Homer,* M. Tarnowski, L. Baylis, and **W. P. Jensen,** Maryland Department of Natural Resources, Tidewater Administration, Fisheries Division, Tawes Building, C-2, 580 Taylor Avenue, Annapolis, MD 21401.

During the last 12 years Maryland's public fishery seasonal oyster landings have declined from over 2 million bushels to less than 200,000 bushels. Although this decline was and is concurrent with severe epizootics of *Dermo* and *MSX* other causal factors such as overharvesting and habitat loss have been suggested. The debate that arose from attempts to attribute causality to the declining fishery led the Maryland Department of Natural Resources to initiate an oyster stock assessment program in 1989. The initial goal of this effort was to produce a sampling procedure and scheme that would give unbiased estimates of oyster abundance. This was accomplished during 1990, and since then over 50,000 acres of oyster bars have been surveyed using a patent tong-based, randomly-initiated, systematic survey. The goal of this effort is to produce a database of quantitative information on oyster stock size

and population structure, and habitat structure and extent. This database is to be dynamic as changes in these parameters can be estimated over time.

Along with survey work in commercially important areas, the stock assessment program has resurveyed over 10,000 acres of oyster bars surveyed during 1975 using similar sampling techniques. This effort has produced results indicating that the primary causal factor in the decline of the public fishery has unequivocally been the infestation of oyster parasites. Although some areas have suffered substantial loss of oyster habitat and stocks in the lower salinity areas have been overharvested, survey results cannot attribute a major role in the fishery's decline to these two factors.

AN OYSTER MANAGEMENT INFORMATION SYSTEM: INTEGRATING BIOLOGICAL, PHYSICAL, AND GEOGRAPHICAL DIMENSIONS. Gary F. Smith,* Stephen J. Jordan, and **Kelly N. Greenhawk,** Maryland Department of Natural Resources, Cooperative Oxford Laboratory, 904 S. Morris St., Oxford, MD 21654.

We have developed a desk-top GIS-based management information system that integrates a wealth of data on Maryland's Chesapeake Bay oyster populations. Biological data include spatfall, mortality, population size structure, disease prevalence and intensity, as well as other stock assessment information. Physical data include locations and extent of all charted oyster bars, bottom characteristics, geographic boundaries and features, bathymetry, salinity and temperature. Management data include shell and seed plantings, seed movement, harvest, lease boundaries, and boundaries of special management areas. The system supports query-based retrieval of all data layers, singly or in combination, with representation by means of maps, statistical graphics, and user-generated tables.

Current and planned applications of the system include automated reporting of monitoring results, integrated information retrieval to support development of Oyster Recovery Area management and monitoring plans, tracking seed plantings, and providing a framework for modeling disease and population dynamics. We are exploring additional applications of the system through discussions with resource managers and scientists, leading to development of specialized system modules for specific uses and users.

SIZE VARIABILITY OF JUVENILE (0+ YR) BAY SCALLOPS IN LONG ISLAND, NEW YORK POPULATIONS. Stephen T. Tettelbach,* Long Island University, Southampton, NY 11968; **Peter Wenczel,** Long Island Green Seal Committee, Southold, NY 11971; **Scott W. T. Hughes,** Long Island University, Southampton, NY 11968.

Size-frequency distributions of juvenile (0+ yr) bay scallops, *Argopecten irradians irradians,* were examined at eight locations in eastern Peconic Bays, Long Island, New York during winter 1990–91. Sampling was conducted via suction dredging until ≥200 juveniles (seed), which all resulted from natural spawning in 1990, were obtained at each site. Shell height (tangential distance from umbo to distal margin) of collected seed ranged from 7–61 mm. Mean shell heights (range: 42.1–50.8 mm) and mean seed densities (range: 5.6–21.5 seed/m^2) varied significantly between sites. The proportions of large seed (≥57 mm), which are legal to harvest in New York, and small seed (defined as ≤20 mm) in sample areas ranged from 0–7.3%, and 0–8.7%, respectively. Examination of growth rings on 268 adult (1 + yr) scallops harvested in October 1992 revealed that 100% had been 2–7 mm seed at the end of their first growing season in December 1991. The potential importance of these findings to bay scallop population dynamics and the fishery are discussed.

MOLLUSCAN FISHERIES OF PANAMA. Janzel R. Villalaz* and **Juan A. Gómez,** Departamento de Biología Acuática, Universidad de Panamá, República de Panamá.

This study will present the history of the fisheries of mollusks in Panamá, including equipment, boats, fishermen and production; also present condition, and an analysis of the future of these resources in Panamá. The shellfisheries captured in Panamá are: the Pacific calico scallop, *Argopecten ventricosus;* mangrove oyster, *Crassostrea rhizophorae;* edible oyster, *Ostrea iridescens;* littleneck clam, *Protothaca asperimma;* grand ark clam, *Anadara grandis;* and queen conch, *Strombus gigas,* for food, and the pearl oyster, *Pinctada mazatlanica,* mainly for pearls. The scallop meats and pearl oysters have been mostly exported to other countries, while the other species are eaten locally.

BIVALVE AQUACULTURE

BLUE MUSSEL AQUACULTURE PARAMETERS. T. Jeffrey Davidson* and **Rod McFarlane,** Atlantic Veterinary College, U.P.E.I., 550 University Ave., Charlottetown, Prince Edward Island, Canada, C1A 4P3.

In order to develop a decision support tool capable of increasing efficiency on blue mussel (*Mytilus edulis*) aquaculture operations utilizing longlines, a number of significant parameters were identified from three general areas of concern; inventory control, production monitoring and health monitoring. Inventory control includes a graphic display of the farm lease and the location and status of the longlines on that lease. The longlines would be identified as to their production status eg. socks, collectors, empty. The type, number and date of deployment of anchors and buoys would assist in the maintenance of longlines and maintain a running inventory of capital equipment.

Parameters identified to monitor production would include: date of longline/sock deployment; date of harvest; average size, source and density of seed stock at the time of deployment; length of shell and meat yield measured throughout the growing period; harvesting and processing data; and growth rate. Health monitoring parameters would incorporate both mortality and predation data. The predation parameters would identify the causative agent

eg. starfish and the extent of losses caused by that agent. The prevalence and cause of mortality would also be identified.

OYSTER (*CRASSOSTREA GIGAS*) CULTURE MANAGEMENT ON THE WESTERN COAST OF COTENTIN, NORMANDY (FRANCE). **Philippe T. Goulletquer, Jean P. Joly, Eric LeGagneur,** and **Francois Ruelle,** Aquaculture Research Laboratory, IFREMER, B.P. 32, 14520 Port en Bessin, France.

The Normandy region is now a leading shellfish rearing area in France yielding 35,000 metric tons of oysters *C. gigas* and 11,000 metric tons of mussels *Mytilus edulis* on a yearly basis.

However, leasing grounds acreage has increased from 625 to 1,000 acres for oyster culture and from 300 to 700 acres for mussel culture since 1970. With increasing demand for additional acres, questions arise about extending the rearing area already considered as saturated.

Using statistical sampling strategy, we estimated the oyster stocks in culture to 33,556 and 35,542 tons in 1990 and 1991, respectively. Concomitant oyster growth monitoring on 24 sites prompted us to study spatial variability and establish relationships to maximize the current spatial management.

A model was established linking overall oyster growth performance to local stocking density, intertidal distribution and distance from the main runoffs inputs.

The goal of the modelling is to provide recommendations in support of management decision-making to improve oyster culture development. This approach is discussed in view of assumptions inherent in the model and used as a basis for further research programs.

PERFORMANCE OF A TIDAL-POWERED UPWELLING NURSERY SYSTEM FOR JUVENILE CLAMS IN SOUTH CAROLINA. **Nancy H. Hadley*** and **Raymond J. Rhodes,** South Carolina Wildlife and Marine Resources Department, Charleston, SC 29422-2559; **R. B. Baldwin,** Low Country Seafood, McClellanville, SC 29458; **M. Richard Devoe,** South Carolina Sea Grant Consortium, Charleston, SC 29401.

A major impediment to small-scale hard clam culturists is the high cost of planting-size seed clams. Raising seed from the post-hatchery size of 1–2 mm to a field size of 8–10 mm requires a protected nursery environment. An inexpensive tidal-powered floating upwelling nursery, modified after a system in use in Maine, was constructed and tested in a South Carolina tidal creek. The system consists of a raft with a wooden scoop on the bow, which directs flowing water into an enclosed compartment containing upwelling units. Tidal currents of 0–1.5 knots produced flow rates through upwelling units of 0–96 lpm, averaging 53 lpm.

Production trials were conducted with seed clams ranging from <1 mm to 4 mm SL, initial size. Growth rates higher than those reported for land-based nurseries were achieved at higher stocking densities. Growth was slower than in bottom trays during the summer but faster during fall and spring.

A tidal upwelling system with a capacity of approximately 400,000 planting-size seed can be constructed for less than $5000, including labor. Operational costs are limited to maintenance, which is much less than for a bottom tray system. The system appears to have considerable potential as an inexpensive nursery system for small- to intermediate-scale hard clam culture.

ENVIRONMENTAL CORRELATES TO EXPLAIN OYSTER FIELD GROWTH OR PICKING THE RIGHT SPOT FOR OYSTER AQUACULTURE. **Mark Luckenbach** and **Valerie L. Shaffer,*** School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

In response to a collapse of the wild fishery, a *Crassostrea virginica* aquaculture industry is developing in the U.S. mid-Atlantic region which is predicated on rapid growth to market size. Considerable spatial variation in growth rates of oysters places a premium on selecting culture sites which support high growth rates.

We have formulated a multi-variate model that explains between site variation in oyster growth based upon quantity and quality of food available. Hatchery-reared juvenile oysters from single cohorts were deployed in off-bottom culture at 6–10 sites in 1990–1993. Growth rates were calculated for 1–3 month periods. Water samples were taken to obtain instantaneous measures of total seston, particulate organic carbon, particulate organic nitrogen, chlorophyll *a*, phaeophytin and suspended sediments. Integrated water movement was estimated using dissolution of chlorine tablets. Using multiple linear regression, the best combination of parameters to explain variance in the data was determined. This process has been used iteratively over several years to develop explanatory models, predict growth rates in subsequent years, and then further refine the models. The result is a relatively simple approach towards obtaining short-term estimates of food flux which can be used, within the confines of caveats related to disease status and nuisance algae blooms, to predict *a priori* growth rates of oysters.

DEVELOPMENT OF A NATIONWIDE FISH AND SHELLFISH CONSUMPTION SURVEY: MODEL DESIGN AND PILOT TEST RESULTS. **Esther C. Peters*** and **Sean B. Donahoe,** Tetra Tech, Inc., 10306 Eaton Place, Suite 340, Fairfax, VA 22030; **Betty M. Hackley,** National Marine Fisheries Service, NOAA, Silver Spring, MD 20910; **Lee J. Weddig,** National Fisheries Institute, Arlington, VA 22209.

Although studies have demonstrated that fish and shellfish consumption rates differ both regionally and within specific subpopulations, there is a lack of sufficient recent data to calculate local consumption rates or to identify special populations at risk as the result of eating fish and shellfish containing chemical contaminants, human pathogens, and biotoxins. Risk assessors and risk managers need this information to determine both acute and

chronic risks and to develop appropriate fish and shellfish consumption advisories and bans. A comprehensive evaluation of previous consumption surveys provided a basis for designing new models to collect scientifically valid data on human consumption of fish and shellfish for use in risk assessments. The types of information needed were identified with the assistance of representatives from federal and state agencies, academia, and the seafood industry. Two survey instruments were prepared and tested to collect demographic data and to examine daily consumption of the types and amounts of all fish and shellfish eaten by all members of a household and the method of preparation of these items by using a diary (first survey) and to examine a site-specific, subpopulation-specific, and species-specific fish consumption issue by using the recall method (second survey). An illustrated portion guide was included with both survey instruments to provide consistency in collecting quantitative data on the amounts eaten. The application of these new models will provide the data necessary to determine the risk to the consumer; to establish realistic guidelines, tolerances, action levels, and advisories for contaminants in fish and shellfish; and to set criteria for safe seafood products.

COST ANALYSIS OF A TIDAL-POWERED UPWELLING NURSERY SYSTEM FOR JUVENILE CLAMS. Raymond J. Rhodes* and **Nancy H. Hadley,** SC Wildlife & Marine Resources Dept., Charleston, SC 29412; **Robert B. Baldwin,** Low Country Seafood, McClellanville, SC 29548; **M. Richard DeVoe,** SC Sea Grant Consortium, Charleston, SC 29401.

The estimated costs of various clam nursery systems have been analyzed by others, but the costs of a passive tidal-powered clam upwelling system have not been documented or compared to the cost of other systems. Starting in 1992, a tidal-powered raft-upweller nursery system was constructed and operated by a commercial enterprise in a South Carolina (SC) tidal creek as part of a demonstration project for the National Coastal Resources Research and Development Institute.

The tidal-powered upweller used in this project consisted of a wooden 20 ft. by 12 ft. raft with a scoop on the bow for directing tidal flow to its 16–2.50 ft^2 seed clam trays. The estimated total cost of materials and labor was $4,500. Based upon costs per clam, a tidal upweller unit appears to be more cost effective than stand-alone land-based nursery systems with similar capacities. Availability of suitable raft sites and other considerations are discussed in view of enhancing opportunities for "small-scale" commercial aquaculture.

THE ECONOMIC FEASIBILITY OF PRODUCING OYSTER SEED USING REMOTE SETTING TECHNOLOGY IN LOUISIANA. John E. Supan,* Office of Sea Grant Development, Louisiana State University, Baton Rouge, LA 70803; **Donald C. Huffman,** Department of Agricultural Economics, LSU Agricultural Center, Baton Rouge, LA 70803; **Charles A. Wilson,** Coastal Fisheries Institute, Center for Coastal, Energy, and Environmental Resources, Louisiana State University, Baton Rouge, LA 70803.

Investment, fixed, and operating costs of producing oyster seed in Louisiana by remote setting of hatchery-reared larvae were analyzed based on a three-tank setting system operating over one- and five-month periods. Data were gathered from previous field demonstration work and interviews with oyster farmers. Three scenarios were budgeted based on combinations of manual labor vs. mechanization, and vessel ownership vs. leasing. Costs per shellbag of seed, potential production of market-size oysters and net profit were estimated. A five-month setting season (May–September), mechanized cultch handling, and vessel ownership constituted the most cost-effective scenario at approximately $6.48/shellbag of seed. Negative net profitability occurred at 5% survival to market size with a $10.00/sack dockside value.

THE TECHNICAL FEASABILITY OF COMMERCIAL HATCHERY AND REMOTE SETTING OPERATIONS IN LOUISIANA. John E. Supan,* Office of Sea Grant Development, Louisiana State University, Baton Rouge, LA 70803; **Charles A. Wilson,** Coastal Fisheries Institute, Center for Coastal, Energy and Environmental Resources, Louisiana State University, Baton Rouge, LA 70803.

A pilot oyster hatchery was constructed and has been operated since 1990 in Grand Isle, La., to test high-volume production of competent, eyed pediveligers for commercial remote setting operations. An industry-university collaboration has facilitated the use of university-based expertise and has provided an operating facility for the Sea Grant program to carry out its research, development and technology transfer mission. The company's objective is to produce high-quality oyster larvae for sale to local oyster farming companies. Approximately 186 million eyed larvae were produced during 1990 and approximately 240 million eyed larvae were produced during 1991. Commercial remote setting efforts produced seed oysters averaging 20 millimeters (mm) ± 8 mm after a thirty-day nursery period, with an average of 15 seed oysters/shell. Efforts have emphasized research and development since 1992 because of an abundance of "wild" oyster seed.

BIVALVE STOCK ENHANCEMENT AND RESTORATION

SHELLFISH STOCK ENHANCEMENT ON MARTHA'S VINEYARD. Richard C. Karney,* Martha's Vineyard Shellfish Group, Inc., Oak Bluffs, MA 02557.

The Martha's Vineyard Shellfish Group, Inc. is a consortium of the shellfish departments of five Island towns attempting to manage the economically important public stocks of quahogs (*Mercenaria mercenaria*), bay scallops (*Argopecten irradians*), and oysters (*Crassostrea virginica*).

Over the past 15 years, the shellfish management program has included efforts to augment natural recruitment through the appli-

cation of aquaculture techniques. Foremost in this stock enhancement effort has been the local production of seed shellfish from native broodstock in a solar assisted shellfish hatchery. In recent years, hatchery seed production has been increased with the use of field nursery systems capable of handling smaller and younger seed. Post set scallops are moved to the field in biodegradable burlap bags. Eyed oyster larvae are remotely set. Scallop "spawning sanctuaries" are also used to encourage spawning of natural stocks in the field.

Genetic shell tags have been helpful in assessing seed survival and the effectiveness of the stock enhancement program.

ENHANCING NEW YORK'S GREAT SOUTH BAY HARD CLAM (*MERCENARIA MERCENARIA*) RESOURCE: DETERMINING WHICH STRATEGY TO USE. Jeffrey Kassner,* Town of Brookhaven. Division of Environmental Protection, 3233 Rte 112, Medford, NY 11763.

Spawner transplants and sanctuaries, planting seed clams, and habitat modification have been undertaken over the past two decades in an attempt to enhance the hard clam (*Mercenaria mercenaria*) resource in New York's Great South Bay. Determining which strategy to undertake, if any, is critical because whatever funds are expended are not available for other enhancement or management options. Determining which strategy yields the greatest benefit is fraught with difficulties: environmental factors controlling abundance are not known, critical hard clam life history stages determining abundance have not been identified, assessing the degree of population enhancement is technically difficult and expensive, and implementation is problematical. Compounding these difficulties are temporal and spatial variability and demands of baymen. However, informed decisions have been made by integrating available environmental data, ecological/life history information from the literature, and a critical assessment of each strategy's objectives and limitations.

ENHANCEMENT OF A SCALLOP BED BY THE NATURAL RELEASE OF SPAT FROM A SCALLOP AQUACULTURE SITE. G. Jay Parsons,* University of Guelph, Guelph, Ont. N0E 1N0; **Shawn M. C. Robinson** and **Jim D. Martin,** Department of Fisheries & Oceans, Biological Station., St. Andrews. N. B. E0G 2X0.

A giant scallop, *Placopecten magellanicus*, (Gmelin, 1791) aquaculture operation located off Tongue Shoal, Passamaquoddy Bay, New Brunswick, Canada has been collecting spat using onion bag collectors for the last eight years. Since the operation started collecting spat, SCUBA divers have observed large numbers of spat and juveniles surrounding the site and local fishermen appear to have realized enhanced landings from the contiguous scallop bed. In the summer of 1993, underwater transects were conducted by SCUBA to determine if the abundance of scallops, through natural spat release, was higher in the area underneath compared to areas adjacent to the site. Scallop density and sizes were recorded every 10 m for 150 m for four transects in a 0°, 90°, 180°, and 270° direction from the center of the site. Scallop abundance was highest within a 10 m radius of the site (mean = 30.4 scallops $\cdot$ m^{-2}) and rapidly declined to 0.4 scallops $\cdot$ m^{-2} at 150 m away. Scallop spat (0+) dominated the size distribution in the 0 to 30 m strata, 2 year olds dominated the 20 to 80 m strata and 3+ scallops where most abundant in the outer strata. This suggests that scallops dispersed in an outwardly radial pattern from the center of the site. An estimated 30,000 scallops encompassed a 30 m radius of the aquaculture site. As they grow and move further afield, the site should provide the local fishing fleet with a modest increase in their annual landings.

HISTORY AND CURRENT STATUS OF NEW YORK STATE SHELLFISH ENHANCEMENT. Gregg Rivara, Cornell Cooperative Extension, 39 Sound Ave., Riverhead, NY 11901.

As early as 1825 shellfish seed were transplanted into New York City waters from Chesapeake Bay. From these extensive efforts Long Island Municipalities have utilized techniques to increase the population of harvestable shellfish. Seed planting, spawner sanctuaries, agreements with private mariculture firms, public and private relays, predator control and management areas are used towards this end. Although many of these methods are not critically evaluated they remain politically and publicly popular in most towns. Resource enhancement strategies used in the marine district of New York State will be summarized and quantified. In addition, a new method for evenly dispersing hard clam seed using a modified agricultural seed planter will be described.

THE POTENTIAL FOR RELAYING AS AN ENHANCEMENT METHOD FOR THE SOFT-SHELL CLAM, *MYA ARENARIA*. Shawn M. C. Robinson,* Dept. Fisheries and Oceans, Invertebrate Fisheries Section, Biological Station, St. Andrews, New Brunswick, E0G 2X0, Canada.

One of the greatest problems being faced by the intertidal fishery for the soft-shell clam, *Mya arenaria*, is the closure of some of the most productive beaches due to fecal coliform contamination. In the fall of 1993, a project was initiated with the local clam industry on the island of Grand Manan, New Brunswick, Canada in the Bay of Fundy to evaluate the potential of relaying clams from a fecally contaminated area to a clean one. Clams were harvested at a contaminated beach with a fecal coliform count of 2400 MPN (most probable number) with a hydraulic harvester and were relayed to another beach approximately 5 km away.

Results from sampling 6 weeks later indicated that the fecal coliform levels had dropped from 2400 MPN at the source beach to 790 MPN while those clams which had been relayed to the new beach were down to 40 MPN (i.e. under the legal level). The survival rate of the relayed clams was size dependent, but for the larger sizes, survival was up to 90%. The implications of this technique for the fishery in Atlantic Canada will be discussed.

SETTLEMENT AND RECRUITMENT OF BAY SCALLOPS, *ARGOPECTEN IRRADIANS*, TO ARTIFICIAL SPAT COLLECTORS IN THE WESTPORT RIVER ESTUARY, WESTPORT, MASSACHUSETTS. **Karin A. Tammi*** and **Michael A. Rice**, Department of Fisheries, Animal and Veterinary Science, University of Rhode Island, Kingston, RI 02881; **Scott Soares** and **Wayne Turner**, Westport Watershed Alliance, 1151 Main Road Box 3427, Westport, MA 02790.

In January 1993, the Westport Watershed Alliance initiated the Bay Scallop Restoration Project as an attempt to restore the once prolific bay scallop population in the Westport River Estuary in Massachusetts. This project is a multiphase endeavor aimed at better understanding recruitment failures of both natural stocks and introduced seed of *Argopecten irradians*. The main objective was to assess larval settlement and juvenile recruitment (survival to >4 mm) to artificial spat collectors placed in historically productive beds and within close proximity to adult spawner rafts. Spat collectors (2 mm–4 mm plastic mesh bags) containing monofilament were suspended on 20–30 ft. floating longlines at 11 locations in the Westport River. A total of 1400 spat collectors were sequentially deployed on 89 longlines from June to August 1993 to determine the timing of peak settlement and recruitment at each study site. The October to November 1993 harvest yielded 4000 scallops of varying shell heights ranging from 4 mm to 60 mm with an overall mean of 36.9. The difference in shell heights was related to the variable harvest times of the longlines which ranged from 68 to 123 days. The most productive longlines were located in the vicinity of Coreys Island, Horseneck Channel, and Canoe Rock. The greatest recruitment was observed at Coreys Island which yielded 1882 scallops averaging 6.1 scallops per collector. However, recruitment to individual longlines at this location was as high as 18.2 scallops per collector.

This study shows that *A. irradians* will settle on artificial spat collectors containing monofilament which may have two major implications in resource management. First, spat collectors may be a means to predict recruitment into the bay scallop fishery. Second, juvenile scallops harvested from spat collectors could be utilized for other grow-out applications to enhance natural stocks. Consequently, the implementation of spat collectors into an overall management plan could be a method employed by coastal communities to improve, stabilize and restore bay scallops in Southern New England and elsewhere.

FEEDING AND NUTRITION OF BIVALVES

THE ROLE OF MUCUS IN PARTICLE TRANSPORT ON BIVALVE PALLIAL CAVITY ORGANS. **Peter G. Beninger*** and **Sylvie St-Jean**, Département de Biologie, Université se Moncton, Moncton, N.B. E1A 3E9 Canada.

The combined techniques of mucocyte mapping and video endoscopy have made it possible to investigate the role of mucus in particle transport on the bivalve pallial cavity organs: gills, palps, lips, and mantle. Although fundamental differences characterize particle handling by the homorhabdic *Mytilus edulis* and the heterorhabdic *Placopecten magellanicus*, several unifying features have also emerged. It will be shown that, regardless of species and organ type, the type of mucus used in particle transport depends on the topography of the transporting surface. Low viscosity mixed (acid + neutral) secretions are characteristic of semi-enclosed transporting surfaces, while high viscosity acidic secretions are used on open surfaces, especially if particle transport is effected counter to the prevailing current (eg. ejection of pseudofeces from inhalent siphon in *M. edulis*), or on semi-enclosed surfaces which terminate in open surfaces (eg. palp trough rejection tracts in *M. edulis*). While in all cases acidic and not mixed secretions are used in particle rejection and pseudofeces formation, transport of particles for ingestion may also involve acidic secretions, depending on the architecture of the transporting surfaces. However, in all species studied, low-viscosity mucus appears necessary for ultimate particle treatment prior to and at ingestion. The accumulated data demonstrate a functional specialization in particle treatment by the pallial organs at two levels: anatomical (transporting surface topography) and biochemical (mucocyte secretion type).

PHYSIOLOGICAL AND BIOCHEMICAL CHANGES ASSOCIATED WITH NORMAL DEVELOPMENT AND NUTRITIONAL STRESS IN POSTLARVAE OF THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*. **Zaul Garcia-Esquivel,*[1,2] V. Monica Bricelj,[2]** and **Marco A. Gonzalez-Gomez,[1]** [1]Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California, Apartado Postal 453, Ensenada, B.C., Mexico; [2]Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794-5000.

Biochemical and physiological changes were assessed during normal development of fed *Crassostrea gigas* postlarvae (0 to 22 days post-settlement), and during enforced starvation (for 2 to 8 days) of one week- and two week-old postlarvae. Larval settlement was induced with epinephrine. Rapid exponential growth of shell and soft tissues following metamorphosis was accompanied by a steady decrease in weight-specific ammonia excretion rates, and 6 to 16-fold increase in the O/N (oxygen consumption to ammonia excretion) molar ratio. Lowest O/N values (2 to 4) coincided with metamorphosis (0 to 2 days post-settlement), and were largely attributed to the increase in ammonia excretion associated with protein catabolism. The most significant change associated with starvation of post-metamorphic stages was a sharp and very rapid reduction in the weight-specific rate of oxygen consumption, which occurred primarily during the first 2 days of starvation (up to 58% and 52% reduction in early and late spat respectively). Although the O/N ratios also declined during starvation, they remained above levels reached during metamorphosis. Changes in the amount of total proteins, lipids and carbohydrates, as well as the activity of the respiratory electron transport

system (ETS) were simultaneously evaluated in order to identify major energy sources and metabolic state of postlarval oysters, in relation to survival, during the first three weeks after settlement.

DEVELOPMENT OF A DIET FOR REARING EARLY JUVENILE FRESHWATER PEARLY MUSSELS. Catherine M. Gatenby,*[1] Richard J. Neves,[1] and **Bruce C. Parker,[2]** [1]Department of Fisheries and Wildlife Sciences, VA Tech, Blacksburg, VA, [2]Department of Biology, VA Tech, Blacksburg, VA 24061.

Nearly 60 species of freshwater mussels (Unionidae) are endangered in the U.S. and another dozen species support a declining commercial harvest of shells for the cultured pearl industry in Asia. Because of these significant declines in abundance, a study was undertaken to develop a diet for rearing juvenile mussels, with the goal of long-term propagation of rare species. Three trials were conducted to test various tri-algal and commercial diets and to determine the influence of silt in survival and growth of the rainbow mussel (*Villosa iris*) and giant floater (*Pyganodon grandis*).

Overall, high survival required the presence of a substratum (silt). Juveniles appear to be pedal-feeding for about 140 days post-metamorphosis; hence, silt provides a substrate for colonizing food materials. At 45 days post-metamorphosis, juvenile rainbow mussels in silt and fed algae exhibited a 2 fold increase in shell length, and 63.5% survival. Juveniles of the giant floater exhibited similar results at 45 days post-metamorphosis. In comparison, juveniles fed algae without silt exhibited no increase in shell length, and only 5.0% survival. Growth of juveniles on an algal diet and in an artificial substrate, kaolin, was similar to growth of juveniles fed algae in silt. Subsequent tests indicated that growth was significantly correlated with the quality of the diet (algae high in oils). Juveniles fed a tri-algal diet, consisting of *Neochloris oleoabundans, Phaeodactylum tricornutum, and Bracteacoccus grandis* (NPB), with silt substratum showed the best growth over time (140 days), with a 9 fold increase in shell length and 30.0% survival at 140 days post-metamorphosis. Commercial yeast diets did not support growth.

GROWTH RESPONSES OF *MYTILUS EDULIS* TO CHANGES IN WATER FLOW: A TEST OF THE "INHALANT PUMPING SPEED" HYPOTHESIS. Raymond E. Grizzle,* Biology Department, Campbell University, Buies Creek, NC 27506; **Richard Langan,** Jackson Estuarine Laboratory, Adams Point Road, Durham, NH 03824; **W. Huntting Howell,** Zoology Department, University of New Hampshire, Durham, NH 03824.

An experiment was conducted during June and July 1993 in a multiple-flume apparatus to determine the effects of a range of flow speeds (0, 1, 2, 4 and 8 cm s^{-1} mid-depth speeds) on shell growth of the blue mussel (*Mytilus edulis*). The experiment was designed to test the recently proposed (*J. Exp. Mar. Biol. Ecol.* 162:213–228, 1992) hypothesis that the relationship between bivalve growth responses and ambient water flow speed can be explained by differences in bivalve inhalant pumping speeds. Maximal individual growth is expected at ambient flow speeds that approximate the inhalant pumping speed, while reduced growth rates are expected at higher and lower ambient flow speeds. Published average inhalant pumping speeds for *M. edulis* of the size used in our experiment (30 to 47 mm shell length) range from 1.2 to 2.6 cm s^{-1}. Mean shell length increases in our 24-day experiment were: 0.1, 1.8, 2.0, 1.9, and 1.5 mm at ambient water flow speeds of 0, 1, 2, 4, and 8 cm s^{-1}, respectively. A preliminary analysis indicates that only the 0-flow growth rate (0.1 mm) differed significantly from the others. However, the overall response pattern closely matches the pattern predicted by the "inhalant pumping speed" hypothesis: maximal growth at about 2 cm s^{-1} ambient flow speed and decreased growth at higher and lower speeds.

SEASONAL VARIATION IN THE RELATIVE UTILIZATION OF DIETARY PROTEIN AND CARBOHYDRATE BY THE MUSSEL, *MYTILUS EDULIS* L. Daniel A. Kreeger,* Academy of Natural Sciences, Division of Environmental Research, Philadelphia, PA 19103; **A. J. S. Hawkins, B. L. Bayne,** and **D. L. Lowe,** Plymouth Marine Laboratory, Prospect Place, Plymouth PL1 3DH, United Kingdom.

Mussels (*Mytilus edulis*) were collected from Whitsand Bay during April, July and September, 1993, and fed a defined diet of pre-killed microalgae and either [^{15}N]protein/[^{14}C]carbohydrate microcapsules (Diet A) or [^{15}N][^{14}C]protein/carbohydrate microcapsules (Diet B). Defecation, excretion, respiration and incorporation of both isotopes were measured to describe the mussel's relative utilization of dietary carbohydrate (^{14}C in Diet A), protein-carbon (^{14}C in Diet B) and protein-nitrogen (^{15}N in Diets A and B). The assimilation efficiency for [^{14}C]carbohydrate (16–18%) was less variable and significantly greater than the assimilation efficiency for [^{14}C]protein (7–15%), and during September a greater proportion of assimilated ^{14}C was respired from protein (29%) than from carbohydrate (17%). These results suggest that protein-carbon was not preferentially conserved from metabolism relative to dietary carbohydrates in these experiments, which were conducted during both spawning and post-spawning periods. In contrast, protein-nitrogen was always assimilated with greater efficiency (25–82%) than either [^{14}C]protein or [^{14}C]carbohydrate. The conservation of protein-nitrogen relative to protein-carbon was particularly noticeable in July when rates of ammonia excretion were lowest and [^{15}N]protein assimilation was ten times more efficient than [^{14}C]protein assimilation. Measured rates of whole-body protein synthesis were also greatest in July, which suggests that seasonal variation in protein-nitrogen conservation was related to the mussel's anabolic demand for nitrogen.

VARIATIONS IN FEEDING BEHAVIOR OF TWO SUBTROPICAL BIVALVES IN RESPONSE TO ACUTE INCREASES IN SEDIMENT LOAD. Bruce A. MacDonald* and **J. Evan Ward,** Department of Biology, University of New Brunswick, P.O. Box 5050, Saint John, NB E2L 4L5, Canada.

Resuspension of bottom sediments, through natural processes or human activities, can greatly alter the concentration of suspended particulate matter (SPM) experienced by benthic organisms. Suspension-feeding bivalves that inhabit areas with high SPM loads are known to have feeding adaptations that allow them to handle high concentrations of sediment. Few studies, however, have examined species that inhabit regions with low ambient SPM concentrations, therefore, questions concerning the feeding adaptations of these species remain.

We examined the responses of two bivalve species (the turkey wing, *Arca zebra;* the Atlantic pearl-oyster, *Pinctada imbricata*) to acute increases in natural sediment load. These two sympatric species inhabit coastal regions of Bermuda, and have fundamentally different gill structures. Our simulated resuspension event caused a four fold increase in the concentration of SPM, which induced a significant decrease in clearance rates, and a significant increase in the rate of pseudofeces production. *P. imbricata* indiscriminately rejected material in the pseudofeces, thereby moderating ingestion rate through bulk rejection. *A. zebra,* however, rejected material with significantly higher carbon and lower nitrogen content, thereby increasing the quality of ingested material by way of particle selection. On average, this selection produced a 40% increase in the quality of material ingested. Our results indicate that different species of bivalves exhibit different strategies when exposed to increases in suspended sediment. We suggest that this may be a consequence of pallial organ structure.

THE EFFECTS OF TEMPERATURE, SALINITY AND FOOD SUPPLY ON OYSTER PRODUCTION IN LOUISIANA: MODEL PREDICTIONS VERSUS FIELD DATA. **Thomas M. Soniat,*** Department of Biological Sciences, Nicholls State University, Thibodaux, LA 70310 and **Eric N. Powell,** Department of Oceanography, Texas A&M University, College Station, TX 77843.

Environmental variables, seston composition and oyster population parameters were sampled from April 1992 to March 1993 at three sites in the Terrebonne Basin of Louisiana. Environmental measures included water temperature, salinity and transparency. The seston was characterized by dry weight (SDW), particulate organic matter (POM), particulate inorganic matter (PIM) and chlorophyll *a* concentration (CHL). Oyster population parameters were size-frequency distribution, condition index (CI), gonadal index (GI), and weighted incidence (WI) and percent infection (PI) of *Perkinsus marinus*. Environmental parameters and seston were measured weekly, whereas oysters were sampled monthly.

Water temperature, salinity, SDW and food supply measures were input into an existing model (developed by ENP). The food measures were POM, CHL, and a value (LCP), which relates CHL to the energy content of the seston measured as the sum of the caloric content of particulate lipid, carbohydrate and protein (regression from data of TMS from Galveston Bay). Model simulations suggest that POM greatly overestimates food supply (oysters accrue biomass at unrealistic rates and grow to unobserved sizes), whereas CHL underestimates food supply (oysters do not accrue biomass fast enough nor grow to sizes observed in the field). With LCP as the food-supply input, the model closely tracked oyster production, size-frequency distribution, WI and GI. Best results were obtained using weekly food supply data as opposed to monthly means.

PALP FUNCTION IN *MYTILUS EDULIS* (FAM. MYTILIDAE) AND *PLACOPECTEN MAGELLANICUS* (FAM. PECTINIDAE), DETERMINED BY MUCOCYTE MAPPING AND VIDEO ENDOSCOPY. **Sylvie St-Jean*** and **Peter G. Beninger,** Département de Biologie, Université de Moncton, Moncton N.B. E1A 3E9 Canada.

As part of the recent resurgence of interest in bivalve feeding mechanisms, the function of the labial palps was studied in *Mytilus edulis* and *Placopecten magellanicus,* which are known to differ markedly in gill structure and function. Mucocyte secretions were divided into categories depending on staining affinity for periodic acid-Schiff and alcian blue. The mucocyte distribution was determined by counts of stained tissue (either whole mounts or histological sections) at pre-determined sites on the palps; functional correlates were provided using video endoscopy on undissected specimens of *M. edulis* and carmine particles on both *M. edulis* and *Placopecten magellanicus*. The *M. edulis* palp shows an efficient separation of the ingestion and rejection functions, at both the anatomical and biochemical levels (mucocyte secretion type). Although pseudofeces production was only studied in the context of ingestion volume regulation, mucocyte distribution and particle treatment on the palp are compatible with the ability to select particles for ingestion. Endoscopic observation was not possible for *P. magellanicus* due to the extreme sensitivity of the peribuccal organs; however, the anatomical organisation and mucocyte distribution suggest that the palps of this species are less specialised compared to those of *M. edulis*. These results are discussed in terms of the functional differences in particle treatment between bivalves of the homorhabdic and heterorhabdic gill types.

DIGESTIVE DIVERTICULA ATROPHY IN EASTERN OYSTERS, *CRASSOSTREA VIRGINICA*, EXPOSED TO SALINITY AND STARVATION STRESS. **James T. Winstead,** U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, FL 32561.

Oysters sampled in February of 1992 from a low salinity site (3 ppt) in Apalachicola Bay, Florida, showed digestive tubule atrophy ($r = .586 \pm .018$ SE) compared with oysters ($r = .186 \pm .034$ SE) sampled from a higher salinity site (18 ppt) 10 miles away. Tubule atrophy has been characterized as an indicator of pollution or environmental stress. Experiments were designed to induce the tubule atrophy in the laboratory. To quantify tubule condition for each oyster, inside to outside tubule ratios (r values) were calculated from 20 tubules per animal using a light micro-

scope equipped with an ocular micrometer. Higher r values indicated greater tubule atrophy than smaller r values. Experiments consisted of one starvation and two salinity stress tests. Oysters were first kept in either static tanks at 3 ppt and 22 ppt and fed once every three days or flow-through tanks. Tubule r values ranged from .233 ± .038 SE in flow-through tanks to .690− ± .012 SE and .594 ± .042 SE in static tanks after 15 days. In replicate static tanks, non-fed animals had tubule r values of .580 ± .053 SE and .665 ± .009 SE compared to animals in flow-through that had .146 ± .009 SE after 15 days. A second salinity test was similar to the first except a 36 ppt tank was added and oysters were fed daily. All tubule r values remained low, and there was no significant difference between static and flow-through tanks after 21 days. These experiments indicate poor nutrition, perhaps due to low salinity, may have played a significant role in the tubule atrophy of Apalachicola Bay oysters.

DECAPOD CRUSTACEAN BIOLOGY

BIOENERGETICS OF INTERTIDAL 0+ DUNGENESS CRAB (*CANCER MAGISTER*): DOES DEMAND EXCEED SUPPLY? David Armstrong,* Miriam Fernandez, and **Oscar Iribarne,** School of Fisheries WH-10, University of Washington, Seattle, WA 98195.

Megalopae of Dungeness crab settle into intertidal oyster shell habitat during early summer and metamorphose into the first juvenile instar (J1) at densities that often exceed 200 m^{-2}. Growth is rapid and individuals reach J5 in about 2.5 mo which is a 3.6× increase in size from 7 to 25 mm carapace width (CW), but a 32× increase in ash-free dry weight (AFDW) from 12.5 to 406 mg. Reflecting natural mortality derived from field data, we estimated energy requirements for an average m^2 cohort of crab growing from the J1 to J5 instar based on the sum of somatic production and respiration, which are about 65% and 35%, respectively, of our estimates of assimilated energy as kJ. Beginning with a conservative J1 density of 150 m^{-2}, cumulative somatic production by the intermolt of J5 is about 530 kJ and respiration about 277 kJ for a total of 807 kJ m^{-2}, or a daily average of 10.4 kJ m^{-2} d^{-1}.

Infaunal and epifaunal prey organisms, including small bivalves and polychaetes, have been quantified as AFDW kJ m^{-2}, but are much less than the estimated energy requirement of the 0+ crab, even accounting for standard production values. We suspect that mobile prey such as gammarid amphipods are one important source of energy within the shell habitat, although interannual density of such prey vary tremendously (highest measured density equated to 5 g AFDW = 97 kJ m^{-2}). A second intermittent source of prey is smaller conspecifics, particularly when later cohorts of crab settle to shell already occupied by larger conspecifics. Cannibalism among cohorts of the 0+ age class has been studied in the lab, and the process may serve both to reduce agonistic interaction and competition, as well as provide an important prey resource.

NOT JUST A STORAGE TANK: ANATOMICAL, BIOCHEMICAL, AND MICROBIOLOGICAL STUDIES OF SPERMATHECA STRUCTURE AND FUNCTION IN SNOW CRAB, *CHIONOECETES OPILIO*. Peter G. Beninger* and **Carole Lanteigne,** Département de Biologie, Université de Moncton, Moncton N.B. E1A 3E9 Canada; **R. W. Elner,** Canadian Wildlife Service, P.O. Box 340, Delta, B.C. V4K 3Y3.

Snow crabs and other Majidae exhibit a complex reproductive biology which includes the possibility of immediate fertilization at copulation and/or storage of spermatophores for subsequent autonomous fertilization. The spermatheca is thus a central organ in the reproduction of this species. Anatomical and ultrastructural studies of the *Chionoecetes opilio* spermatheca reveal two functionally distinct parts: the dorsal (glandular) and the ventral (chitin-lined) regions. Although unsubstantiated conventional wisdom states that the glandular secretions are utilized as metabolites for stored spermatozoa, an alternate role is proposed: as a metabolic substrate for the dense, morphologically homogeneous bacterial population observed in all spermathecae examined. These bacteria may act to exclude opportunistic microbes from the spermatheca lumen, which stores spermatozoa. *In vitro* spermatophore dehiscence experiments support the hypothesis of spermatophore partitioning according to physical properties: spermatophores used for immediate fertilization rupture as a result of physical stress during transfer through the first gonopod and exposure to seawater introduced during copulation, while those stored for subsequent autonomous fertilization are more resistant to these stimuli. Storage of these spermatophores in the spermatheca reduces their resistance to dehiscence, such as the conditions assumed to take place during autonomous fertilization. The importance of spermatophore storage and maintenance of integrity is discussed in terms of sperm viability, copulation danger, brood success, and last-male precedence in sperm competition.

HABITAT SELECTION BY YOUNG OF THE YEAR DUNGENESS CRAB *CANCER MAGISTER* DANA AND PREDATION RISK IN INTERTIDAL HABITATS. Miriam E. Fernandez,* Oscar Iribarne, and **David A. Armstrong,** School of Fisheries (WH-10), University of Washington, Seattle, WA 98195, U.S.A.

Habitat selection by megalopae, and habitat preference and relative mortality of young-of-the-year (YOY) Dungeness crab, *Cancer magister*, were evaluated in four habitat types: bivalve shell middens (*Crassostrea gigas*), eelgrass (*Zostera marina*), mud with scattered shell and bare mud. Under laboratory conditions shell was the habitat most preferred by megalopae and YOY, eelgrass ranked second. Field tethering experiments showed that shell habitat provided the best protection from predation, and that the proportion of crab eaten was highest on bare mud. Field tethering experiments using small hooks attached to tether lines and glued to the crab showed that the sculpin *Leptocottus armatus* was

the most important fish preying on YOY crab in this area. Field and laboratory experiments showed that cannibalism rate by 1+ and 2+ Dungeness crab on YOY conspecifics decreases as habitat complexity increases. However, cannibalism among YOY is not affected by habitat complexity, and may be even higher in complex habitats where YOY density is higher. Most evidence suggests that intertidal shell habitat enhances Dungeness crab survival during the first several months of benthic life. It has led to use artificial shell habitat as a technique to increase juvenile crab abundance and compensate crab losses due to dredging.

TOXICITY AND PATHOGENICITY TESTS USING GRASS SHRIMP EMBRYOS. **William S. Fisher*** and **Steven S. Foss**, U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, FL 32561.

A simple test, using embryos of the grass shrimp, *Palaemonetes pugio*, has been employed to determine the toxicity of oil, commercial oil dispersants, oil biodegration products and microbial pest control agents (MPCA). The test system has several advantages for toxicity and pathogenicity testing: Embryos are held separately in glass tubes so there is no interaction among individuals, they do not require feeding, can be examined with little disturbance, and require low volumes of test toxicant. Moreover, adult *P. pugio* can be cultured through successive reproductive cycles year-round in the laboratory and embryo tests can be performed away from the marine environment by using artificial sea salts.

Tests of toxicity to marine organisms from oil and oil/water fractions are often characterized by highly variable results. However, *P. pugio* embryos were especially responsive to oil products, exhibiting high sensitivity and exceptionally low variability. Tests with water-soluble fractions of Number 2 Fuel Oil demonstrated that oil dispersants increased tenfold the toxicity of nondispersed oil. Exposure to neutral products formed by microbial degradation of artificially weathered Prudhoe Bay crude oil (ANS 521) showed toxicity at relatively low concentrations. Exposure of embryos to oil degradation products caused virtually 100% mortality within a relatively narrow time interval. In contrast, results from exposure to fungi and fungal toxins (MPCAs) were highly variable.

In all tests a consistently high (90–100%) percentage of unexposed, control embryos survived beyond hatching at a variety of temperature and salinity conditions. Even more notable was the extraordinary synchrony among embryos in rate of development and age of hatching. Even though individually placed in glass tubes 2–3 days after oviposition, embryos held at the same temperature hatched after 11–14 d usually within 24 h of each other. This implies a strong endogenous control of embryonic development.

PROTANDRIC HERMAPHRODITISM IN THE BAY SHRIMP *CRANGON FRANCISCORUM* (DECAPODA, CARIDEA). **Maria A. Gavio,* Jose M. Orensanz,** and **David Armstrong,** School of Fisheries, University of Washington, Seattle, WA 98195.

Secondary sexual characters and the reproductive system of the bay shrimp, *Crangon franciscorum*, are described, and evidence of protandric hermaphroditism is presented. This study was based on detailed examination of the external morphology and gonadal histology of large samples collected in Grays Harbor (Washington). Direct evidence of sex change was provided by two males which molted in captivity to become immature secondary females. Externally there was a change in the shape of the endopodite of the first pair of pleopods, and loss of the *appendix masculina*. Histological study of the gonad showed an immature ovary and presence of atrophied *vas deferens*.

The adult population is composed of: 1) individuals that first mature as females (*primary* females), 2) males in two different stages (*mature* and *transitional*), and 3) individuals that mature as females after having passed through a male stage (*secondary* females). Results from previous studies on the population dynamics of this species, based mostly on the analysis of sequential size frequency distributions, were reinterpreted in the light of the findings outlined above to explain sexual size dimorphism and the apparent disappearance of males as a year-class ages.

PERKINSUS MARINUS DISEASE OF OYSTERS

PERFORMANCE VARIATION AMONG NATIVE AND SELECTIVELY-BRED EASTERN OYSTER STRAINS IN NORTH CAROLINA. **Bonnie L. Brown** and **Arthur J. Butt,** Chesapeake Scientific Investigations Fdn, Inc., Richmond, VA 23220; **Kennedy T. Paynter,** University of Maryland, College Park 20742.

Little is known of the potential performance of the many naturally occurring and selectively bred strains of eastern oyster, *Crassostrea virginica*, each responding differently depending upon local environmental variables and upon innate genetic and physiological differences. This study evaluated production of two strains of eastern oyster under floating tray-culture conditions in Pamlico Sound where performance of the two strains differed markedly between sites of high and low salinity.

Growth, disease, and survival of native NC oysters were compared to a domestic strain of Chesapeake Bay oysters selectively bred for rapid growth. Introduced in September 1992, spat of both strains of oyster (10 mm) were cultured side-by-side at two sites selected on the basis of salinity (low: 10‰ and high: 30‰), water quality, and accessibility. Through fall, winter, and spring the selectively bred strain grew significantly faster than the native strain at both sites. In May 1993, *Perkinsus marinus* infection exceeded 80% in both strains held at high salinity at which time the selectively bred strain ceased to grow, and began to experience accelerated mortality. Meanwhile, despite high levels of infection, the native NC strain held at the high salinity site experienced <20% mortality and continued to grow; exceeding harvest size of 76 mm by October 1993. In contrast, at the low salinity site, while

overall growth was lower and despite *P. marinus* infection, selectively bred oysters out-performed the native strain. Mortality of both strains at the low salinity site was negligible (<5%). The trend in growth for the two strains suggests that performance of oysters is related to genetic makeup, despite our inability to detect such differences, while the trend in mortality suggests a relationship to other factors such as acclimatization and pathogenicity of *P. marinus*.

FIELD EXPOSURE OF TRIPLOID *CRASSOSTREA GIGAS* TO *HAPLOSPORIDIUM NELSONI* (MSX) AND *PERKINSUS MARINUS* (DERMO) IN THE LOWER CHESAPEAKE BAY. **Eugene M. Burreson*** and **Roger Mann,** School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Pt., VA 23062; **Standish K. Allen, Jr.,** Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349.

To determine the disease susceptibility of the Pacific oyster, *Crassostrea gigas,* under natural conditions in the lower Chesapeake Bay, 200 individually-typed triploid individuals from Rutgers University were placed in mesh bags in four replicate trays in the lower York River at VIMS. Control oysters consisted of 200 diploid *C. virginica* from Ross Rock in the upper Rappahannock River and 200 diploid *C. virginica* from Worlds End Aquaculture, Queenstown, Maryland, placed in separate mesh bags in the same trays as the *C. gigas*. Mean shell height of the *C. gigas* and Maryland controls was approximately 45 mm: Ross Rock controls were slightly larger. All oysters were deployed on 29 June 1993. Salinity averaged 20 ± 2 ppt. and temperature was greater than 25°C during the sampling period. Samples of 25 oysters from each group were removed for disease diagnoses on 10 August, 8 September and 14 October. Maximum prevalence of *H. nelsoni* was 84% in the Ross Rock controls and 92% in the Maryland controls with a high proportion of heavy and moderate infection intensities; no *C. gigas* was infected with *H. nelsoni*. Maximum prevalence of *P. marinus* was 96% in the Ross Rock controls, 100% in the Maryland controls and 24% in the *C. gigas*. A high proportion of heavy and moderate infection intensities occurred in both control groups, but all *P. marinus* infections in *C. gigas* were low intensity. Mortality was greater than 90% in both control groups by 1 November; mortality was 25% in *C. gigas* and was not attributable to disease. Shells of *C. gigas* were heavily infested with the polychaete *Polydora* sp. Results suggest that *C. gigas* of the size range tested are not susceptible to the major oyster diseases of the Chesapeake Bay.

SYNERGETIC EFFECTS OF TEMPERATURE AND SALINITY ON THE RESPONSE OF OYSTERS (*CRASSOSTREA VIRGINICA*) TO THE PATHOGEN, *PERKINSUS MARINUS*. **Fu-Lin E. Chu,* Aswani K. Volety,** and **Georgeta Constantin,** School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

The response of oysters challenged by two different doses (2.5×10^3 or 2.5×10^4 trophozoites oyster^{-1}) of *Perkinsus marinus* (Dermo) was examined at nine salinity-temperature combinations using 10, 15, and 25°C and 3, 10 and 20 ppt. The significance of the effects of temperature, salinity, and doses of infective particles and their interaction on *P. marinus* susceptibility and progress of infection was assessed using a log linear model and logistic regression analyses. Results reveal that increased infection prevalence occurred at high temperatures and salinities and there was a dose dependent response to infective particles. Temperature was the most important factor followed by the dose of infective particles influencing the susceptibility to *P. marinus* and subsequent disease development in oysters. Salinity was the least influencing factor compared to temperature and trophozoite doses. However, the effect of interactions among these three factors on disease prevalence was insignificant.

Intensity of *P. marinus* infection in oysters increased with increasing temperatures, salinities and trophozoite dose. There was a significant effect of interaction between temperature and salinity, and between temperature and trophozoite dose on intensity of *P. marinus* infection.

PERKINSUS MARINUS* AND OYSTER POPULATIONS: MODELING THE DISEASE.** **Eileen E. Hofmann and **John M. Klinck,** Center for Coastal Physical Oceanography, Old Dominion University, Norfolk, VA 23529; **Eric N. Powell,** Department of Oceanography, Texas A&M University, College Station, TX 77843.

Perkinsus marinus is one of the primary factors controlling population abundance and productivity of oyster (*Crassostrea virginica*) populations. A numerical model, which includes *C. virginica* and *P. marinus* biology and physiology, was used to examine the factors controlling *P. marinus* prevalence and infection intensity and the effects of these on oyster populations. The simulations show that temperature is an important environmental variable that regulates *P. marinus*. In particular, increased temperature promotes highest summer prevalences and infection intensities at mid-latitudes, the northern Gulf of Mexico and much of the southeastern U.S. coast, modifies the time span and degree of the winter lows in infection intensity, and varies the phasing of recruitment and the fall decline in infection intensity. The simulations also suggest that *P. marinus*-produced mortality as a fraction of the adult population may be most important at mid-latitudes because increased reproductive potential at lower latitudes balances out the tendency towards an increased rate of mortality from disease and, as a consequence, infection intensities and prevalences average higher in the central part of the latitudinal range. The cap on population infection intensity in the range of Mackin's 3 or 4 (moderate to moderately-heavy), present in most field data sets in populations having high prevalence, accrues from two processes. First, slowing rates of cell division and slowing rates of cell mortality allow oysters to remain moderately to moderately-heavily

infected for extended periods of time. Second, the rate at which these individuals reach a lethal infection level is slow enough that less heavily-infected oysters of submarket size can grow up to replace those that die. The density effect on cell division serves to retain infection in oyster populations since cell death (by oyster death) is also minimized. High host infection rates do not necessarily result in high host mortalities in contrast to many host/parasite systems. Changing the timing of phytoplankton blooms dramatically affects the degree of oyster mortality. An early spring bloom produces less mortality because a larger fraction of the net production goes into somatic growth and one of the oyster's primary defenses is to outgrow its disease.

LONG-TERM CHANGES IN THE DISTRIBUTION OF *PERKINSUS MARINUS* IN GULF OF MEXICO OYSTERS, A PRODUCT OF CLIMATIC CYCLES? Yungkul Kim* and **Eric N. Powell,** Department of Oceanography, Texas A&M University, College Station, TX 77843.

As a part of NOAA's Status and Trends Mussel Watch Program, prevalence and infection intensity of the protozoan *Perkinsus marinus* were measured in oysters from a minimum of 50 sites from the Laguna Madre of Texas to the Everglades of Florida each winter for the last 7 yr. Gulf-wide mean prevalence was highest in 1986, but remained above 70% in most years. Gulf-wide mean infection intensity was high in 1986, declined in 1987 and 1988, rose to a seven year high in 1989–1990, and then declined again. Between 1986 and 1989, prevalence increased in the northeastern Gulf and declined in the southern and western Gulf. Between 1989 and 1992, prevalence dropped in the northeastern Gulf and increased in south and central Texas and the central panhandle of Florida. Similar trends occurred in infection intensity. Concordant interannual changes in infection intensity occurred in bays up to 1000 km apart. In contrast, interannual changes in prevalence varied considerably between adjacent bays. Concordant interannual changes in infection intensity over a large regional scale suggests the importance of Gulf-wide changes in temperature and rainfall in determining apparent focuses of infection. The El Niño cycle affects the Gulf on a regional scale of this magnitude by modifying precipitation and consequently salinity. Transmission rate seems to be less affected by large scale climatic phenomena.

PHYSIOLOGICAL EFFECTS OF PROTOZOAN PARASITISM ON THE EASTERN OYSTER *CRASSOSTREA VIRGINICA*: FEEDING AND METABOLISM. Roger I. E. Newell* and **Ken Paynter,** Horn Point Environmental Laboratory, University of Maryland, Cambridge, MD 21631; **Gene Burreson,** Virginia Institute of Marine Science, Gloucester Point, VA 23062.

The eastern oyster, *Crassostrea virginica* is highly susceptible to infection by the parasite *Perkinsus marinus* which causes the oyster to cease growing and eventually die. This disease progression suggests that the parasite may interfere with routine physiological functions, as has been shown to occur with another major oyster parasite, *Haplosporidium nelsoni*. Thus, we hypothesized that oysters infected with *P. marinus* may have a reduced food intake, an elevated metabolic rate and decreased assimilation efficiencies compared with uninfected oysters.

We have been measuring these physiological functions in oysters transplanted in June 1992 to three locations within Chesapeake Bay which have differing salinity regimes and consequent differences in *P. marinus* infection intensities. Oysters at two sites became infected with *P. marinus* during the summer 1992. In August 1992, at the high salinity site, experimental oysters ceased growing shell, and in September exhibited a 35% mortality rate as a consequence of these infections. In 1993, the disease infections at the high salinity site intensified causing all oysters to die by late July. During this entire disease progression we detected only on a few occasions a decline in feeding activity in the most severely infected oysters. We could detect no differences in metabolic rate between infected and uninfected oysters at each of these locations. Unexpectedly, there were also no significant changes in assimilation efficiency (measured using the Connover ratio method and radiotracer techniques) between uninfected and heavily infected oysters. We hypothesize that one mechanism whereby *P. marinus* causes the host to cease growing is that parasite cells in the hemolymph and tissue outcompete the host for absorbed nutrients.

PERFORMANCE OF VARIOUS OYSTER POPULATIONS IN CHESAPEAKE BAY: GROWTH AND DISEASE TOLERANCE. Kennedy T. Paynter, Department of Zoology, University of Maryland, College Park, MD 20742.

The oyster population in Chesapeake Bay is decreasing at an alarming rate. Most of the decline is believed to be the result of mortalities caused by two protozoan parasites. The most prevalent disease is dermo caused by the protozoan *Perkinsus marinus*. For the last three years, oysters have been deployed in floating rafts in various regions of Chesapeake Bay in order to better understand the effects of dermo at different salinities. In conducting these studies, oyster cohorts produced from a variety of broodstocks have been employed. While genetic differences have not been quantified between these different groups, great variation in growth, disease acquisition, disease progression and mortality was observed between groups. Some differences were associated with variation in size at the time of deployment, others associated with history of infection, but many of the differences were not associated with any particular differences in seed quality except the origin of the broodstock.

Broodstocks from various natural oyster bars and/or from leased bottom stocks were conditioned at the Horn Point Environmental Lab oyster hatchery and spawned. Typically, newly set oyster seed were immediately moved to an upwelling facility on the Wye R. MD, and raised there in a low salinity environment to

avoid infection by *P. marinus* or *H. nelsoni*. Two groups of oysters produced from North Carolina broodstocks during two different years performed much better than several native Chesapeake Bay oyster groups in high salinity disease prevalent areas. North Carolina animals grew more vigorously when they became infected with *P. marinus* and grew longer into the winter season than Chesapeake Bay groups which resulted in significantly larger oysters at the end of the growing season. These genetic or physiological traits may be valuable in enhancing production in the Chesapeake Bay region. (Supported by Maryland Agricultural Experiment Station grant #10-5-25101).

PERKINSUS MARINUS*: TRIGGERING MECHANISMS FOR EPIZOOTICS. Eric N. Powell, Department of Oceanography, Texas A&M University, College Station, TX 77843; **Eileen E. Hofmann** and **John M. Klinck,** Center for Coastal Physical Oceanography, Old Dominion University, Norfolk, VA 23529.

Perkinsus marinus is one of the crucial factors controlling population abundance and productivity of oyster (*Crassostrea virginica*) populations. A computer model was used to examine the factors which trigger *P. marinus* epizootics. Epizootics are triggered by three general classes of environmental and biological perturbations: factors affecting food supply, factors affecting environmental characteristics, and factors affecting the supply of juveniles in the populations. Most simulated epizootics followed a typical time course. The conditions triggering the epizootic occurred and disappeared well before, as much as 18 months before, the initiation of mortality in the population. Once started, most epizootics progressed more or less rapidly toward population extinction. No internal mechanism was available to limit their time course. Stopping an epizootic required reducing the infection intensity in the submarket-size adults and subadults in the population. Our simulations suggest that a principal mechanism is a large recruitment event which would dilute *P. marinus* in that portion of the population, although it would not affect the infection intensity of the market-size adults which is maintained at a relatively stable level by the death of heavily-infected individuals. Infection intensity of the market-size adults does not need to be reduced to stop an epizootic nor does it need to be raised to start one. Reducing the rate of replacement of market-sized individuals that die is sufficient. In the common case where prevalence exceeds 60% and infection intensity rises to 3 or more during the summer months, most oyster populations suffer adult mortality due to *P. marinus*. Stability is maintained by an adequate rate of replacement of adults to minimize the effect of these losses on adult density and population fecundity. Accordingly, our simulations suggest that the key to triggering an epizootic is to raise the infection intensity in the subadult and submarket-size adult portion of the population and the key to stopping an epizootic is to reduce infection intensity in that same portion of the population.

CHARACTERISTICS OF THE DISEASE AGENT *PERKINSUS*

RACE-SPECIFIC INTERACTIONS BETWEEN *CRASSOSTREA VIRGINICA* AND *PERKINSUS MARINUS*. David Bushek and **Standish K. Allen, Jr.,** Haskin Shellfish Research Laboratory, Rutgers University, RD#1 Box B-8, Port Norris, NJ 08349.

Little is known about the population structure of the oyster pathogen *Perkinsus marinus*. For example, pathogens often develop races that vary in virulence which may lead to race-specific resistance in the host. The existence of *P. marinus* races has considerable implications for management, breeding and testing resistance of alternative species. This study represents the first attempt to examine race-specific interactions between *P. marinus* and *C. virginica*.

F1 generation oysters from Maine, New Jersey, Virginia and Texas were reared in a common environment for two years. Putative races (isolates) of *P. marinus* from New Jersey, Virginia, Louisiana, and Texas were injected into the shell cavity of individual oysters (80 to 192/stock). Each oyster received 5×10^5 *in vitro* cultured cells/g oyster meat weight. No *P. marinus* was detected in any oysters prior to inoculation. Oysters inoculated with different isolates were maintained in separate tanks for 3 months with 2 replicate tanks per isolate. Preliminary analysis of *P. marinus* body burden (# cells/g wet wt.) indicates differences exist across isolates and among oyster stocks. Atlantic isolates appear more infective than Gulf isolates (mean log(body burden) = 2.13, se = 0.36, n = 45 vs. 1.00, se = 0.25, n = 45). Mean log(body burden) for oyster stocks was: TX = 0.35 (se = 0.11, n = 33); VA = 1.97 (se = 0.52, n = 19); NJ = 2.11 (se = 0.59, n = 19); ME = 2.76 (se = 0.55, n = 19). Resistance seems to correlate with the length of time oyster populations have coexisted with *P. marinus*. NJAES Pub. # K-32100-13-93.

USE OF A COLORIMETRIC CELL PROLIFERATION ASSAY WITH IN VITRO-PROPAGATED *PERKINSUS MARINUS* TO OPTIMIZE CULTURE CONDITIONS, ASSESS PATHOGEN CHEMOSENSITIVITY, AND DETERMINE PHYSICAL TOLERANCES. Christopher F. Dungan* and **Rosalee M. Hamilton,** Maryland DNR, Cooperative Oxford Laboratory, Oxford, MD 21654.

Recent development of in vitro propagation methods for the protozoan oyster pathogen, *Perkinsus marinus*, has permitted the design of controlled experiments to evaluate previously untestable hypotheses concerning pathogen physiology, virulence, and genetics. While in vitro pathogen propagation has been reported by several authors as routine under a variety of culture conditions, use of such cultured cells in vitro to measure effects of metabolic substrates, physical conditions, or drugs, require methods for quantification of effects. We have adapted a commercial, spectro-

photometric cell proliferation assay for use with *P. marinus* cultures. The method provides an elegant, quantitative means for rapid assessment of in vitro effects on cell proliferation, using small volume assays compatible with high levels of treatment replication and modern micropipetting technology. We report here data on adaptation and standardization of the assay method, as well as examples of its use in optimizing culture conditions and chemotherapeutant efficacies.

DETECTION OF PROTEASES IN THE SUPERNATES OF *PERKINSUS MARINUS* CULTURES. **Mohamed Faisal,* Jerome F. La Peyre,** and **Doris Y. Schafhauser,** School of Marine Science, Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, Virginia 23062, USA; **Esam H. Rizkalla,** Central Laboratory for Aquaculture Research, Abassa, Sharkia, Egypt.

Perkinsus marinus causes severe mortalities in eastern oyster (*Crassostrea virginica*). Little is known, however, about the virulence factors produced by this deadly protozoan. The recent development of a continuous culture system of *P. marinus* in our laboratory has permitted the identification of a variety of parasitically-derived proteins. These proteins became detectable within the supernates as early as one week postinoculation. These extracellular proteins (ECP) require extensive analysis as preliminary work indicates their possible roles as virulence factors.

Initially, protease activity was demonstrated in *P. marinus* supernates by radial diffusion in casein-agar gel plates. Also sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) revealed the presence of parasitic ECP ranging from 15–65 kDa in size. Analysis of substrate impregnated SDS gels showed that some of these ECP possess proteolytic activity. Although originally detected on gelatin and casein, of particular importance was the demonstration of this activity in oyster hemolymph and oyster tissue homogenate-gels. Fibronectin, laminin, and bovine serum albumin were also hydrolyzed. Some degree of substrate specificity has also been observed. These results demonstrate that the parasite is synthesizing and secreting proteases that are capable of hydrolyzing oyster proteins as well as general substrates.

USING MOLECULES TO DIAGNOSE *PERKINSUS* SPECIES AND DETERMINE THE AFFINITIES OF PARASITES OF UNCERTAIN PHYLOGENETIC AFFINITIES FROM FISH AND SHELLFISH. **C. Louise Goggin*** and **R. J. Cawthorn,** Atlantic Veterinary College, Dept of Pathology and Microbiology, University of Prince Edward Island, 550 University Avenue, Charlottetown, PEI, Canada C1A 4P3.

The status of *Perkinsus* parasites continues to challenge scientists. Are they fungi or protistans? Are they apicomplexans? How many species of *Perkinsus* exist? Molecular biology is a powerful tool which has been used to address these questions of taxonomy and phylogeny. In particular, nucleotide sequence data from the internal transcribed spacers in the ribosomal RNA gene cluster differ between some described *Perkinsus* species and data from the small subunit of this gene cluster have indicated the phylogenetic affinities of the Perkinsea.

We will discuss our current project which uses data from the ribosomal RNA gene cluster to investigate the taxonomy of *Perkinsus* parasites found in Canadian and American shellfish and determine their affinities to protistan parasites of uncertain phylogeny, including *Dermocystidium* spp. from fish and *Labyrinthuloides haliotidis* from abalone.

COMPARISON OF EFFECTIVENESS OF POTENTIAL CHEMOTHERAPEUTIC AGENTS AGAINST *PERKINSUS MARINUS* CULTURED IN THIOGLYCOLATE MEDIUM AND IN A COMPLETE GROWTH MEDIUM. **G. Krantz,** Maryland Department of Natural Resources, Cooperative Oxford Laboratory, 904 S. Morris St., Oxford, MD 21654.

Recent successes of *in vitro* propagation of *Perkinsus marinus* at the Oxford Laboratory, and the technical report of La Peyre et al. (1993), proved a new system for screening chemotherapeutic agents for their ability to kill or inhibit *P. marinus*. Previous work at the Laboratory evaluated several organic and inorganic compounds for the suppression of hypnospore development by *P. marinus* in a thioglycolate culture system. This system used the hemolymph from heavily infected oysters to provide a sheet of *Perkinsus* cells in a culture plate that then transformed into hypnospores under the stimulatory conditions of the thioglycolate or "Ray medium." Some of the chemicals added to the Ray media suppressed hypnospore development, and replicated dilution tests allowed definition of concentrations that were effective against the hypnospore development. These same compounds and concentration dilutions were applied to the *in vitro* propagation technique for vegetative *P. marinus* isolates which were grown in tissue culture flasks using a complete growth media.

Perkinsus cells were exposed to 10 inorganic salts and 25 organic compounds. Silver nitrate at 200 ppm concentration was the only inorganic salt to suppress proliferation. Fourteen (14) commercially available organic compounds inhibited *Perkinsus* cell growth at concentrations similar to those that presented hypnospore development in the "Ray medium."

Test results provide information on effective chemotherapeutic concentrations that must be achieved in living oysters for these compounds to be an effective treatment for "Dermo" disease.

INTERANNUAL ABUNDANCE VARIATION OF ENVIRONMENTAL *PERKINSUS MARINUS* CELLS IN CHESAPEAKE BAY WATERS. **Tong Li** and **Bob S. Roberson,** University of Maryland, College Park, MD 20742; **Christopher F. Dungan,*** Cooperative Oxford Laboratory, Maryland DNR, Oxford, MD 21654.

Efforts to enumerate *P. marinus* cells in water samples from the Tred Avon River have continued with results accumulated for a second year. Flow cytometric parameters for detecting the patho-

gen in liter water samples were adjusted after the first year's sampling to include initial gating on fluorescein labelling with anti-*P. marinus* antibody and nucleic acid staining. Further discrimination for counting was based on forward angle (FALS) and side light scatter (90LS). With this gating, particles that morphologically resemble *P. marinus* could be sorted in high purity, and provide a conservative estimate of abundance in that sample. The numbers of pathogen cells falling outside of these gates is significant, and attempts to estimate counting efficiency in this conservative window have been made. Adjacent windows were sorted and counted by fluorescence microscopy to estimate the percentage of particles that appear to be *P. marinus*.

Second year data show an extended period of elevated *P. marinus* counts by comparison with the previous season. Abundancies in both years peaked at the end of May, and continued at lower levels with subsequent minor peaks. Salinities and temperatures were collected throughout the sampling period for correlation with abundancies.

ACUTE OSMOTIC TOLERANCE OF CULTURED CELLS OF THE OYSTER PATHOGEN *PERKINSUS MARINUS*. **Lisa M. Ragone Calvo*** and **Eugene M. Burreson,** School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Pt., VA 23062; **Kennedy T. Paynter, Jr.,** Department of Zoology, University of Maryland, College Park, MD 20742.

To determine the osmotic tolerance of cultured cells of *Perkinsus marinus*, cells in log-phase growth from cultures maintained at 22 ppt. in modified JL-ODRP medium were exposed for 24 h to salinities of 0, 3, 6, 9, 12 and 22 ppt. The various salinities were prepared by dissolving seawater synthetic basal salt mixture in culture grade water. Cells were also exposed to the same salinities but with the osmotic concentration adjusted to the equivalent of 22 ppt. with sucrose. Four replicate experiments were conducted. Viability was determined by neutral red uptake. Mortality increased as salinity decreased. Mean mortality was 5% at 22 ppt., 20% at 12 ppt., 43% at 9 ppt., 70% at 6 ppt., 94% at 3 ppt. and 100% at 0 ppt. Variability among replicates was greatest at 6 and 9 ppt. Mortality was low and equal to that at 22 ppt. in all treatments where osmotic concentration was adjusted with sucrose indicating that mortality is caused by a decrease in osmotic pressure, not a decrease in salts. In subsequent experiments to examine viability after exposure times of 5 min to 24 h, mortality occurred rapidly, within 5 minutes of exposure to hypoosmotic conditions. Additional experiments were conducted to determine the effect of temperature on osmotic tolerance. Cells in log-phase growth were exposed for 24 h to 0, 3, 6, 9, 12, and 22 ppt. at 1, 5, 10, 15 and 28°C. There was a general trend of higher mortality at lower incubation temperature within a salinity treatment, but this temperature effect was statistically significant only at 9 ppt. Results suggest that cultured *P. marinus* cells are relatively intolerant of salinity below 9 ppt. and are somewhat surprising considering the present natural distribution of *P. marinus* in low salinity. Within hemocytes of the host, the pathogen may be somewhat protected from hypoosmotic stress.

A COMPARATIVE STUDY OF ACID PHOSPHATASE IN THE PARASITE *PERKINSUS MARINUS* AND ITS HOST *CRASSOSTREA VIRGINICA*. **Aswani K. Volety*** and **Fu-Lin E. Chu,** School of Marine Science, Virginia Institute of Marine Science, College of William & Mary, Gloucester Point, VA 23062.

Acid phosphatase (AP) in parasites, has been postulated to play a role in avoiding the host defense through dephosphorylation of host phosphoproteins and/or inhibition of the superoxide radicals released by the host phagocytes. The AP activity (1 unit = 1 μM p-nitro phenol released/hr/mg protein) in two lifestages of *Perkinsus marinus*, namely, cultured meronts and freshly isolated prezoosporangia, as well as hemocytes and serum of the host *Crassostrea virginica* from different regions were compared. Hemocytes of oysters from James River, Virginia, showed significantly ($p < 0.05$) higher AP activity (4.73 ± 0.17 at 10°C, 6.43 ± 0.13 at 15°C, and 7.62 ± 0.07 units at 25°C) than those from Damariscotta River, Maine (0.37 ± 0.03 at 10°C, 0.5 ± 0.03, and 0.58 ± 0.03 units at 25°C). AP activity was higher ($p < 0.05$) in hemocytes compared to meronts (0.49 ± 0.04 at 10°C, 0.91 ± 0.2 at 15°C, and 0.94 ± 0.08 units at 25°C) and prezoosporangia (0.03 ± 0.02 at 10°C, 0.05 ± 0.06 at 15°C and 0.03 ± 0.01 units at 25°C). Only trace amounts of AP were detected in sera from both Virginia and Maine oysters. The difference of AP activity in hemocytes from different regions might be related to the differences in food, habitats or genetic factors. Based on these results, the role of AP in avoiding host defense is inconclusive. Further study is needed to examine the processes of AP secretion by the parasite.

BIVALVE DISEASES AND DEFENSE

COMPARISON OF OXYRADICAL GENERATION BY HEMOCYTES FROM COMMON ESTUARINE BIVALVE MOLLUSKS. **Robert S. Anderson*** and **Lisa L. Brubacher,** Chesapeake Biological Laboratory, University of Maryland System, P.O. Box 38, Solomons, MD 20688; **Kennedy T. Paynter,** Department of Zoology, University of Maryland System, College Park, MD 20742.

Phagocytic leukocytes of many animal species respond to appropriate stimuli with a burst of respiratory activity and the generation of cytotoxic reactive oxygen intermediates (ROIs). In concert with certain lysosomal enzymes, ROIs function as effector molecules in antimicrobial defense mechanisms. Luminol-dependent chemiluminescence (LDCL) and nitroblue tetrazolium (NBT) reduction assays are often used to quantify ROI production by oyster hemocytes; LDCL is particularly useful in studying modulation of ROI production caused by intracellular parasitic disease

or exposure to environmental toxicants. Therefore, it was thought that these tests would be equally applicable for use with hemocytes from other bivalves. However, preliminary results with several species of clams indicated otherwise.

In the four bivalve species tested, untreated hemocytes produced low-level ROI activity. In *Crassostrea virginica* and *Geukensia demissa,* phagocytosis or stimulation with phorbol myristate acetate triggered significantly augmented, but kinetically dissimilar, ROI responses; however, no induction was recorded in *Mya arenaria* or *Mercenaria mercenaria*. These conclusions were supported by both LDCL and NBT reduction assays which measure myeloperoxidase/hydrogen peroxide system activity and intracellular superoxide anion production, respectively. The failure of standard ROI-eliciting procedures in certain bivalves suggests variability of cellular ROI responsiveness, possibly indicative of interspecies differences in hemocyte-mediated antimicrobial defense mechanisms.

CHARACTERIZATION OF REACTIVE OXYGEN INTERMEDIATE PRODUCTION IN OYSTER HEMOCYTES. **Kellie A. Austin*** and **Kennedy T. Paynter,** Dept. of Zoology, University of Maryland, College Park, MD 20742.

Hemocytes of the oyster, *Crassostrea virginica,* produce reactive oxygen intermediates (ROIs) to destroy foreign cells. Recent evidence indicates that ROI production is enhanced in hemocytes isolated from oysters infected with *Perkinsus marinus*. Typically, enzymes such as NADPH oxidase and myeloperoxidase give rise to a variety of ROIs such as O_2^-, H_2O_2, $^1O_2$ and ·OH. Therefore, increased ROI production induced by parasitic infection is likely due to increased enzyme activity.

To determine how specific enzyme activities may change with infection, ROI production in isolated hemocytes was characterized by measuring chemiluminescence (CL) with two different luminescent probes, luminol and lucigenin. Luminol detects a variety of ROIs while lucigenin is selective for superoxide radicals. CL was stimulated with zymosan or phorbol 12-myristate 13-acetate (PMA). The lucigenin CL stimulated by PMA was used as a specific measure of superoxide production by NADPH oxidase. To identify the ROI species detected by each luminescent probe, the following ROI scavengers were employed: SOD (O_2^-), catalase (H_2O_2), azide ($^1O_2$) and mannitol (·OH). Azide is also a strong inhibitor of myeloperoxidase. CL was also determined for hemocytes from infected and non infected oysters to evaluate increased enzyme activity related to *P. marinus* infection.

A significant correlation existed between infection intensity and zymosan stimulated luminol CL ($p = .0003$) and PMA stimulated lucigenin CL ($p = .0001$). Results of inhibitor studies suggest that luminol CL represents myeloperoxidase and NADPH oxidase activity which are both stimulated by zymosan. Lucigenin CL, however, appears to represent only superoxide production most likely by NADPH oxidase. Azide inhibition of zymosan stimulated luminol CL indicated that myeloperoxidase is responsible for the majority of increased ROI production associated with infection. Therefore, infection appears to increase the ROI production by both NADPH oxidase and myeloperoxidase.

PREVALENCE OF *BONAMIA OSTREAE* IN *OSTREA EDULIS* POPULATIONS IN MAINE. **Bruce J. Barber*** and **Chris V. Davis,** Department of Animal, Veterinary & Aquatic Sciences, University of Maine, Orono, ME 04469.

The European oyster, *Ostrea edulis,* was introduced to several coastal Maine locations, beginning in 1949. Small populations became established by 1962 and in 1983, commercial populations were discovered. Harvests reached a maximum in 1985 (623,000 oysters), but have fallen since then. Disease is a factor possibly contributing to this decline. The parasite *Bonamia ostreae* was found in oysters from the Damariscotta River in both 1991 and 1992. The objective of this work was to determine the distribution of *B. ostreae* in *O. edulis* populations throughout Maine.

Seven populations were sampled between 10 August and 27 September 1993. 25–35 individuals from each site were fixed in Davidson's, processed histologically, and stained with hematoxylin and eosin. Prevalence of *B. ostreae* ranged from 0% to 20%. All infected oysters had a shell height of 67 mm or greater.

Thus we know that *B. ostreae* is more widely distributed than previously documented. Further sampling at these and other sites and times of year will help determine the spatial and temporal distribution of this oyster pathogen in Maine.

CYTOLOGY AND BEHAVIOR OF HEMOCYTES OF THE ZEBRA MUSSEL, *DREISSENA POLYMORPHA:* A TIME-LAPSE VIDEO STUDY. **Albert F. Eble*** and **Jill Sampson,** Dept. Biology, Trenton State College, Trenton, NJ 08650-4700.

Four hemocyte types were identified: *Type I*—25 × 15 um, with a large nucleus, an endoplasm filled with clear vacuoles and a large cortex; *Type II*—similar in most aspects to Type I but endoplasm with 3–10 large, colored (yellow to orange) crystalline inclusion bodies; *Type III*—35 × 20 um, a large nucleus surrounded by a small endoplasm containing few granules with a large, clear cortex; *Type IV*—10 × 8 um with a nucleus that almost fills the cytosol. Types I and II displayed pronounced membrane ruffling; granules in the endoplasm showed much activity.

Frequently, these cell types would advance and pinch off anucleate pieces of the cytosol which would remain active and intact for the duration of the examination (3–4 hours). *Flaring* of the endoplasm was frequently observed: a portion of the endoplasm would gather itself and suddenly extend to the cell membrane, then just as suddenly retreat back to the confines of the endoplasm. This same-type behavior has also been observed with *Mya* hemocytes.

***IN VITRO* CULTURE OF *HAPLOSPORIDIUM NELSONI*.** **Stephen J. Kleinschuster,* Susan E. Ford,** and **Sharon L. Swink,** Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349.

A considerable body of information concerning the relationship of the pathogenic organism, *Haplosporidium nelsoni* (MSX) and it's American oyster host *Crassostrea virginica* (Gmelin) has accumulated primarily from the study of stained and sectioned material. Studies utilizing long-term *in vitro* culture of this parasite could also contribute to understanding the cellular physiology, genetics, life cycle and host interaction and specificity of this organism. Utilizing standard sterile technique and medium consisting of a 25% *Perkinsus marinus* medium previously described (1), 35% sterile oyster hemolymph and 40% sterile sea water, cultures of MSX were maintained for several weeks allowing observation of several transitional stages of the parasite while in culture.

This is NJAE publication No. K-32100-11-93 and Institute of Marine and Coastal Science publication No. 9341.

1. Kleinschuster, S. J. and S. L. Swink. 1993. A simple method for the in vitro culture of *Perkinsus marinus*. The Nautilus 107:76–78.

RESPONSE OF OYSTERS TO A CULTURE OF TOXIN-PRODUCING "PHANTOM" DINOFLAGELLATE. G. Krantz,* Maryland Department of Natural Resources, Cooperative Oxford Laboratory, 904 S. Morris St., Oxford, MD 21654, and **J. M. Burkholder** and **H. B. Glasgow,** Department of Botany, North Carolina State University, Raleigh, NC 17695.

A laboratory investigation described the interaction between adult and pediveliger oysters (*Crassostrea virginica*), and a culture of the recently isolated "phantom" dinoflagellate that has been identified as the causative agent in fish kills along the east coast of the United States. Oysters were exposed to both the vegetative (toxic) and resting, encysted stages of the dinoflagellate. Observations were made on the alteration of behavior, morbidity, and lethality in oysters as induced by the dinoflagellate.

There was no observed impact on adult oysters even after 2 weeks of continuous exposure to the vegetative dinoflagellate cultures that were actively killing finfish. Histological studies described sublethal effects. Pediveliger larvae were affected by the toxins secreted by the vegetative dinoflagellate cells. They became narcotized with a cessation of swimming and fell to the bottom of the containers, but were not directly killed by the toxin or by attached vegetative cells. Cultures of the vegetative stage of the dinoflagellate did not appear to utilize oyster tissue as the source of nutrients, and rapidly transformed into resting stages. Oysters did not appear to stimulate transformation of the dinoflagellate resting stages into vegetative, toxic stages.

The most significant observation of the interaction was the potential impact of the waterborne toxic secreted by the dinoflagellate on the pediveliger larvae and subsequent reproductive success of oysters in an estuary that experienced a fish kill during periods of oyster spawning. These observations also suggest that the dinoflagellate toxins may have a similar effect on other plankton. Therefore, blooms of the phantom dinoflagellate could be a significant ecological factor in the trophic dynamics of the estuarine system.

MOLECULAR CHARACTERIZATION OF CELLULAR MECHANISMS INVOLVED IN *MERCENARIA* SPP. GONADAL NEOPLASIA. Maureen K. Krause,* Duke University Marine Laboratory, Beaufort, NC 28516; **George Gardner,** EPA Rhode Island, Narragansett, RI 02882; **Rebecca J. Van Beneden,** University of Maine, Orono, ME 04469.

Bivalves are unusual among marine invertebrates in their capacity for neoplasm production and growth, but virtually nothing is known about the specific biochemical and molecular mechanisms responsible for these disorders, or their evolutionary relationship to those in other taxa. We are investigating the molecular basis for gonadal tumors in 1) natural populations of *Mercenaria* spp. from the Indian River, Florida, a zone of hybridization between *Mercenaria mercenaria* and *M. campechiensis*, and 2) hatchery-produced reciprocal hybrids of *M. mercenaria* and *M. campechiensis* from South Carolina. Gonadal tumor incidence may reach a prevalence of 60% and 84%, respectively, in these clams. The goal of our research is the identification and characterization of cellular oncogenes and tumor suppressor genes involved in these neoplasms. We have screened tumor tissue for the presence of a suite of known oncogenes and tumor-suppressor genes, and have also explored the relationship between species hybridization and tumor incidence. The results of our study suggest that the mechanisms of carcinogenesis may be conserved from man to clam. The use of *Mercenaria* as a model organism to study environmental carcinogenesis will be discussed.

CYTOMETRIC ANALYSES OF OYSTER HEMOCYTES FROM VARIOUS BODY LOCATIONS. Dale S. Mulholland* and **Frank E. Friedl,** Department of Biology, University of South Florida, Tampa, FL 33620.

Hemocytes of *Crassostrea virginica* are in solid tissues, the digestive system, blood vessels, sinuses and shell liquor. Do hemocytes from different body regions show different functional morphology?

Some measurable characteristics indicate a cell is functionally active (*e.g.*, many granules; nucleus which is large, decondensed, perhaps irregular; spreading cytoplasm). Other characters suggest a quiescent hemocyte (*e.g.*, dark, condensed, rounded nucleus and cytoplasm). Hemocytes were taken from (a) pericardial cavity and adductor muscle sinuses (b) exterior epithelium and shell liquor, and (c) tissue sources (*i.e.*, palps, mantle edges, gills, and body wall). Fixed cells were stained for image-analysis on BioQuant™ System IV.

Highly significant differences ($P < 0.01$) were shown by analyses of variance for six of seven characteristics (number of granules, nuclear area, nuclear shape, nuclear density, cytoplasmic area and cytoplasmic shape). Differences in these characteristics imply differences in function and activities. Differences in cyto-

plasmic density were not significant, probably due to multiple factors in the more dense measures (cytoplasmic condensation and numerous granules).

Multiple-range tests determined probabilities of relatedness among hemocyte sources. Hemocytes from body wall and gills were strongly related in all characteristics and different from hemocytes of pericardial cavity, adductor muscle sinuses and shell liquor. Hemocytes from the latter three sources were closely related in most characters.

COMPARATIVE FORM AND FUNCTION OF OYSTER *CRASSOSTREA VIRGINICA* HEMOCYTES FROM APALACHICOLA BAY (FLORIDA) AND CHESAPEAKE BAY (VIRGINIA). Leah M. Oliver,* Avanti Corporation, U.S.E.P.A. Environmental Research Laboratory, Gulf Breeze, FL 32561; **W. S. Fisher,** U.S.E.P.A. Environmental Research Laboratory, Gulf Breeze, FL 32561.

Oysters (*Crassostrea virginica*) from Chesapeake Bay, Virginia, and Apalachicola Bay, Florida, were collected in March 1992, to determine relationships among hemocyte number, morphology and size with putative defense-related activities, including hemocyte mobility, particle binding and superoxide anion (O_2^-) production. Hemolymph drawn from Chesapeake Bay oysters contained an average of 1.08×10^6 hemocytes mL^{-1} hemolymph, significantly lower than the average 1.63×10^6 hemocytes mL^{-1} hemolymph obtained from Apalachicola Bay oysters. An average 84% of Chesapeake Bay oyster hemocytes were granular in type, significantly exceeding the 54% granular hemocytes found in oysters from Apalachicola Bay. Hemocytes from Chesapeake Bay oysters demonstrated a higher percentage of mobile cells and a faster rate of locomotion, a greater capacity for binding yeast particles, and increased O_2^- production compared to Apalachicola Bay oyster hemocytes. Hemolymph levels of the protozoan parasite, *Perkinsus marinus*, did not differ significantly between oysters from the two sites. Percentage granular hemocytes showed a significant positive correlation with percentage of mobile hemocytes, particle binding activity, and hemocyte O_2^- production for oysters from both sites. Results substantiate previous reports of heightened phagocytic and locomotive activity by granular hemocytes, and indicate that granular hemocytes possess greater potential to produce O_2^- than agranular cells.

BIVALVE MOLLUSK BIOLOGY

ATRIAL NATRIURETIC PEPTIDES IN HEMOLYMPH AND TISSUES OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*. Frank E. Friedl,* Department of Biology, University of South Florida, Tampa, FL 33620; **David L. Vesely,** University of South Florida and James A. Haley Veterans Hospital, Tampa, FL 33612.

Vertebrate atrial natriuretic peptides (ANF) have dramatic diuretic and natriuretic effects. Utilizing proANF 1-30 (amino acids (a.a.) 1-30), proANF 31-67 (a.a. 31-67), and ANF (a.a. 99-126) radioimmunoassays (RIA), we have found that these peptides exist in oyster hemolymph, heart, and gill and that their levels respond to ambient salinity changes.

Tampa Bay oysters were maintained at 20°C and 27 ppt salinity. For time and salinity experiments, they were kept at 25°C for 3, 7, and 14 days at high (HS), medium (MS), and low (LS) salinities (947, 623, and 234 mosmolal). Peptides measured by ProANF 1-30, proANF 31-67 and ANF RIAs in centrifuged pericardial hemolymph were 458, 156, and 66 pg/ml at 7 days in MS. The above RIAs also measured ANF peptides (at 7 days in MS) in heart (59, 16, and 19 ng/g wet wt.) and gill (8.6, 2.6, and 2.8 ng/g wet wt.). During 11 days at HS the peptides detected by the respective RIAs in heart and gill increased (40 → 102, 11 → 37, and 11 → 39 ng/g for heart; 4.5 → 20, 1.3 → 6.1, and 1.1 → 5.8 ng/g for gill) whereas those in hemolymph did not (1034 → 940, 240 → 247, and 94 → 95 pg/ml). For animals in MS and LS, amounts in heart and gill did not change as notably, and hemolymph levels dropped in general. The biological function of ANF peptides in oysters is unclear at present, however, the responses to external salinity imply a relationship to salt or volume homeostasis.

VITELLOGENESIS IN THE HARD CLAM, *MERCENARIA MERCENARIA;* SYNTHESIS AND UTILIZATION OF VITELLIN. Richard F. Lee,* Skidaway Institute of Oceanography, P.O. Box 13687, Savannah, GA 31416; **Mary Sweeney** and **Randal Walker,** University of Georgia Marine Extension Service, Shellfish Laboratory, P.O. Box 13687, Savannah, GA 31416.

The accumulation of large amounts of vitellin by the developing oocyte takes place during vitellogenesis in clams and other bivalves. This vitellin serves as a source of amino acids for the synthesis of proteins by the non-feeding clam embryos. We have isolated and purified a major vitellin peptide (clam egg peptide 1) from M. mercenaria. Several monoclonal antibodies have been prepared against this peptide. Using these antibodies an indirect competitive enzyme linked immunosorbent assay (ELISA) has been developed to quantify clam vitellin. These antibodies were also used in immunohistochemical studies of clam tissues when clam ovaries were in a mid-maturation stage of development. In the developing oocytes the immunoreactivity was localized in yolk granules. No immunoreactivity was observed in the surrounding follicle cells. We speculate that vitellin peptides are synthesized within the oocytes and that follicle cells and extra-ovarian tissues play a minor role in vitellin synthesis. Proteinases, associated with lysosomes, are released after fertilization and act on vitellins to produce small peptides and amino acids. We found that after fertilization there was rapid utilization of vitellin so that after 48 hours there was little detectable vitellin peptide. We propose that successful induction of spawning and good embryo survival both require a critical concentration of vitellin in the eggs.

GRAZING OF NATURAL PARTICULATES BY THE MUSSEL, *MYTILUS EDULIS:* A SPATIAL AND TEMPORAL PERSPECTIVE. Carter R. Newell, Great Eastern Mussel Farms, Inc., Tenants Harbor, Maine 04860 U.S.A.

The production of suspension feeders such as mussels may be limited by processes ranging from cm to km scales, and time periods ranging from seasonal changes in temperature to tidal cycle changes in the flux of seston. Field observations have shown that the processing of phytoplankton, organic detritus and inorganic particulates by shellfish beds results in kilometer scale effects on particle concentration (depletion) which may be simulated by computer modelling. Over 4 years (1986–1990), biological and oceanographic processes were examined at 4 commercial mussel bottom leases in Maine, USA, in order to build and test mussel production models and determine the optimal seeding density. Several key factors examined during the study include: height of the mussel feeding zone; fine vertical gradients in the food (seston) supply; spatial variations in benthic boundary layer particle flux; temporal variations in feeding rates on natural particulates; and estuary-scale variations in food supply. The data suggest that the estuary-scale processes of tidally-supplied diatom blooms are a major forcing function in the production of benthic filter-feeders such as the mussel, *Mytilus edulis*. The implications of these studies on site selection and the management of shellfish bottom leases are discussed.

GAMETOGENIC CYCLE IN THE NON-NATIVE ATLANTIC SURFCLAM, *SPISULA SOLIDISSIMA* (DILLWYN, 1817), CULTURED IN THE COASTAL WATERS OF GEORGIA. C. Spruck,* M. Sweeney, D. Hurley, and **R. L. Walker,** Shellfish Research Laboratory, Marine Extension Service, University of Georgia, P.O. Box 13687, Savannah, GA 31416-0687.

The Georgia Department of Natural Resources currently bans the importation of exotic bivalve seed into the state. Thus, seed to support any potential aquaculture enterprise must be produced in state. If yearling clams cultured under field conditions mature and produce viable offspring, then a major biological hurdle to the development of a commercial enterprise will be achieved.

Hatchery produced clams (19.4 + 0.12 (SE) mm in shell length) were planted at the mean low water mark on an intertidal sandflat located at the mouth of House Creek, Georgia. Monthly samples (N = 30 clams) were collected from October 1992 to June 1993. Shell lengths were measured and gonadal tissue was prepared using standard histological techniques.

Early active stages of gametogenic development commenced in November with the majority (83%) of animals being in the early active phase by December. Gonadal indices increased to late active stages by March with ripe individuals occurring in April. Spawning commenced in May and continued into June.

The results of this study show that clams grew from 19.4 mm to 42.6 mm in shell length when field cultured in Georgia from October to June and that clams achieved sexual maturity and spawned under field conditions. Thus, a commercial enterprise in Georgia can obtain broodstock for the following seasons seed crop from animals prior to harvesting.

SURFACE AND TISSUE OXYGEN TENSIONS FOR MONOVALVE PREPARATIONS OF THE FRESHWATER BIVALVE *ELLIPTIO BUCKLEYI* (LEA). Michael A. Vitale,* Department of Biology, Daytona Beach Community College, Daytona Beach, FL 32120; **Frank E. Friedl,** Department of Biology, University of South Florida, Tampa, FL 33620.

Oxygen tensions, measured with a microelectrode, on the surfaces of monovalve preparations (animals with one valve removed) of *Elliptio buckleyi* ranged from 47.6 mm Hg on the gill to 8.1 mm Hg on the posterior adductor muscle. Surface oxygen tensions followed the pattern gill = mantle > kidney = anterior adductor muscle = posterior adductor muscle. The higher oxygen tension on the gill and mantle is thought to be related to ciliary mixing and flow at organ boundaries. Linear oxygen gradients were measured above the surfaces of the kidney, posterior and anterior adductor muscles. These gradients were altered by exposure to potassium cyanide indicating that the oxygen diffusing directly across the exposed surfaces into the animal is related to its oxygen metabolism.

Oxygen tensions measured within selected tissues were all low but did not differ significantly from one another ($P > 0.05$). An oxygen tension of 6.43 mm Hg was measured within the visceral mass, foot muscle and anterior adductor muscle while the kidney and posterior adductor muscle had a slightly higher internal oxygen tension of 6.69 mm Hg.

POSTERS

MITOCHONDRIAL DNA VARIATION IN THE CULTURED BAY SCALLOP, *ARGOPECTEN IRRADIANS*. Sandra G. Blake,* School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Samples of cultured bay scallops were obtained from the Virginia Institute of Marine Science lab at Wachapreague, Virginia, and from the Institute of Oceanology, Academia Sinica in Qingdao, China. The Chinese bay scallop culturing operation was initiated in 1983 with 26 individuals imported from the U.S. Atlantic coast. It is believed that most of the surviving broodstock originated from the VIMS Wachapreague lab. A comparative analysis of variation in the mtDNA of cultured scallops from the Wachapreague lab and the Chinese operation should indicate the degree of genetic variability remaining after ten years of culturing under extreme inbreeding conditions.

Mitochondrial DNA was isolated from scallop gonad and/or mantle tissue by cesium chloride density-gradient ultracentrifugation. MtDNA was then subjected to digestion with a series of

restriction enzymes, and RFLP analysis was performed. DNA of scallops from the Wachapreague source was isolated and analyzed in the summer of 1993. In September of 1993, DNA isolation from Chinese cultured bay scallops was initiated at the Qingdao laboratory. Stable preparations were transported back to the VIMS genetics lab in Gloucester Point, Virginia, where ultracentrifugation and RFLP analysis were completed.

FATE OF PARALYTIC SHELLFISH TOXINS IN THE AMERICAN LOBSTER *HOMARUS AMERICANUS*. **Allan D. Cembella,*** Institute for Marine Biosciences, National Research Council, Halifax, Nova Scotia, Canada B3H 3Z1; **Michel Desbiens,** Direction de la recherche scientifique et technique, Ministère de l'Agriculture, Pêcheries et Alimentation, Gaspé, Québec, Canada GOC 1R0.

On the Atlantic coast of North America, paralytic shellfish poisoning (PSP) has had a major effect on the harvest fishery and aquaculture of filter-feeding bivalve molluscs. However, scavenging benthic crustaceans including lobsters can also accumulate PSP toxins through vectorial transfer. In eastern Canada, a regional public health advisory against the consumption of more than two lobster hepatopancreas per day was issued after toxicity levels >1500 μgSTXeq [saxitoxin equivalents] $100g^{-1}$ hepatopancreas were detected in the Gaspé area of Quebec along the Gulf of St. Lawrence. Adult lobsters were caught in lobster traps in Gaspé Bay in late spring of 1990 and analyzed for PSP toxin composition. The PSP toxin content of lobster tissues (hepatopancreas, tail muscle, haemolymph) was determined by AOAC mouse bioassay and by high-performance liquid chromatography with fluorescence detection (HPLC-FD). Co-occurring benthic fauna, which may have served as potential toxin vectors, including mussels, whelks, crabs, periwinkles, sea urchins, sand dollars, were collected by diving and their toxin content was also determined. A sub-set of toxic lobsters were maintained in a flow-through seawater system at 4°C without feeding to establish detoxification rates over 53 days under long term cold storage conditions. Another group of lobsters was fed on toxic softshell clams, *Mya arenaria*, to determine PSP toxin accumulation in the hepatopancreas. To investigate thermal decomposition or loss of toxins, heat treatment experiments involving steaming were performed to simulate the cooking conditions employed encountered by consumers at home. Under all conditions, the lobster hepatopancreas contained the highest toxin concentrations among the tissues; only traces were found in the tail muscle and no toxin was detected in the haemolymph. The PSP toxin composition was markedly different in hepatopancreas and muscle tissue, although gonyautoxins (GTX2, GTX3), saxitoxin (STX), decarbamoyl STX and N-sulfocarbamoyl toxins were present in both tissue compartments. The individual toxicity levels of raw hepatopancreas of lobsters harvested from the same site varied considerably (from <42 to 1550 μgSTXeq $100g^{-1}$), thereby complicating the establishment of a reasonable toxin monitoring strategy. The storage of lobsters in cold seawater without feeding did not significantly decrease the toxicity over almost two months. There were circumstantial indications from field populations that the PSP toxin retention time in hepatopancreas could extend over several months under prevailing environmental conditions.

ANNUAL VARIATION OF HEMOLYMPH COMPONENTS AND DERMO INFECTION IN OYSTERS SAMPLED FROM DEEP WATER SHOAL, JAMES RIVER, VIRGINIA. **Fu-Lin E. Chu,* Aswani Volety,** and **Jerome La Peyre,** School of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

The seasonal variation of hemolymph components and *Perkinsus marinus* (Dermo) infection in oysters (*Crassostrea virginica*) sampled (August 1990–July 1991) from Deep Water Shoal of James River (DWS), Virginia and from oysters which were collected from the same site at the beginning of the study and maintained in a flow-through flume receiving raw York River water (YRW) were investigated. Total number of hemocytes (TC), percent of granulocytes (PG), serum protein (P) and lysozyme (L) of individual oysters were measured. Dermo infection and condition index (CI) were then determined in oysters. In both DWS and YRW oysters, higher TC (and number of hemocytes of different sizes, ranged from 2.5 μm to 13.0 μm), and PG occurred in the months of higher water temperatures. L concentration was highest in the months of lower water temperatures and in the case of DWS oysters, concomitantly with low salinities. P fluctuated, maximum P was found in DWS oysters in April and in YRW oysters in January and April. The highest CI of DWS oysters was in June and of YRW oysters was in May. *P. marinus* prevalence was greatest in DWS oysters in October (85%) and in YRW oysters in June (65%) and July (75%), the months of relatively high temperatures (23–28°C). *P. marinus* intensity coincided with infection prevalence. No infection was detected in DWS oysters in the months of low temperatures and salinities. Prevalence in DWS oysters in June (5%) and July (25%) was unusually low, due to the consistent low salinity (0–3 ppt) from January to May.

VISUAL AND HISTOLOGICAL SEMI-QUANTITATIVE REPRODUCTIVE SCALES DEVELOPED FOR SHRIMP AND CRABS AS PART OF THE GULF OF MEXICO OFFSHORE OPERATIONS MONITORING EXPERIMENT (GOOMEX). **Matthew S. Ellis,* E. A. Wilson-Ormond,** and **Eric N. Powell,** Department of Oceanography, Texas A&M University, College Station, TX 77843-3146.

The Gulf of Mexico Offshore Operations Monitoring Experiment is a study designed to detect chronic and sublethal effects of offshore oil and gas production. A component of this project involves the assessment of macroinvertebrate reproduction. Species of interest include the shrimps *Penaeus aztecus* and *Trachypeneus similis* and the crabs *Callinectes similis* and *Portunus gibbesii*. Reproduction for these species was evaluated by two methods;

visual inspection and histological analysis. Evaluation by visual inspection occurred immediately after collection. Individuals were sexed and a visual determination of the reproductive stage of females was made based on the development and color of the ovary. Whole specimens were preserved and returned to the laboratory for complete histological analyses. The reproductive cycle of most of the species involved in this study has not heretofore been studied; therefore a semi-quantitative reproductive scale was developed for each species based on the appearance of the gonads and the quantity of gametes present for both methods. Some animals examined visually in the field were also subjected to histological analyses to cross check the two methods. We have found visual inspection to be an important tool because it is an inexpensive method easily performed in the field, allows a larger number of animals to be inspected more rapidly than by histological analyses, and the animals do not have to be returned to the lab for analyses. Although histological analyses provide a more detailed description of the reproductive state of an individual, visual inspection allows a greater quantity of data to be collected at lower cost which is important in identifying statistically significant trends in highly variable data; and should be the method of choice for monitoring programs.

OYSTER SETTLEMENT: A SYNTHESIS OF OLD AND NEW DATA. **William K. Fitt,** Institute of Ecology, University of Georgia, Athens, Georgia 30602, **Steven L. Coon,** LDN, NICHD, National Institutes of Health, Bethesda, Maryland 20892.

Settlement of oysters of the genera *Crassostrea* and *Ostrea* involves stereotypical behaviors first described over 60 years ago. These settlement behaviors, exhibited by pediveligers while swimming above and crawling on the substrate, end in irreversible cementation to the substrate providing the appropriate cues are received by the larva. What these "appropriate cues" are has been the topic of numerous research projects. Larval recognition of soluble and substrate bound chemical cues, as well as tactile and other physical cues, has been investigated extensively, often times resulting in conclusions that are poorly integrated with previously published data. Evaluation of recent published and unpublished data with older studies suggests that there are multiple cues that act either in series or parallel, to trigger settlement behavior, and under the right combination of conditions end in cementation and subsequent metamorphosis.

APPLICATION OF THE POLYMERASE CHAIN REACTION (PCR) IN GENETIC ANALYSIS OF LARVAL OYSTERS, *CRASSOSTREA VIRGINICA*. **Yaping Hu, David Foltz,** Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803.

Seeking additional gene markers for genetic analysis of larval and juvenile oysters (*C. Virginica*), we used the PCR technique to amplify specific nuclear DNA fragments (575-1440 base pairs) from individual larval and juvenile oysters. Five PCR products of anonymous single-copy nuclear DNA were made using five pairs of oligonucleotide primers: CV-07, CV-19, CV-32, CV-195 and CV-233 (Karl and Avise, 1993) and were screened with restriction enzymes for polymorphic restriction sites. Approximately eight Mendelian polymorphic loci have been identified. With the present PCR techniques, the DNA extract from a larva (200-330 μm in shell length) would provide sufficient templates for five different amplifications. In the present study, we sampled larval and juvenile oysters from both natural and commercial hatchery populations in Grand Isle, Louisiana, and analyzed allele frequencies and heterozygosities across the early life history stages of *C. Virginica*.

FACTORS OF BAG MESH SIZE, STOCKING DENSITY, AND QUAHOG STOCKING SIZE, WHICH AFFECT GROWTH AND SURVIVAL OF SECOND YEAR *MERCENARIA MERCENARIA* (LINNAEUS, 1758), IN A COASTAL GEORGIA GROWOUT APPLICATION. **Dorset H. Hurley*** and **R. L. Walker,** Shellfish Research Laboratory, Marine Extension Service, University of Georgia, Savannah, GA 31416-0687.

Growth and survival of one-year quahogs were tested against growout bag mesh diameter and stocking density in the second year of a two-year study in coastal Georgia. After the first year's growout, quahogs were restocked in commercially used oyster bags 1.0×0.5 m^2. Bag mesh diameters were changed from 3 mm and 6 mm to 6 mm and 12 mm, respectively. Clam densities (10 replicates each) were 250, 325, 500, 675, 750, 975, 1500, 2025 and 2250 per bag. Initial quahog stocking sizes were 4.7 mm, 6 mm, 9.5 mm and 13.7 mm in shell length. Second year growth and survival data was collected from August 1992 to October 1993.

In December 1992, all high density bags stocked with 13.7 mm seed (975 to 2250) were thinned to 750 quahogs per bag. At termination, no significant differences in growth ($\bar{x}$ = 33.7 mm) or survival existed in the high density stockings of 975 to 2250 quahogs per bag. The 750 stocking exhibited equal survival, however, greater growth ($\bar{x}$ = 35.7 mm) as compared to the other high density stockings.

Clams stocked at a size of 4.7 mm showed significantly lower survival (9.6%), as compared to 6 mm (51.7%), 9.5 mm (55.4%), and 13.7 mm (58.8%) stocking sizes.

For 9.5 mm clams stocked in densities of 250 to 750 in 6 mm and 12 mm mesh diameter bags, quahogs exhibited a trend toward greater growth at lower density stockings, with survival between densities being equal, and with no significant difference in growth between mesh treatments.

No survival differences existed between stocking densities of 250 to 750 in quahogs of the 6 mm initial stocking size; however, growth differences existed with a trend toward larger growth at lower densities in the 6 mm bag mesh stocking of this seed size.

EFFECTS OF INTERTIDAL DUNGENESS CRAB HABITAT ENHANCEMENT ON BIVALVE SETTLEMENT AND GROWTH, CRAB FORAGING RATE, AND CRAB-BIVALVE INTERACTIONS. **Oscar O. Iribarne, David A. Armstrong,** and **Miriam E. Fernandez,** School of Fisheries WH-10, University of Washington, Seattle, WA 98195.

Intertidal oyster shell habitats have been created and used to mitigate the impact of dredging of Grays Harbor navigational channel (WA) on the subtidal Dungeness crab (*Cancer magister*) population. We studied the effect of this artificial shell habitat on soft bottom species focusing in particular on: 1) settlement and survival of the bivalve *Macoma balthica*, 2) growth of the filter feeder soft-shell clam *Mya arenaria*, and 3) the effects of habitat heterogeneity, and clam and crab density on the foraging rate of juvenile Dungeness crab preying on *M. balthica*.

Epibenthic shells affected the growth rate of small *M. arenaria* (11–17 mm total length), but not of larger clams (18–24 mm total length) transplanted into shells habitat. Settlement of the bivalve *M. balthica* was not affected by shell, but mortality rate was higher in shell habitat than on open mudflats. The pattern of clam survival may be due to crab predation, and patterns of this predator's density. Tethered juvenile Dungeness crabs showed a higher mortality rate in the open mud, intermediate at the border of shell plots and lowest in the center. As a consequence, 0+ crab (J1–J2) are scarce over open mud but up to 200 m^{-2} inside shell plots where predation reduces 0+ clam density to a level several times lower than measured on open mudflats. A positive density-dependent (type III) functional response indicates that *M. balthica* finds refuge at low clam density. Agonistic interactions among juvenile crabs affect consumption rates of *M. balthica* because clam handling time increases significantly as a function of crab density. Intertidal shell habitats have been shown to efficiently increase Dungeness crab density during the first months of their benthic existence, but our results suggest that some changes in the infaunal species composition and abundance should be expected.

THE NORTHERNMOST GIANT CLAMS (*TRIDACNA* SPP.). **R. Kilada,** University of Texas, Marine Science Institute, Port Aransas, TX 78373.

Giant clam populations were compared in five different sites, on the southern coast of the Sinai peninsula, at the north part of the Red Sea. The sites varied in currents and substrata. Eight depths (between the reef flat and 20 m deep) were surveyed in each site. *Tridacna maxima* was the dominant species, while *T. squamosa* was very rare. There were only 45 clams of the latter species, compared with 6709 individuals of the former. Clam abundance varied significantly among sites, depths and the sites-depths interaction (two-way ANOVA, $P < 0.0001$). Mean clam density varied between 1.58 and 0.09 individual/m^2 with decreasing current strength. These densities were comparable to other places in the Indo-Pacific region. About 95% of the recorded clams were found between the reef flat and 5 m. This is explained by the clams symbionts (the zooxanthellae) dependence on the light intensity at that depth. Clam sizes were between 0.40 and 32.2 cm. There was a significant difference in sizes among different sites ($P < 0.01$), and depths ($P < 0.05$). To study the reproduction behavior, gonad samples were taken from *T. maxima* in site 2, between October 1989 and December 1990. All-male clams were between 5.50 and 12.00 cm in length, but some immature ova were noticed in clams of sizes 8.96 and 11.38 cm. No clams were all-females. Although the area occupied by the ovaries increased with increasing shell length. Spawning took place between June and October 1990. Early spermatogenesis and oogenesis were not seen in any of the samples. Late gametogenesis stages in both ovaries and testis were found in every month over the study period. No sections were seen in which mature spermatozoa were accompanied with primary spermatocyte. By contrast, fully ripe egg stages were not accompanied by early egg stages. Testis and ovary development was synchronous within monthly samples of the population. No correlation was found between the gonad weight and other body parts weight in different months. This northernmost population of tridacnidae, is different than that in the Great Barrier Reef in the timing of spawning, but it is similar in the distribution pattern within the reefs. This data suggest that mariculture potential exists in the Red Sea.

BARATARIA AND TERREBONNE ESTUARIES' OYSTER RESOURCE MAPPING PROJECT. **Earl J. Melancon, Jr.,* Tom M. Soniat,** and **Vincent J. Cheramie,** Department of Biological Sciences, Nicholls State University, Thibodaux, LA 70310; **Ronald J. Dugas,** Louisiana Department of Wildlife and Fisheries, 400 Royal St., New Orleans, LA 70130.

This project is in support of the Louisiana Barataria-Terrebonne National Estuary Program staff's efforts to develop a comprehensive conservation and management plan for the two estuarine systems. The goal of this project is to develop for the plan a reference map (1:100,000 scale) which delineates the areas within the estuaries with oyster resources (*Crassostrea virginica*). This is being accomplished in several phases: (1) Develop a preliminary map by an advisory group of oystermen, (2) document the location, size distribution, and physiological condition of the oyster populations within the estuaries in order to verify the map, (3) inclusion as part of the map development process the Louisiana Department of Wildlife and Fisheries' biologists who manage the resource, (4) Hold public meetings to show a preliminary version of the map, (5) develop a final map stored in a GIS format for use by federal and state agencies, and (6) overlay of the map with other GIS data sets in order to develop a preliminary ranking of areas according to environmental influences.

Preliminary data analyses indicate that oyster population distributions correlate well with the preliminary map. Four primary zones have been initially delineated: areas that are consistently too fresh for oysters to exist, areas where oyster populations are killed during a drought or excessive rainfall period, areas where salinities

are consistently too high for oysters to survive for extended periods because of associated predators and disease, and areas where oysters can consistently survive, irrespective of environmental flux.

SUSPENSION FEEDING AND UPTAKE OF DFAA BY NORTHERN QUAHOGS *MERCENARIA MERCENARIA*: MAGNITUDE OF ORGANIC NITROGEN ASSIMILATION BY THE NARRAGANSETT BAY POPULATION. **Michael A. Rice,*** Dept. of Fisheries, Animal and Veterinary Science, University of Rhode Island, Kingston, RI 02881.

Since the 1950s, studies have been undertaken in Narragansett Bay to assess the population structure of northern quahogs *Mercenaria mercenaria*. Narragansett Bay is 38036 ha in area and has an average quahog density of 8.9 individuals/m^2. The distribution of quahogs is not homogenous, with highest average densities in the Providence River (41.2 ind/m^2), and the lowest densities in the East Passage (<0.1 ind/m^2). Standing crop biomass of quahogs estimated from area densities, length-frequency analyses and allometric estimation of shell-free weight is approximately 41300 metric tonnes. The population filtration by the quahogs (6.25×10^6 m^3/hr or 6.21×10^{16} phytoplankton cells/hr) was estimated by using published weight-specific filtration rates at environmentally realistic phytoplankton concentrations. Assuming 1.5 pg dry weight/cell and an average nitrogen content equal to 4.65% of the dry weight, uptake of particulate organic nitrogen by the quahogs is 3.76 kg-N/hr. Using published values for the uptake of dissolved free amino acids by quahogs (5.5 nmol/g * hr from 500 nM ambient concentrations), uptake of dissolved organic nitrogen by the Narragansett Bay population of quahogs is estimated to be 3.31 kg-N/hr. Uptake of dissolved organic nitrogen is potentially a significant source of nutrition to quahogs at the population level, and may be a significant contributor to nitrogen cycling within estuarine ecosystems. RI Agricultural Experiment Station Contribution #2916.

THE GAMETOGENIC CYCLE OF THE SCORCHED MUSSEL, *BRACHIDONTES EXUSTUS* (LINNE, 1758), AT WASSAW ISLAND, GEORGIA. **M. Sweeney*** and **R. L. Walker,** Shellfish Research Laboratory, Marine Extension Service, University of Georgia, P.O. Box 13687, Savannah, GA 31416-0687.

The gametogenic cycle of a population of scorched mussels, *Brachidontes exustus*, was studied from January 1993 to January 1994 at the north beach of Wassaw Island, Georgia. Mussels occur at the base of dead trees within the intertidal zone of the beach. Gonadal tissue was extracted from mussels (N = 15) sampled biweekly ranging in size from 1–2 cm. Tissue samples were fixed in Davidson's solution, sectioned, stained, and rated according to gametogenic development. Staging criteria of 1 to 5 were employed for Gonadal Indexing (G.I.): Early Active (EA = 3), Late Active (LA = 4), Ripe (R = 5), Partially Spawned (PS = 2), and Spent (S = 1). Since male and female G.I. showed little variations over time, values were averaged per sample period. Rapid gonadal development occurred from March ($\bar{x}$ G.I. = 1.75) to mid-May (x G.I. = 4.47) and follicles were fully ripe by mid-July ($\bar{x}$ G.I. = 4.79). The sampled population may have had a minor spawn in late July ($\bar{x}$ G.I. went from 4.79 to 4.25) with a major spawn occurring from September ($\bar{x}$ G.I. = 4.93) to the first of November ($\bar{x}$ G.I. = 2.38). For a sampling of 236 animals, the sex ratio was significantly different (1.00 female:1.45 male) from parity and 8% were undeveloped. The greatest percentage of undeveloped mussels occurred in March (50%), with some undeveloped mussels occurring from January to April. No incidents of hermaphroditism were observed.

SEX RATIO AND REPRODUCTION POTENTIAL FOR THE NORTHERN QUAHOG, *MERCENARIA MERCENARIA*, ACCORDING TO AGE, SIZE, AND HABITAT FROM COASTAL GEORGIA. **R. L. Walker,*** Shellfish Research Laboratory, Marine Extension Service, University of Georgia, P.O. Box 13687, Savannah, GA 31416-0687.

Due to the lack of intensive commercial fishing for the northern quahog, *Mercenaria mercenaria* (Linnaeus, 1758), in coastal waters of Georgia, the Georgia clam population is dominated with larger and older-aged individuals (chowders). Many fishermen toss the chowders into the high marsh to die in belief that they are making room for recruitment of the more valuable littleneck size group. Although these chowders are of no commercial value, they may be of importance in terms of reproductive potential. This study determines the sex ratio of various age and size groups of quahogs and determines if that ratio alters according to habitat. Sex ratios were determined for 16 quahog beds from various habitats. Sex ratios did not significantly differ from a 1:1 ratio, regardless of habitat. For pooled data from all sites (N = 2637 quahogs), no significant departure from parity occurred according to age for clams aged to 38 years (excepting the one and 23 year-old classes) or size for clams sized to 118 mm (except the 66–70 mm size class). No difference in growth rates occurred between female and male quahogs as determined by the Hotelling's T^2 procedure for multivariate analysis of parameters for the von Bertalanffy growth model. The results of this study show that the quahog population in Georgia occurs in sexual equilibrium. Ongoing experiments show that no reproductive senescence occurs during the embryonic phase for gametes produced by older-aged quahogs (up to 38 years). Thus, chowders should be protected for their reproductive potential rather than destroyed.

INFLUENCE OF PARTICLE DENSITY AND SURFACE CHARACTERISTICS ON SUSPENSION-FEEDING IN TWO SPECIES OF BIVALVES (*ARCA ZEBRA* & *MYTILUS EDULIS*). **J. Evan Ward*** and **Bruce A. MacDonald,** Department of Biology, University of New Brunswick, Saint John, NB E2L 4L5, Canada; **Nancy M. Targett,** College of Marine Studies, University of Delaware, Lewes, DE 19958, USA; **Nick H. Vrol-**

ijk, Center of Marine Biotechnology, University of Maryland, Baltimore, MD 21202, USA.

Many species of bivalves have the ability to select and preferentially ingest certain particle types from the diverse range of particles present in the seston. It is not clear, however, whether this preferential retention and ingestion is brought about by active selection based on the chemical components of the particles, or simply a passive phenomenon based on the physical properties of the particles. We addressed this question by delivering two bivalve species (the turkey wing, *Arca zebra;* the blue mussel, *Mytilus edulis*) microspheres with different densities and surface characteristics, including silica, alumina, octadecyl silane (ODS), and polystyrene based spheres. Surface properties of some sphere types were also modified by treating them with metabolites of several phytoplankton species, or a 2% solution of methylcellulose. Bioassays were then performed to examine the effects of sphere type and treatment on the retention efficiency and selection in *A. zebra* and *M. edulis*. Retention and selection were quantified by means of video endoscopy and direct enumeration of particles rejected in pseudofeces.

Preliminary results indicate that retention and selection for the test microspheres were unaffected by sphere type or treatment. This trend was true for both no choice (i.e., one sphere type delivered at a time) and choice (i.e., two sphere types delivered at one time) assays. Our study suggests that density and surface characteristics play little role in particle retention or selection, and is the first to examine the factors that mediate selection processes *in vivo*.

THE EFFECT OF PROXIMITY TO GAS PRODUCING PLATFORMS ON SIZE, STAGE OF REPRODUCTIVE DEVELOPMENT AND HEALTH IN SHRIMP AND CRABS. **E. A. Wilson-Ormond,* M. S. Ellis,** and **E. N. Powell,** Department of Oceanography, Texas A&M University, College Station, TX 77843.

One objective of the Gulf of Mexico Offshore Operations Monitoring Experiment (GOOMEX) is to document the impacts of contaminant exposure related to proximity to gas producing platforms. Differences in size frequency distribution, stage of reproductive development and health, determined by the presence of parasites and pathologies, were used as indicators of possible sublethal effects of exposure to chemical contaminants in target species. Shrimp and crabs were collected by trawling from locations close to (near-field) and far from (far-field) the platforms to test the contaminant gradient hypothesis. After collection, individuals were measured and sexed. Stage of reproductive development was determined in all females by visual inspection or gross dissection. Ten individuals were preserved and returned to the laboratory for histological analysis. Tissue sections were analyzed for sex, stage of reproductive development, and presence and intensity of parasites and pathologies. Results suggest that when a difference in size-frequency distribution did occur between populations from the near and far-field stations, the individuals collected at the far-field station were typically larger. Differences in the stage of reproductive development are not as clear, however, preliminary results show that more crabs from the far-field stations were gravid. Shrimp at the near-field stations had an increased presence of parasites and pathologies suggesting variations in population health on a scale of 3 km. These results indicate that detectable differences exist, possibly due to chronic exposure to environmental contaminants, in populations living in close proximity to and removed from gas producing platforms.

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ABSTRACTS OF TECHNICAL PAPERS

Presented at the 14th Annual Meeting

MILFORD AQUACULTURE SEMINAR

Milford, Connecticut

February 22 — 24, 1994

CONTENTS

OVERVIEW OF THE MILFORD AQUACULTURE SEMINAR. Walter J. Blogoslawski, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460.

The 14th Milford Aquaculture Seminar was held in Milford, Connecticut from February 22–24, 1994. The Seminar is intended to provide a forum for persons interested in aquaculture where they might discuss advances and ideas in the field and seek answers to common problems. The major themes of this fourteenth seminar included hatchery management techniques, shellfish habitat ecology and diseases, shellfish and selected finfish culture methods by locale, effects of dinoflagellate blooms, recruitment and restoration efforts and a review of regulations affecting the aquaculture industry. Over 140 participants from ten states provided expertise in both areas of shellfish and finfish aquaculture.

DISEASE STUDIES IN MAINE—1993. Bruce J. Barber and **Christopher B. Davis,** Department of Animal, Veterinary & Aquatic Sciences, University of Maine, Orono, ME 04469.

The goal of this study was to determine the present status of 4 diseases of 3 species of commercially important bivalves in the State of Maine. They were: Juvenile Oyster Mortality and MSX (*Haplosporidium nelsoni*) in eastern oysters, *Crassostrea virginica;* Bonamiasis (*Bonamia ostreae*) in European oysters, *Ostrea edulis;* and neoplasia in softshell clams, *Mya arenaria*. Samples were collected between June 11 and September 27, 1993 and processed for histological examination at the Department of Animal, Veterinary and Aquatic Sciences, University of Maine, Orono. The major findings were as follows:

1) MSX was not seen in eastern oysters from either the Damariscotta River or the Piscataqua River.
2) Juvenile Oyster Mortality did affect eastern oysters in the Damariscotta River in 1993, and mortality was inversely related to spat size. Oysters that had a mean shell height of 12.1 mm in mid-July had a cumulative mortality of 30.9% by the end of September; oysters that averaged 16.2 mm had a 16.75% cumulative mortality; and oysters that averaged 25.9 mm in July had a cumulative mortality of only 5.0%.
3) Bonamiasis was found in European oysters for the third consecutive year in the Damariscotta River (20% prevalence), and in two previously undocumented locations, Winnegance Bay (14.3% prevalence) and Yarmouth Island (2.9% prevalence). *B. ostreae* was not found in 4 other locations sampled. Prevalence was size related, as only oysters having a shell height greater than 67 mm were infected.
4) Neoplasms (both gonadal and hematopoietic) occurred in 5 of 8 populations of softshell clams at prevalences ranging from 3.6 to 42.9%.

EFFECTS OF SEAGRASS (*ZOSTERA MARINA*) COVER ON GROWTH AND SURVIVAL OF SOFTSHELL CLAMS (*MYA ARENARIA*). Brian F. Beal, Division of Science and Mathematics, University of Maine at Machias, Machias, ME 04654.

In eastern Maine, intertidal beds of eelgrass, *Zostera marina*, (area = 100–400 m^2) are common near the mid intertidal on mudflats that receive considerable ice scour during severe winters. Water-filled depressions are colonized in the spring by seeds of this species and, once established, beds may remain stable for more than ten years. Like subtidal seagrass beds or meadows in the mid-Atlantic and further south, species richness, evenness, and total density of large (>0.5 mm) macrofauna is higher inside these beds than outside in the unvegetated, muddy sediments. In addition, the root/rhizome mat associated with these beds is dense and relatively impenetrable compared with sediments surrounding the beds. Seagrass beds have been shown to enhance the growth of deep-burrowing bivalves such as *Mercenaria mercenaria* as well as provide a spatial refuge from large gastropods and decapods because the heterogeneous mat complex reduces predator foraging efficiency. The hypothesis was tested that intertidal eelgrass beds would, similarly, provide individuals of *Mya arenaria* a similar escape from predation as well as enhance their growth.

During the summers of 1991 and 1992, hatchery-reared softshell clams were placed at a density of 666/m^2 within both open and protected (12.7 mm flexible plastic netting) circular enclosures (0.18 m^2 × 15 cm deep) inside and outside intertidal eelgrass beds at Duck Brook flat in Holmes Bay, near Cutler, Maine. Sediments from the extant mudflat and grassbed were added to enclosures and then each was pushed into the substrate so that it was nearly flush with the sediment surface. (Controls for the physical disturbance of placing discrete sections of the bed within the experimental enclosures on the health of those plants demonstrated that the integrity of the eelgrass blades, roots, and rhizomes was maintained throughout the experimental interval). In 1991, ten replicates of each of the four treatments were deployed in and around three randomly chosen, medium-sized beds (total number of experimental units = 120). Clams used in that experiment (12 August to 9 October) were 11.4 mm ± 0.39 SE (n = 70). In 1992, the test was conducted from 2 July to 23 October using slightly smaller clams (10.0 mm ± 0.14 SE; n = 79), five replicates of each treatment were used and six beds ranging in size from 130 m^2 to 360 mm^2 were chosen (number of experimental units = 120).

Seagrass beds provided no protection to softshell clams from their predators and significantly depressed clam growth when compared with survival and growth rates in the adjacent, unvegetated sediments. In both years, survival of clams within open enclosures inside the beds was significantly ($P < 0.0001$) reduced compared with survival in the same treatment outside the beds (inside vs. outside—[1991: 46.4% ± 4.3 SE vs. 74.7% ± 5.1 SE] [1992: 38.1% ± 3.7 SE vs. 58.6% ± 4.6 SE]). Greatest clam losses were attributed to crustacean predators which accounted for 5 to 25% of the mortality inside the beds in open enclosures in 1991 and 10 to 35% in 1992. Traps baited with salted herring, *Clupea harengus*, during October 1993 and placed inside and out-

side eelgrass beds at the study site demonstrated that green crabs, *Carcinus maenas*, were abundant only in the beds and rarely were caught on the open mudflat. Growth of clams was not influenced by the mesh netting in either year ($P > 0.25$); however, growth rates and final lengths were significantly depressed, regardless of treatment, within the beds versus outside the beds in both years ($P < 0.0001$). Growth rates were depressed by 10 and 50% in 1991 and 1992, respectively, within the beds. For example, average final shell length of animals outside the beds in 1992 was 21.1 mm ± 0.27 SE (n = 60) and was 14.1 mm ± 0.34 SE (n = 60) inside the beds. Increased siphon nipping or other predator-mediated disturbances within the beds are likely mechanisms.

These results suggest that the observed enhancement of natural densities of softshell clams within high-latitude eelgrass beds probably occurs at the time of settlement as a result of an interaction between larval supply, larval densities, and the physical hydrodynamic baffling created by the eelgrass beds.

BLUEFIN TUNA: BLUEWATER BEEF CATTLE OF THE FUTURE. Sebastian Belle, New England Aquarium, Central Wharf, Boston, MA 02110.

Interest in the culture of bluefin tuna is growing rapidly. Work in Japan has established bluefin's potential as a major marine aquaculture species in the twenty-first century. Market growout of bluefins requires the development of specialized husbandry techniques designed to handle large, highly athletic fish. Beginning in 1989, the New England Aquarium (NEAq) developed methods for the capture, transport, and husbandry of sub-adult bluefin tuna. NEAq is presently the only group in North America actively growing bluefins. Current work focuses on the development of growout techniques, documentation of growth and conversion rates, studies of captive pathologies, and the development of fertilization, incubation and juvenile rearing techniques. Working with the National Marine Fisheries Service and commercial and recreational fishing groups, NEAq has established a network of field samplers to gather basic data on the reproductive biology of Western Atlantic, bluefin tuna stocks. A systematic study of the commercial feasibility of bluefin culture is underway and the possibility of a pilot scale demonstration project is being considered.

CULTURE OF SUMMER FLOUNDER *PARALICHTHYS DENTATUS*: RESEARCH ON HATCHERY AND GROWOUT PHASES. David A. Bengtson,[1] Gustavo Bisbal,[1] Heidi Iken,[2] and **Ronald P. Athanas,[2]** [1]Department of Zoology, University of Rhode Island, Kingston, RI 02881; [2]Marine Station, University of Massachusetts, Gloucester, MA 01930.

Given the declining finfish stocks in the northeastern U.S. and the commercial aquaculture of Japanese flounder in Asia and turbot in Europe, the culture of summer flounder is worthy of investigation. Our Saltonstall-Kennedy-funded program of research over the last three years has answered several questions about summer flounder culture and identified some remaining problem areas.

Broodstock flounder can be hormonally induced to produce gametes both in and out of their natural spawning season. The spawning process is difficult in that milt is not abundant and sperm are active for only about 2 minutes upon contact with seawater. Hatching occurs in about 55 hrs at 20°C. Larvae begin to feed at 3 d post-hatch and are raised on the rotifer *Brachionus plicatilis* until 16 d post-hatch. Varying quantities (10–40%) of embryos are lost during development and similar quantities of larvae are unable to successfully initiate feeding. Once feeding commences, mortality remains low through metamorphosis. Larvae are weaned off rotifers and onto brine shrimp nauplii from days 16–23, then are fed nauplii alone through metamorphosis. As the larvae increase exponentially in weight, their consumption rates on rotifers and brine shrimp nauplii also increase exponentially. A histological study of the development of the larval digestive tract indicates that a stomach and pyloric caecae are present prior to metamorphosis, so that weaning to an artificial diet should be possible prior to settlement. Like most marine fish larvae, summer flounder require 20:5(n-3) as an essential fatty acid for optimal growth, but they do not appear to require 22:6(n-3). Batches of 200–1500 metamorphosed juveniles have been produced at laboratory scale. Interestingly, about 4% of them were "reversed," i.e., the eye migration produced right-handed rather than left-handed individuals.

Flounder were transferred immediately after metamorphosis to the University of Massachusetts for rearing in a recirculated artificial seawater system. Substantial mortality occurred during the weaning to a prepared diet. However, of the fish that survived, growth rate was quite good when the fish were fed a diet formulated from cod-frame hydrolysate as a protein source. Fish grew to 25–30 cm in their first year in the rearing system, a growth rate similar to that of young turbot in European culture systems. The dorsal surface of many fish was incompletely pigmented, which could be a marketing problem. Weaning mortality and pigmentation problems remain to be solved and estimates of maximum stocking density need to be obtained. Nevertheless, many similarities appear to exist among summer flounder, turbot, and Japanese flounder culture, so that the potential for commercial summer flounder aquaculture appears promising.

PRELIMINARY RESULTS ON *PERKINSUS* PREVALENCE IN OYSTER POPULATIONS FROM LONG ISLAND SOUND (WESTERN). Diane Brousseau, Christopher Orsine, Maria Rios, and **William Zavadoski,** Biology Department, Fairfield University, Fairfield, CT 06430.

Prevalence rates for *Perkinsus marinus* were determined for three intertidal populations of *Crassostrea virginica* from Long Island Sound (Black Rock Harbor, Bridgeport, CT; Saugatuck River, Westport, CT; Milford Point, Milford, CT) from Septem-

ber 1993 to January 1994. The Ray thioglycollate assay was used for *P. marinus* diagnosis. Temperature and salinity patterns were determined by us for one site (Black Rock Harbor) and obtained from the HarborWatch program (Harris et al., unpubl.) for the Westport sampling location.

Results indicate that parasite burdens during these months are highest in oysters from Bridgeport (20–100%) and Westport (10–60%). Positive infections were found in Milford oysters during September and December only with prevalence rates <25%. The majority of the infections at all sites were classified as light. Water temperatures were favorable for parasite proliferation from mid-June to September and salinities were above 15‰ throughout the study period.

DOSE RESPONSE OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*, TO CULTURED CELLS OF *PERKINSUS MARINUS*, THE AGENT OF DERMO DISEASE. **David Bushek, Standish K. Allen, Jr., Kathryn A. Alcox, Richard Gustafson** and **Susan E. Ford,** Haskin Shellfish Research Laboratory, Institute of Marine and Coastal Sciences, Rutgers University, RD#1 Box B-8, Port Norris, NJ 08349.

Recently *in vitro* culture methods were developed for *Perkinsus marinus*. We investigated the dose response of adult *Crassostrea virginica* to cultured *P. marinus* and the fate of *P. marinus* cells delivered via 3 different methods.

To examine dose response, oysters were challenged with 0 to 10^7 *in vitro*-cultured *P. marinus* cells by feeding, shell cavity injection and adductor muscle injection. After 47 days, no "fed" oysters were patently infected, but oysters receiving shell cavity or adductor muscle injections had *P. marinus* body burdens roughly proportionate to the dose delivered. Between 10^3 and 10^4 cells were required for development of infections when cells were injected into the shell cavity. Fewer were required for adductor muscle injections.

The fate of *in vitro*-cultured cells delivered by each method was determined by inoculating oysters with 10^7 *P. marinus* cells labelled with the vital stain neutral red. Feces, pseudofeces and water were collected daily for 4 days and examined for stained cells. Eighty-nine percent of the parasites discarded in this manner were found within 24 hrs; many had been engulfed by phagocytes. On average, 3, 9 and 11% of cells delivered were found from fed, shell cavity injection and adductor muscle injection treatments, respectively. The path of elimination varied with exposure method. Stained cells were most abundant in feces when *P. marinus* was fed to oysters, equally abundant in feces and pseudofeces after shell cavity injections, and most abundant in pseudofeces after adductor muscle injections. Body burdens determined after sacrificing oysters on day 4 were highest from adductor muscle injections and lowest from feedings. Total recovery of *P. marinus* from all sources combined averaged 3, 11 and 20% for fed, shell cavity and adductor muscle treatments, respectively.

To examine more closely the fate of phagocytosed *P. marinus*, we injected 7×10^6 live *P. marinus* cells into the adductor muscles of 3 oysters. Control oysters received equal doses of chlorine-killed *P. marinus*. Transmission electron microscopy revealed that less than 1% of hemocytes contained *P. marinus* 6 hrs post-injection. Even fewer were found after 18 hrs. No free *P. marinus* cells were found. Compared to chlorine-killed *P. marinus*, live injected *P. marinus* cells within hemocytes appeared unaffected at 6 hrs post-injection. After 18 hrs most cells appeared dead and were being digested. In contrast, similar preparations of hemolymph from naturally infected oysters found a high percentage of hemocytes packed with up to 10 healthy *P. marinus* cells.

Crassostrea virginica either destroyed or eliminated a large percentage of *in vitro*-cultured *P. marinus* cells. Cultured *P. marinus* appears to be less virulent/infective than *P. marinus* obtained directly from infected oysters and more susceptible to phagocytic digestion. Finally, these results demonstrate that the method of delivery used to infect *C. virginica* with *P. marinus* in the laboratory alters the course of infection. NJAES Publication #K-32100-1-94.

POTENTIAL VIRULENCE FACTORS OF *PERKINSUS MARINUS*. **Mohamed Faisal, Jerome F. LaPeyre,** and **Doris Y. Schafhauser,** School of Marine Science, Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062; **Esam H. Rizkalla,** Central Laboratory for Aquaculture Research, Abassa, Sharkia, Egypt.

The apicomplexan protozoan *Perkinsus marinus* has been reported to be the cause of mortalities in eastern oyster (*Crassostrea virginica*) populations. Earlier studies have shown that infected oysters suffer from extensive tissue lysis. However, the factors causing these degenerative changes in the muscles and organs of infected oysters are unknown. Recently, we have identified extracellular proteins (ECP) that *P. marinus* synthesizes and secretes.

In order to characterize the production of ECP by *P. marinus*, culture flasks were seeded at a density of 10^7 cells/ml and sampled weekly over a six-week period. Analysis of the culture medium by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining revealed the presence of as many as fifteen bands ranging in molecular weight from 23 to 155 kDa. Most bands were present at one week post-inoculation. Interestingly, bands of molecular weight 24, 30, 46 and 100 kDa appeared at 2, 3, 3 and 4 weeks, respectively.

The presence of proteases in ECP was detected using SDS-PAGE with the protease substrate gelatin in the gel. Six proteases within the molecular weight range of 35 to 54 kDa were detected after two weeks post-inoculation, some of which increased in intensity with time. ECP also contained a protease which was capable of lysing oyster hemolymph proteins which had been incorporated into the gel. The possible role of the protozoal ECP in the pathogenicity of the disease will be discussed.

STUDIES OF JUVENILE OYSTER DISEASE, 1993. **C. Austin Farley** and **Earl J. Lewis,** National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Cooperative Oxford Laboratory, 904 S. Morris Street, Oxford, MD 21654.

Weekly samples of cultured juvenile oysters were obtained from Frank M. Flower and Sons Oyster Co. prior to, during, and after the 1993 onset of juvenile oyster disease (JOD). Cultured and wild juvenile oysters from Oyster and Peconic Bays on Long Island, NY, were also collected in July and September. All were examined for mortality, size, and presence of conchiolin in live and dead oysters. Samples of *Mercenaria* (mean length 15.1 mm) and *Mya* (mean length 18.5 mm) were examined and showed no evidence of disease.

Flower's oysters, spawned in January, February, March, April, and May, were placed overboard in trays by June. Ambient water temperature reached 20°C on June 15. As expected by our previous studies, mortalities began 5 weeks later and peaked at 30% by the end of July. Mortality was low in oysters spawned before March and increased significantly in those spawned later. Mortality was higher in oysters under 25 mm, but a clear relationship with age was also noted. Small oysters (19 mm mean length) older than 20 weeks showed significantly lower mortalities. Mortalities at the Flower's hatchery in 1993 were half those observed in 1991 and 1992. In contrast, heavy mortalities were seen in previously uninfected Bluepoint oysters moved to the Peconic Bay facility. Mortalities also began in mid-July and peaked at approximately 70% after 5 weeks. Stocking density of these oysters was found to have no effect on mortality, but high water flow increased growth significantly and reduced mortality.

A clear relationship was seen between the presence of conchiolin and mortality. The highest prevalence was seen in dead oysters (70–100%), but high levels were also seen in surviving oysters (14–60%). The presence of conchiolin diminished after mortality stopped and calcified lesions which matched shell checks were apparent. Primary shell checks usually measured 15–19 mm, comparable to size at the initial mortality period. Secondary and tertiary shell checks were evident in some older oysters. Conchiolin and healed shell lesions also were found in fresh boxes 70–112 mm in length. Four percent or less of the 1992 natural set examined from oysters in Peconic Bay exhibited conchiolin. No evidence of JOD was seen in any 1993 natural seed examined in September.

External shell surfaces of live JOD-affected oysters were sterilized with chlorox. Mantle and internal shell were then cultured for ciliates. More than 20 species of ciliates have been recognized, some showing possible internal parasites. Oysters cultured from non-JOD affected populations yielded very few species of ciliates.

Studies continue to reveal intracellular organisms in mantle tissue, characterized ultrastructurally by the presence of tubular cristae, vesicles, and dense bodies not consistent with oyster cell organelles.

A COMPARISON OF JUVENILE OYSTER DISEASE IN THE USA AND BROWN RING DISEASE OF MANILA CLAMS IN EUROPE. **Susan E. Ford**[1] and **Christine Paillard,**[2] [1]Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349 USA; [2]Laboratoire de Biologie Marine—URA CNRS 1513, Université de Bretagne Occidentale, 29287 Brest, France.

Juvenile eastern oysters (*Crassostrea virginica*) and juvenile Manila clams (*Tapes philippinarum*), cultured in western Europe and northeastern USA, respectively, have experienced repeated, high mortality episodes (50–90%) during the past 6–7 years. The disease begins while the small bivalves are held in high density culture containers and is characterized by an abnormal deposition of conchiolin ("Brown Ring") on the inner shell, which precedes mortality. The conchiolin deposit is found adjacent to the entire mantle in oysters, which have little or no pallial muscle attachment to the shell, but is typically found external to the pallial line in clams, which have a tight attachment of the mantle to the inner shell at the pallial muscle. Juvenile oysters die over a period of 4–6 weeks during the late summer (temperature >20–22°C), while they are still held in containers. Manila clams develop the brown ring while in bags, but mortalities tend to occur in winter and early spring (temperature 5–20°C) after the clams have been planted in culture parks. In contrast, the disease appears to recede in oysters that are removed from containers and planted on the bottom. Juveniles of both species are affected by the disease (i.e., exhibit the brown ring syndrome), but mortalities are far more severe in the smaller individuals (<25 mm). For both clams and oysters in the vulnerable size range, the prevalence of heavy brown ring deposits is directly related to mortality. Both Brown Ring and Juvenile Oyster Diseases inhibit shell growth, affect animals at salinities of about 25 ppt or higher, and have not been associated with protozoan or metazoan primary parasites. A marine bacterium (*Vibrio* P1) has been isolated from Manila clams with Brown Ring Disease and causes the disease when injected into healthy clams. Preliminary studies indicate that total *Vibrio* spp. counts rise exponentially in juvenile oysters 1–2 weeks before the start of mortality, suggesting a similar etiology.

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FISH PROCESSING WASTES AS HIGH PROTEIN INGREDIENTS IN SALMONID DIETS. **Susan H. Goldhor,** Center for Applied Regional Studies, 45-B Museum St., Cambridge, MA 02138; **Linda J. Kling,** Dept. of Animal, Veterinary & Aquatic Sciences, University of Maine, Orono, ME 04469; **Robert E. Levin** and **Radu A. Giurca,**[1] Department of Food Science, University of Massachusetts, Amherst, MA 01003; **Michael A. Rice,** Department of Fisheries Animal & Veterinary Science, University of Rhode Island, Kingston, RI 02881.

Feed represents a major expense to salmon growers, comprising close to half of the production costs. Typical post-juvenile salmonid diets contain up to 45% crude protein supplied as high

cost fish meals used at inclusion levels exceeding 50% of the total ingredients. Increasing costs of fish meal over the last five years has spurred the search for alternative fish protein sources. One source of inexpensive fish protein might be fish processing wastes. Fish processors are often paying for the disposal of wastes composed largely of fish protein, while fish culturists are paying for feeds rich in fish protein. For example, in New England the spiny dogfish has become an alternative to the traditional cod and flounders. Fish processors will utilize the fins, belly meat and fillets, with the rest for disposal. These processors pay $80 to $100 per ton for landfill or ocean dumping.

Semi-moist test diets were formulated using hydrolysates from cod (*Gadus morhua*) and dogfish (*Squalus acanthias*) processing wastes. Hydrolysates were obtained by proteolytic digestion of processing wastes followed by concentration and stabilization by acidification. Cod, which had been gutted at sea, were digested by papain; dogfish were autolysed by their own visceral enzymes. Semi-moist test diets containing the hydrolysates were fed to Atlantic salmon (*Salmo salar*) and compared to a control diet containing ground raw herring as the moist protein source. Results showed that while diets containing acidified cod hydrolysates were less palatable than the control, diets containing acidified dogfish hydrolysates provided feed ingestion, growth and conversion rates comparable to the controls. Further preliminary experiments on dogfish hydrolysate, pH adjusted and co-dried with either fish meal or poultry by-product meal show that each of these ingredients was able to replace a significant fraction of high quality fish meal in Atlantic salmon diets without loss of performance. Blind taste tests of salmon reared on hydrolysates showed that salmon fed hydrolysed dogfish were rated equal to controls, while those fed hydrolysed cod were rated lower than the controls.

This project was funded by the Northeast Regional Aquaculture Center and is publication number 2938 of the Rhode Island Agricultural Experiment Station.

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GROWTH RESPONSES OF *CRASSOSTREA VIRGINICA*, *MERCENARIA MERCENARIA* AND *MYTILUS EDULIS* TO CHANGES IN WATER FLOW: A TEST OF THE "INHALANT PUMPING SPEED" HYPOTHESIS. **Raymond E. Grizzle,[1] Richard Langan,[2]** and **W. Hunting Howell,[3]** [1]Biology Department, Campbell University, Buies Creek, NC 27506; [2]Jackson Estuarine Laboratory and Department of Zoology, University of New Hampshire, Durham, NH 03824; [3]Zoology Department, University of New Hampshire, Durham, NH 03824.

Experiments conducted in the summer of 1992 in a multiple flume apparatus to determine the effects of a range of flow speeds on the growth rates of oysters (*Crassostrea virginica*) and hard clams (*Mercenaria mercenaria*) revealed very different growth patterns for the two species of bivalves at flow rates of 0, 1, 2, and 4 cm/sec. Clams showed a consistent positive relationship between flow speed and growth (maximal growth at 4 cm/sec), and oysters showed maximal growth (average three-fold increase over other flow speeds) at 1 cm/sec with decreased growth at 0 cm/sec and >1 cm/sec. We hypothesized that the differences in growth may be explained by differences in inhalant pumping speeds, with maximal individual growth expected for suspension-feeding bivalves at ambient flow speeds that approximate the inhalant pumping speed. In 1993, an experiment was conducted with the same apparatus, flow speeds of 0, 1, 2, 4, and 8 cm/sec, and *Mytilus edulis* as the experimental animal. The experiment was designed to test the aforementioned "inhalant pumping speed" hypothesis. Published average inhalant pumping speeds for *M. edulis* of the size used in our experiment (30 to 47 mm shell length) ranged from 1.2 to 2.6 cm/sec. Mean shell length increases in our 24-day experiment were: 0.1, 1.8, 2.0, 1.9, and 1.5 mm at flow speeds of 0, 1, 2, 4, and 8 cm/sec, respectively. A preliminary analysis indicates that only the 0-flow growth rate (0.1 mm) differed significantly from the others. The overall response pattern, however, closely matches the pattern predicted by the "inhalant pumping speed" hypothesis: maximal growth at about 2 cm/sec with decreased growth at higher and lower speeds.

PROPOSED MULTISPECIES AQUACULTURE DEMONSTRATION FACILITY FOR NEW JERSEY. **John N. Kraeuter,** Haskin Shellfish Research Laboratory, Institute of Marine and Coastal Sciences, Rutgers University, RD#1 Box B-8, Port Norris, NJ 08349.

Rutgers University and Cumberland County College have been planning a large scale aquaculture center that will act as the equivalent of the Agriculture Experimental Farm. The overall goal of the center is to provide the aquaculture industry with access to appropriately scaled techniques that will enhance their competitiveness. The center consists of three parts: The Aquaculture Training and Information Center, Aquaculture Extension, and the Multispecies Aquaculture Demonstration Facility. Specific roles have been developed for each of the three parts and an industry board is to be utilized to guide the center education, demonstration and extension programs. Site selection, and construction of the demonstration facility have emphasized the integration of aquaculture into New Jersey's intensely used and heavily regulated coastal zone.

POTENTIAL FOR AN AQUACULTURE-BASED OYSTER FISHERY IN NEW HAMPSHIRE. **Richard Langan,[1] Stephen H. Jones,[2] George E. Frick,[3] Douglas E. Morris,[3]** and **Jerrard J. Whitten,[3]** [1]Department of Zoology and Jackson Estuarine Laboratory; [2]Department of Natural Resources and Jackson Estuarine Laboratory; [3]Department of Resource Economics and Development, University of New Hampshire, Durham, NH 03824.

Despite a limited coastline, the State of New Hampshire contains substantial oyster resources in the bays and tributaries of the

Great Bay/Piscataqua River Estuary. Due to a limited shellfish sanitation program, however, only a small percentage of the oyster beds are harvested for recreation. Recent proposals and legislative action, based on economic and biological analyses, may result in the creation of an aquaculture-based oyster fishery in some of the areas currently classified as "restricted." Scientific studies of bacterial reduction conducted at the Jackson Estuarine Laboratory and Spinney Creek Shellfish Co. have shown that relay and depuration techniques may be used to dramatically increase shellfish safety. With this in mind, we gathered information supporting potential oyster bed rehabilitation, combined with aquaculture techniques and relay and depuration strategies, and have estimated the production and revenue potential for several of the closed shellfish growing areas.

THE ROLES OF *VIBRIOS* AND *GYMNODINIUM SANGINEUM* IN UNEXPLAINED JUVENILE OYSTER MORTALITY AT THE F. M. FLOWER AND SONS OYSTER HATCHERY IN OYSTER BAY, NEW YORK. Mijin Lee, Gordon T. Taylor, Stephen Zahn, and **Monica Bricelj,** Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794-5000; **Susan E. Ford,** Haskin Shellfish Research Laboratory, Rutgers University, RD #1 Box B-8, Port Norris, NJ 08349.

Since 1988, episodic unexplained mass mortalities of hatchery-reared eastern oysters, *Crassostrea virginica,* have been documented in Long Island, New York. Previous studies suggested that these devastating events are caused by a toxin, probably of bacterial or microalgal origin. Suspected organisms were *Vibrio* spp. and *G. sangineum*. To test this hypothesis, water, oyster and sediment samples were analyzed weekly from the F. M. Flower and Sons oyster hatchery in Oyster Bay, New York during the period from May to September, 1993. Membrane filtration and *Vibrio*-selective media were used to isolate and enumerate bacteria. Of 1700 suspected *Vibrio* isolates, 200 were further identified by BIOLOG identification system. Oyster mortality showed significant correlation with suspected *Vibrio* concentration up to bacterial concentrations of ~15,000 CFU per gram of oyster. Abundances of *G. sangineum* were poorly correlated with oyster mortality and may only play a secondary role. Confirmation of the identity of the pathogenicity of *Vibrio* isolates awaits challenge experiments with healthy oyster broodstock.

EFFECTS OF SALINITY AND SELECTED TREATMENTS ON JUVENILE OYSTER DISEASE. Earl J. Lewis and **C. Austin Farley,** National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Cooperative Oxford Laboratory, 904 S. Morris Street, Oxford, MD 21654.

The unidentified causative agent of east coast juvenile oyster disease (JOD) is known to be a temperature-dependent transmissible agent. Studies reported herein evaluate the effects of salinity and selected standard saltwater aquarium medications on transmission and treatment of JOD. Disease transmission to uninfected Horn Point hatchery-reared oysters, mean length 20 mm, was attempted at salinities of 10–26 ppt. Transmission occurred at 20 and 26 ppt, with 60% and 75% cumulative mortalities after 6 weeks exposure. Dead oysters exhibited conchiolinous shell lesions typical of JOD. Mortalities declined with decreased salinity. At 10 ppt, 16% of the oysters died, but no conchiolin was observed.

Naturally infected oysters were used to test the effects of salinity and medications on JOD in 2 experimental trials. Two hundred oysters, mean length 16 and 18 mm, were placed in each aquarium at salinities of 10–30 ppt. Oysters were also placed in aquaria 26 ppt and treated with erythromycin, minocycline, the antibiotics in combination, or commercial medications for fungus and external parasites. The fungal medication was found to be toxic to oysters. All oysters treated with this medication died by week 5 of treatment. Unmedicated control oysters held at 26 ppt experienced mortalities of 85% and 65% after 6 weeks. Oysters which experienced least mortality in trial 1 were those treated with the combined antibiotic (43%) and unmedicated oysters held at 15 ppt (44%). Examination of daily mortality rates for all aquaria in trial 1 showed an initial mortality peak at the beginning of the experiment and a second peak at week 3. In the second trial, medications were administered initially and again at week 3 of the experiment. This was found to be important in further reducing mortalities. Oysters treated with erythromycin (9%) and combined antibiotics (9%) showed the lowest mortality in trial 2.

Unmedicated oysters held at 26 ppt, and oysters treated with erythromycin and minocycline were cultured for bacteria 1 week after completion of the experiment. One unidentified non-*Vibrio* sp. was isolated from 1 oyster in each group. No vibrios were isolated from oysters treated with erythromycin. Four species of *Vibrio* were isolated from unmedicated and minocycline-treated oysters. None of the isolates were found to be susceptible to erythromycin. Conversely, all but 1 *Vibrio* isolate was found to be susceptible to minocycline. Some cultured protists were found to be susceptible to erythromycin. There was no association of a specific bacterium and JOD infections in this study.

THE CASE FOR RECIRCULATING SYSTEM AQUACULTURE FOR FINFISH IN THE NORTHEAST. Scott Lindell, AquaFuture, P.O. Box 783, Industrial Road, Turners Falls, MA 01376.

The advantages and disadvantages of recirculating system aquaculture for the production of finfish will be presented. Specific examples of operations in the Northeast will highlight the advantages of this type of aquaculture, particularly as it pertains to marketing. The high capital cost of construction and maintenance, and the complexity of managing high-tech biological systems will be discussed.

REGULATORY LANDSCAPE FOR SHELLFISHERMEN. Michael Ludwig, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Habitat and Protected Resources Division, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460.

Increased use of shellfish aquaculture and a corresponding increase in concern about the "use" of public lands have accelerated the frequency and ferocity of confrontations over the issues. These confrontations are furthered by competition for access to habitat of declining populations. Confrontations are often in inverse relationship to the level of government involved although all are experiencing difficulty. The Federal community has been or is attempting to be deferential to state agencies in managing these confrontations in every state in the Northeast. State application of legislation and the associated regulation of aquatic activities has required progressively more interaction with in-house and other regulatory bodies. The result is increasingly more complex reviews as operational advances, previously unconsidered issues and the various regulatory elements are brought together to review aquaculture requests. After an initial gearing up, this consolidation usually has proven beneficial to both applicants and regulators.

The same cannot be said of local regulation. With varying degrees of government authority but a frequently negative attitude toward shellfishing in general, local government has become the front line of regulatory confrontation. A combination of insufficient information, misconceptions and alienation of affection have created a rapidly deepening morass of local involvement. Legal action over noise or potential degradation of water quality (with an associated hysteria over disease or pollutant dispersion) and the questioning of shellfish ownership has stimulated feelings of resentment. The issues raised are not reconcilable at the regulatory level and they are more and more frequently adjudicated. Unfortunately, there is very little case law or use of technical expertise on which to base decisions so the process creates few definitive conclusions. This failure to reach closure on specific issues leads to more adjudication and more confusion. It also costs time and money.

These circumstances, individually and collectively, indicate a need for a national policy on aquaculture. It should reflect the "state-of-the-art" on this topic and contain an appreciation of traditional fishing activities. The policy should provide guidance for siting, design and operation of aquaculture operations and assurances that competing uses are given full and equal consideration. To establish this policy, proponents of aquaculture should network and develop the skills necessary to influence public opinion.

DESCRIPTION OF AN UNUSUALLY HEAVY SET OF SOFTSHELLS (*MYA ARENARIA*) AND OTHER BIVALVES IN NORTHERN NEW JERSEY, LONG ISLAND SOUND AND SOUTHERN NEW ENGLAND IN 1993. Clyde L. MacKenzie, Jr., National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, James J. Howard Marine Science Laboratory, 74 McGruder Road, Highlands, NJ 07732.

An unusually dense and widespread set of softshells, *Mya arenaria*, occurred in the Navesink and Shrewsbury Rivers and the south shore of Raritan Bay, New Jersey, in June, 1993. The juvenile softshells set in bottoms mostly from the low tide mark to a distance at least 60 m from shore to depth of at least 1 m; further depths were unexplored. In the Navesink River, densities were high (up to 7,000/m^2) nearly everywhere in early July, but declined gradually by about 40% by November, 1993. Densities in the Shrewsbury River were about ⅓ as high. The reduction in densities was not caused by predation, but by many softshells emerging from the bottom and living on the surface for a few weeks before dying. By November, mean sizes of the clams at different sites in the rivers and Raritan Bay ranged from about 15–24 mm. Besides the softshells; oysters, *Crassostrea virginica;* amethyst gemclams, *Gemma gemma;* and Atlantic jackknife clams, *Ensis directus*, also set in unusually high densities. The sets resulted from relatively small spawning stocks of softshells, oysters, and jackknife clams. Setting was also heavy of softshells; oysters; northern quahogs, *Mercenaria mercenaria;* and bay scallops, *Argopecten irradians*, in some bays on Long Island and in southern New England.

Weather conditions in 1993 probably were responsible for the good setting. During May and June, the weather was unusually warm and dry, water temperatures increased steadily, skies were usually clear, winds were mostly westerly and were gentle, and there were no northeast storms. In contrast, the weather in 1992, when setting of the shellfish was much poorer, was unusually cold and rainy, winds were mostly easterly, and there were two northeasters in May, but none in June. The weather in 1993 was also excellent for the production of garden vegetables and fruit crops, whereas the weather in 1992 was poor for them.

COMMERCIAL FARMING OF SHRIMP IN THE PHILIPPINES: A CASE STUDY. Victor J. Mancebo, University of Massachusetts Dartmouth, Research 201, North Dartmouth, MA 02747.

A commercial shrimp farm was built on the island of Cebu in the Philippines. Construction began in 1985 and the first ponds were harvested in 1986. Production totalled 36.4 metric tons (mt) of heads-on shrimp in 1986, 215.2 mt in 1987 and 305.4 mt in 1988 when pond development totalled a net area of 34.5 hectares. Ex-farm sales of shrimp totalled $2.36 million in 1988, an average selling price of $7.73 per kilogram. During the 1986–1988 period stocking densities of shrimp in ponds ranged from 10 up to 25 animals per square meter, with an average stocking density of 18.2 animals per square meter. Production averaged 10,300 kilograms per hectare per year. Ponds averaged 2.2 crops per year, with the following average statistics: survival = 79.7%; body weight of shrimp = 35.7 grams; FCR = 1.68; crop length = 136 days and

pond preparation time = 30 days. Feeding techniques, water management, harvesting and pond preparation will be discussed.

NORTHEASTERN REGIONAL AQUACULTURE CENTER: AN UPDATE. Victor J. Mancebo, University of Massachusetts Dartmouth, Research 201, North Dartmouth, MA 02747.

The Northeastern Regional Aquaculture Center (NRAC), headquartered at the University of Massachusetts Dartmouth is one of five Regional Aquaculture Centers (RACs) established by the U.S. Congress. Funded by the U.S. Department of Agriculture at an annual level of approximately $750,000, and representing 12 states and the District of Columbia, NRAC develops and sponsors cooperative regional research, development and extension projects in support of the aquaculture industry in the northeastern United States.

A Board of Directors representing the region's aquaculture industries, academic institutions, and government agencies provides overall direction and management of NRAC. NRAC programs are industry-driven, i.e., industry communicates research and technology transfer priorities to NRAC through bi-annual industry summits and through NRAC's 12-member Industrial Committee. A 12-member Technical Committee provides technical oversight for NRAC's projects. Projects supported by NRAC are developed and carried out by Cooperative Regional Work Groups with researchers, extension specialists and industry representatives working together with multi-state and multi-institutional participation on each project. Projects are evaluated annually for achievement of technical and industry objectives.

Thirteen regional projects are ongoing in the following areas: 1) Commercial Field Trials of MSX-Resistant Strains of the Eastern Oyster *Crassostrea virginica;* 2) Alternative Marketing Options to Improve Profitability of the Northeast Aquaculture Industry; 3) Domestication of Striped Bass for Aquaculture; 4) A comprehensive Investigation of Larval Development and Mortality in the Eastern Oyster, *Crassostrea virginica;* 5) Northeastern Regional Aquaculture Center Newsletter; 6) Assessment of the Impact of Stray (Neutral-to-Earth) Voltage on Finfish Aquaculture in the Northeast; 7) Development of a Northeastern Regional Aquaculture Extension Network; 8) Alternatives to Lessen the Economic Impact of Aquaculture Regulations; 9) Computer Network Communication System; 10) The Role of Bacteria and Microalgae in Unexplained Juvenile Oyster Mortalities; 11) Possible Cytotoxic Effects of the Dinoflagellate, *Gyrodinium aureolum* on Juvenile Bivalve Molluscs; 12) Analysis of Genetic Purity of Captive and Wild Striped Bass; 13) A Proposal for the Study of A Protozoan Disease Agent(s) Associated with Mortalities of Hatchery-Reared Juvenile Oysters in the Northeastern United States. Total NRAC funding commitment to projects in progress is approximately $1,183,322.

In the upcoming year NRAC plans to modify the Cooperative Regional Work Group approach to project development by initiating a Request for Proposal (RFP) process. Quality proposals will be selected for funding in a competitive process wherein research, extension and industry or any combination thereof may respond to a call by submitting preproposals for targeted areas of research. A team building approach to proposal and project development is highly encouraged and successful proposals will be cooperative and collaborative in nature.

HISTORY AND CURRENT STATUS OF HARD CLAM CULTURE IN NEW JERSEY. George Mathis, Mathis & Mathis Enterprises, Northfield, NJ 08225; **Richard F. Crema,** R. F. Crema & Family, Oceanville, NJ 08231.

The commercial culture of hard clams (*Mercenaria mercenaria*) is a relatively new aquaculture endeavor in New Jersey, the oldest operations dating back to about 1970. Earlier research on clam culture and growth potential, financed by Campbell Soup Co., was initiated by T. C. Nelson and H. H. Haskin in the late 1940s. The development of hatchery and growout techniques, a private initiative with technical input from various sources, is summarized with highlights of key events, both disasters and successes. Early marketing impediments and strategies are discussed. Presently there are seven hatchery/nursery facilities, plus numerous individuals who grow plantable seed clams purchased from third party suppliers. A synopsis of current technology, onshore and field, and a discussion of operational problems (fouling, ice, poor winter survival and other unexplained mortalities), vexing questions and "solutions" are presented. The changing market picture is examined, with observations on volume/value factors and tentative approaches for improving stability, or living with instability. Critical areas of research, including reduction of overwintering mortalities, and need for remediation of administrative and regulatory impediments are elucidated and prioritized. Cautious projections concerning future developments in the industry are proffered.

NOAA'S AQUACULTURAL ACTIVITIES. Dean Parsons, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, 1335 East West Highway, Silver Spring, MD 20910.

Aquaculture production worldwide has grown tremendously in the past 25 years. Over 50% of seafood harvested from inland waters is from aquaculture, while less than 10% of seafood production from marine waters is of an aquacultural origin. The aquaculture industry continues to grow in the United States at a rate lower than that worldwide. While overall seafood consumption in the U.S. has declined since reaching a peak in 1987, the percent of that consumption contributed by farm-raised seafood products has risen. The National Oceanic and Atmospheric Administration (NOAA) has been involved in aquaculture since the late 1800's. Extensive fishery management responsibilities assumed by the National Marine Fisheries Service (NMFS) led to neglect of com-

mercial aquaculture research. A review of recent policy actions by NOAA and the Department of Commerce show that this decline has been checked. NOAA is now developed an Implementation Plan for Aquaculture which will serve as a planning guide for the next several years. The Implementation Plan will (1) identify opportunities and recommend strategies to strengthen enhancement capabilities, (2) establish criteria for identifying appropriate candidate species for use in commercial and enhancement aquaculture systems, (3) identify opportunities for collaborative system design studies with the private sector or other Federal agencies, (4) identify research parameters for nearshore and offshore culture systems which minimize environmental perturbations, and (5) develop experimental plans to verify the effectiveness of enhancing wild populations with releases of hatchery-reared finfish.

POTENTIAL FOR *CORBICULA* IN AQUACULTURE. Harriette L. Phelps, University of the District of Columbia, Washington, DC 20009.

In Fall 1993 a short trip was made to Taiwan to collect information on the aquaculture and food use of the freshwater Asiatic clam, *Corbicula fluminia*. At present, *Corbicula* is indigenous to rivers and freshwater estuaries of all but the most northern U.S. states. *Corbicula* has been found to be heavily fished locally by Asians but is not present in the U.S. market. For that to happen *Corbicula* will have to be raised in aquaculture. Aquaculture of *Corbicula* could be considered at many U.S. locations.

In Taiwan *Corbicula* is not a high-value aquaculture product and is consumed mostly as a side dish and in soups. It is considered a health food and has the highest (50%) glycogen content of any shellfish. In 1987 *Corbicula* had the fourth highest total shellfish market value in Taiwan with around 8000 mt produced at 3.7 mt/ha. The shelf life of fresh *Corbicula* is marked at one week. It is mostly cooked fresh or can be found "pickled" with hot peppers and soy sauce (which apparently does not prolong shelf life).

Corbicula is raised in shallow farm ponds and farm wastes are used to cultivate food algae. Seed production of *Corbicula* required shallower ponds with a sand bottom. Mature clams produce marketable seed (0.3–0.5 cm) within two months, which are sold to farmers for grow-out. Three crops can be marketed a year from farm ponds, but the necessity for flowing fresh water causes official discouragement. The short poster-video presentation shows some of the raising and cooking methods of *Corbicula* in Taiwan.

A COMPARISON OF TAXONOMIC CLUSTERING METHODS TO AID IN THE IDENTIFICATION OF BACTERIAL PATHOGENS OF FISH AND SHELLFISH. Steven Pitchford and **Richard Robohm,** National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460.

Over the past 15 years a number of bacterial isolates presumptively pathogenic for fish and/or shellfish have been collected and characterized by Milford laboratory personnel. Classification of these isolates to genus or species level was often difficult; application of bifurcating keys using bacterial phenotypic traits frequently produced results that were contradictory to other identification methods. In an attempt to resolve these problems, a database of phenotypic characteristics of known pathogenic species was compiled from the literature. Ninety-six permutations of pairs of computer-aided, numerical-taxonomy programs and clustering techniques contained in the NTSYS-PC software package (Exeter Software, Setauket, New York) were analyzed for their ability to group known organisms in the database into correct phylogenetic groups. The most accurate combinations proved to be K2 or UN5 coefficients clustered with WPGMA or UPGMA. The reliability of the various computer programs will also be discussed.

MODIFYING OYSTER HATCHERY MANAGEMENT TO AVOID LOSSES DUE TO UNEXPLAINED MORTALITY OF JUVENILE OYSTERS. David Relyea, Frank M. Flower & Sons, Inc., P.O. Box 1436, Bayville, NY 11709.

Starting in July of 1990, juvenile oysters in the growout raft system at Frank M. Flowers and Sons in Bayville, NY, showed unusually high mortalities. Hatchery production declined by 50%. This pattern continued in 1991 and 1992 while experienced marine scientists analyzed the symptoms. To date, no definitive cause of the problem has been found. It is now well known, however, how and when the problem progresses through the hatchery season. With this knowledge, it has been possible to adjust the hatchery schedule and procedure to substantially increase hatchery output. Hatchery production for 1993 was the best since 1989 and this presentation will show how that came about.

INVESTIGATING ALLELOPATHY BETWEEN THE MARINE MICROALGAE: SOME SPECIES FAMILIAR TO AQUACULTURE. Barry C. Smith, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460.

Nine species of marine unicellular microalgae were tested for intra- and interspecific allelopathic activity using a petri dish and paper disk method. Cultures in the logarithmic and stationary growth phases were each tested in three ways: 1) Live cells against live cells; 2) Live cells against medium previously used to grow each algal strain; 3) Live cells against medium-soluble cellular extracts of each algal strain.

Two of the species also were tested using a test tube method which employed "used media" as the exposure vector. Stationary phase cultures of *Tetraselmis maculata* (TTM) and *Dicrateria inornata* (Dicrat) were inoculated into test tubes containing 50%

fresh E Medium and 50% that had previously been used to grow one of the two algal strains. Optical density and cell numbers were monitored for over 21 days.

Dunaliella tertiolecta (DE), TTM, and *Pyramimonas grossii* (78) were the only algae to exhibit clear evidence of allelopathy. All three of these strains caused *Chlorella autotrophica* (580) to form inhibition zones when growing on agar medium. However, 580 displaced *Isochrysis galbana* (ISO) and *Pavlova lutheri* (MONO). In the test tube experiments, *Dicrateria inornata* (Dicrat) was repressed by one-third by itself and by TTM. A 'pecking order' begins to emerge of most successful to least successful strains; DE, TTM, and 78 followed by *Phaeodactylum tricornutum* (PHAEO), 580, MONO, ISO, Dicrat, and *Chaetoceros gracile* (Chaet-B). The species that grew the most vigorously were usually those that exhibited the highest levels of allelopathy.

In shellfish aquaculture, the most favorable of situations would be to grow algal strains that are nutritious to shellfish and that grow densely. Algae that exhibit allelopathy should be more successful at resisting culture contaminants and therefore would be more dependable to culture for shellfish food. Further, by selecting out of these the strains that exhibit antibiotic abilities, against undesirable and contaminating bacteria, the shellfish cultured should be healthier.

THE EASTERN OYSTER, HISTOPATHOLOGY OF SETS WITH LOW SURVIVABILITY. Roxanna M. Smolowitz, Laboratory for Marine Animal Health, University of Pennsylvania, Marine Biological Laboratory, Woods Hole, MA 02543.

Remote setting of late-stage larval oysters could greatly increase the total production of oysters on the east coast. Attempts to replicate the remote setting success of the west coast hatcheries that raise *Crassostrea gigas*, on the east coast using *Crassostrea virginica*, have been unsuccessful and have resulted in setting successes of 5% or less. However, oyster hatcheries of the northeast have demonstrated setting efficiencies of up to 50%. The work presented today is part of a larger multi-disciplined study undertaken to determine the reason for poor setting success in mid-Atlantic oysters used in remote setting attempts.

A northern and southern broodstock were spawned, reared and set at a northern hatchery, Aquaculture Research Corporation (ARC) and at Horn Point Environmental Laboratory (HPEL), a southern hatchery. Live animals were collected from each experimental group from day-4 postspawn to day-40 postspawn. Animals were fixed and processed in paraffin and/or plastic for histological examination and electron microscopic examination.

Histological evaluation of larval animals showed a subtle but significant difference in growth between animals spawned, reared and set at ARC as compared to animals spawned, reared and set at HPEL. Animals from ARC showed good development of all organ systems and a high level of lipid storage in the digestive system. Animals from HPEL, however, showed moderate development of organ systems with moderate lipid storage primarily only in the intestine.

Postset animals showed significant differences in survivability with ARC setting between 27.6 to 33.0% of the larvae and HPEL setting between 4.2 and 7.4%. Histological evaluation of these animals showed poor organ development of animals from HPEL as compared to those from ARC.

These results indicate that organ development and lipid storage are good indications of future setting success. They also suggest that nutritional quality and/or the amount of food supplied to larvae during development plays a key role both in the setting readiness of those oysters, and in their ability to survive setting.

PROSPECTS FOR THE GENETIC IMPROVEMENT OF SHELLFISH FROM LONG ISLAND SOUND. Sheila Stiles and **Joseph Choromanski,** National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460.

Significant progress has been made in the intensive and extensive culture of shellfish from Long Island Sound. Genetics and biotechnology studies offer additional opportunities for improving desirable characteristics and for investigating heritable and molecular bases of processes including growth, development, survival, and responses to stress, such as pollution and disease. Moreover, in enhancement efforts, there is a need for development of methods to increase the reproductive potential, conserve gene pools, and improve genetic adaptation of species in stressed environments. For example, oysters bred selectively for fast growth at the Milford Laboratory on Long Island Sound are being employed in studies of survival and disease resistance in Maine and New Jersey.

A similar systematic approach to breeding of the bay scallop from Long Island Sound for increased growth rate and meat (muscle) yield is planned. Prior to breeding, quantitative and qualitative measurements are being made of approximately 350 bay scallops from Connecticut. Measurements made to date of characteristics such as height, length, depth, weight, and age seem to be positively associated, that is, the older and larger (height, length) the scallop, the greater the weight and the depth. If desirable traits are linked, selecting for one would simultaneously improve another. Conversely, if traits are negatively associated, selecting for one could lead to a diminution in another. An additional feature, rib number, reported to be characteristic of some geographic populations, was more-or-less consistent at 18–20, irrespective of size. Qualitative characteristics such as shell and mantle edge or fringe color showed a considerable range of variability. Shell color has been reported to be associated with viability, i.e., growth and survival. Overall results from the preliminary profile data of a population of Connecticut bay scallops indicate favorable prospects for genetic gains based on the experience with oysters from Long Island Sound.

SETTLEMENT AND RECRUITMENT OF BAY SCALLOPS, *ARGOPECTEN IRRADIANS*, TO ARTIFICIAL SPAT COLLECTORS IN THE WESTPORT RIVER ESTUARY, WESTPORT, MASSACHUSETTS. **Karin A. Tammi,[1] Scott J. Soares,[2] Wayne H. Turner,[2]** and **Michael Rice,[1]** [1]Department of Fisheries, Animal and Veterinary Science, University of Rhode Island, Kingston, RI 02881; [2]The WATER WORKS Group, General Delivery, Westport Point, MA 02791.

In January 1993, The Water Works Group initiated the Bay Scallop Restoration Project as an attempt to restore the once prolific bay scallop population within the Westport River Estuary in Massachusetts. The project is a multiphase endeavor aimed at better understanding recruitment failures of both natural stocks and introduced seed of *Argopecten irradians*. The main objective was to assess larval settlement and juvenile recruitment (survival to >4 mm) to artificial spat collectors placed in historically productive scallop beds and within close proximity to adult spawner rafts. Spat collectors (2–4 mm plastic-mesh bags) containing monofilament were suspended on 20–30 ft floating longlines at 11 locations in the Westport River.

A total of 1400 spat collectors were sequentially deployed on 89 longlines from June to August 1993 to determine the timing of peak settlement and recruitment at each study site. The October to November 1993 harvest yielded 4000 scallops of varying shell heights ranging from 4 to 60 mm, with an overall mean of 36.9 mm. The difference in shell heights was related to the variable harvest times of the longlines which ranged from 68 to 128 days. The most productive longlines were located in the vicinity of Coreys Island, Horseneck Channel and Canoe Rock. The greatest recruitment was observed at Coreys Island which yielded 1882 scallops averaging 6.1 scallops per collector. However, recruitment to individual longlines at this location was as high as 18.2 scallops per collector. This study shows that *A. irradians* will settle on artificial spat collectors containing monofilament which may have two major implications in resource management. First, spat collectors may be a means to predict recruitment into the bay scallop fishery. Second, juvenile scallops harvested from spat collectors could be utilized for other grow-out applications to enhance natural stocks. Consequently, the implementation of spat collectors into an overall management plan could be a method employed by coastal communities to improve, stabilize and restore bay scallops in Southern New England and elsewhere.

THE USE OF BIOCHEMICAL INDUCERS FOR ASSESSING AND MANIPULATING LARVAL COMPETENCE, SETTLEMENT AND METAMORPHOSIS IN SELECTED SPECIES OF COMMERCIALLY IMPORTANT BIVALVES. **Kim W. Tetrault** and **Michael A. Rice,** Department of Fisheries, Animal and Veterinary Science, The University of Rhode Island, Kingston, RI 02881.

Within the last decade, it has been demonstrated and is now possible to assess the onset of competence in marine larvae with confidence. By applying certain naturally occurring chemicals in low, precisely defined concentrations, induction of settlement and metamorphosis of competent molluscan larvae can be safely and routinely achieved, although further work is required to determine which chemicals, and which concentrations of these chemicals are most effective on bivalve larvae of present and potential commercial interest. A study is currently in progress at the University of Rhode Island Shellfish Aquaculture Vetlab which will examine the role of biochemical inducers of metamorphosis in *Mercenaria mercenaria, Argopecten irradians* and *Spisula solidissima*.

Once larvae of these species are produced or obtained, selected biochemicals will be employed in order to determine the effectiveness of each potential inducer on triggering metamorphosis of each species. The minimum concentration and exposure time will be established without decreasing post-metamorphic survival and growth rate. The results from these tests will be used to address the relationship between larval growth rate, rate of becoming competent to metamorphose, and success of juvenile development. The growth and survival of juveniles whose larvae were chemically induced to metamorphose will then be compared to juveniles whose larvae demonstrated a settlement behavior and/or experienced a delay of metamorphosis.

The ultimate goal of this project is to determine whether the use of biochemical induction is of any beneficial consequence to the spat being produced. Does triggering metamorphosis as soon as the larvae become capable of metamorphosing produce higher juvenile survival and growth? Do larvae that become competent to metamorphose sooner show more successful post-metamorphic development? If so, the technique can be used as a tool for maximizing larval metamorphosis, juvenile survival and juvenile growth in order to increase the economic efficiency of bivalve mariculture.

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AN OVERVIEW OF ECONOMIC AND INSTITUTIONAL ISSUES CONFRONTING UNITED STATES MARINE AQUACULTURE DEVELOPMENT. **Eric M. Thunberg,** National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Woods Hole Laboratory, 166 Water Street, Woods Hole, MA 02543.

Compared to international experience in marine aquaculture, the United States is still in a relatively early state of development. While aquaculture promises to play a significant role in international food and nonfood product markets, the role that aquaculture may play in the United States and an international economy is still unfolding. In this presentation an overview of economic and institutional issues that will influence the pattern and rate of development of marine aquaculture in the United States are discussed. Particular emphasis is placed on how these issues may affect the global competitiveness of United States aquaculture products in international markets.

THE BAY SCALLOP RESTORATION PROJECT IN THE WESTPORT RIVER. **Wayne H. Turner** and **Scott J. Soares,** Bay Scallop Restoration Project, The WATER WORKS Group, General Delivery, Westport Point, MA 02791.

The time has come to reverse the trend of the deteriorating water quality in the Westport River. To this end, it has become increasingly evident that a positive way to mitigate the effects of pollution is to make it economically advantageous to do so.

In an effort to focus public attention on the problems facing communities like Westport, states like Massachusetts and Rhode Island, and the economic well-being of the entire coast of the United States, The WATER WORKS Group has spawned the *Bay Scallop Restoration Project*. This undertaking is the first in a series of projects initiated under the umbrella of *The Watershed Reclamation Project*.

The *Bay Scallop Restoration Project* was launched in January 1993 with the aim of increasing public awareness about the plight of the Westport River. By virtue of its economic value and universal appeal, the bay scallop was selected as the vehicle through which resources could be mobilized and public support and local commitment garnered. From its inception, the *Bay Scallop Restoration Project* has rallied an unprecedented outpouring of community and regional involvement centered around the effort to return the bay scallop resource to the Westport River.

Faculty, graduate, and undergraduate students from the University of Rhode Island, the University of Massachusetts–Dartmouth, the Massachusetts Institute of Technology, and the Marine Biological Laboratory at Woods Hole have been brought aboard to address technical aspects of bay scallop propagation. In support of these initiatives, a substantial number of local businesses and volunteers from the Westport Fisherman's Association, local high schools, and the general public have provided necessary building materials and equipment while investing more than 10,000 volunteer hours in the effort.

Currently, the *Bay Scallop Restoration Project* is analyzing the vast amount of data collected by graduate, undergraduate, and high school students from nearly one year's worth of field work. To their credit, the investigations of these professional, academic, and volunteer researchers have revealed the importance of pure and applied science.

A *GYMNODINIUM* ISOLATED FROM THE F. M. FLOWER HATCHERY CAUSES NO APPARENT ILL EFFECTS ON JUVENILE OYSTERS. **Gary H. Wikfors**[1] and **Roxanna M. Smolowitz,**[2] [1]National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460; [2]Laboratory for Marine Animal Health, Marine Biological Laboratory, Woods Hole, MA 02543.

A *Gymnodinium sp.* culture was established from an Oyster Bay water sample, taken near the F. M. Flower oyster hatchery on 17 August, 1992. At this time, mortalities from "juvenile oyster disease" were occurring. An experiment was conducted to determine if juvenile oysters exposed to this dinoflagellate exhibited characteristic symptoms of either 1) juvenile oyster disease, ie, conchiolin deposits within the shell, wasting of mantle tissue, and mortality, or 2) degeneration of absorptive cells of the digestive diverticula, as seen in shellfish feeding on certain other dinoflagellates.

Laboratory spawned and reared juvenile oysters of about 10 mm shell length were starved for four days and then given daily feedings of the *Gymnodinium* culture (designated Gymno-F) or one of several control diets. The Gymno-F culture remained contaminated with a small (ca. 2 μm) gold-pigmented microalga, therefore, controls included: 1) a unialgal culture of the algal contaminant (strain GBF), 2) *Tetraselmis maculata,* an alga known to support growth of oysters, and 3) starved oysters. Twenty-five oysters were included in each experimental treatment. The fed controls were given .012 ml packed cells d^{-1}; however, packed-cell volumes could not be obtained for fragile *Gymnodinium* cells. Daily feedings of Gymno-F ranged from 40–100 cells $oyster^{-1}$ d^{-1}; cell densities in feeding chambers were 1500–4000 cells l^{-1}. Gymno-F rations were lower than for other diets.

For the first two weeks, oysters fed Gymno-F filtered actively, but produced abundant pseudofeces; thereafter, normal fecal strands were produced. The other two diets were ingested normally. Shell growth was noted qualitatively in all treatments except the unfed control. After 36 days—approximately the time required for development of juvenile oyster disease symptoms—oysters were sacrificed, observed for abnormal conchiolin deposits, and evaluated histologically. No abnormal conchiolin deposits were found, nor was there any histological evidence of poor health in any fed treatments. Digestive diverticula showed good development in all fed controls, with Gymno-F appearing in slightly better condition than the other fed controls. From these observations we conclude that this isolate of *Gymnodinium* is not a causative agent of juvenile oyster disease. The refractory period during which oysters produced pseudofeces when fed Gymno-F is consistent with our previous studies of oysters feeding on another dinoflagellate, as is the eventual resumption of normal feeding and growth; this may be related to changes in oyster digestive enzymes.

DINOFLAGELLATE AUTOLYSOSOMES AND RESPONSES OF DIFFERENT BIVALVES FEEDING ON *PROROCENTRUM*—IS THERE A CONNECTION? **Gary H. Wikfors**[1] and **Roxanna M. Smolowitz,**[2] [1]National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460; [2]Laboratory for Marine Animal Health, Marine Biological Laboratory, Woods Hole, MA 02543.

Differences between response of the bay scallop, *Argopecten irradians,* and the eastern oyster, *Crassostrea virginica,* to the dinoflagellate *Prorocentrum minimum,* led us to consider possible

mechanisms by which dinoflagellates affect different bivalves. Juvenile scallops suffered rapid mortality accompanied by damage to absorptive cells in the digestive diverticula and systemic pathologies characteristic of toxin effects when fed a diet including *P. minimum*. By contrast, larval and newly post-set oysters grew and developed poorly, but did not experience acute toxic effects, when fed algal diets including *P. minimum*. Large, dense accumulation bodies (persistent phagolysosomes) were present in digestive diverticula absorptive cells of young oysters fed *P. minimum*. When older juvenile oysters were exposed to the dinoflagellate, initial response was to filter, but not ingest *P. minimum* cells for approximately two weeks, after which oysters fed and grew normally, and digestive diverticula appeared healthy. Thus, presence and contents of accumulation bodies should hold clues about digestive interactions between bivalves and this dinoflagellate.

In TEM preparations, accumulation bodies in oyster absorptive cells were similar to dinoflagellate autolysosomes, organelles that are thought to catabolize cellular products to provide energy; these organelles may contain high concentrations of catabolic enzymes. A recent study (Zhou and Fritz, 1994, *J. Phycol.*) demonstrated that *Prorocentrum* autolysosomes react strongly with PAS, a carbohydrate stain. We have demonstrated that both *P. minimum* cells and accumulation bodies in oyster absorptive cells are PAS-positive. In oysters, it appears likely that *P. minimum* autolysosomes are not degraded in the digestive lumen, but are phagocytized by absorptive cells, accumulate in characteristic phagolysosomes, and interfere with normal digestive function. Older juvenile oysters appear to be able to produce digestive enzymes that degrade dinoflagellate autolysosomes intracellularly. In contrast, scallop digestion occurs primarily in the lumen, and dinoflagellate autolysosomes may be degraded in the lumen, thereby releasing dinoflagellate enzymes which may then have toxic effects upon scallop absorptive cells. We hypothesize that differences in responses of bivalve mollusks to dinoflagellates, such as *Prorocentrum*, may result from differences in digestive function, specifically the site at which dinoflagellate autolysosomes are digested.

THE DEVELOPMENT OF CLAM AQUACULTURE THROUGH DEMONSTRATION PROJECTS ON THE GULF COAST OF FLORIDA. David E. Vaughan and **Leslie N. Sturmer,** Harbor Branch Oceanographic Institution, Inc., 5600 U.S. Highway I North, Fort Pierce, FL 34946.

The potential of hard clam, *Mercenaria mercenaria*, culture in Florida was described and initiated along the Gulf of Mexico coast over 20–30 years ago by several researchers, most notably Dr. Winston Menzel. However, successful implementation of hard clam production was achieved only during the past 10 years along the Atlantic coast, primarily in the north east and east central counties. According to the Florida Agriculture Statistics Service, 41 growers reported sales of 5.9 million clams in 1989 and 8.8 million clams in 1991. Value of production rose to $1.2 million dollars.

Recently, federally-funded retraining programs have provided an infrastructure for establishing shellfish aquaculture as a means of economic growth for the Gulf coast. These community-level programs showed excellent growth rates and have demonstrated culture technologies. The successful availability of new 10-year-term, aquaculture leases, has made the potential of a shellfish aquaculture industry on the west coast of Florida a reality.

Project OCEAN, the Oyster and Clam Educational Aquaculture Network, located in Cedar Key, was initiated in the spring of 1991 to train displaced seafood workers in shellfish cultivation techniques. Over 170 eligible participants from 4 counties received services during the past 2½ years. Each trainee was given shellfish seed, equipment, and training necessary to establish small-scale farms. The technology utilized for the growout of clams was the soft tray, which is made of a flexible mesh material in the form of a bag. Although it provides top and bottom protection from predators like a tray, the 1.5 square meter bag is planted directly on the bottom with naturally accumulating sediments serving as substrate. When lifted or harvested only the product and mesh are removed from the bottom. This method, developed by HBOI researchers on the east coast of Florida, was adaptable to west coast conditions.

A total of 5 million clam seed, ranging from 2 to 12 mm in shell length, were planted by 109 Phase I participants in their in-water classes during the summer of 1991. Since then, over 1.4 million littleneck clams have been harvested. Trainees, who maintained culture practices such as cleaning and sieving, have each harvested over 28,000 clams, resulting in 55–60% of their crops reaching marketable size, or about 50 mm shell length, in the 2-year training period. Water temperatures, reported as monthly means, ranged from a low of 10.4°C in January of 1992 to a high of 32.3°C in August of 1992. The overall mean for salinity was 24 ppt, with a monthly maximum of 28 ppt in December of 1991 and minimum 18 ppt in April of 1993.

Project OCEAN concluded last month. At that time, 750 acres of submerged land had been permitted, surveyed and marked for lease areas. One hundred and thirty seven graduates of the training program were provided on shellfish aquaculture leases, each person receiving 4 acres. In the past 8 months, over 7 million clam seed have been purchased by new leaseholders. These accomplishments could not have been met without the support of county governments, almost all state agencies, local agencies, and civic organizations. Citing this exceptional level of cooperation and collaboration required to make Project OCEAN a reality, Cedar Key was named Florida's Outstanding Rural Community of 1993 by proclamation by the Governor.

STRATEGIES FOR SUCCESSFUL AQUACULTURE DEVELOPMENT: THE CONNECTICUT EXPERIENCE. John H. Volk, Connecticut Department of Agriculture, Aquaculture Division, P.O. Box 97, Milford, CT 06460.

Connecticut is a leader in aquaculture production in the north-

east and is the nation's second largest producer of the eastern oyster (*Crassostrea virginica*). The State's oyster aquaculture industry has grown from approximately 30,000 bushels in 1972 to nearly one million bushels in 1992 with a farm gate value of over $63 million. Most of this dramatic production increase has occurred within the last five years.

Oysters are farmed by the industry on leases and grants off the Connecticut coastline in Long Island Sound utilizing extensive bottom-culture methods. Oyster seed is obtained by planting cultch (clean oyster shell) on private grounds or by purchasing seed harvested from managed public grounds. Over 46,000 acres are currently under cultivation by Connecticut aquaculturists.

The remarkable growth of Connecticut's oyster farming business is economically significant and serves to demonstrate several strategies that are beneficial in fostering aquaculture as an industry. Connecticut's recent success is primarily due to a cooperative effort between State government and the oyster industry. A joint venture was legislatively created in 1987 establishing an aggressive management program for the State-owned public beds. During the past five years through this program, the State has funded the purchase of over 5 million bushels of cultch for the public beds. The industry provides the equipment and personnel to plant the shells. A 10 percent tax on the sale of the seed oysters is dedicated to the program.

State laws and supportive programs provide a well established foundation for the shellfish aquaculture industry in Connecticut allowing the business to flourish. Conflicts with competing uses have been minimal.

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ABSTRACTS OF PAPERS

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"Pearls '94"
by C. Richard Fassler
Conference Chairman

The scarcity of a gem and the degree of quality control by its producers or processors play major roles in determining its value. Pearls are no exception to this rule. For the past century, the Japanese, similar to De Beers with diamonds, have enjoyed a monopoly on cultured pearls—even on those produced outside of the country, because they were marketed through Kobe or Tokyo. This dominance has benefited the global industry, as both price and product quality have been preserved at high levels. However, in recent years, the situation has changed drastically, resulting in a crisis.

This change has come about because of several significant factors, including the meteoric rise of Chinese freshwater (and now saltwater) pearls; the decline of Japanese production, due to environmental considerations; and the spread of pearl farming to other areas of the world, mainly Southeast Asia and the South Pacific. Like De Beers, Japan now finds itself losing control. Without the traditionally strong Japanese involvement, the crucial question facing the industry is: can product quality and high prices be maintained? Another inportant concern is the endangered status of more than half of the American mussel species which provide nucleus material. A call to address these issues went out to pearl farmers, jewelers, scientists, equipment suppliers and government officials.

The response was most enthusiastic. The State of Hawaii and the Hawaii Jewelers' Association hosted, and the International Pearl Association sponsored, the largest and most diverse assemblage of the world's pearl community ever held. The meeting; from May 14 to 19 at the Sheraton Waikiki Hotel in Honolulu, attracted speakers and technical presentations from Japan, China, French Polynesia, Bahrain, Kuwait, India, Colombia, Canada, the U.S.A., French Polynesia, Australia, the Solomon Islands, Mexico, the Cook Islands, Myanmar, Vietnam, New Caledonia, Bangladesh, the Philippines, France, India, Taiwan, and Iran. Another 20 nations sent delegates. There was a wide range of topics, which included; the future of the American freshwater mussel; starting a pearl farm; pearl culture in India; the pearl resources of Bahrain; conch pearls; and abalone pearls.

Conference participants discussed both obstacles and opportunities. Marketing strategies were presented, and the exposition featured a vast number of pearls of all colors and sizes for sale. The Jewelers' Forum assisted the jeweler in selling more pearls through sessions that included pearl grading, stringing, peeling, design and quality control, and overviews from all major pearl producing countries. Importantly, the meeting provided an in-depth understanding of all aspects of the pearl business, and offered the participant the chance to influence the course of the industry over the next decade.

Pearls '94 was for persons who love pearls, and who wanted to be a part of the history—and future—of this ancient and most treasured jewel.

CONTENTS

INVASION AND IMPACTS OF THE ZEBRA MUSSEL IN THE UNITED STATES. Steven A. Ahlstedt, Clean Water Initiative, Tennessee Valley Authority, Norris, Tennessee 37828.

The zebra mussel, *Dreissena polymorpha,* was first discovered in North America in Lake St. Clair, Michigan in 1988. The species is native to Europe and is believed to have been introduced in 1983 or 1984 from transoceanic ships which discharged freshwater ballast containing planktonic larvae or young adults. Since its introduction, zebra mussels have quickly spread throughout the Great Lakes and into interconnected navigable waterways of the St. Lawrence, Hudson, and Mississippi River drainages. To date, zebra mussels are documented as far south as New Orleans, Louisiana.

This small mussel with zebra-like stripes has the potential to become the most serious biofouling pest of any exotic species introduced to North America, and also has the capability of seriously altering the ecology of lakes and streams where it occurs. Zebra mussels are sexually mature by the time they reach 8 to 10 mm shell length and can grow to 50 mm in length. Females are capable of producing as many as 1 million eggs in 2 years. The animal is able to colonize new areas quickly because planktonic larvae (veligers) drift or are carried by water currents great distances before they settle on firm substrates. Another mode of dispersal is attachment on commercial boat tows, barges and recreational boats. The mussels produce byssal threads which enable the animal to attach to a substrate and remain firmly anchored.

Zebra mussels pose serious economic and ecological threats because of the high population densities they have attained in the Great Lakes area. Municipal, industrial and power plant water intakes are especially vulnerable because of the mussels' ability to settle and clog raw water pipelines. Operating budgets for controlling zebra mussels at water-using utilities in the Great Lakes area are costing millions of dollars annually and are projected to reach billions in the next decade. Ecologically, the zebra mussel is a serious threat to native freshwater mussel populations because of the tremendous filtering capacity which strips the water column of food and the smothering effect of colonization on native mussel beds. The pearl industry would also be threatened since it is dependent upon North America commercial mussel species which provide the nuclei for pearl formation. Because native mussels are the preferred substrate for zebra mussel colonization, the damage and losses to the fauna have already begun in the Great Lakes and losses are starting to occur in the rich mussel beds of the Mississippi River drainage. Indirectly, native mussel host-fish species essential for mussel life-cycle/reproduction may also be affected during early life stages from disruption of the food chain by zebra mussels.

THE CURRENT FISHERY, POPULATION DENSITY, AND CULTURE FEASIBILITY OF PEARL OYSTER, *PINCTADA RADIATA*, IN KUWAIT WATERS. S. M. Almatar, Xucai Xu and S. H. Alhazeem, Mariculture and Fisheries Department, P.O. Box 1638, Salmiya, Kuwait 22017

The current fishing areas for pearl oyster, *Pinctada radiata* (Leach), in Kuwait waters are distributed between 29°06'N latitude, 48°12'E longitude and 28°33'N latitude, 48°30'E longitude at depths ranging from 10 to 20 m. The fishery was monitored daily from January 1989 to May 1990, twice a week from May to December 1992, and six to ten days per month in 1993. Pearl fishery in Kuwait relies on natural pearls only, with estimated annual worth of about KD 770,000 (US$ 2.5 million), of which approximately KD 330,000 (US$ 1 million) were paid to purchase pearl oysters from divers. The total landings of pearl oysters in 1989, 1992 and 1993 were 313 t, 129 t and 102 t, respectively. Average CPUEs in 1989, 1992, and 1993 were 117.6, 75.0 and 41.9 kg, respectively. There has been no apparent fluctuation in fishing efforts over the past decade.

From a total of 4,414 oysters sampled, 96 were found to bear one or more pearls, i.e., a bearing rate of 2.2%. Oysters with at least two pearls accounted for 17.7% of all oysters bearing pearls. All the pearls collected from the sample were smaller than the commercial size. The mean diameter of pearls in the sample was 1.53 ± 0.88 mm, ranging from 0.5 mm to 2.6 mm. A survey from 5.9×10^6 oysters landed from June 1989 to January 1990 indicated that only 400 large pearls (>4 mm) and 984 small pearls (3–4 mm) were sold in the market. Thus the probability of landing a commercial-sized pearl is one of 4,200.

Eleven major pearl oyster beds, varying in size from a little more than 1 km^2 to several km^2, were scattered in the fishing area. 110 sites were investigated by diving, short dip or grab. 27 of 59 dives observed no oysters. The densities in the remaining diving sites varied significantly from site to site, ranging from 0.3 to 832 pearl oysters per 200 m^2. High densities of pearl oysters were observed at an exploited bed which is located in the industrial area near the south of Mina Abdulla. The abundance and biomass of pearl oysters in the whole survey area was estimated to be 8.3 millions and 391 t, respectively.

The pearly oyster fishery in Kuwait has not been officially regulated, although several recommendations were proposed for the fishery management. The recommendations include: 1) establishment of a limited entry licensing regulation to prevent rapid increase in effort and to provide divers with some official sanction; 2) the number of licenses should be kept at the current level; fishing hours per day should be restricted to the currently working hours of 08:00 to 12:00; 3) a minimum size limit of hinge length of 40 mm was suggested to be established on a trial basis for one year, and it could be increased to 45 mm later.

The technical and economical feasibilities of pearl culture in Kuwait were studied in 1990. The study revealed that Al-hiran area in the south of Kuwait was a suitable site for oyster culture. Pearl farming can rely on wild oysters taken from the oyster beds. An experiment on pearl seeding was carried out during the study. A preliminary cost was estimated to evaluate the pearl culture in Kuwait, and the labor cost seems to be a large part of farm operating budget. A pilot-project study is strongly suggested to determine the technical aspects and also to evaluate the quality and the market price of the products.

INCIDENCE OF PEARLS OF VARIOUS SIZES IN THE PEARL OYSTER OF BAHRAIN. Mohammed Al-Rumaidh, Scientific Research Department, Bahrain Center for Studies & Research, Building 70, Flat 12, Road 30, Manama, State of Bahrain.

A pearl oyster resources survey was carried out in Bahrain waters during 1986 to 1989 to determine the yields of pearls and size ranges in oysters from different beds. A total number of 54,338 pearls were obtained from 1,145,420 oysters collected from 14 different oyster beds. Most of the pearls harvested (94.4%) were very small—less than 3 mm. 38.1% of the pearls were less than 0.9 mm; 37% were 1.0–1.9 mm; and 18.3% were in the 2.0–2.9 mm group. Larger pearls 5.0–8.9 constituted less than 0.4%.

VARIATION IN ABUNDANCE OF BLACKLIP PEARL OYSTER SPAT IN THE SOLOMON ISLANDS. Johann Bell, ICLARM Coastal Aquaculture Centre, P.O. Box 438, Honiara, Solomon Islands.

The Australian Centre for International Agriculture Research (ACIAR) has provided ICLARM's Coastal Aquaculture Centre with funding for two years to assess the feasibility of farming pearl oysters in Solomon Islands.

The project stems from the observation that reasonable quantities of blacklip pearl oysters have been harvested from many areas within the Solomon Islands on a regular basis. In view of the success of the blacklip pearl industry in Tahiti and the Cook Islands, ACIAR, ICLARM, and the Solomon Island Fisheries Division are collaborating to determine whether it is possible to establish blacklip pearl oyster farms in other types of coral reef habitats in the Pacific, e.g. the more open lagoon complexes of the Solomon Islands. The most important question in this regard is, "Are there sufficient wild spat of the blacklip pearl oyster in the Solomon Islands to set up a viable industry?" To answer this question, staff from the Coastal Aquaculture Centre have designed a sampling program to measure spatial and temporal variation in abundance of blacklip spat over a wide area of the Solomon Islands.

Spat of blacklip pearl oysters will be collected from three sites in each of five main areas (i.e., a total of 15 sites). At each site, a longline 100 m in length will be set up. Spat collecting bags will be suspended from the longline every three months and left to soak for six months to allow enough time for the spat to grow to a size where they can be identified easily. Two types of spat collecting material will be used in the spat collecting bags: shadecloth and black plastic sheet.

The five main areas to be sampled are Ngela, South Malaita, Seghe, Munda and Gizo. All these areas provide access to a range of sheltered reef habitats. Selection of the three sites in each area was based on aerial photographs and historical levels of blacklip harvests.

Blacklip spat collected at each site will be grown-out in nearby coastal villages using conventional methods.The hope is that the sampling program will identify areas where villagers can reliably catch and grow enough spat to establish their own farms, or to sell live oysters to an overseas pearl farming company.

GENETICS OF BLACK-LIPPED PEARL OYSTER (*PINCTADA MARGARIFERA*). John A. H. Benzie, Australian Institute of Marine Science, PMB No 3, Townsville MC, 4810, Queensland, Australia.

Genetic data now available from black-lipped pearl oyster stocks from Japan, French Polynesia, Kiribati, the Cook Islands, the Great Barrier Reef (GBR) and Mauritius are reviewed. *P. margaritifera* stocks have high levels of genetic variation within populations and high levels of gene flow between populations widely separated geographically. Early work emphasised the lack of geographic differentiation. However recent surveys of populations from the west and central Pacific have shown significant genetic differences, not only between the Great Barrier Reef and central Pacific populations, but between local populations within island groups. The implications of these findings for restocking and stock transfer are discussed.

POTENTIAL OF PEARL OYSTER CULTURE ON THE COLOMBIAN CARIBBEAN. Francisco J. Borrero. INVEMAR, Instituto de Investigaciones Marinas de Punta de Betín, A.A. 1016, Santa Marta, Colombia.

As part of a preliminary, but comprehensive program to assess the feasibility of culturing several species of bivalve mollusks from the Colombian Caribbean, we have initiated studies on the potential for cultivation of the mother-of-pearl oyster (*Pinctada imbricata*), and the winged pearl oyster (*Pteria colymbus*). We are carrying out five major studies related to elucidating aspects of the biology and ecology of local pearl oyster populations, which are critical to any culture plans: 1) we are surveying the extent and status of natural stocks of pearl oysters, including cartography of bottom types, abundance and oyster size distribution, and density; 2) we are studying the spatial (across bays, and bathymetric) and temporal variation of intensity in spat settlement to artificial collectors made of two different materials, and placed at several depths; 3) we are monitoring temporal changes in abundance of planktonic bivalve larvae, including those of pearl oysters, both at the surface and on deeper waters, with the goal of elucidating possible relationships between changes in environmental conditions and abundance of planktonic larvae, as well as examining the relation between changes in abundance of larvae in the water and of spat on collectors; 4) to elucidate the sexual system of these species, we are studying the relationships between size/age and sex, as well as fecundity of the two pearl oysters, and are monitoring the occurrence of the main spawning seasons; 5) in an effort to identify important seed collection areas, we are studying the local hydrographic patterns, and their relation to major seasonal changes of climate. In addition, we have built a small hatchery for artificial seed production, which will prove useful due to possible variability in spat settlement. These studies will result in a recom-

mendation to the government of Colombia regarding the biological feasibility of pearl oyster cultivation.

GEMMOLOGY OF THE ABALONE AND OTHER GASTROPOD PEARLS. Grahame Brown, ALLGEM Services, 14 Allamanda Cres. Albany Creek Q 4053 Australia.

The pearls of commerce—nacreous aragonitic and/or calcitic concretions, of shell-like composition, secreted by specialised mantle epithelial tissues of the molluscan pearl sac—have traditionally been considered to be products of a few species of nacre lined fresh or salt water bivalves that belong to the Pelecypoda class of phylum Mollusca. Commercial pearl-producing bivalves are popularly termed pearl oysters or pearl mussels. In nature, pearls form in these bivalves as either free (whole), or attached (blister) pearls.

Gastropod and much rarer cephalopod pearls are less well known in both commerce and fashion. These quite unique univalve or 'sea snail' pearls are often non-nacreous, with smooth external surfaces either attractively coloured or aesthetically patterned. Both gastropod and cephalopod pearls are also secreted within mantle tissue-derived pearl sacs.

Although gastropods represent about 80 per cent of all living molluscs, pearl-producing species of the class Gastropoda are few—being restricted to those families of marine snails of the subclass Prosobranchia that secrete a calcareous shell. Gastropod pearl-producing molluscs can be further subdivided, on the basis of their evolutionary document, into three orders of genera:

- *Haliotis* (abalone), *Patella* (rainbow limpets), *Trochus* (top shells), *Turbo* (turban shells) . . . belonging to the nacre-secreting primitive order Archaeogastropoda;
- *Littorina* (periwinkle), *Strombus* (conch), *Cassis* (helmet shell) . . . belonging to the non-nacreous order Mesogastropoda; and,
- *Murex* (murex), *Xancus* (sacred chank shell), *Melo* (bailer shell) . . . non-nacreous shells belonging to the highest order, Neogastropoda.

Rare non-nacreous whitish cream pearls have also been reported to have been recovered from one species of caphalopod—the *Nautilus pompilius* or chambered nautilus.

In this presentation, the gemmology of abalone, trochus, conch, and bailer shell pearls will be described and illustrated. Those identifying features and properties, that contribute gem-like desirable attributes of beauty, rarity and durability to these gastropod pearls, will be presented for naturally occurring pearls, as well as pearls cultured in the abalone. In addition, methods used either to value-enhance these pearls, or to imitate them, will also be discussed. Available historical evidence, supporting recovery of extremely rare pearls from limpet, turban, periwinkle, helmet, murex, sacred chank, and nautilus shells will also be presented.

"Since pearly concretions partake of the characteristics of the shell within which they are formed, it follows that practically all species of mollusks whose shells have a well developed nacreous lining yield pearls to a greater or lesser extent. But the number of these species is small." (Kunz and Stevenson 1908).

CONTRIBUTION TO THE KNOWLEDGE OF THE DYNAMICS OF POPULATION OF THE BLACK PEARL OYSTER IN FRENCH POLYNESIA. Nathalie Cheffort-Lachhar, O.R.S.T.O.M. Papeete/Brest, P.O. Box 2089, Papeete, Tahiti.

Four atolls with different levels of pearl farming exploitation and geomorphology were investigated. Several dives were made in 1990 to estimate the density and size frequency distribution. A tagging procedure enabled us to estimate the growth and mortality coefficients.

COMMERCIAL HARVEST OF FRESHWATER MUSSELS IN THE UNITED STATES. Nelson Cohen, SEA, INC, Shell Exporters of America, PO Box 235, Terre Haute, Indiana 47808-0235.

The shell exporters of America, Inc. is an organization of the major exporters of freshwater shell in America. We have organized to be better able to follow the harvesting of the shell, to help in the preservation of this resource, and to assist the various state and federal agencies in their attempt to manage the freshwater shell and to eradicate the zebra mussel. The exporters banded together in this organization to better manage the resource and to sustain the industry's survival here in the United States, and also to assure our customers overseas that they will have a reliable source of this raw material. This industry in the last 15 years has become a major factor in the economics of many states with commercial fisheries. In many cases, the dollar volume now exceeds the dollar volume of commercial finfishing in these states. For these reasons and for the employment of approximately 10,000 persons in the commercial musseling industry, it is imperative that we develop a proper concept of shell harvesting management with all the agencies concerned. This will assure the ability of the resource to be harvested on a managed and sustained basis, giving employment to those involved, allowing ongoing research for the mussel populations and to maintain our ability to generate dollars for the United States in the international trade market.

EVALUATING PEARL SHELL HABITAT IN TORRES STRAIT AND THE ARAFURA SEA. Kathy Colgan, Bureau of Resource Sciences, John Curtin House, P.O. Box E11, Queen Victoria Terrace, ACT 2600, Australia.

Stocks of pearl shells have declined markedly in the Torres Strait and the Arafura Sea. Extensive surveys of historically important pearling beds were carried out in 1989. Environmental factors associated with presence/absence and abundance of pearl shell were monitored and relationships modelled.

QUEEN CONCH PEARLS—A UNIQUELY CARIBBEAN GEM. R. Leroy Creswell[1] and Megan Davis-Hodgkins[2], [1]Harbor Branch Oceanographic Institution, Inc., 5600 US Highway 1 North, Fort Pierce, FL 34946, and [2]Caribbean Marine Research Center, 805 46th Place E., Vero Beach, Florida 32963.

The queen conch, *Strombus gigas*, is an important fisheries resource and an icon of the Caribbean culture. This large marine

gastropod produces a porcelainous pearl that is variable in shape from extremely baroque to very symmetrical, and ranges in color from beige, to yellow or pink; dark pink pearls are the most sought after. Conch pearls are sometimes enhanced by a characteristic called "flame structure," which has been described as having the appearance of watered silk. The deep, rich color of the conch pearl, in conjunction with these "flame structures" makes these unusual gems very attractive.

Unfortunately, the occurence of natural conch pearls is extremely rare—less than one in 10,000. A commercial supplier in the Lesser Antilles recovered only four large (5 to 10 carat), and a few dozen smaller pearls, from over 54,000 conch. In addition, only about 10% of the pearls found can be considered gem quality. But the rarity of conch pearls, and its unique linkage to Caribbean culture has enhanced their mystic. During the late 1800's and early 1990's, conch pearls played an integral role in fashioned jewelry of the Edwardian style, and they are found in the Crown Jewels of England. There has been a dramatic upsurge in interest for conch pearls during the past 10 years. A 17-carat oval conch pearl sold for $12,000 at a Paris auction in 1984, and in 1987, a 6.41-ct fine conch pearl, unmounted, sold at auction for USA $4,400 at Christies, London.

The beauty, rarity and unique marketing niche of queen conch pearls create an attractive opportunity for their cultivation. Preliminary research conducted by the authors indicates that queen conch can be easily anaethetized and the mantle manipulated for making surgical implants. Concretions of shell around implants appear to occur at a rapid rate, consistent with shell development of adult queen conchs. Finally, the benthic feeding strategy of the queen conch is conducive to adding pigments to formulated feeds to control and enhance pearl coloration.

There is a unique opportunity to develop a culture pearl industry in the Caribbean. Wild stocks of conch are plentiful in most locations, they are hardy and relatively easy to maintain, and the conch pearl can have a unique market niche by targetting tourists who wish to take home a symbolic memory of their Caribbean experience.

DEVELOPMENT OF THE PEARL CULTURE INDUSTRY IN INDIA. Daniel S. Dev, Pearl Culture Project, Tamilnadu Fisheries Development Corporation, 95A Kennedy Street, Nagercoil, Tamilnadu, Pin. 629 001, India.

Topic discussed includes: collection of pearl oysters (*Pinctada fucata*) from the wild; production of spats in the hatchery for commercial farming systems; culture of pearl oysters in different ecosystems; operation methods and management; harvest, pearl drilling, processing, and stringing; and marketing practices in India.

COMMERCIAL PRODUCTION OF INDIAN PEARL OYSTER (*PINCTADA FUCATA*) SPATS IN HATCHERY. Daniel S. Dev, Pearl Culture Project, Tamilnadu Fisheries Development Corporation, 95A Kennedy Street, Nagercoil, Tamilnadu, Pin. 629 001, India.

This paper discusses the infrastructural facilities for a commercial pearl oyster (*Pinctada fucata*) hatchery, induced spawning techniques, mass production of micro-algal feed for the larvae, open-mixed culture of algae for spat rearing and nursery rearing in farm implantation.

ON THE INDIAN PEARL OYSTER RESOURCES & CONSERVATION. Daniel S. Dev, Pearl Culture Project, Tamilnadu Fisheries Development Corporation, 95A Kennedy Street, Nagercoil, Tamilnadu, Pin. 629 001, India.

The paper discusses the history of the Indian pearl fishery; the present status of pearl oyster resources of different species and their suitability for pearl production; replenishment and conservation of pearl oyster beds; and ecological factors influencing the oyster beds and oysters.

INFESTATION OF THE PEARL OYSTER BY THE BORING AND FOULING ORGANISMS IN THE PEARL CULTURE FARM AND NATURAL BEDS FROM NORTHERN COAST OF PERSIAN GULF. M. S. Doroudi, Persian Gulf Shellfish Fisheries Research Center, P.O. Box 1416, Bandar-e-Lengeh, Iran.

Infestation of the pearl oysters, *Pinctada margaritifera* and *Pinctada fucata*, by the fouling and boring organisms in the pearl culture farm and natural beds have been studied from January to November 1993. Barnacles, spat of edible oysters and tubiculous polychaete were major fouling organisms in the pearl culture farms. In the natural beds, however, sponges, algaes and ascidians were considered to be the main fouling organisms. The boring sponges, *Cliona vastifica*, *Cliona margaritifera*, *Cliona carpenteri* and the boring mussels *Lithophaga hanlyana*, *Lithophaga malaccana* were the most important boring organisms among studied species, causing considerable damage to the shells. Pearl culture farms indicated more infestation than the natural beds. The pearl oyster, *Pinctada margaritifera* farmed in Kish Island were found to be the most affected. The maximum rate of mortality of the pearl oysters was apparently related to the invasion by predators. The relationship between frequency of cleaning and growth of the pearl oysters also was investigated during this study. The oysters were divided into four groups and cleaned after every 23 + 2, 45 + 2, 90 + 2 and 180 days, respectively. Statistical analysis indicated no significant difference in growth rate between the various experimental groups for a period of 6 months. There is a significant difference, however, in growth rates between the summer and winter seasons.

ABALONE PEARLS: PAST, PRESENT AND FUTURE. Peter V. Fankboner, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6

Natural abalone pearls have a history which extends to at least 8th century Japan, and the data resulting from recent archeological digs in California suggest that natural abalone pearls may have been used as trade items by aboriginal peoples over 7000 years

ago. The first recorded reference to abalone pearls occurs in one of Japan's oldest historical writings, the *Kojiki*, and in the ancient city of Nara, large, bluish, crescent-shaped abalone pearls appear in the crown of the Buddhist Goddess of Mercy (installed in 748 A.D.) located in the Sangatsu-do of the temple Todai-ji.

Tooth or tusk-shapes are a distinctive characteristic of many natural abalone pearls collected within the mollusk's reproductive organs, and this configuration likely reflects the physical limitations imposed by the horn-like tip of the abalone gonad. Other shapes occur in natural abalone pearls including ovoid, spherical and baroque forms. The largest known natural abalone pearl is the "Big Pink," a 470-carat baroque pearl owned by Californian Wesley Rankin. This pearl has been valued in the United States at $4,700,000.

It is evident that the development of natural abalone pearls follows the pattern of natural pearls found in the soft tissues of other Molluska; to wit, the stimulus for pearl formation is linked to the mistaken infection of the abalone's soft parts by a swimming cercaria larva of a parasitic flatworm. The invading worm larva is from a species which has evolved a parasitic relationship with a host of other than the abalone it has infected. When the worm enters the soft parts of the abalone, it is recognized by host tissues as material foreign to the abalone. The abalone's defense systems (mostly undifferentiated cells and amoebocyte blood cells) may react to an invading cercaria by entombing this larval flatworm in concentric layers of nacre. By so doing, a natural abalone pearl is formed. Invasive parasitic worm larvae likely originate from the feces of coastal sea birds. It is suggested that as coastal habitat for migrating sea birds becomes lost to development and other causes, the frequency of cercaria infections of wild abalone will decrease. As a consequence, fewer natural abalone pearls may be available in the future to the market place, and this situation may drive the already high price of natural abalone pearls higher.

Cultured abalone pearls are of far more recent origin. At Roscoff's Laboratory during the late 1890s, the French scientist Louis Boutan successfully cultured spherical pearls in the abalone *Haliotis tuberculata* using a technique (nucleus on the end of a hair or wire) similar to that used by the famous Swedish biologist Carl von Linnaeus during the mid-1700s to produce pearls in freshwater mussels. Bouton went on to successfully culture small blister pearls in abalone, but it was not until the pioneering work of the Japanese scientist Dr. Kan Uno, during mid-1950s, that any remarkable advances were made in the technology of cultured abalone pearls. It was Dr. Uno who realized that in order to precisely position (the site of optimal nacre deposition by the shell mantle) and to stabilize a mabe nucleus against dislodgement by the abalone's powerful foot muscle, an opening had to be milled through the abalone shell through which the nucleus could be positioned and cemented in place. In the course of his investigations at the Tateyame Fisheries Station in Japan, Dr. Uno was very successful in his culture experiments on growing semi-spherical pearls in several abalone species including *Haliotis discus*, *Haliotis gigantea* and *Haliotis seiboldii*. Thirty years later, Mr. W. H. Jo of Korea Abalone Pearls began producing keshi (graft-nucleated) free pearls within abalone mantle tissues, and the author, working at the Bamfield Marine Station in British Columbia, developed the biotechnology (now described in 56 patent claims) for commercial production of both blister and free pearls in several abalone species. During 1986–1987, nucleus implants by the author in the pinto abalone *Haliotis kamtschatkana* led to the culture of the first gem quality marine pearls to be produced in North America. Abalone pearls are currently being cultured in Canada, the United States, and Korea. In addition, there is interest and/or research in abalone pearl culture technology occurring in Australia, China, Greece, New Zealand, and South Africa.

The use of hatchery produced abalone versus wild abalone stocks insures consistently higher nacre quality and success rates in producing gem quality cultured abalone pearls. In California alone, there are over 25 permits issued for abalone culture and the largest growers may inventory nearly 1,000,000 adult abalones. Species currently under intensive cultivation in California include the red abalone *Haliotis rufescens* (the largest abalone species in the world), and the green abalone, *Haliotis fulgens*. When transplanted to the cooler coastal waters of British Columbia, Canada, *Haliotis rufescens* still retains a very high rate of growth and the production of high quality nacre; in both Canada and the United States, this abalone has proved to be the species of choice for culture of abalone pearls. Current wholesale prices on grade C to grade AAA cultured mabe (10 mm–14 mm) abalone pearls typically range from $25 to $300 (U.S. dollars).

Whether of natural or cultured origin, abalone pearls express an orient which may include unique multihued tones of silver, orange, pink, green, blue and lavender. Natural abalone pearls of gem quality are very rare, and consequently costly. Abalone are technically difficult to nucleate, and recent mastery in producing gem quality abalone pearls owes its emerging success to unique implantation methods, modern nucleus materials, superb adhesives, flourishing abalone hatchery production and the perseverance of culturists.

PERSPECTIVES AND OPPORTUNITIES OF PEARL OYSTER CULTURE DEVELOPMENT ON THE COAST OF SONORA, GULF OF CALIFORNIA, MEXICO. Sergio Farell, Douglas McLaurin, and Enrique Arizmendi, AP 484, Goaymas Sonora, Mexico CP 85400

The potential of pearl oyster (*Pinctada mazatlanica* and *Pteria sterna*) culture in Mexico has been mentioned by various authors.

These two species inhabit the Sonora coastline in the Gulf of California, as a part of their natural distribution. In the 1950's there was a huge pearl oyster fishing effort done by the Seri Indians, especially on Tiburon Island and other pearly oyster banks along the Sonoran coast and consequently both species became overexploited. The general objective of this paper is to present preliminary results and advances on experimental spat collection

and hanging culture of the mother-of-pearl, *Pinctada mazatlanica* and the mother-of-nacre, *Pteria sterna*.

Pearl oyster spat is collected in experimental mesh bags, used for scallop spat collection. The seed is placed in a nursery system consisting of plastic trays and pearl nets, and later transferred to lantern nets hanging in a long line system at a depth of 2.0, 2.5 m. Preliminary spat collection results show that an average of 220 *Pteria sterna* spat per collector can be achieved in the Fall season. The growth rate (in height) for this species was 9.48 mm/month, from 6.63 mm seed to 45.57 mm in 4 months. Intermediate growth of *Pinctada mazatlanica* has been from 6.88 mm seed to 22.20 mm in a 4-month period. The results of these experiments are preliminary, but clearly show that both species grow rapidly in suspended culture. The culture of these two species is important from a commercial point of view, and they offer good possibilities for promoting pearl culture in the Gulf of California, but further research is still needed.

The culture of the pearl oysters, *Pinctada mazatlanica* and *Pteria sterna* on the Coast of Sonora, Gulf of California, Mexico, shows a good potential as preliminary data demonstrates. Spat collection and growth in the hanging culture for these species are promising. The culture of these two species is important from a commercial point of view.

HAWAII'S IMPACT ON THE INTERNATIONAL PEARL INDUSTRY. Richard Fassler, Aquaculture Development Program, State of Hawaii Department of Land and Natural Resources, 335 Merchant Street, Honolulu, HI 96813.

The pearl oyster, *P. radiata*, occurs in close-in waters in Hawaii. Pearl Harbor, for example, was reputed to have an abundant supply of "pipi," which the early Hawaiians used for food. The deeper water *P. margaritifera* was also in abundance, especially in the northern Hawaiian island region. These oysters yielded few pearls, but the Hawaiians utilized the mother-of-pearl for implements, such as fishhooks.

Commercial pearling in the Island was initiated in 1927 and 1928 when an American fisherman harvested 100 tons of *P. margaritifera* from Pearl and Hermes Atoll, 1100 miles northwest of Honolulu. Concern over the possible depletion of the resource led to a joint State/Federal commission in 1930, which surveyed the oyster throughout the islands. The members concluded that conservation measures should be initiated, and these have remained to this day.

Modern pearl farming in the state has been impeded by environmental constraints, which have made utilization of the open ocean extremely difficult. Therefore, efforts have been directed to on-land operations. These have occurred at the Natural Energy Laboratory Authority of Hawaii (NELHA) site at Keahole Point, on the Big Island.

In 1990, Hawaii Cultured Pearl, Inc. attempted to raise the Japanese pearl oyster, *P. fucata*, by culturing algae in tanks and feeding this algae to oysters in raceways. A lack of success led to a halt in this experiment. Efforts have been made to resume operations.

The next attempt to raise pearls in Hawaii focused on utilizing freshwater lakes and reservoirs to raise American mussels. In 1992, Cross-Pacific Pearls of California was investigating this possibility, and applied for permits to import various species of mussels, but financial problems terminated the firm's plans.

In 1992, Black Pearls, Inc. developed hatchery methods for the Hawaiian blacklip pearl oyster, *P. margaritifera galtsoffi*, and is now examining the feasibility of commercial pearl culture in land-based or ocean-based systems in Hawaii. In addition to establishing commercial culture techniques, the company is testing methods for a stocking program to help re-establish the Hawaiian pearl oyster.

Black Pearls, Inc. is also using their hatchery technology to supply spat to other South Pacific islands. Black-lip pearl oysters from the Marshall Islands have been spawned, and the larvae successfully raised to settlement in the deep-OTEC water available at NELHA. Use of this pathogen-free water ensures that quarantine concerns are met. Spat recently returned to the Marshall Islands have shown good growth and survivorship.

The Black Pearls, Inc. effort has significance for islands and atolls in the South Pacific, like Namdrik, which are lacking a major source of mature oysters. Moreover, other areas that are rapidly depleting their oyster resource, may need to rely on firms, like Black Pearls, for future supplies.

In 1993, the Biosystems Engineering Department of the University of Hawaii, began experiments with raising algae to feed to *P. margaritifera* for on-land oyster culture. The successful production of three key species of diatoms directly from an ocean intake of seawater, without having to maintain expensive laboratory cultures, has pointed to cost-effective land-based culture. The University is attempting to repeat this success with *P. maxima*.

The University's experiments may lead to pearl culture on atolls or islands, like the Hawaiian Islands, or even in Mainland locations, where access to the open ocean is either difficult or impossible.

Perhaps Hawaii's most valuable contribution to the world pearl industry is *Pearls '94*, the largest and first truly international gathering of pearl farmers, researchers, government officials, jewelers and equipment suppliers. As originally conceived in 1991, the meeting would bring aquaculturists together to discuss ways to accelerate pearl farming in the South Pacific. In subsequent years, strong interest from other areas of the world considerably expanded the scope of the gathering. More than thirty nations will be represented in Hawaii.

Pearls '94 is expected to have a profound impact on international pearling by offering important opportunities, which include: 1) stimulating sales, through identifying and solving industry problems; 2) creating marketing strategies; 3) disseminating information on the latest farming techniques; 4) encouraging investments in farms; 5) heightening awareness of quality control; 6) informing pearl producers and buyers of the most recent develop-

ments in pearl production throughout the globe; and 7) formulating plans for future international pearl conferences.

CONCEPT OF THE SOUTH SEA PEARL AND ITS FUTURE FROM LESSONS OF THE PAST. C. Denis George, P.O. Box 5811 Cairns, Qld. 4870 Australia

The pearls from the South Seas are a later addition to the ones since antiquity from the legendary pearl fisheries of Arabia, India, the Americas and elsewhere. With the exploration of the Indo-Pacific region, the much larger pearls discovered attracted more admiration—especially the black ones from Polynesia. As the fisheries were declining, advances in the alluring mystery of pearl-formation were promising new horizons in reproducing them at will.

After the ingenious Chinese pearly-images and the Linneous pearl in 1893, W. Saville-Kent published a half-pearl he had developed earlier, created an impetus with his first South Sea pearl farm in 1906 and a Royal Commission, he created a round pearl in situ. In 1894, K. Mikimoto made his first half-pearl and by-passed the controversy on the origination and Saville-Kent's influence. The fact is that by 1920 the Japanese mastered the cultivation of a pearl when Australia, with better resource potential and which had started to develop it, outlawed it as illegal.

Dr. Sukeyo Fujita, in visualising a better pearl, after years of trials, by 1928 achieved it at Celebes. By 1932, the Japanese initiated six pearl farms at Palau and an industry started. The concept of the South Sea pearl was created:

"A pearl equal to the natural one but at a ¼ of its value and, with a nucleus of ⅓ only to the overall diameter".

The standard was maintained in the post-war ear with the renown Burmese pearls until the late 60s, when Japanese in Australian joint ventures flooded the market with inferior pearls selling them as cheaply as $10.00 each. An all-around catastrophe took place, and a woman's "beloved-pearl" was devalued to her dismay.

There were similar crises: repetitive production calamities from continuing shell mortalities; a quality decline; uncontrollable production increases with deficient approach; whatever was produced was sold for as much as possible; new aspiring producers looking for profit; the over-priced thin-skin-large pearl; lack of overall coordination and expertise guidance. There was, then, an overall decline of the concept of the South Seas Pearl, with looming calamities and an uncertain future.

TRIBULATIONS OF PEARL CULTIVATION IN AUSTRALIA. C. Denis George, P.O. Box 5811 Cairns, Qld. 4870 Australia

At the termination of the Pacific War, Australia declared the Japanese pearl technique as a war reparation and decided to establish a pearl industry for Australians. The obstructive pearl law was repealed, aspiring farmers by 1956 made pearls and Australia, with an ideal ecology and a vast pearl-shell resource, was on the right track of developing a national pearl industry.

The Japanese, fearful of "Australia's Declaration," reacted with the 1952 Overseas Pearl Law stipulations. For example, a Celebes farm in 1952 was confiscated for exploiting. A Burmese Joint Venture in 1954 was also confiscated in 1965 for exploiting. In aiming at Australia and by maneuvering to placate anti-Japanese sentiments with "promises of good will," in 1956 a joint venture started in Kuri Bay—under the stipulations of the Japanese pearl-law.

Nineteen months later, Kuri Bay stopped the pearlers taking the shell by bankrupting them. Yet, unknown to Australia, button manufacturers were going bankrupt by shell shortages; the Japanese Arafura pearlers also went bankrupt and their 26 boat fleet was bought by the Kuri Bay; in 1960, the Japanese Government, in breach of their own law, granted illegally a second pearl licence to Kuri Bay—on behalf of the Arafura Fleet. Kuri Bay pearl production expanded enormously and the "plastic button" is generally still blamed as the sole culprit.

As the Australian farmer was resisting the Japanese policy in 1960 the Japanese Government, in abusing their own law ("One Joint Venture for each country"), rushed another six ventures to snowball him, and after six years of a fierce obstruction, he was eliminated. In the ensuing 17 years to 1983, the Japanese policy resulted in:

A. Japanese: Free-for-all exploitation of Australia's resources and in breach of the Japanese pearl-law limit of the 30,000-shell yearly quota, by exporting 1,500,000 shells—a vast quantity of pearl shells—to Japan at US $27,000 a kilo—in disregard of sizes and quality. Pearl shells were dying by the millions. By 1970, the Australian partners were short-changed and bankrupt and in 1983, Kuri Bay shut down. Australian farms sold cheaply and became 100% Japanese in breach of the Fisheries Acts. And, after *38 years of pearl-making,* Australia is still dependent on Japanese technicians, etc.

B. Australian Farmer: In between starting farms and losing them, the author motivated pearl awareness by television documentaries, round pearls and the training of the first two Western Australian farmers; gradually Indo-Pacific farmers have emerged.

JAPANESE PEARL POLICY LAW FOR OVERSEAS PEARL CULTIVATION; ITS IMPLEMENTATION AND EFFECTS ON THE INDO-PACIFIC. C. Denis George,

In the post-war reconstruction of the Japanese pearl industry by the Allied Powers, the Japanese Government enacted the "Pearl Raising Industry Law" No. 9 of 1952, particularising all components essential to promote their pearl industry: from mother-shell to pearl sizes, rafts and guidance; research, finance, inspection and exports; a Pearl Council for planning, etc. It was a unique law for development, yet with the ever-increasing pearl targets (e.g. 100 tons for 1962, but harvested 120 tons mostly of poor quality pearls) the law would cause a decline of the "Pearl Standing," repetitive production calamities and severe marketing crises.

Because of the Japanese Law's aim to "Secure reputation of

their pearl by '*checking*' unhealthy development in the industry," and, because of their precept as "Originators of the pearl-principle," other countries were not entitled to make a pearl. Japanese went too far by legalising "means and method," that is, how to stop other pearlers and pearl-farmers and take their pearls for their own end.

The overseas policy, known as "The Three Principles," dictated: *1*. Techniques secretive to Japanese; *2*. all pearls produced to be exported to Japan; *3*. production amount regulated by Japan.

To round out their control, the policy also dictated a "Secret obligation to be undertaken privately" by the overseas partners in joint ventures and as it was expressed by Hatsuro Sonahara of the Japanese Fisheries Agency in February 1958 (Special Report of Fisheries Agency "The Pearl," p. 83 Clause V), to the effect:

"Overseas partners to make their utmost effort to find ways and means to stop their rival local pearlers and farmers".

As it happened in the well-documented events of Australia since 1956, the Australian partners adhered to "The Three Principles" and in executing their secret obligation, bankrupted Australia's pearling industry in 1958. The original Australian farm was forced to become a Japanese one and for the ensuing 15 years to 1978 not one Australian could start a farm without a Japanese partner.

After Japanese law fulfilled its objective and was discarded as of no further use, the aftermaths continue in various forms to suppress true pearl development of Indo-Pacific farmers: "As entity of their own, and for their own best benefit and of their future."

ECOLOGICAL CHARACTERIZATION OF THE TONGAREVA LAGOON. Maria Haws, Ben Ponia, Daniel Cheney, and **Hugh Thomforde,** RDA International, Inc. % TMRC, Ministry of Marine Resources, Omotea, Tongarea, Cook Islands.

The ecological monitoring program has three objectives—1) to collect baseline data on the physio-chemical and biological parameters needed to form a database containing information on the hydrological and biological processes of the Tongareva lagoon, 2) to collect data relevant to oyster culture to benefit development of management plans, and 3) to monitor possible environmental impacts of farming or other human activities. Baseline water quality data was collected over the year prior to the start of intensive pearl farming. Periodic sampling included measurements at 40 stations around the lagoon, at both shallow and deep depths, of temperature, salinity, pH, dissolved oxygen, orthophosphate, silicates, ammonia, total dissolved nitrogen, chlorophyll, total organic carbon, and total dissolved phosphorus. The values obtained are typical of a tropical coral atoll with oceanic water exchange. Values for most parameters were highly variable throughout the lagoon and no spatial trends were detected. No indications of deleterious human impact were found. None of the values were suggestive of nutrient loading or eutrophication. This database will serve as a reference to assess environmental changes associated with farming and human activities in the future. Several patch reefs were surveyed for an assessment of coral type and fish abundance/diversity.

A stock assessment was conducted to estimate the standing stock of the *Pinctada margaritifera* population. It also established permanent sites to monitor the mortality and recruitment rates of the pearl oyster fishery. Total standing stock for the lagoon was estimated at 2 to 3 million oysters. Alternatives to collection of wild stock for farming purposes, such as spat collection and hatchery production, will be emphasized since these will reduce fishing pressure on the wild stock in the future.

Experiments were conducted to evaluate the feasibility of using spat collectors to obtain oysters for farming. Results to date are inconclusive and spat collection trials continue.

Oysters were collected for histopathological examination in 1992 and 1993. The *P. margaritifera* population appears to be generally healthy with no prevalent pathogenic or parasitic infection.

GROWTH AND MORTALITY OF *PINCTADA MARGARITIFERA* IN FRENCH POLYNESIA. Andre Intés, Institut Francais de Recherche Scientifique pour le Developpement en Cooperation (ORSTOM), Centre de Brest, B.P. 70.29.280, Plouzane, France.

In the early 80's, pearl culture was still exclusively dependent on natural populations prosperity and also providing livestock for farms. At least 70% of pearl oysters were caught by skin diving and the remainder came from the rearing of collected spat. But most of the required biological parameters were lacking to promote new management of these natural stocks: reproduction, growth, mortality, stock assessment.

To assess growth and mortality, among the most important parameters of the population dynamics, a tagging experiment was initiated. This species is particularly suitable, as in theory, individuals can be measured when wished. Individuals (505) were tagged from April 1983 to April 1984, and only 37 were present and still alive in June 1987.

The data collected over 4 years could have provided very valuable information on growth and mortality if some unpredictable climatic and biological events had not occurred.

During the late 1982 and early 1983, six hurricanes struck the Tuamotu archipelago, destroying most of the shallower bottoms and pearl farms. Two years later, from mid-1985 to 1986, high mortality affected both the farmed and natural populations, but no evident explicative factor, such as disease of hydrological perturbation, could be taken into account. As the mortality developed mostly in the more pearl productive lagoons, an overload of the carrying capacity was considered as a hypothesis. The year following the hurricanes, stock reproduction was exceptional and natural recruitment combined with rearing of the collected spat could have enhanced the biological trophic demand to an unsustainable level for the ecosystem.

Observing growth, the monthly mean length increment for individuals greatly decreased during the year with maximal mortality, but recovered prior levels at the end of the experiment. Examining growth of age classes, it seems that the maximum length increments do not occur during the same period for adults over three years old and for juveniles. Most of the trophic energy captured by adults is used in the maturation of gonads from January to March, when the juvenile growth rate is highest.

RESEARCH FOR WILD STOCK MANAGEMENT OF *PINCTADA MAXIMA*. L. M. Joll, W. A. Marine Research Laboratories, P.O. Box 20, North Beach, Western Australia 6020

Although the Western Australian fishery for the silver-lipped pearl oyster *Pinctada maxima* has a history going back to the 1860's, there is little published information on the biology of the species. A research programme has been underway since 1989 to examine the status of the wild stock and to provide a sound understanding of the reproduction, growth and recruitment of wild stocks.

Examination of the reproductive cycle over a large part of the species' range in Western Australia showed that, in the Broome/Eighty Mile Beach area, there was an extended period of gonad development with at least two spawinings (early and late summer). At Exmouth, near the southern end of the species' range, there was a shorter period of gonad development, with a single spawning in mid-late summer.

Natural growth rates of spat have been derived from spat caught on artificial collectors as well as from shifts in modal frequencies. Mark and recapture techniques have been used to examine the growth of larger (>30 mm DVL) animals. Over 4000 pearl oysters have been tagged and released in an area of 80 Mile Beach, with over 2000 recaptures or re-recaptures over a four year period. The growth rate data indicate that, in the main Eighty Mile Beach area, pearl oysters are about three years old at the legal minimum size of 120 mm.

Measurement of recruitment has been attempted using artificial spat collectors and the examination of catch rates of 'piggy-back' shell (spat attached to adult pearl oyster shell). However, the artificial spat collectors trialled were found to only have a low efficiency for collecting spat or, alternatively, were poorly located for spat settlement. The examination of catch rates of 'piggy-back' spat is a potentially more useful technique for the measurement of recruitment, as it has the capacity to integrate settlement events over a much wider area than spat collectors. However, high variability in 'piggy-back' spat rates means that large numbers of adult pearl oysters must be sampled to reliably differentiate between real changes in overall recruitment levels and random events.

Fishery data detailing the catch, effort and location of the vessel for every dive have been provide by all vessels in the fishery since 1979. These data show that CPUE remained relatively steady over the 1980's, at about 20 shells per diver hour, but that in the early 1990's catch rates increased by about 50%. Although there have been technological changes over the the period, the recent increase in CPUE appears to be the result of a real increase in the abundance of culture-sized pearl oysters rather than an increase in vessel or diver efficiency. Analysis of the fishery data indicates strongly that the Western Australian stock of *P. maxima* is being exploited at a level less than or equal to the production of the stock.

SOME RESULTS OF FRESHWATER PEARL CULTURE IN WEST LAKE (HANOI, VIETNAM). Phan Ngoc Kim, Ngo Thi To and **Vu Ngoc Tuan,** Ho Tay Fishery Development Investment and Exploitation Centre, 79 Yen Phu, Hanoi, Vietnam

Freshwater oysters are usually used to produce non-nucleated pearls in various countries of the world. In Vietnam, the freshwater oysters, *Hyriopsis cumingii (Lea)*, *Cristaria bialata (Lea)* and *Anodenta jourdyi (Morlet)*, are being reared to produce nucleated pearls.

The implantation of nuclei into the mantle of the *hyriopsis cumingii (Lea)* oyster has brought the best results.

Hyriopsis cumingi (Lea) is only distributed in some of the rivers in the north of Vietnam, such as the Cau River, Thuong River, Chau Giang River, and Day River. The bottom of these rivers is mud-sandy, with a slow flow of water. The oyster lives in the river's bottom at a depth of 4 m to 15 m. Its growth rate is slow, as it takes from 3 to 4 years to reach 14 cm to 16 cm in length, with a weight of 120 gr to 140 gr. However, this oyster grows rapidly when reared in a lake or fishpond where abundant phytoplankton is available. The shape and color of its shell can be changed slightly.

HoTay Lake (West Lake), located to the north of Hanoi City, has an area of 413 ha, and an average depth of 2 m, with a muddy and mud-sand bottom. The lake's water is supplied by rain water and waste water from the city. Four 5 mm to 6 mm nuclei were implanted in an oyster at the bottom of its mantle. These nuclei were manufactured from the shell of *Lamprotula* species. They were then suspended on rafts, and 40 days later, dead and nuclei expelled oysters were discarded. In a 1993 experiment, the survival rate of the implanted nuclei was 57.7%, with a mortality rate of 6.8%.

By means of the selection and treatment of the graft tissue, it is possible to change the color of the pearl to: pink (60%), gold (20%), white pink (10%), and cream (10%).

The pearl culture of Vietnam is only at a starting point, but we feel that the results of pearl culture of *Hyriopsis cumingii* in lakes or fishponds has good potential for creating a new industry for rural areas of Vietnam. Essential is the marketing of Vietnam's freshwater pearls to the rest of the world.

NATURAL ABALONE PEARLS. Sarabeth Koethe, KCB Pearls, P.O. Box 1737, Santa Monica, CA 90406-1737.

The paper presents an in-depth history of the Pacific Coast natural abalone pearl—distribution, statistical data, etiology of pearly rariety and future implications of current population decline. The discussion will also include examples of color, and type

and size of natural abalone pearls, with emphasis on those specimens particularly suited for the gem industry.

TRENDS AND DEVELOPMENT OF THE PEARL OYSTER INDUSTRY IN THE PHILIPPINES. Daisy F. Ladra, Bureau of Fisheries and Aquatic Resources, 860 Arcadia Bldg., Quezon Ave. Q.C., Philippines.

The Philippines has substantial areas that support the favorable growth of pearl oysters. Despite the availability of several species of pearl-producing oysters, including freshwater mussels, the pearl industry has focused only on South Seas pearls using *P. maxima*. As of 1991, the industry was valued at $1.4 million, which the Philippines obtained through the export of pearls, live mother of pearl, pearl oyster shells and shell buttons.

Despite the value of the resource to the Philippine economy, the industry is undeveloped as very little information is known about the pearl oysters. Currently, there are 23 pearl farms situated in Mindoro, Palawan, Masbate, Cebu, Tawitawi and Pangasinan. This paper attempts to describe the pearl and pearl oyster industry, its development status, as well as trade and market situation. Traditional farming and pearl culture technology will also be discussed, as well as spat collection practices, utilization and post-harvest handling of pearl oyster products. The income potential of pearl production will be considered, together with prospects for development. Recommendations for the development of the industry will also be presented.

RECENT DEVELOPMENTS IN ARTIFICIAL PROPAGATION OF THE GOLD- OR SILVER-LIPPED PEARL OYSTER *PINCTADA MAXIMA (JAMESON)*. R. G. Lawyer, Palawan Pearl Project, Inc., P.O. Box 878, Manila, Philippines.

The paper discusses recent developments at the hatchery of the Palawan Pearl Project, with specific descriptions of improved induced spawning techniques, spat settlement and grow-out techniques for artificial propagation of the *Pinctada maxima*.

ACIAR/JCU BLACKLIP PEARL OYSTER PROJECT. John S. Lucas, Zoology Department, James Cook University, Townsville, Queensland 4811 Australia

This three year project, 1993–1996, is funded by the Australian Centre for International Agricultural Research. It involves James Cook University, Queensland Department of Primary Industry and the Ministry of Natural Resources, Kiribati. There will also be some collaboration with ICLARM's Coastal Aquaculture Centre, Honiara.

The project is focused on the Republic of Kiribati, a Pacific nation consisting of a series of coral atolls. *Pinctada margaritifera*, through shell and/or cultured pearls, is one of a limited rangeof potential export commodities for this country. However, the pearl oyster stocks appear to be low, either intrinsically or from overfishing. Thus, the overall objective of this project is to build up the *P. margaritifera* stocks in selected atoll lagoons in Kiribati as a means to an appropriate pearl industry.

Initially, pearl oyster stocks will be systematically surveyed to establish in which atoll lagoons they occur and their abundances. Spat collectors on long-lines will be deployed at selected sites in lagoons to determine levels of natural settlement and the potential of settlement on these artificial substrates as a source of pearl oyster stocks.

The alternative source to spat collection is hatchery production of spat, and simplified hatchery methods, suitable to a coral atoll environmental, are being investigated. These are based on the system of flow-through culture with artificial diets that were developed for successful hatchery culture of giant clams. The objectives are to get away from air-conditioned culture rooms and sophisticated algal culturing facilities.

One further aspect of the project is related to the pearl bead insertion process. It addresses the trauma and infections of the operation, and considers how these can be reduced.

The first aspect of this research program, related to pearl oyster stocks and settlement in Kiribati atoll lagoons, is particularly applicable to that country; but if there are significant advances in the other two aspects, low-technology hatchery culture and aspects of bead insertion technology, these will have general application.

POTENTIALS OF FRESHWATER PEARL CULTURE IN THE PHILIPPINES. Virginia S. Luyun, Aquaculturist II, Bureau of Fisheries and Aquatic Resources, 860 Arcadia Bldg., Quezon Avenue, Quezon City, Philippines.

Pearl production in the Philippines has mainly centered in marine pearls. The Philippines has sizable quantities of Freshwater *Cristaria plicata* and *Anadonta* spp. which were accidentally introduced into the country and would be tapped for pearl production.

Freshwater pearl production is currently in its experimental stage where research expenses reached $1,176.50 for 750 pieces of operated mussels. Local production is sourced from Hong Kong, People's Republic of China and Thailand. Demand for freshwater pearl is strong in the domestic market due to the low prices of freshwater pearls and pearl jewelry as compared with South Sea pearls.

This paper attempts to present an overview of the freshwater pearl industry, domestic demand and market prospects in the country.

The results of the experimental pearl culture initiated in the country will also be discussed in the paper.

PERSPECTIVES FOR THE INSTALLATION OF A PEARL CULTURE ENTERPRISE IN BAHIA DE LA PAZ, SOUTH BAJA CALIFORNIA, MEXICO. Mario Monteforte[1], [1]Grantee International Foundation for Science, Centro de Investigaciones Biológicas del Noroeste, P.O. Box 128, La Paz, B.C.S. 23000, México. FAX (112)5.36.25.

Pearl resources in La Paz region have been overexploited since early XVI century, both by international companies and local fishermen. In 1940, the natural populations became almost extinct and a permanent banning was decreed, but furtive fisheries have con-

tinued until today. However, Bahia de La Paz is famous for the richness and high quality of its natural pearls, which are present in many royal treasures, personal collections and museums.

There have been many attempts to cultivate pearl oysters and produce pearls, but the only successful one was that realised by Don Gastón Vives in 1903. He founded a prosperous enterprise, the "Compañia Criadora de Concha y Perla" at Bahía San Gabriel in Isla Espíritu Santo, near La Paz city. This enterprise was known internationally and many pearls were sold by Mr. Vives in Paris and New York. Although the process of pearl oyster cultivation was extensive using an advanced technology, all the pearls were natural.

After the destruction of Mr. Vives' enterprise during the Mexican Revolution in 1914, there have been at least 15 or more different projects on pearl oyster culture and pearl production by grafting and nucleus implanting. All these projects failed or were interrupted and abandoned. So far, no positive results have been obtained.

At present, a research programme carried out in the Centro de Investigaciones Biológicas del Noroeste has developed an efficient technology for the extensive culture of *Pinctada mazatlanica* and *Pteria sterna* and has obtained positive results in the production of fine cultured mabes on both species. Production of round pearls is under study.

This project is feasible at commercial scale and has the advantage of being technologically independent. Several elements favor the possibility of a joint-venture enterprise on pearl production in La Paz.

First of all, the quality of the nacre of both pearl oysters is recognized as being one of the best in the world. Moreover, the new economic politics established in México concerning international investments, the facilities for obtaining territorial concesions and the inauguration of the NAFTA, make pearl production in La Paz a strategic activity.

Our results on pearl oyster culture and pearl production are starting to spread among the public and private sectors. Pearl culture is now being considered as an interesting alternative for regional socioeconomic development, and a joint-venture proposition would be most welcome.

Our figure of a pearl culture center could have an economic potential of up to $5 million U.S. annually from the 4th year of activities, starting with an investment of $1.5 million U.S. which would include the construction of a laboratory and other infrastructure in a site that would be the center of the operations. This benefit comprises the production of mabes only, without considering the aggregate value of jewelry, handicraft with shells and the use of nacre for cosmetology and dermatology. Besides, we have detected at least 15 other sites in Bahia de La Paz where additional "standard" pearl farms could be installed.

On the basis of our technology, a "standard" farm could produce a steady annual production between 120,000 and 300,000 mabes under a scheme with large margins of security in spat collection, mortality, mabe incidence, etc. We are estimating a price between $15 and $20 U.S. per piece, which we believe to be more than realistic, taking into account the quality we are able to attain.

Nevertheless, a potential pearl culture enterprise must consider the recovery and conservation of natural populations through aquaculture and repopulation activities and support research studies. The resource is fragile and scarce and will not resist long and massive grafting operations. Since both species are under legal protection, projects involving the management of wild individuals cannot be sustained.

SPAT COLLECTION, GROWTH AND SURVIVAL OF PEARL OYSTER *PTERIA STERNA* UNDER EXTENSIVE CULTURE CONDITIONS IN BAHIA DE LA PAZ, SOUTH BAJA CALIFORNIA, MEXICO. **Mario Monteforte,**[1] and **Cynthia Aldana,**[2] [1]Boursar International Foundation for Science, [2]Universidad Autónoma de Baja California Sur, Centro de Investigaciones Biológicas del Noroeste, P.O. Box 128, La Paz, B.C.S. 23000, México. FAX (112)5.36.25.

The present work resumes the experiences on spat collection and extensive culture of *Pteria sterna* since winter 1988. Because of repeated accidents and operative obstacles, the research sequence has suffered interruptions and several experiments were restarted. The general results for each phase of the culture process are the following:

1) Spat collection: the analyses and comparisons of spat yield were performed in control collectors (square bags 30 × 30 manufactured with black plastic screen 2 mm mesh, filled with gillnet). So far, three stations have been tested for spat collection of this species. Bahía Falsa, Isla Gaviota and Caleta El Merito, all of them are located at the continental coast near La Paz city. Gaviota and Merito have given the best results.

Spatfall of this species was generally continual during all the years but showed erratical patterns in its start, duration and abundance. Annual averages in control collectors during the main season (winter) have varied from 35 in to 120 individuals per bag in 1992–1993 and 1989–1990 respectively, while the start and duration of recruitment have been detected with differences of up to two months between years. Nevertheless, the start of the main spatfall season always coincided with a sharp decrease in water temperature.

Most spat always occurred between surface and 5–7 m depth during winter. When a recruitment was present in summer, the spat were collected mainly below 8 m and as deep as 15 m. Average spatfall in summer has also varied widely, from 0 to 65 individuals.

Concerning substrate selection for spat settlement, we tested natural (palm leaves, coconut halves and a local shrub called "chivato") and artificial substrates (gillnet, and black, green, red, yellow and white onion bags). The "chivato" was a good collector, followed by black onion bags. The rest of the artificial substrates gave diversified results which depended mostly on the

abundance of spatfall. With the exception of the black color, no evident preference of spat settlement was detected for a special one among the others.

2) Growth and survival during fattening: several variables were studied during 5 months: density of individuals in trays (50, 75, 100 and 150), depth (in suspension and at bottom), and site (stations Bahía Falsa and Merito). Measurement of individuals includes shell height, width, length, wing length and weight. For the purpose of the present work, we will use shell height for the description of growth.

The best growth was recorded in the trays placed at Bahía Falsa with 100 individual density (from 13.4 mm to 42.2 m of average shell height). There were no significant differences of growth between suspension and bottom fattening, but the latter presented much less accumulated mortality (17% and 5% respectively). Fouling was also less in bottom. Bahía Falsa gave better results than Merito considering growth, but fouling and mortality was higher in the first.

3) Growth and survival during culture: this phase was performed at station Merito in bottom structures using individuals with different periods of fattening (2, 4, 6 and 8 months) at 80 individuals per tray. At the 12th month, individuals with 4 and 6 months of fattening presented the best growth during culture (average increment of 50.4 to 53 mm of shell height). No significant differences were detected between these groups, but it seemed that fattening during 6 months favored the increase in shell thickness, which represents and important factor for the production of mabe.

Individuals with 2 months of fattening also had good growth (increment of 44.6 mm), but total mortality attained 75% in the first two months of culture. However, we cannot conclude a clear relationship between this duration of fattening and the mortality since we had other problems that could have influenced this parameter.

Individuals fattened for 8 months presented small sizes, shell weakness and deformities in the wing, and mortality rates started to increase dramatically from the 7 of fattening.

So far, we suppose that the best elements for cultivating *Pteria sterna* in Bahia de La Paz are the following:

1. Spat collection: collectors should be installed in alternated monthly series from November to March, each series remaining at least 65 days in the water, from surface to 6–7 m depth. The best collectors could probably be "cloth-lines" with series of three "curtains" per system filled with dark colored netting, such as black onion bags.
2. Fattening: four to six months of fattening at 80 to 100 individuals per tray seemed adequate. Fattening should be carried out in protected, non-polluted sites. Bottom structures are desirable. Cleaning and maintenance every two months is probably enough, but the periodicity depends on the site where the cultivation is carried out. We have observed that this species stands better the manipulations and resists higher fouling than *P. mazatlanica*.
3. Culture: previous experiments realized in 1987 and 1988 tested suspended and bottom culture structures in Bahía Falsa. No significant differences were found. Nevertheless, our experiences using innovated bottom culture structures at Merito have yielded better results.

The site of culture appeared to be the main factor determining growth and survival. We believe that the oceanographic conditions prevailing at Caleta El Merito favored these parameters. Significant differences on growth, mortality and fouling rate were found between this site and Bahía Falsa, which receives the direct influence of the Pichilingue commercial port.

As seen, the information on this species is somewhat fragmented if compared with that of *P. mazatlanica*. This has been mainly the consequence of several accidents, vandalism and the lack of support for maintaining a continuous sequence of activities and supervision. However, we feel that *Pteria sterna* is as suitable as *P. mazatlanica* for a large scale commercial production. More studies are in progress for this species.

SPAT COLLECTION TRIALS FOR PEARL OYSTER *PINCTADA MAZATLANICA* AT BAHÍA DE LA PAZ, SOUTH BAJA CALIFORNIA, MEXICO.

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The first spat collection experiments on *Pinctada mazatlanica* started in spring–summer 1989 with the deployment of several series of "envelopes" (square bags 30 × 30 cm manufactured with black plastic screen 2 mm mesh, filled with 30 to 40 g of monofilament gillnet) at six different sites in Bahía de La Paz. From these results, we selected station Gaviota as a propitious site for further spat collection. Although spat yield was not the best, the site is more accessible and presents better facilities for surveying and operation.

From 1990 to 1993, a total of 11 different spat collectors for *Pinctada mazatlanica* were tested during the maximum recruitment season, from surface to 6 m depth at station Gaviota. The devices were named after the main variables to be tested, which were the structure, architecture and position. All the devices were filled with old gillnet as asubstrate, except in 1993 when we tested dark onion bags as a substrate. Evaluation of spat abundance between collectors considered a standard amount of these substrates which was adjusted to 40 or 50 g.

In 1990, we tested "envelopes" (square bags 30 × 30 cm manufactured with black plastic screen 2 mm mesh), onion bags of assorted colors (no special design for color testing was followed in this occasion) and HYSEX film protected with screen bags.

In 1991, we evaluated the effect of volume on spat settlement. The devices were "curtains" (envelopes of 1 m^2), "cylinders" (cylindrical structures manufactured with rigid plastic 1.5 m high

per 0.5 m diameter) and "lanterns" (prismatic structures manufactured with rigid plastic 0.5 × 0.5 × 1.5 m containing five cubic boxes of black plastic screen).

In 1992, we tested the effect of the position and color of collectors. The devices were "pillows" (curtains positioned horizontally in series of 5), "cloth-lines" (curtains positioned vertically in series of 5) and plastic onion bags of different color (black, white and red).

In 1993 we also tested "pillows" and "cloth-lines" but in series of 3 curtains filled with dark onion bags.

Spat abundance was recorded into "curtains" and into each curtain of the "pillows" and the "cloth-lines" (1991, 1992 and 1993) under a strategy addressed at detecting differences of spat settlement between the outside and the inside of each "curtain" individually, and also between the outer and inner curtains of the "pillows" and "cloth-lines".

"Envelopes" were installed as controls from 1991 to 1993 to detect annual differences in spatfall. These results were taken into account when comparing the efficiency of the devices between years.

The criteria for the selection of the devices to be tested from one year to the following were dictated by the elements which showed propitious features for spat collection during each trial.

Comparing the spat yield in "envelopes," average spatfall presented small differences between years, but 1990 and 1992 seemed to be best with 18 to 23 individuals per bag.

In 1990, the "envelopes" yielded the best results for spat collection. Onion bags were rather poor collectors (average of 5 to 7 individuals), but we detected some preference for dark colors. The HYSEX films were ineffective. These results suggested the use of larger envelopes, and we produced the "curtains" in 1991, together with volumetric collecting devices.

The "curtains" collected an average of 25 to 30 individuals. An important proportion was recorded mainly on the black inner surfaces of the container. "Lanterns" and "cylinders" showed averages of 12 to 15 individuals. We observed that in "lanterns" most individuals appeared in the outer faces between the cubic boxes inside, suggesting that shaded sites enhance spat settlement. With these results, in 1992 we constructed the "pillows" and the "cloth-lines" (with series of 5 curtains), also introducing a test for color selection using dark and light onion bags as containers.

This time, the average spat yield increased to 137 individuals in the "pillows" and 132.4 in the "cloth-lines." Considering the average abundance on each curtain individually and comparing these results with the curtains of 1991, we observed a clear increase of spat yield (43 to 50 individuals per curtain). However, there were differences in spat abundance, depending on the place of each curtain into the system. The abundance was higher in the outer curtains, while the one in the middle recorded zeros quite often. These results suggested that larvae are "filtered" through the sucessive screens and are trapped mainly in the outer curtains. We also observed a clear preference for dark onion bags.

Based on these results, in 1993 we tested "pillows" and "cloth-lines" in a series of 3 curtains filled with dark onion bags. The spat abundance between the curtains was more uniform. The "pillows" yielded an average of 105 individuals, while the "cloth-lines" had 98. No significant differences were registered between the center and the outer curtains. Although these results seemed lower than in the previous year, we have to consider that the general recruitment in 1993 was also low as shown by the records in the control envelopes. Adjusting the equivalences, the best devices were definitively the "pillows" used in 1993.

However, we decided to retain the "cloth-lines" with 3 curtains filled with dark onion bags as the best device for massive spat collection because they presented better handling and facility in manufacture and spat harvest. This device uses less material than the "pillows," it is lighter and cheaper, and can be installed in vertical series of up to four systems. Besides, if the curtains are properly sewn, most of the material can be recycled, twice or more, after harvest operations.

We suppose that similar results could be achieved with *Pteria sterna*. A special study considering these variables is in progress for this specie.

ECOLOGY OF PEARL OYSTER SPAT COLLECTION IN BAHIA DE LA PAZ, SOUTH BAJA CALIFORNIA, MEXICO: TEMPORAL AND VERTICAL DISTRIBUTION, SUBSTRATE SELECTION, ASSOCIATED SPECIES. **Mario Monteforte**[1] and **Humberto Wright**[2,3], [1]Grantee International Foundation for Science, [2]Boursar PIFI of CICIMAR, [3]Boursar CONACYT, Centro de Investigaciones Biológicas del Noroeste, P.O. Box 128, La Paz, B.C.S. 23000, México. FAX (112) 5.36.25.

Experimental spat collectors (square bags 30 × 30 cm manufactured with black plastic screen 2 mm mesh, filled with different materials) were deployed at station Gaviota from 1991 to 1993, from surface to 15 m. The effect of exposure (2, 4, 6, 8 and 10 weeks of immersion) was tested in 1991 using gillnet only as substrate. Six substrates were evaluated in 1992: gillnet, onion bags (black, yellow and red), and two natural substrates (dry palm leaves and a Mimosoidae bush called "chivato"). These collectors had 8 to 10 weeks of exposure. The objectives were to determine, for pearl oysters: 1) temporal and vertical spat distribution, 2) substrate selection, 3) predators, competitors, noxious species and species that could be useful as index of spatfall start, and 4) adequate immersion time in function of spat size and mortality.

In 1991, the recruitment of *Pinctada mazatlanica* was short and scarce, starting in late August and finishing in late October with a maximum in September. In 1992, we detected two recruitments: June–July (intense) and September–October (moderate). These coincided with a water temperature of 28 to 30°C. Most spat were collected over 4 m depth.

Pteria sterna spat were present the whole year in 1991 and during winter only in 1992–1993. In 1991, the maximum recruitment occurred in December, which was much more intense than in January–February 1993. These coincided with low water temperature (22–24°C). During winter, spat appeared between surface and 6–7 m depth. In summer 1991, the few spat were collected only below 8 m.

Substrate selection analyses were performed during the seasons of maximum recruitment. For both species, the best substrate was the "chivato" bush followed from nearby onion bags. Palm leaves were poor collectors.

Pinctada mazatlanica seemed to prefer the dark colored netting, while no differences between colors were detected for *Pteria sterna*. Although "chivato" bush was the best material, it is difficult to handle in massive operations and cannot be recycled. We have chosen dark colored onion bags for future spat collection activities, but new substrates will be tested.

An exposure of at most 8 weeks seemed adequate for harvesting spat larger than 5 mm of shell height. This size is easier to manage and ensures higher survival during the first phases of cultivation. Spat mortality was 12 to 20% into this period. At an exposure of 10 weeks, spat attained nearly 11 to 13 mm of shell height, but mortality increased to 45% or more.

Concerning the associated species, we have identified about 120 different forms, but only 98 have been identified at species level. The rest (families and genera) were treated as "items." This strategy was applied because many of the collected organisms were present in juvenile forms or were incomplete. Crustaceans, polychaetes and bivalves amounted to more than 63% of the total species/items collected. There were 7 to 9 species of bivalves with actual or potential economic importance such as *Pinctada mazatlanica*, *Pteria sterna*, *Argopecten circularis*, *Pecten vogdesi*, *Pinna rugosa*, *Anadara* spp., *Spondylus princeps*, and *Lyropecten subnudosus*.

Among the species considered as predators, the most important were the Brachyurans *Pilumnus towsendi* and *Portunus xantusii*. During massive spat collection with larger collectors, many broken shells bore clear marks of chelipeds. Covariance analyses showed a strong inverse relationship between the abundance of any of these crabs and that of live pearl oyster spat.

Sponges, ascidians and barnacles were spatial competitors. In summer, these species are abundant and clog the collectors avoiding the arrival of new pearl oyster spat and reducing food availability for those that are already settled.

Noxious species coat the juveniles impeding valve opening. These are mainly sponges (*Hymeniacidon* sp.) and ascidians which have an important contribution to pearl oyster spat mortality.

The associated species were also used for the design of a strategy for improving spat collection efforts. We have selected the index conditions that could be easily surveyed.

The start of spatfall of *Pinctada mazatlanica* coincides with the decrease in abundance of *Pteria sterna* and *Argopecten circularis*, and a sharp increase in water temperature of at least 4°C in two months. The presence of the hydroid *Obelia* sp. (which is well-known by the local fishermen and divers), and *Pinna rugosa*, *Pecten vogdesi*, *Lyropecten subnudosus*, *Anadara* sp. and *Isognomon* sp., is also related with the recruitment of *P. mazatlanica*.

The start of spatfall for *Pteria sterna* is announced by a steep decrease in water temperature and the absence of *Pinctada mazatlanica*, *Pinna rugosa* and the stingy hydroid. The recruitment of *Argopecten circularis*, *Anadara* sp., *Laevicardium sp.*, *Glycimeris gigantea* and *Anomia peruviana* is contemporaneous to that of *P. sterna*.

These indexes are not definitive. However, we have applied this strategy during massive spat collection operations with positive results.

GROWTH AND SURVIVAL OF PEARL OYSTER *PINCTADA MAZATLANICA* IN EXTENSIVE CONDITIONS AT BAHÍA DE LA PAZ, SOUTH BAJA CALIFORNIA, MÉXICO.

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Growth curves and survival rates of *Pinctada mazatlanica* were evaluated in two related experiments testing different conditions of fattening at 8 m depth at station Merito. The individuals used in these experiments were collected in July 1992.

In the first experiment, 640 individuals were placed in Nestier trays during 2, 4, 6 and 8 months at a density of 40 individuals per tray. Each batch had two repetitions and an additional group of 160 individuals was reserved (in the same fattening conditions) to replace the dead juveniles of the main batches in order to maintain a constant density.

In the second study, the individuals were placed at densities of 25, 50, 75 and 100 per tray during 5 months of fattening. Each batch also had two repetitions. A group of 100 individuals was reserved for replacement of dead juveniles in the main batches to maintain constant conditions. This was placed in two trays each with 50 individuals.

The individuals were distributed into the trays until there were no differences in the starting average size, which was approximately 7.5 mm of shell height. Average weight was 0.32 g. This corresponds to an age of approximately 65 days.

Temperature, salinity and oxygen were recorded every month. Records of growth and mortality were also taken. We measured the height, width, length and weight on samples of 25% of total alive individuals in each tray. All deads were counted and measured (except weight) and replaced with the reserves. Unfortu-

nately, there were some losses of batches during the experiment, and this problem had to be managed during the analyses. In some cases the repetitions were suppressed.

The first trial was followed in late bottom culture for an additional 6 months. The second trial considered the fattening period only.

For the purpose of the present work, we will analyse the shell height and the weight only, since these dimensions describe better the growth of juvenile individuals.

At the 12th month in the first experiment, the growth of *Pinctada mazatlanica* with 6 months of fattening was the best (increment of 45.36 mm and 24.5 g). The shells of these individuals showed better shapes (no deformations) and seemed to be more solid than those of the other groups.

In the second, after 5 months of fattening, the growth in densities of 25, 50 and 75 individuals did not show significant differences (increment in 18.02 to 18.62 mm). In 100 individuals density, the growth was only 15.5 mm. Increment in weight seemed to be better in densities of 25 and 50 (3.8 and 3.2 g respectively). At density of 100, weight increment was only 1.7 g. In the individuals placed at 50 and 75 per tray, we could detect that shells presented better shapes and seemed to be more solid. Some of the individuals at 25 per tray were quite large in shell height, but this was very heterogeneous. Individuals at 100 per tray had thin shells and presented some deformations. Shells were generally larger in length than in height and had less growth projections over the margin.

Mortality in both experiments did not seem to be influenced by the variables tested. We suppose that handling during spat harvest was the main element affecting survival, specially during the first month after this operation. Records of 25 to 35% of mortality were always observed in the first month and then it decreased to less than 3% (often 0% in some batches) from the 4th month on. Another element which seemed to affect survival was the handling of individuals during the monthly measurements. Average sizes of deads in one particular month were very similar to the sizes of alives of the previous month, suggesting that mortality took place just after the manipulation.

These results suggest that the best fattening conditions for *Pinctada mazatlanica* are 6 months of fattening at a density between 50 and 60 individuals per tray. It would be desirable to reduce handling to every 2 months and avoid excessive manipulation of individuals, specially during the first 3 or 4 months of fattening. A possible alternative would be to place the newly harvested individuals at densities of 100 individuals per tray during the first two months and then reduce the numbers to 50 or 60 until the transfer to culture structures.

We still need to compare growth and survival in other sites and depths (in suspension for instance), but this would be hazardous until we could count with protected sites where the installations could be properly surveyed. For the moment, station Merito and the underwater culture installations we use are giving satisfactory results.

RESULTS ON THE PRODUCTION OF CULTURED PEARLS IN *PINCTADA MAZATLANICA* AND *PTERIA STERNA* FROM BAHIA DE LA PAZ, SOUTH BAJA CALIFORNIA, MEXICO. **Mario Monteforte,[1] Horacio Bervera,[2] Sandra Morales,[3] Victor Pérez,[4] Pedro Saucedo,[4]** and **Humberto Wright,[4,5]** [1]Grantee International Foundation for Science, [2]Universidad Autónoma de Baja California Sur, [3]Universidad Nacional Autónoma de México, [4]Boursar PIFI of CICIMAR-IPN, [5]Boursar CONACYT, Centro de Investigaciones Biológicas del Noroeste, P.O. Box 128, La Paz, B.C.S. 23000, México. FAX (112)5.36.25.

Half and round nuclei implants were performed exclusively on cultured individuals of *Pinctada mazatlanica* and *Pteria sterna*. Two batches of 120 individuals of *P. mazatlanica* (22 to 30 months old), and two of 105 individuals of *P. sterna* (18 to 24 months old) were used. Half nuclei were hand-made from porcelain, shells of *P. mazatlanica* and *Strombus galeatus*, marble and plastic. Sizes of half nuclei were between 10 and 13 mm in diameter per 7 to 9 mm height. For round nuclei (also hand-made, between 5 and 7 mm in diameter) we only used shells of *P. mazatlanica* and *Strombus galeatus*.

The operations on *P. mazatlanica* were performed in late summer. For *P. sterna*, they were applied in late spring. This strategy ensures that gonads would be empty in every case, even if the implant experiment was to be made with half nuclei.

Several essays for pre-surgery sedation and recovery were applied. Treatment #1 was ineffective and produced frequent mortality; treatment #2 gave good results, while treatment #3 also gave good results, but the individuals produced too much mucus which made implants difficult to perform. With treatment #2, the organisms became almost unresponsive to surgery and implanting after 25 min of exposure. They produced practically no mucus and it was quite easy to open the valves. The organisms were completely recovered after 5 to 7 minutes in running water.

Once the sedation and recovery treatment was defined, we proceeded to the nuclei implantings. Three, and up to five half nuclei per individual, were implanted using a special cement. Distribution of these into the valves were 2–1 and 3–0 for the three nuclei, 2–2 for four and 3–2 for five. The sizes of half nuclei were selected in function of the size of the receptor, therefore, to avoid obstacles when the valves close. Several locations on the valves were also evaluated for mabe formation: muscular (very near the abductor muscle), paleal (in the center band of the valve) and marginal (near the margin of the nacre layer). This last site was not used when the half nuclei would obstruct valve closing. For *Pteria sterna*, we placed the larger number of half nuclei on the "flat" valve. No special design in this sense was followed for *Pinctada mazatlanica* since both valves are almost equal.

Round nuclei insertions (one per individual) were performed with our own techniques. We are using the traditional surgical instruments but some transformations were necessary, specially for the base, to adapt them to the shape, size and anatomy of our species. The insertions were made in the floor of the gonad and in the tip of the pearl-sac. Concerning this organ, *P. sterna* has a simple bag, similar to *Pinctada margaritifera* from French Polynesia and *P. fucata* from India, while in *P. mazatlanica* it is bi-lobulated. It is probable that this species is the only pearl oyster presenting this characteristic.

Donors of the grafting tissue were selected on the basis of the color of their marginal nacre layer. We chose those having the best iridescence (''aile de mouche''). For mabes, we selected assorted individuals.

Both types of implants were realised in not more than 40 sec for round nuclei and 55 sec for half nuclei. We did not try to reduce this time, therefore, to ensure as much as possible a good insertion.

After the operations, the organisms were immediately replaced into the water, each one into a numbered bag. Since we do not have access to a laboratory (all the operations were performed at the field), it was not possible to realise a better convalescence treatment.

After 8 months, retention of half nuclei was 100% and accumulated post-implant mortality was 3%. Nearly 85% of nuclei were completely covered by nacre (1.2 to 1.7 mm width). Quality of mabes was better and more uniform in shells with 3 half-nuclei. Those with 4 and 5 were heterogeneous. Shell and porcelain nuclei had better response than mabe, while plastic gave poor results.

There were not particular differences on nacre covering between nuclei placed in different valves. However, the quality and homogeneity of nacre layers was higher in mabes formed on the paleal band. Mabes had different colors depending on the species and also on the site of implanting. Hues were varied in *Pteria sterna* (silvery, golden, purple, blue, gray), while blue-gray and dark-gray predominated in *Pinctada mazatlanica*. Mabes tended to be darker when they were formed near the margin of the valves, but imperfections were noted in the surfaces of the mabe facing this area, particularly when it was too near the margin. It was also noted that the mabes placed in the central sector of the valve had better quality. We suppose that at least 10 to 12 months are needed to obtain superior quality. From these preliminary results, we can expect a gem incidence (beautiful mabes) of more than 55% applying the best propitious elements obtained in this experiment.

Concerning round pearls, we obtained modest results. Post-surgical mortality after 8 months nearly approached 75%, but graft retention was 100% in survivants. Only four round pearls of standard quality (dark gray, 7.5 to 7.8 mm diameter) were obtained in *P. mazatlanica* and three (blue-gray to green-gray, 6.8 to 8.2 mm diameter) in *Pteria sterna*. There is 3.3% and 2.9% of round pearl incidence respectively. No further analysis is possible with these results. We can only say that much more work must be done about this aspect.

PROGNOSIS FOR THE FUTURE: CRISIS MANAGEMENT OF AN IMPERILED MUSSEL FAUNA. Richard J. Neves, National Biological Survey, Virginia Cooperative Research Unit, Virginia Polytechnic Institute, 100 Cheatham Hall, Blacksburg, VA 24060-0321

The recorded declines in both rare and common species of freshwater mussels have sensitized federal and state regulatory agencies to the need for conservation and management of this renewable resource. Up until now, the distribution of most federally protected species and commercially harvested species was allopatric; hence the take of protected species was inconsequential. However as the list of federally endangered mussels continues to increase beyond the 56 species currently protected, the co-occurrence of harvested and protected species will create management problems for state wildlife resource agencies. Similarly, the spread of the zebra mussel (*Dreissena polymorpha*) into rivers with commercially exploited native mussel species is beginning to generate reactionary responses to conserve and protect native species. The impact of zebra mussels on native mussels in rivers is currently being documented in the Illinois River. Zebra mussels were first collected in this river in summer 1991, and after 2 years, most of the native mussels are encrusted with zebra mussels. As judged by the infestations recorded in the Great Lakes and the extirpation of native mussels from Lake St. Clair, western basin of Lake Erie, and the Detroit River, I project a major die-off of native mussels in the Illinois River in summer 1994. If this die-off occurs, then many states will respond to the zebra mussel threat by restricting or closing harvest of live mussels in their respective waters. Many mussel species, once common in rivers, will become candidates for federal protection. The future of commercial musseling in the U.S. is in jeopardy.

STATUS OF THE FRESHWATER MUSSEL FAUNA IN THE UNITED STATES. Richard J. Neves[1] and James D. Williams,[2] [1]Virginia Cooperative Research Unit, Virginia Polytechnic Institute, 100 Cheatham Hall, Blacksburg, VA 24060-0321, [2]National Biological Survey, National Laboratory, 7920 NW 71st St., Gainesville, FL 32606, USA.

A comprehensive review of the conservation status of the 297 species and subspecies of native freshwater mussels in the U.S. was completed to assess present and future trends for the fauna. Distributional data, historic and recent collection records of biologists, and literature reviews provided sufficient information to categorize the status of each species. Twenty-one taxa (7%) are listed as endangered but presumed extinct; 77 (21%) are endangered but extant; 43 (14%) are threatened; 72 (24%) are of special concern, 14 (5%) are of undetermined status; and only 70 (24%) are considered stable at this time. The primary reasons for the

decline of freshwater mussels are habitat destruction from dams, channel modification, siltation, contaminants, and the introduction of exotic mollusks. Construction of dams within the Tennessee River system by the Tennessee Valley Authority, and dams and navigation projects in large rivers by the U.S. Army Corps of Engineers created impoundments and tailwaters that were unsuitable for many indigenous species. Nonpoint source pollution from agriculture and urban runoff, and point source discharges have contributed pollutants and contaminants to degrade water quality. Competition from non-native mollusks such as the Asian clam (*Corbicula fluminea*) has seemingly affected some mussel populations in streams, and the zebra mussel (*Dreissena polymorpha*) appears poised to decimate commercially important mussel populations occurring in large rivers. The high numbers of imperiled freshwater mussels in the U.S., which harbors the most diverse mussel faunal globally, indicate an impending extinction crisis that will severely reduce an important component of aquatic biodiversity. The harvest and export of mussel shells for the cultured pearl industry in Asia will be affected by the decline in mussel populations in the U.S.

THE DEVELOPMENT OF BLACK PEARL FARMING IN MANIHIKI. Raymond Newnham, Terone Pearls LTD, Tauhunu Manihiki, Cook Islands.

This paper looks at the development of black pearl farming in Manihiki, a coral atoll in the North Group of the Cook Islands.

The focus is on the three areas of the industry: access to technicians, material supplies, and marketing. The functions of the development agencies responsible for pearl farming in Manihiki are discussed.

Some considerations are offered to other countries comtemplating implementing pearl development programs.

NATURAL PEARL FARMING IN THE EARLY CENTURY AT BAHIA DE LA PAZ, SOUTH BAJA CALIFORNIA, MEXICO. Martha Micheline Cariño Olvera, Universidad Autónoma de Baja California Sur, Departamento de Humanidades, Km 5.5, Carr. al Sur, La Paz B.C.S., 23080, México. FAX (112)1.18.80.

The Bay of La Paz, during the first two decades of the century, was the stage of a unique event in the pearl world history: a large scale production of natural pearls through the extensive culture of *Pinctada mazatlanica*. The performance of this production was accomplished by Don Gastón Vives, based on his technological innovations and deep knowledge of the local environment.

In 1903, Vives founded the "Compañía Criadora de Concha y Perla de Baja California S.A." (CCCP), that employed more than 1000 workers and possesed a large infrastructure in the islands Espiritu Santo, San José and Cerralvo.

The success of Don Gastón has to be explained in the regional, national, and world historical context, because of the particular work and pearl/pearl oyster trade conditions of the early century and the close relations existing between the economic development and the political control of the Porfirian dictatorship.

The CCCP grew in exponential proportions until July 1914, when under the flag of the Mexican Revolution, a very staunch enemy of Vives destroyed his installations. Therefore, the study of these event in the pearl world history has to be analysed parallel to the historical and the scientific domains.

The technology of the CCCP was founded on the three traditional steps of the extensive culture of bivalves, although Vives adapted the process to the conditions of the local environment by designing original artifacts and installations.

The devices used for spat collection were wooden cases (2 m^3) with metallic net inside forming compartments that contained a substrate composed of branches and pearl oyster shells. These collectors were placed afloat near the natural banks, and they remained in the water for ten months. The juvenile pearl oysters were carefully harvested one by one and transported to a new site for the fattening phase.

It was in this intermediary stage when Vives utilized one of his most original inventions. The pearl oysters were arranged into baskets (15 × 30 cm) manufactured with metallic net that had individual compartments. These baskets were placed in the channels of a large nursery located at San Gabriel Bay in Espiritu Santo Island.

For the construction of this nursery, Vives made good use of the physographic features of San Gabriel Bay and its coastal lagoon that he had separated by a rubble-work dam 1 km long; on one side, he built 36 channels disposed in a zig-zag pattern. The water circulated through these channels by a flood-gate system that operated with the tides. These channels were protected by a palm roof that offered cool shade to the young oysters avoiding the negative effects of excessive illumination and high temperature.

When the individuals grew up to adult size, they were taken to the open sea and placed at the bottom on hand-made substrates. These had been previously prepared using huge quarry stones taken from the nearest islands. Each adult pearl oyster, before it was established on its permanent habitat, was covered by a metallic armour-plating with sharp points around the margin, and provided with a cork bark that helped the whole to have an orientation towards the substrate. In these artificial stone beds, and with their highly efficient protection against predators, the development of pearl oysters was set under a rigorous vigilance for two to three years until pearl harvest time.

The CCCP employed the traditional *armada* system for the harvest-fishery procedure. This system used one large boat (45 ft) and several canoes provided with 6 or 8 scaphander pearl divers. All the pearl oysters were opened in the boat or on the Espiritu Santo installations, under a strict surveillance.

All the product of these molluscs was used: the meat fed the workers, the shells were packed in wood cases ready for their

export to the European and American markets, and the natural pearls that were found were taken by Don Gastón Vives to the Paris jewellers that appreciated very much their high quality orient, exotic colors, regular shapes and huge size. The profits, always very rich, were invested in the enterprise, either in the pearl oyster culture installations and operations, or in other economic activities developed with the purpose of making more profitable the pearling industry. These economic strategies are important factors that explain the fast improvement and growth of the CCCP activities.

The political activities and relations of Don Gastón, plus his personal business administration management, also led to the large expansion of the CCCP. From 1894 to 1911, he was the municipal president of La Paz—the second most important political post in the region—which offered him a privileged situation for eliminating almost every obstacle or enemy that the CCCP would meet. At that time, the socioeconomic local structure offered him numerous and cheap workmanship, from which he could obtain a maximum profit employing them in the day-labourer system that was current at that time. We cannot talk about any abnormal exploitation of workers: he paid them a good salary, fed them and their families during the time they were employed, and contributed to the education of their children; but the rules were very strict and so were the punishements when they were not respected.

In the sight of reducing the expenses of nourishing and transportation of the workers, Don Gaston developed, parallel to the CCCP, other economic activities, such as agriculture, in a farm called "Las Cruces," and cattle-raising in the natural enclosed fields of the islands included in the territorial concession. He also employed some resources undertaking commercial and shipping activities with the two steam boats of the enterprise. Through this diversification of economic activities, and considering that the CCCP employed (between 1912 and 1914) more than 16% of the whole population of La Paz, we may affirm the significant role that pearl oyster culture and pearl trading have had in the socioeconomic local structure.

Another positive consequence of these rational strategy of pearl oyster proficiency, was that the massive culture of this highly important resource had temporarily caused the overexplotation of the natural beds. After the destruction of the CCCP, no other culture experience was undertaken, but the unmoderated pearl oyster fisheries continued under the misunderstood principle of free exploitation of the national natural resources by any Mexican who wished. The consequence of 25 years of overfishing of the natural pearl oyster beds was that the most important local resource came to exhaustion and the Mexican Government declared a permanent banning in 1939. Nevertheless, furtive and uncontrolled extractions have continued until the present days. In several cases, these extractions are carried out by local institutions under the excuse of research purposes which have not produced any positive results.

We know that only one recent research program on pearl oyster culture, pearl production and repopulation, under the direction of Dr. Mario Monteforte from the Centro de Investigaciones Biológicas del Noroeste, has achieved positive and promising results. This program has brought new and solid hopes for La Paz to recover its worldwide reputation that once gave to this region the name of "Island of Pearls".

BLACK PEARL CULTURE DEVELOPMENT IN THE PACIFIC ISLANDS. Gary L. Preston, South Pacific Commission, Noumea, New Caledonia.

The paper describes the potential for, and constraints to, the development of black pearl culture industries in those South Pacific island countries where such industries do not yet exist. The paper also describes the international institutional arrangements presently in place to support marine resource development efforts in Pacific Island nations, and suggests ways in which they might be strengthened specifically to support pearl culture industry development.

PEARL OYSTER CULTURE IN MEXICO. Dr. Carlos Rangel-Dávalos and Carlos Caceres, P.O. Box 18-B, La Paz, Baja California Sur., Mexico C.P. 23081.

From 1900 to 1914, pearls were produced in Mexico by culturing oysters to obtain natural occurrence. Government formerly prohibited pearl oyster fisheries in 1940, in order to recuperate natural beds from strong catches. A group of professors researching in Baja, California Sur State University is doing research and development on the subject, together with training students. Aim is to initiate extension projects and offer technical advice.

SOCIOECONOMIC AND POLITICAL ASPECTS OF THE TUAMOTUAN BLACK PEARL INDUSTRY. Moshe Rapaport, University of Hawaii, Department of Geography, Porteus Hall, Honolulu, HI 96822.

Black pearl farming in the Tuamotus has experienced dramatic growth in recent years. However, among the atoll communities, there have been deep divisions on the criteria to be applied for allocating lagoon concessions. Management efforts by the Tahitian administration have been frustrated because of their insufficient attentiveness to local concerns.

PEARLS AND ECONOMIC DEVELOPMENT. John T. Rowntree, RDA International, Inc., 801 Morey Drive, Placerville, CA 95667.

Several international development agencies and South Pacific governments are actively encouraging the expansion of the cultured pearl industry as a technique for promoting economic development. This presentation will explore the role of pearl farming in stimulating economic development and in generating employment, income, and foreign exchange for developing island economies. It will also address the monetary and fiscal implications and some of

the socio-economic consequences of expanding pearl production to promote economic development.

AN OVERVIEW OF PEARL PRODUCTION TECHNIQUES IN AUSTRALIA. David (Dos) O'Sullivan[1] and Derek Cropp.[1,2] [1]Key Centre for Teaching and Research in Aquaculture, University of Tasmania at Launceston, PO Box 1214, Launceston, Tasmania, 7250, Australia. Tel: +61 03 243-448, Fax: +61 03, 243–449 [2]Aquatech Australia Pty Ltd and Abalone Pearls Pty Ltd, 15 Wignall St, North Hobart, Tasmania 7000, Australia. Tel: +61 02 349-337, fax: +61 02 311-627.

The pearl culture industry in Australia has been operating since the mid 1950s. The main production comes from the northwestern coast of Western Australia, although production is increasing in the Northern Territory and north Queensland, especially in the Torres Strait region. Shell are harvested at licensed collecting areas under a quota system to prevent overfishing of the stocks. Seeding is undertaken at the harvesting leases or on the farms.

On almost all the farms, the seeded pearl shells are held in specially designed net panels, each with about 6 or 8 mesh pockets in which to hold the shell. Farms are moving away from the traditional raft culture into the use of surface longlines or bottom fences, mainly as a preventative measure to avoid losses from cyclones. The shells are regularly cleaned using high-pressure water and are X-rayed after 6–8 months to check for the presence of the nucleus.

Harvesting of the pearls takes place about 2 years after implantation and some shells may be reseeded up to 3–4 times before being used for mabe production. With the advent of hatchery production of juveniles, technology is being developed for nursery culture and subsequent growout of these small shell until they reach a seedable size (1–2 years old).

STATUS AND POTENTIAL OF PEARL FISHERY OF BANGLADESH. Manamatha Nath Sarker, Marine Fisheries Survey, Management & Development Project, Cox's Basar, Bangladesh.

Bangladesh is famous for her natural pink pearl. These are obtained from the freshwater mussels, like *Lamellidens spp.* and *Ferreysia spp.* Another kind of small white pearls is obtained from marine windowpane oyster (*Placuna placenta*). Pearl oyster (*Pinctada fucata*) is reported to be available, but there is no information on collecting pearls from them. The species of *L. marginalis* reaches up to 10 cm and produces pink coloured pearls, whereas *P. daccaensis* grows up to 6 cm and produces golden coloured pearls. *L. marginalis* is abundant in almost all freshwater bodies of Bangladesh, particularly in lowland areas where water is available throughout the year. The main area of distribution comprises the greater district of Sylhet, Dhaks and Mynensingh. The natural pearls collected annually are estimated to be 150 kg, with negligible amount from farming. Mussel meats are used for poultry feed, while the shells are mostly used for producing shell craft and lime. Some shells are also used as a mineral source for poultry feeds. Although there is a great potential for pearl culture in both freshwater and marine water bodies, pearl culture has not yet flourished commercially in the country, due to lack of technical know how and financial constraints.

BREEDING CYCLE OF PEARL OYSTERS *PINCTADA MAZATLANICA* AND *PTERIA STERNA* IN BAHIA DE LA PAZ, SOUTH BAJA CALIFORNIA, MEXICO. Pedro Saucedo[1] and Mario Monteforte,[2] [1]Boursar PIFI of CICIMAR-IPN, [2]Grantee International Foundation for Science, Centro de Investigaciones Biológicas del Noroeste, P.O. Box 128, La Paz, B.C.S. 23000, México. FAX (112)5.36.25.

This study intends to increase our knowledge of the reproductive biology of the mother-of-pearl *Pinctada mazatlanica* and the rainbow mabe *Pteria sterna* from Bahia de La Paz. It is aimed at completing the spat collection strategies and enhance nucleus implanting operations for the production of cultivated pearls.

Between June 1992 and July 1993, gonad samples of cultured individuals of both species were collected monthly (10 individuals of each specie) and preserved in 10% formalin. They were then embedded in parafin, sectioned at 7 μm, colored with hematoxylin-eosin and observed at 10 and 40× magnifications. The reproductive cycle was studied by dividing it into five main gonadal stages: 1) indeterminate, 2) gametogenesis, 3) maturity, 4) spawning and 5) spent. The histological observations were supported by a gonadic index.

The sample size might seem rather small for the purpose of histologic studies. However, since this work was carried out using cultured individuals, and not many were available for being sacrificed, we had two options to choose from: either we increased sample size covering only a restrained temporal period, or we used smaller samples but we could cover an annual cycle. We decided for the second alternative which we believe to be richer in information.

In both species, gametogenesis was a continuous process through the year. *P. mazatlanica* bred once a year between August and September (summer), when water temperatures reached 28 to 30°C. It is a protandrous hermaphrodite which matured as female at a shell height greater than 100 mm (approximately 22 to 24 months old). Below this size, all organisms were males. Because we used young-adult organisms in this study, the sex-ratio we found was 0.12:1. Finally, the first gonad maturity was observed at a shell height of 50–55 mm (11 to 13 months old).

The breeding season for *P. sterna* was bimodal with two spawnings in the year, the main one in January–February (winter) when the water temperature was about 20 to 22°C, and another short one in May (spring) at 23 to 25°C. There was not enough evidence to conclude the species to be a protandrous hemaphrodite, but the highest proportion of females was found over 55 mm of shell height (older than 10 to 11 months). The sex-ratio also tended to male at young stages (0.38:1). The first gonad maturity

of this species seemed to occur at a shell height of 40–45 mm (7 to 9 months old).

From these results, we could deduce that a periodical determination of the gonad maturity of the population by sampling some wild and/or cultured organisms (selecting medium to large sizes) could improve the strategy for massive spat collecting operations by previewing the right moment to install the collectors.

Additionally, from these observations we could advance that the best season for nucleus insertion (round pearls in particular) is between November and January for *Pinctada mazatlanica* and between April and June for *Pteria sterna*. We suppose that in these periods most of the receptors would present spent and/or indeterminate gonadal stages which is ideal for the surgery.

REPOPULATION OF NATURAL BEDS OF PEARL OYSTERS *PINCTADA MAZATLANICA* AND *PTERIA STERNA* IN BAHIA DE LA PAZ, SOUTH BAJA CALIFORNIA, MEXICO. **Pedro Saucedo,[1] Mario Monteforte,[2] Horacio Bervera,[3] Victor Pérez,[1]** and **Humberto Wright,[1,4]** [1]Boursar PIFI of CICIMAR-IPN, [2]Grantee International Foundation for Science, [3]Universidad Autónoma de Baja California Sur, [4]Boursar CONACYT, Centro de Investigaciones Biológicas del Noroeste, P.O. Box 128, La Paz, B.C.S. 23000, México. FAX (112) 5.36.25.

The natural beds of pearl oysters of Bahia de La Paz have been overexploited for more than 400 years. *Pinctada mazatlanica* and *Pteria sterna* are considered as endangered and have been under legal protection since 1940. Nevertheless, furtive fisheries are still carried out by fishermen and tourists who are influenced by the same Pearl Myth that motivated the Spanish empire and large fishing enterprises to colonize and exploit this aggressive and isolated territory.

Today, the resource is rare and the local populations are discontinuous along the coast. The "banks" present low densities and natural recruitment is also low. In addition to this, there are uncontrolled extractions, some of them performed by local institutions which use research purposes as a false excuse without any consideration about the fragile equilibrium of the resource and no intention to realize repopulations.

In our research program on pearl oyster culture, the repopulation of natural beds is considered as a main objective. With recovery and conservation of the resource in mind, the study on repopulation is aimed at defining adequate seeding techniques using cultured individuals and repeating these actions as frequently and extensively as possible.

In the present work, we report the results of two years of repopulation experiences.

Different seeding conditions were tested using two groups of 300 cultured individuals of *Pinctada mazatlanica* and *Pteria sterna* each year. Trials were carried out in one site (station Merito) in 1991 on protected and unprotected, natural and artificial substrates. Natural substrates were the *in situ* rocks; artificial substrates were boards (45 × 30 cm) made with cement, asbestos and fiberglass.

Protection was provided by the use of large soda plastic cases (45 × 30 × 14 cm) which had the bottom cut out and the top covered with 5 mm mesh net. Each individual was placed in the spaces originally used for bottles therefore to ensure direct contact with the substrate. In the unprotected experiment, the individuals were distributed into large-mesh net bags and placed in direct contact on the substrates. We also evaluated two additional variables: depth of seeding (3, 6 and 9 m) and organism size defining three groups (larger than 80 mm shell height, between 60 and 80 mm and smaller than 60 mm).

The observations were made at the second, seventh and fifteenth day after seeding. From the 15th day on, observations were taken monthly and bimonthly. We wished to assess mortality, settlement speed and strength and in situ growth of organisms.

Settlement strength was evaluated using the following indexes:

Index 0: there are practically no byssus threads attached to the substrate.

Index 1: some byssus threads are attached but the settlement is weak.

Index 2: there are more byssus threads attached to the substrate and the settlement is almost normal. The organism can still be moved side to side but possesses resistance.

Index 3: practically all the byssus threads are perfectly attached to the substrate. The organism is settled normally and possesses strong resistance of movements.

The degree of protection against predators was a very important factor for the survival of seeded individuals. Those placed on unprotected repopulation cells were rapidly consumed since the first or second day of seeding. From the observation of some of the broken shells we were able to recover, we suppose that large malacophage fishes (Tetrodontidae, Serranidae, Lutjanidae), as well as portunid crabs and lobsters could be the most common predators.

Protected natural substrates enhanced settlement speed and strength and organism growth, whereas artificial substrates did not. Among the artificial substrates, the asbestos gave better results than cement and fiberglass. This last was completely ineffective.

A seeding depth of 6 m gave the lowest mortality, the highest settlement speed and strength, and the best individual growth. Individuals of small to medium size, particularly the latter, gave the best responses to all the variables tested.

In 1992, from the results of the first experience, we used special large cages manufactured with rigid plastic 5 mm mesh with medium size flat stones (10 to 15 cm in diagonal dimension) arranged on the bottom. These cages are being used now as repopulation cells in the coast of El Merito and Bahía Falsa. So far, the results are highly positive. Every spat collecting season of *Pinctada mazatlanica* and *Pteria sterna*, we reserve half of the harvest for repopulation actions. We hope that the gradual recov-

ery of pearl oysters in Bahía de La Paz is starting with our modest effort.

HATCHERY CULTURE OF THE BLACK-LIP PEARL OYSTER IN HAWAII—STOCK RE-ESTABLISHMENT AND EXPANSION OF COMMERCIAL PEARL CULTURE THROUGHOUT THE REGION. Neil Anthony Sims and **Dale J. Sarver,** Vice-President (Research), and President, Black Pearls, Inc., P.O. Box 525, Holualoa, HI 96725.

The establishment of a commercial hatchery for black-lip pearl oysters (*Pinctada margaritifera*) at the OTEC facility in Kona, Hawaii, has significance for the preservation of threatened populations, as well as opening up commercial pearl culture potential for Hawaii and other Pacific Islands.

The Hawaiian variety of black-lip (*P.m.galtsoffi*) was overfished in the past, and is now rare to the point of being protected by the State. Relict stocks in Pearl and Hermes Reef, Kaneohe Bay, and along the Kona Coast show no signs of recovery. Hatchery culture would allow a stock re-establishment program. Ocean-based pearl farming options are being explored at several sites throughout the Hawaiian Islands. Land-based pearl culture is also being developed at the OTEC plant in Kona.

The feasibility of using pathogen-free deep-OTEC water for broodstock maintenance, larval culture and early spat rearing has been proven in trials with Marshall Island pearl oysters. These techniques remove the risks of inadvertent transfer of exotic organisms (pathogenic or benign) and genetic mixing between stocks. Pacific Islands with small quantities of broodstock can now use this system to provide spat for stock re-establishment or development of commercial pearl culture.

With this technology, the natural scarcity of pearl oysters in a lagoon is no longer a principal constraint to the development of pearl farming. The Kona facility can operate as a regional hatchery for the central Pacific, obviating the need for expensive construction and operation of pearl oyster hatcheries on each island group.

FRESHWATER PEARL CULTURE IN INDIA. Ajai Kumar Sonkar, 557/470 Old Katra, Allahabad, UP 211 002, India.

Since time immemorial, the Indian Ocean was famous for natural pearls. In the late eighties, some natural pearls were found in a lake in Agartalla (capital of 'Tripura' State). Until then, pearl cultivation from fresh water was not well known in India.

Scientists from the Central Institute of Fresh Water Aquaculture (CIFA) have examined the feasibility for production of cultured pearl in fresh water.

A research project of the Dept. of Biotechnology on fresh water pearl culture was initiated in CIFA in the year 1982, when several successful experiments were undertaken on three species (*Iemellidens-marginalis, L. Corrianus, & Parreysia-corrugata*) for nucleated spherical pearls.

The author's involvement in fresh water pearl culture began in May 1991. In two fish ponds owned by his family, a natural stock of fresh water mussels was available.

In a small laboratory constructed on the farm premises, experiments on pearl culture were initiated. Initially, the author fabricated his own nuclei of caldiferous substance for implantation.

In the early trials, more than 20% operated mussels would to die due to surgery, and about 50% animals would reject the implantation.

After short term training at the Central Institute of Fresh Water Aquaculture in 1992, the author started experiments to determine the causes of mortality and rejections. Generally, species are not differentiated in pearl mussel surgery, often one species mantle tissues were implanted in other species.

In fact, two species (*L. marginalis & L. carrianus*) available in India are suitable for gonadal implantation and are often found together in the same pond or lake.

In experiments, the author used the same species' mantle for grafting in the same species, and sprayed steroid on the operated mussels to stimulate their immune systems and antibiotics to counter possible infections.

After surgery, the recipient mussels were kept in laboratory conditions up to 14 days for post-operative care under proper aeration and aseptic conditions. During the period feeding and movement were restricted.

Through the experimental process, 70% efficiency was achieved with a mortality rate of zero.

It is observed that an ideal pearl takes about six months to one year to form depending upon the size of the implanted nuclei. It is found that the Indian climatic condition in several parts of the country is favorable for pearl culture, and this field offers considerable commercial possibilities for India and neighboring countries.

PEARL FARMING IN VIETNAM. Ha Duc Thang, Marine Product Research Institute, 170 Le Lai Street, Haiphong, Vietnam.

Vietnamese understood the use of pearls a very long time ago, but scientists of the Marine Product Research Institute started research on pearl culture only in 1966.

In 1967, the first pearl farm was set up at Co-lo Island (belonging to Quang Ninh province of North Vietnam). It had produced many round pearls from *Pinctada martensi* mussels. However, in 1973, because of the war, it had stopped. In recent years, the pearl culture project was set up with assistance of government in Cat Ba Island (belonging to Haiphong Province). Some experts from Australia and Japan came and started producing pearls at Nhatrang Province and Halong Bay (in the north).

There are many species of pearl oysters in Vietnam namely: *Pinctada martensii, Pinctada margaritifera, Pteria penguin, Pictada maxima* and *Pteria formosa*. Vietnamese are farming *Pinctada martensii* mussels at Halong bay and Australians are farming *Pinctada maxima* at Nhatrang Sea.

In fresh water, there are 38 species of freshwater mussel, but only 5 species are used to produce pearls. They are: *Hyriopsis cumingi, Cristaria bialata, Anodonta eliptical, A. jondui, Lamprotula, sp.* All of them are distributed in rivers and ponds with a depth of 7 to 16 m.

The *Hamprotula* especially has a shell thickness ranging from 8 to 12 mm. All nuclei in Vietnam are made of Lamprotula mussels.

All culture systems and culture techniques are based on the techniques of China and Japan. The hanging method using rafts and long line is widely employed.

The nuclei implantation technique in marine pearl oysters is almost the same as that used for *Pinctada jucata* in Japan. There are 5 methods employed in the fresh water pearl culture. They are: Inserting graft tissue into the mantle of mother oysters (producing seed pearl). Implanting nucleis with graft tissue into the gonad of mother oysters (producing round pearl). Implanting nucleis with graft tissue into the mantle of mother oysters (producing round pearl). The results of research show that: implanting a nuclei with graft tissue into the mantle of a mother oyster is best because we can insert about 8 to 10 pieces of nuclei in each mussel (diameter of nuclei is 3 to 5 mm and the expulsion rate is 30 to 40%). After 18 months of culture, pearls grow to 7–8 mm (diameter of pearl). The color of the pearl depends on the tissue inserted.

Our research has found that if one uses the graft tissue of *Cristaria*, one will get pearls of silver or blue colors.

Graft tissue of *Anodonta* gives pink and cream pink pearls.

Graft tissue of *Hyriopsis* gives cream pearls and some gold. The mother oysters are *Hyriopsis cumingi*, and experiments were done at the same place and many times from 1986–1990 in Ho-Tay Lake and ponds of our Institute of Haiphong.

Halong Bay is not only famous for its beauty with thousands of small islands rising from the sea surface, but is also a good place for pearl culture.

The water depth ranges from 5 to 10m and the water temperature is about 20°C in winter 29–32°C in summer. The salinity ranges between 23–25 ppt during the rainy season, and between 31–35 ppt in the dry season. The density of phytoplankton is about $2–3\times10^6$ cell ml. There are many rivers and large ponds in the north which can be used for good pearl culture sites, and the resources for pearls are also rich.

PEARL CULTURE ON TONGAREVA, COOK ISLANDS: IMPACT OF COMMUNITY BASED MANAGEMENT. **Hugh Thomforde, Rorangi Tonitara,** and **Amelita Tabique,** RDA International, Inc., Tongareva Marine Research Ctr., Ministry of Marine Resources, Omoka, Tongareva, Cook Islands.

The authors live on Tongareva (also known as Penrhyn) in the Cook Islands. They review the development of the cultured pearl industry and the effects of traditional fishing and recent economic factors on that development.

Prior to 1992, the majority of the residents on Tongareva were either uninterested or opposed to pearl farming. A large share of the adult population feared loss of local control over lagoon tenure rights and increased control in all lagoon affairs by the central government. As many as 5% of adults in the village of Omoka feared pearl seeding would induce a biological catastrophe on the scale of a nuclear explosion with the consequent necessity to evacuate their homeland. This was apparently due to the incorrect association of the word "nucleus"—used to refer to the beads used in spherical pearl seeding—and the word "nuclear" in regards to the contamination experienced at Bikini and Enewetak atolls in the Marshall Islands. In March, 1993, the Penrhyn Island Council shifted from a policy of opposition to supporting pearl seeding, although public opinion remained highly divided on the issue. By November, 1993, pearl seeding licenses were initiated and prospective pearl farmers were required to apply for a permit from the Island Council. The economic potential of pearl farming was the overriding factor which influenced people to support commercial farming. The recent collapse in the copra industry due to reduced world market prices, and the steadily declining price for pearl shell have obviously been influential. From November, 1993 to January, 1994, support for pearl farming and commercial seeding ran at about 95% of the adult population in the village of Omoka. There is still lower support for pearl culture at the village of Tetautua because of a greater reliance on regular harvests of pearl shell for basic subsistence. It is anticipated that opposition for establishment of reserve areas, or for a total ban on wild harvesting, could come from Tetautua.

A BRIEF ACCOUNT ON MYANMAR'S PEARL CULTURE INDUSTRY. **Tint Tun,** 267 Bargaya Road, Myaynigone, Sanchaung P.O., Yangon, MYANMAR.

The history of Myanmar's pearl culture industry is 40 years old. It is significant that the whole operation of pearl cultivation, from fishing, through seeding, to harvest, has been carried out by Myanmar citizens for three decades. In accordance with its market economy system, local and foreign companies are interested in pearl cultivation in Myanmar.

According to Myanmar literature, the pearl is one of nine precious stones, and grace is an attribute of the pearl. Union of Myanmar is a South Sea Pearl (SSP) producer and pearls are cultivated by Myanmar culturists. It has been 40 years since Japanese technicians introduced pearl cultivation to Myanmar and four decades is considerable time since the birth of the pearl culture industry. Today's production of precious South Seas Pearls (SSP) is by Myanmar technicians and Myanmar citizens.

Pearl cultivation was commenced in Myanmar in 1954, although pearl oysters were exploited for mother-of-pearl shell trading since the 1890s. The Burma Pearl Syndicate was established in 1954 as a consequence of negotiations between Myanmar and Japanese (Takashima Pearl Company) businessmen to form a joint venture pearl culture industry. The syndicate started pearl cultivation and MOP shell collection at Mergui archipelago in Myanmar.

Myanmar citizens were appointed to work at the pearl culture station, but seeding was done by Japanese only. Japanese did not show or instruct Myanmar about seeding while they were working at the syndicate. The syndicate brought in modern diving equipment and diving boats. It is known that all pearls cultured by the syndicate were brought to Japan and they were sold under the name of "South Sea Pearl."

A new chapter in the history of Myanmar's pearl culture industry was started in 1963. On 16th, August, 1963, Burma Pearl Syndicate was nationalized and pearl cultivation became a branch of the People's Pearl and Fishery Board. Later, the Board was transformed into the state-run People's Pearl and Fishery Corporation.

A visit to Pearl Island was arranged in April, 1964, by an authority concerned for teachers and students of biology of Yangon (Rangoon) University. They were encouraged to do pearl cultivation at the Island. A group of four teaching staff was interested and were allowed to transfer from the University to Pearl Island. By appointing them as pearl culture officers, they became the first group of Myanmar pearl culturists.

As they were not taught anything about seeding and cultivation, pearl culturists tried to produce SSP with an all-out effort. Fortunately, they had the opportunity of thorough investigation on pearl cultivation themselves. As a result of coordination and cooperation among the technicians, doctors of medical college and military hospital, and personnel and department concerned, Myanmar culturists did, at last, succeed in seeding. Since that time, the whole operation of pearl cultivation has been controlled by Myanmar citizens. It is a significant fact in the history of Myanmar pearl culture industry. The first pearls—a hundred momme of SSP cultured by Myanmar technicians—was harvested in the 1967–68 fiscal year.

The relocation of the pearl culture branch was done in 1986. It was attached to the Salt Industries Corporation. However, in 1989, it became a separate state-run enterprise. The name was "Myanma Pearl Enterprise" and it was the only pearl cultivator in Myanmar until 1992. (Note: Myanma is an adjective form of Myanmar in Myanmar language).

Pearls were put on sale at successive auctions held at Yangon. A total of 40 lots of pearls fetched 413,034 at the 31st Myanma Gems Emporium which was held in March, 1994.

A VIEW ON SEEDING. Tint Tun, 267 Bargaya Road, Myaynigone, Sanchaung P.O., Yangon, MYANMAR.

Seeding can determine the quality of a pearl and seeding technicians play the most important role in seeding. Various grafting methods and nucleus sizes can be used commensurate with mother-of-pearl oysters. Research is important in all aspects of pearl cultivation.

To harvest a pearl, there are many processes in the way of its cultivation. Fishing, seeding and cultivation can be regarded as major phases and seeding can be said metaphorically as the heart of the whole process. Seeding can determine the quality of a pearl and, consequently, can determine the benefit of a farm.

Graft tissue plays an important role in pearl formation as it is responsible for secretion of nacre and it influences colour and lustre. Tissue transplantation can be achieved by three methods: autograft, homograft or heterograft.

In the autograft method, the operation, cutting graft tissue and transplantation, is done in each individual. In homograft method, a number of oysters are sacrificed so as to get mantle pieces to transplant in other of the same species. Homograft is a desirable method for small oysters such as *Pinctada funcata* but, autograft method is not preferable in such species. It is difficult to cut a piece of mantle from an oyster and then insert it in the same individual. It is certain that it will weaken the oyster much more than by the homograft method.

Autograft method is preferable in larger oysters such as *Pinctada maxima*. it could save oysters from undue killing just for mantle pieces although it takes much more time to do seeding for a large quantity of oysters. Since autograft and homograft methods are applied in the same species, there are no problems between graft tissue and host oyster. But, in heterograft method, it is important to know whether grafting is accepted by the host or not, because graft tissue is taken from other species. Heterograft method should be tried in experimental culture, as it can influence colour and lustre of a pearl.

Various sizes of nuclei are being used in seeding. Generally, small oysters can accept small beads whereas large oyster accept larger beads. However, seeding technicians use a range of size of nucleus for a species. There is no doubt about the fact that a large nucleus can produce larger pearls if the mother oyster can bear up to harvest. Therefore, the size of inserted nucleus strongly contributes to the size of pearl to be harvested.

Despite the fact that a higher rate of nucleus rejection or mortality of mother shells is more possible in large beads than small ones, technicians must be encouraged to use larger nuclei in order to produce larger pearls. Sometimes, targeting a quantity set for production can be a kind of constraint to seeding because technicians may not dare to use larger nuclei because it can affect the rate of success in seeding. A pearl farm must therefore consider a preference basis for production: quality or quantity.

Seeding technicians play an important role in seeding and the success or failure of the farm hinges on them. Skill and keen interest are significant qualification for seeding technicians. It is essential to know anatomy of the pearl oyster and to understand the theory of pearl formation and concept of seeding. They must be interested in seeding with heart and soul. Undesirable competition must not be observed among them and they must acknowledge each other. Discussion is an effective way to improve seeding techniques, although it is quite certain that each has his own secret. However, success rate is highly variable, especially in *Pinctada maxima*, in seeding in accordance with size or age or condition of mother oysters. *P. maxima* can grow beyond 25 centimetres in dorsoventral measurement.

Research is important in all aspects of pearl cultivation. Neglecting the important role of research or just having the satisfac-

tion of previous achievement can lead to bring a once buoyant industry to hit rock bottom.

It will be best if seeding technicians are research-minded and like to collect data of their research. A number of oysters should be appropriated for research as it can provide positive or negative results. The results are invaluable indeed. Some experiment, such as the quality of the pearl, will take about three years to get a result and it is unpredictable whether it may be positive or negative. Pearl culturists should know that research cannot always give positive results. Trying to do research and to utilize the results will lead to a successful industry for many years.

LABORATORY GROWTH *RATES* OF PINCTADA *MARGARITIFERA*—A PRELIMINARY REPORT. Jaw-Kai Wang, Biosystems Engineering Department, University of Hawaii at Manoa, Honolulu, HI 96822.

Two groups of oysters were used in this preliminary experiment. In the first experiment, 75 *Pinctada margaritifera* were imported from Palau on March 15, 1993. Ten of the seventy-five were taken by the state quarantine officer. The oysters appeared to be healthy. The remaining 65 oysters went to a small integrated oyster-shrimp production facility on Coconut Island, Oahu. To meet the quarantine requirement, the facility was designed to sterilize its effluent before discharge into a drainage pit. Nutrients that came from shrimp production were used to produce algae to feed the oysters. As the water quality in the Kancohe Bay, where Coconut Island is located, varied greatly, we were unable to maintain a stable condition at the Coconut Island facility and the oysters growth rates varied greatly. On August 16, 1993, the oysters were moved back to the Aquacultural Engineering Laboratory, Biosystems Engineering Department, University of Hawaii at Manoa. Forty-six oysters died before we were able to stabilize them. Our growth experiment started on November 15, 1993 using the remaining nineteen oysters. The oysters were kept in 30 degree centigrade un-filtered sea water enriched with cultured algae at a density of 5×10^5 cells per milliliter. Algae were produced using sea water obtained from Kewalo Basin, Oahu, without inoculation. Under appropriate management practices, eighty percent Chactoceros dominate culture can be established without inoculation.

PEARL CULTURE IN CHINA. Qiang Wang and Hualiang Wu, Department of Agricultural and Resource Economics, Department of Biosystems Engineering, University of Hawaii at Manoa, Honolulu, HI 96822.

China is the world's largest potential pearl culture country. In 1992, China's export value of pearl reached 2.78$ million, which basically was primary pearl export. After the successful artificial pearl culture technique in *Pinctada fucata* in 1958, China set up the first pearl culture farm in 1964. However, China really developed the pearl culture at large scale after the end of 1970s. China's pearl culture can be classified into marine pearl culture and freshwater pearl culture. The major marine culture species of pearl oyster is *Pinctada fucata*, *P. margaritifera*, and *P. maxima*, which mainly distributes in Guangdong, Guangxi, and Hainan provinces of south China areas. The major freshwater culture species of pearl oyster is freshwater mussel, *Cristaria plicata* and *Hyriopsis cumingii*, which is generally distributed in the most East China, South China, and Central China areas. This paper will describe the history of pearl culture, especially oyster culture, present current principal culture methods, and examine main culture policies and problems, including the investment policy, culture technique, and market.

RECENT DEVELOPMENTS IN PEARL OYSTER RESEARCH IN INDIA. A. C. C. Victor,[1] A. Chellam,[1] S. Dharmaraj,[1] and **T. S. Velayudhan,** [1]Tuticorin Research Centre of CMFRI, 90, North Beach Road, Tuticorin-628 001, India. Central Marine Fisheries Research Institute, Cochin-682 014, India.

The occurrence of pearl oysters in the beds of Gulf of Mannar is known from time immemorial. The oysters were fished for their pearls whenever they were found to bear pearls and the fishing operations found to be economically viable. Research on such aspects of pearl oysters as farming, production of cultured pearls and pearl oyster seed, are of recent origin and in a period of 20 years, much information particular to Indian conditions has been collected and published in various journals.

Pearl oyster seed are produced in the hatchery. The scaling up of the seed production in the hatchery of the Central Marine Fisheries Research Institute at Tuticorin is achieved through investigations on oyster breeding, larval rearing, larval food production, spat-setting and in the rearing of juveniles.

The inshore waters in the southeast coast of Indian bordering the Gulf of Mannar are shallow and unprotected to a large extent from the vagaries of nature. A farming technology to suit local conditions has been developed and the juveniles and mother oysters farmed.

The formation of the nacreous coating on the implanted nuclei is rapid in the tropical Gulf of Mannar waters. Even though seasonal variation is not greatly pronounced here, experimental production of pearls during different periods has shown differential nacre growth and pearl quality. Unlike in temperate waters, all gonadial developmental stages can be encountered in the oysters throughout the year.

Many of these research and development efforts carried out on the pearl oysters at Tuticorin have streamlined cultured pearl production to develop into an industry in India.

A PEARL FARMING FAMILY. Peter William, William Family Pearl Farm, Manihiki Cook Islands.

The William family owns and operates the first and the largest local pearl farm in the Cook Islands. This paper reviews the history of the farm's development, and outlines the present status of the William family farm in Manihiki. The history of differences in development approaches and industry management between the local and the central governments are discussed. The paper de-

scribes the role these difficulties played in hindering development of the William farm, and the whole industry.

The importance of good seeding technicians is highlighted. Recent marketing strategies for Manihiki pearls are presented.

The socio-economic, cultural and political changes wrought by pearl farming are profound. These changes are described, and possible solutions are outlined.

STATUS OF PEARL CULTURE IN INDONESIA. Tjahjo Winanto, Seafarming Development Centre, PO Box 74/TK, Teluk Betung 35401, Indonesia.

Indonesia is an archipelago country with an extensive area of 13,667 islands. It has numerous bays and coves, and many sheltered areas which have good potential for pearl culture. Indonesia also has abundant pearl oyster resources. When properly developed, pearl culture can be one of the major non-oil commodity-generating industries, which can produce substantial revenues for the nation. The potential area suitable for pearl culture in Indonesia is at least 5,600 hectares.

At present, these wide areas for pearl culture are not yet managed properly. Most of the pearl companies are still managed by foreign investors and foreign experts. Just recently, some pearl culture has been handled by national investment and local, skilled experts. This situation has happened because of no special government institutional work in this activity. Considering this situation, Seafarming Development Centre, under the Directorate General of Fisheries, is the one government institution which has responsibility for pearl oyster farming and pearl culture, and also for transferring pearl culture technology. The results of this, compared to original expectations, leave much room for improvement, especially when the number of private pearl producers is taken into consideration.

The government action through the Seafarming Development Centre is not, however, of little value. The Centre helps to accelerate the process of the transfer of technology. This is demonstrated through the annual number of pearl exports (rounds, half-pearls, and mother-of-pearl shells), which is gradually increasing each year. In addition to that, many more pearl oyster companies have been successful in the mass production of seeds through hatcheries.

COVER PHOTO: Juvenile pearl oysters *Pinctada margaritifera galtsoffi* produced at Black Pearls, Inc. hatchery in Kona, Hawaii. Approximate shell diameter 5–8mm. Photo by Dale J. Sarver.

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JOURNAL OF SHELLFISH RESEARCH

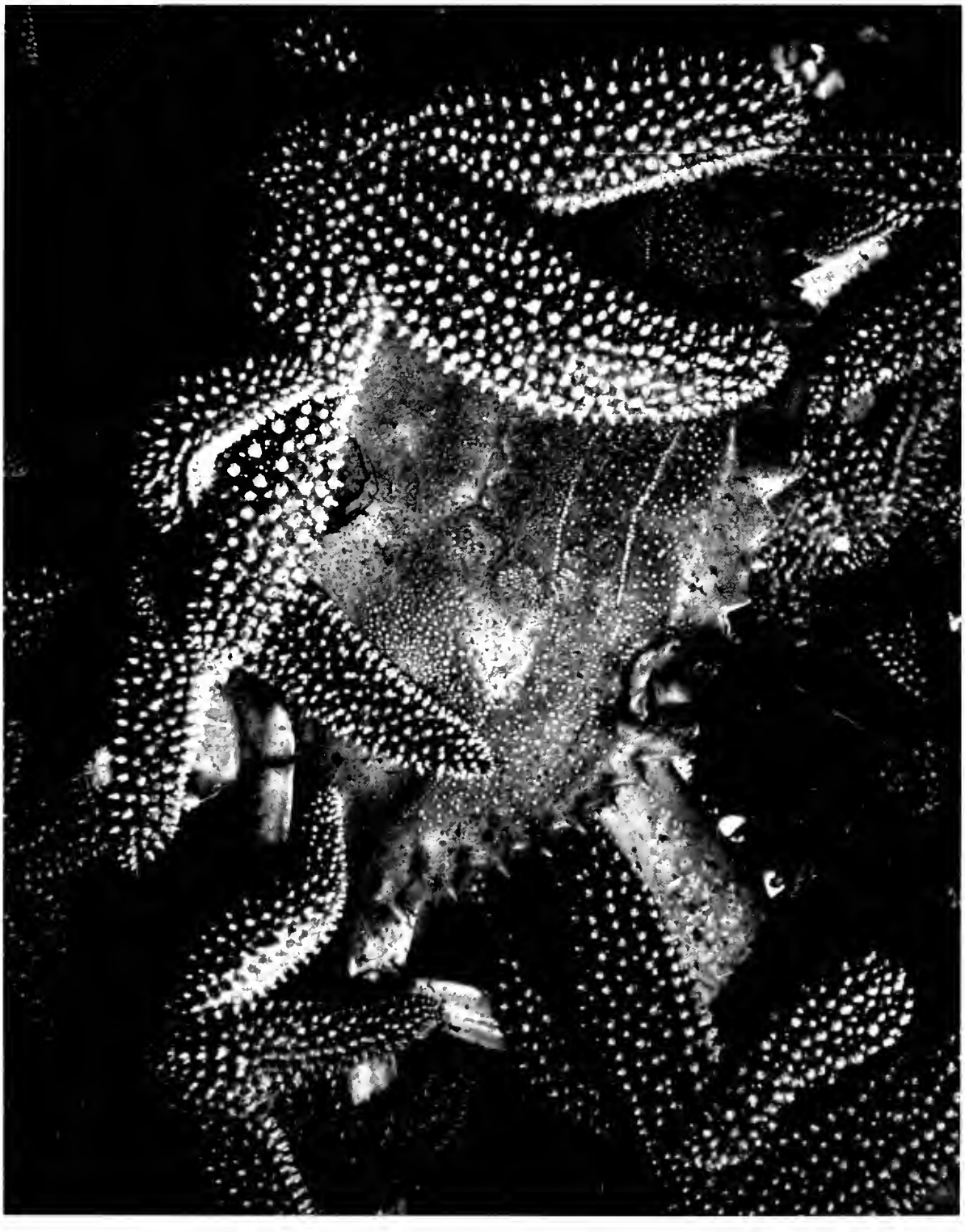
Vol. 13, No. 1 JUNE 1994

CONTENTS

CONTENTS CONTINUED ON INSIDE BACK COVER

JOURNAL OF SHELLFISH RESEARCH

VOLUME 13, NUMBER 2 DECEMBER 1994



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IN MEMORIUM
RAVENNA "RENEE" UKELES

Dr. Ravenna Ukeles, a recognized authority on phytoplankton culture and nutrition of bivalve mollusks, died July 11, 1994, in Milford, Connecticut after a long illness.

Renee was born in New York City. Her early education at Walton High School in Manhattan instilled in her diverse interests in art, design, and the natural world. While painting and photography remained free-time creative outlets throughout her adult life, with some striking, original works produced, she chose biological science for undergraduate and graduate studies.

Renee entered Hunter College in New York City in 1943 to major in Biology. Many of her classmates at the time were older students—WWII Veterans studying under the GI Bill; she described them as ". . . some pretty tough guys who had seen a lot." Despite the challenges these circumstances provided, she was graduated with honors in 1947. Her M.S. research project at New York University involved studies of carbon sources for culturing the saprophytic cryptophyte, *Chilomonas paramecium*. Interestingly, Renee's last senior-authored paper (*J. Protozool.*, 39: 399–405), coming after a long and varied career, also investigated her first major research subject, "CP," as she called it.

Following completion of the M.Sc. Degree in 1954, Renee began her doctoral studies with R. P. Hall at New York University. At the time, competition was intense for student positions with more established faculty, and Renee was proud to be accepted for study with the eminent protozoologist. Renee was awarded the Ph.D. Degree from NYU in 1958.

Dr. Ukeles began her professional career in 1959 when she was hired by Dr. Victor Loosanoff—a colorful character in the history of shellfish research—to work at the Milford Marine Biological Laboratory, then under the jurisdiction of the US Department of the Interior, Bureau of Commercial Fisheries. Loosanoff, Harry Davis, and others on the Milford staff had developed protocols for conditioning and spawning a number of shellfish species in the laboratory. There was, however, a missing step in what has come to be known as the "Milford method" of shellfish aquaculture—how to feed the larvae? Previous work at both Milford and in Great Britain had demonstrated that larval bivalves could be grown on diets of selected phytoplankton isolates, but there was not a reliable method for culturing marine phytoplankton in sufficient amounts for further development of molluscan aquaculture, at the scale necessary for research or commercial enterprise. This became Dr. Ukeles's assignment at the Milford Laboratory.

Being a protozoologist by training, Dr. Ukeles began addressing her algal research problem on two fronts: first, by assembling and characterizing—in many ways—a collection of phytoplankton strains that had been or were in use for feeding invertebrates, and second, by acquiring a profound knowledge of the published literature on algal culture and molluscan feeding. Recognition of this knowledge led to her selection by Dr. Otto Kinne to write the chapter, "Cultivation of Plants: Unicellular Plants," for his Treatise on Marine Ecology in 1975. The Milford Culture Collection contained an endless trove of research questions, which Dr. Ukeles addressed in her eclectic and imaginative way. Effects of contaminants, myxotrophic growth, and temperature tolerance were just several of the algal questions that provided fodder for her experiments. The main objective of developing culture methods was, however, always foremost in the work of her laboratory. Assembling easily-obtained components in new and imaginative ways, Dr. Ukeles designed and built culture assemblies such that 5-gallon carboy cultures could be kept bacteria-free. After much experimentation, a semi-continuous culture management strategy was found to be most successful, in terms of biomass production and contaminant exclusion. The apparatus and management scheme were published as a chapter in the first volume of the Handbook of Phycological Methods (1973). With this model, and Dr. Ukeles's patient personal consultation, hatchery production of shellfish seed advanced from a theoretical goal to a viable commercial venture. The facility she designed at the Milford Laboratory for these culture units is still producing sufficient algal biomass to feed many millions of shellfish seed and to generate useful research information.

Dr. Ukeles's career at the Milford Laboratory was long and productive, as the partial publication list that follows will attest. She retired in 1989 as Investigation Chief of the Microbiology Program at the Milford Laboratory, that had been transferred to NOAA's National Marine Fisheries Service in 1970. She continued to contribute to algal research efforts after retirement. What those of us who worked with her remember about her, though, is the relentless tenacity with which she pursued "THE TRUTH." "The bugs don't lie," she would say, "so it's our job to figure out what they are trying to tell us." Experiments were repeated, alternative hypotheses were explored—every detail was examined carefully before a conclusion would pass muster. She once told me with a mixture of pride and shyness that she had never had a submitted manuscript rejected by a journal. The attention to detail and thorough evaluation of ideas are easily recognizable in her written works.

A remarkable aspect of Dr. Ukeles's career is the ease with which she interacted with both algal and molluscan researchers. She was fluent in the language and lore of both traditions, and was in a unique position to bring them together. In conversations at meetings and on the phone, Dr. Ukeles often would explain algal ideas to shellfish biologists and shellfish feeding and nutrition to algal researchers. Thus, although she was not affiliated with an academic institution, teaching was integral to her interactions with her peers as well as with her co-workers at Milford. She leaves us an influential and uniformly excellent body of written work, an appreciation for the truth that underlies life in all its forms, and a sense of curiosity and skepticism that compels us to continue probing living things for the truths they hold.

Gary H. Wikfors and Elizabeth N. Wikfors
Milford, CT
1994

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IN MEMORIAM
Dr. Jürgen E. Winter
1937–1994

With the death of Dr. Jürgen E. Winter on June 30 the aquatic science community lost a colleague and a dear friend. Dr. Winter was born on October 23, 1937, in Schneidemühl, Germany. In 1969 he received his doctorate from the University of Hamburg, Germany. His doctoral study of feeding in *Arctica islandica* and *Modiolus modiolus* was favorably received by the international academic community and attracted a lot of attention. It was one of the foundations upon which later work on bivalve ecophysiology was built. In the same year he joined the "Institut für Meeresforschung" in Bremerhaven, Germany, as an assistant researcher, moving through the ranks to become director of the Zoology Department of this institute between 1974 and 1977. In 1977 he emigrated to southern Chile at the invitation of the Universidad Austral de Chile, located in Valdivia, where he thrived in his new environment and began his enormous academic activity in the field of aquaculture. It was during this period (1979) that I met Jürgen for the first time. As one of his students I really became captivated by his enthusiasm and passion for the development of aquaculture in Chile. How can I forget the adventurous trip in his VW minivan from Valdivia, Chile, to Cartagena, Colombia, driving and sharing with two other colleagues more than 15,000 km in just 3 weeks, to attend the III Latin American Aquaculture Simposium in 1980? Also all of the field trips to Chiloé, when after a long working day, he showed himself to be an expert in German cuisine while talking about the miracles of the marine environment.

From 1982 to 1986 Jürgen was the president of the "Comité de las Ciencias del Mar-Chile," the most important association of marine scientists in Chile. He established the "Centro de Investigaciones Marinas" in 1981 with two experimental aquaculture stations in Chiloé Island (Quempillén and Yaldad), southern Chile. These two aquaculture stations have received most of his attention during the past 10 years, because through them the university had the capability to develop aquaculture activities in southern Chile. The stations were also used for academic purposes such as research and teaching. Jürgen was very ingenious and successful in raising funds to support the mollusc aquaculture research at the Universidad Austral, particularly its research stations. Much of this funding came from well-known international agencies. He was also very supportive of those of his faculty members who wished to study abroad. In 1985 Jürgen founded the School of Marine Biology (undergraduate marine biology program) at the Universidad Austral de Chile and became its director, a position he held until his recent death. In 1989 he also founded the "Instituto de Biología Marina" of the Faculty of Science, which he headed until 1992.

In the academic world, where the reign of egos is so predominant and where it always seems possible to find someone with an even bigger ego than the biggest one you've seen before, Jürgen Winter was something of an enigma. He was, at least during the years I knew him (over 15), among the most humble of my acquaintances. His manner—always friendly—and speech—energetic—was associated with enthusiasm, keen wit, and outstanding intellectual ability. Jürgen always spoke kindly of his former students and was intensely interested in their careers.

While he was not much dedicated to classroom teaching, outside the lecture rooms, the students had the fortune to find in him (the "doc" as they referred to him) an inexhaustible source of information. Jürgen was knowledgeable in virtually every aspect of marine biology, and in a wide variety of subject areas outside that extensive discipline. He was a compulsive worker, who led by example and earned the respect of his colleagues. In his many conversations with students, who always surrounded him, he always had some tidbit of information that none of us had previously known. Working till very late at night in the university, he always left his door open for everyone who asked for advice, literature, or some specialized instrument. He always had time for those who needed him.

He had a gentle, self-deprecating charm that enabled him to move as easily among fishermen and fishing industry representatives, the general public, students, and government officials, as among his colleagues. I doubt that Jürgen ever failed to respond to a request for information or advice; he was incredibly helpful to everyone.

In addition to his administrative responsibilities, Jürgen remained active in the academic field. He worked on the editorial boards of Marine Biology, Marine Ecology Progress Series, Vie Marine, Revista Chilena de Historia Natural, Estudios Oceanológicos, and Investigación Pesquera. His involvement with aquaculture activities in southern Chile was multifaceted, and he worked very closely with

artesanal fishermen, in educational programs for rural teachers on aquaculture. Several projects concerning social and basic research were in progress when Jürgen died.

His life's work and accomplishments point to a significant lesson: dedicated individuals have to struggle to get their scientific plans and intellectual messages through to politicians, managers, and the lay public, and have to overcome the inertia of the system. Only in this way can ideas persist, to the benefit of future researchers and society. If we can make just a small fraction of the contribution to science and society that Jürgen made in his time, we will have done well. Those of us who were Jürgen's students can only hope that some of the knowledge, dedication, high ethical standards, and love of life that he possessed have been kept alive in us and passed on to our students.

I'll miss you, Jürgen.

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PUBLICATIONS OF DR. JÜRGEN E. WINTER

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WINTER PREDATION ON BLUE CRABS, *CALLINECTES SAPIDUS*, BY STARFISH *ASTERIAS FORBESI*

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ABSTRACT Blue crabs, *Callinectes sapidus*, burrowed into the sand-silt bottom at a site in the Mystic River estuary, Connecticut, at temperatures below 5°C. Crabs were torpid at these low temperatures. Starfish, *Asterias forbesi*, were observed to prey on burrowed crabs. The proportion of crabs preyed upon increased as water temperatures declined through the winter, reaching up to 81% of crabs observed during a single dive. There was no significant difference in the size or sex of crabs preyed on by starfish and those which were not preyed upon. Starfish that were collected preying on crabs were not significantly different in size than those found on the surrounding bottom. Low temperatures, which affect the physiology of normally motile megafauna, can greatly alter predator-prey dynamics.

KEY WORDS: *Callinectes*, *Asterias*, predation, temperature, census, winter

INTRODUCTION

The blue crab, *Callinectes sapidus* Rathbun, 1896, is distributed in the coastal waters of the western North Atlantic from northern Argentina to Nova Scotia and from the shoreline to a depth of approximately 90 m (Williams 1984). The waters around Cape Cod, Massachusetts, are generally considered to be the northern limit of the species range except during periods of higher than normal water temperatures (Scattergood 1960, Harris 1982).

Studies of mortality of juvenile and adult blue crabs have generally focused on fish predators. For example, sandbar sharks (*Carcharhinus plumbeus*), Atlantic croaker (*Micropogonias undulatus*), American eel (*Anguilla rostrata*), and striped bass (*Morone saxatilis*) have been identified as major predators of blue crabs (e.g., Manooch 1973, Wenner and Musick 1975, Overstreet and Heard 1978, Medved et al. 1985). Extreme environmental factors have also been shown to cause high mortalities in blue crabs. Van Engel (1982) correlated low temperature conditions during January 1977 and February 1978 in the Chesapeake Bay with high mortalities of crabs observed in Virginia's winter dredge fishery. He also reported that strong wind-driven onshore surface currents caused strong offshore nearbottom currents in the southern Chesapeake Bay which swept blue crabs over the bottom and abraded their carapaces.

Long Island Sound is near the northern limit of distribution of *C. sapidus*. Nearshore bottom water temperatures in this region decline to near freezing during winter and produce extreme conditions for resident species. This is a period of the year when little *in situ* work has been conducted in this area. Herein we describe predation by starfish, *Asterias forbesi*, on blue crabs during periods of low water temperatures when the crabs were torpid.

MATERIALS AND METHODS

An overwintering aggregation of blue crabs was studied using scuba diving at a site in the Mystic River Estuary (41°19.5′N, 71°59′W) off the coast of eastern Connecticut in Fishers Island Sound (Fig. 1). The study site is a sloping sand-silt substrate, on the east side of a natural channel, which grades into a flat mud bottom at 3–5 m depth. The channel is adjacent to a shallow eelgrass (*Zostera marina*) bed. Qualitative observations were made during winter at the primary study site, as well as nearby areas, from January 1981 through March 1983, in order to describe the relationships between predator and prey. Estimates of temporal variation in predation intensity, by starfish on crabs, were made by conducting six visual censuses during January and February 1984, as water temperatures declined. Approximately 16.4 hours during 22 dives were spent observing crabs and starfish in the primary study area. Two hours of additional observations were made during 4 dives at another area within the Mystic River Estuary, around Ram Island, and at Ellis Reef.

Each census, one per sampling date, was conducted by making a single random swim over the area occupied by overwintering crabs. The census was conducted in an upcurrent direction (during either ebb or flood tides) and took a circuitous path in order to increase the probability of encountering crabs and increasing the sample size. We were careful not to disturb the sediment surface around crabs in order to avoid exposing buried individuals and making them more susceptible to predation by starfish, which would bias subsequent surveys.

Multiple censuses were not conducted on any single date because of the relatively small area occupied by overwintering crabs (ca. 500 m^2) and because it was not possible to determine if replicate censuses surveyed the same area. Transect lines could have been deployed for each census but we felt that the lines would have disturbed the sediment cover over crabs. Permanent lines could also have been used but these would have accumulated drift macroalgae and also had the effect of increasing exposure of buried crabs.

Buried crabs were identified by observing their anterior carapace margin and eyes at the sediment-water interface. Occasionally, only a subtle outline of the carapace was visible as a slight topographic discontinuity in the otherwise smooth sediment surface. We are confident that virtually all crabs along the census path were counted. (We dragged knives through the mud during several portions of previous observational dives and did not encounter any crabs which we could not identify from surface features.) Individual crabs were identified and counted, noting if they were preyed upon by starfish. Dead crabs (empty carapaces) with no starfish were not counted. Predation intensity for each census date was expressed as the percent of total crabs preyed upon by starfish (i.e., the number of crabs preyed upon divided by the total number of crabs observed, multiplied by 100).

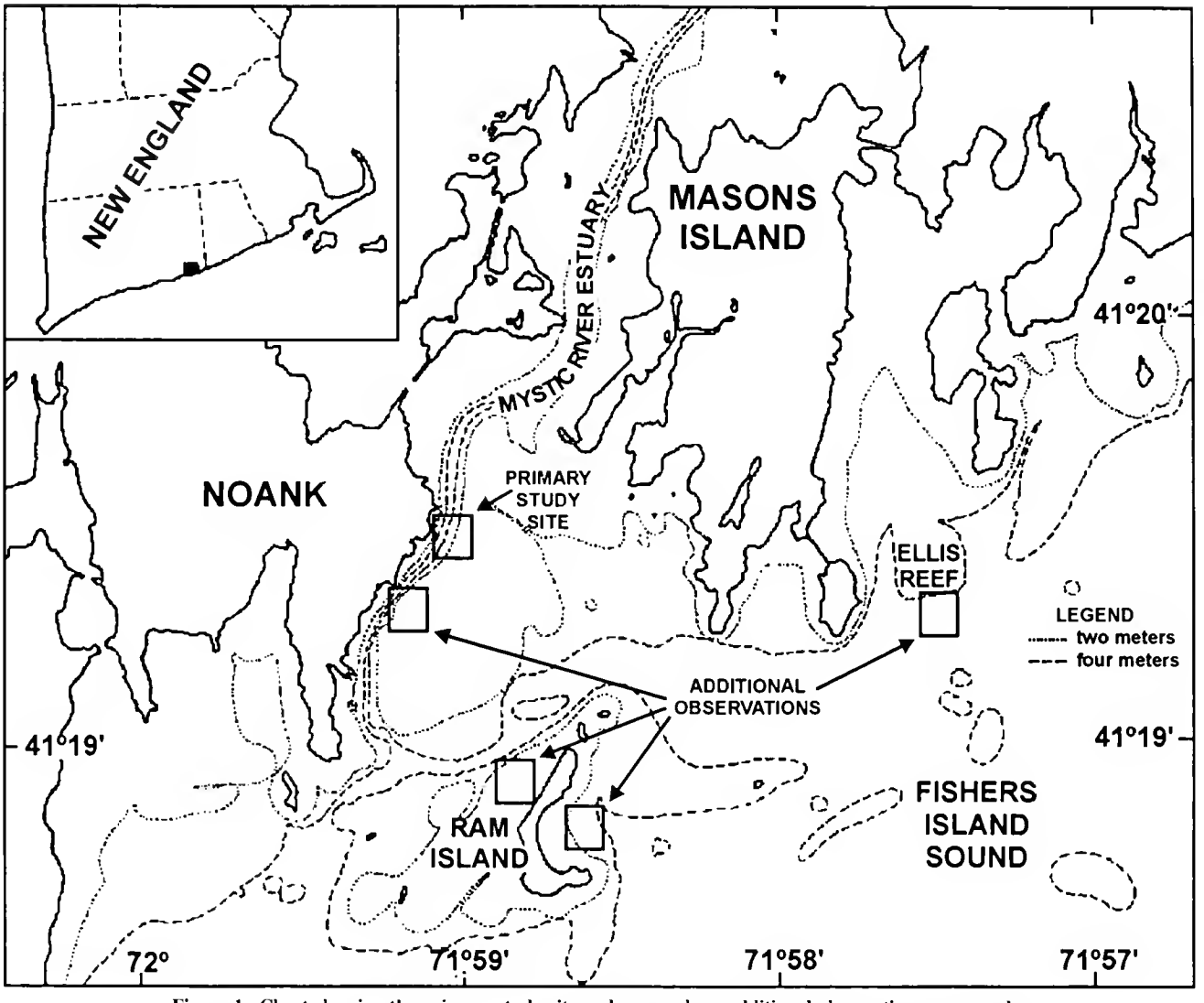

Figure 1. Chart showing the primary study site and areas where additional observations were made.

After the final census was completed on 20 February 1984, all live and dead crabs were collected, carapace width measured (including lateral spines), and sex determined. Starfish were also collected and classified into two groups: those found on crabs and those from the surrounding bottom. Starfish size was measured as the longest distance between the distal ends of opposite arms. All measurements were made to the nearest 0.1 mm. Crabs which were preyed upon early in the survey may have been missing from the collection due to export from the site by moving rafts of drift macroalgae.

Water temperature data were taken almost daily from the seawater intake at The University of Connecticut's Marine Research Laboratory in Noank, Connecticut. Temperature was measured to the nearest 0.5°C. The laboratory is approximately 100 m south of the study site. Temperatures from the seawater intake were always within 0.5°C of temperatures measured with a mercury thermometer while diving.

RESULTS

Winter 1981–1983 Observations

When water temperature declined to approximately 5°C, crabs moved to the eastern slope and bottom of the Mystic River channel

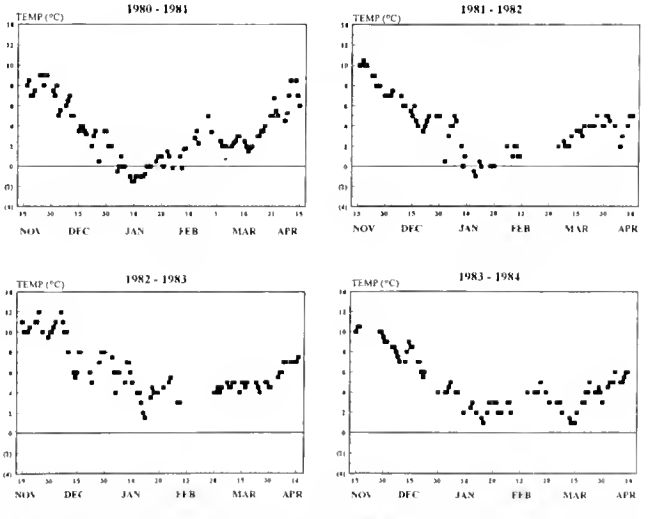

Figure 2. Water temperature recorded at The University of Connecticut's Marine Research Laboratory in Noank, November–April of 1981–1984.

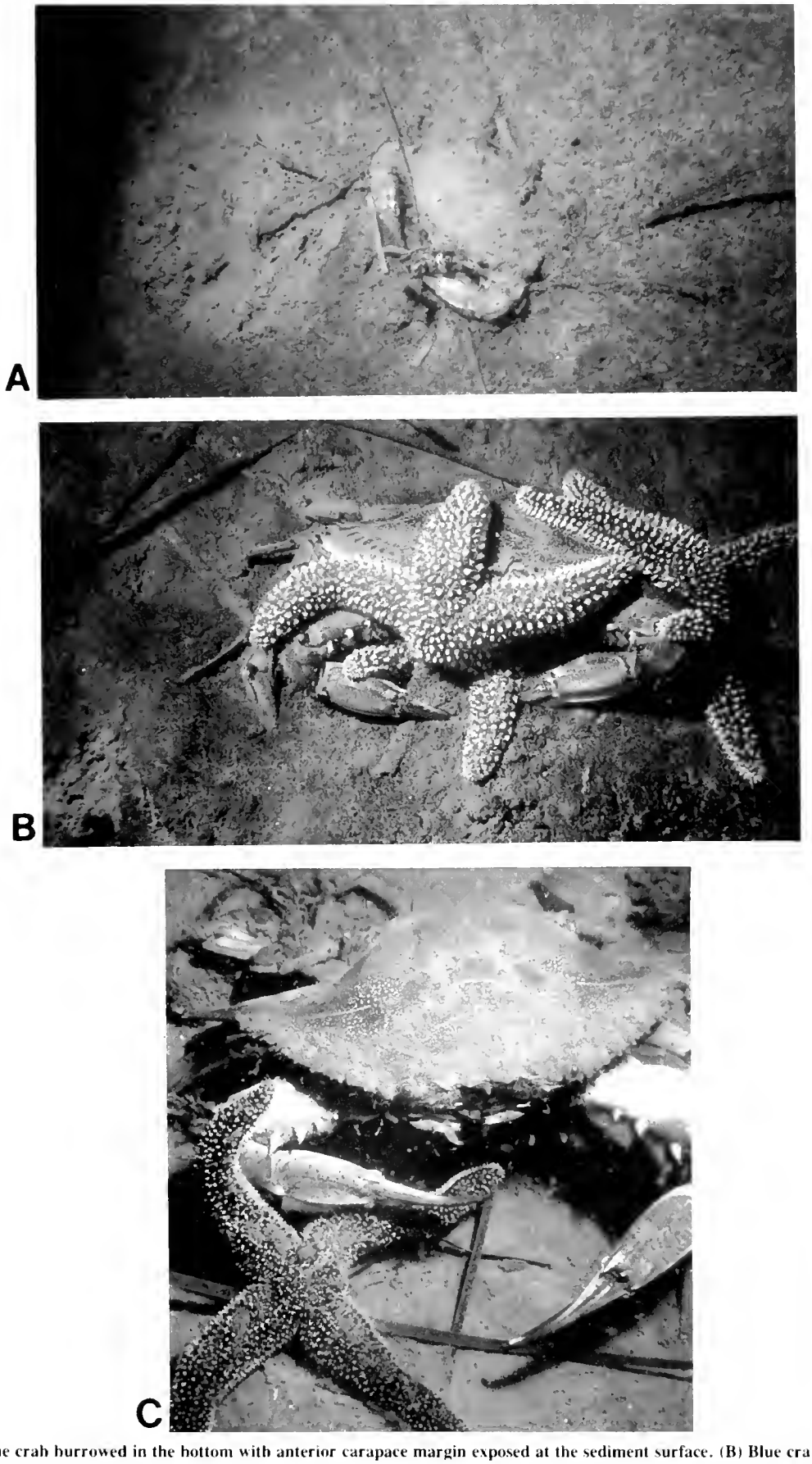

Figure 3. (A) Blue crab burrowed in the bottom with anterior carapace margin exposed at the sediment surface. (B) Blue crab being attacked by starfish. (C) Blue crab attempting to remove a starfish from the chelae (water temperature 5°C). Ultimately, the starfish was removed.

and burrowed into the substrate. Temperatures ranged to as low as −1.5°C after burrowing (Fig. 2). Crabs were generally unreactive (i.e., torpid) if not physically prodded. The typical orientation of an individual crab while burrowed was at an upward angle along the posterior-anterior axis with only the eyes and a narrow portion of the anterior carapace margin exposed. Chelae were held close to the body and were just below the sediment surface. A small number of crabs were burrowed into the bottom so no parts of the carapace were visible and were identified by slight but recognizable discontinuities in the sediment surface. Some crabs were unable to burrow deeply into the bottom leaving a greater portion of the anterior carapace exposed (Fig. 3A); these comprised no greater than approximately 10% of the observed individuals. Tidal currents in the channel (ca. 25 cm s^{-1} maximum), bioturbation by winter-active benthic organisms (e.g., *Cancer irroratus*, *Carcinus maenus*, *Pagurus longicarpus*, Mysidacea), and drift macroalgae eroded and scoured the substrate cover over the crabs exposing more of the dorsal carapace and chelae. As temperatures rose to 5°C crabs became active, emerged from the sediment, and left the area.

The common starfish, *A. forbesi*, was found to be active during all observation periods, even at water temperatures of −1.5°C. Tracks across the sediment surface and through benthic microalgal mats indicated recent movement. Starfish approached and successfully attacked burrowed crabs at temperatures below 5°C. Crabs were seen partially extricated from the substrate, as well as fully extricated and sometimes lying on their dorsal carapace, indicating that crabs attempted to move or that starfish were able to pull individual crabs out of the bottom. Starfish were generally observed feeding along the posterolateral region of freshly killed crabs, gaining access to the internal organs at the telson and through the walking leg and chelae joints (Fig. 3B). Given that crabs were burrowed before an initial attack, a starfish would have to be successful at attaching to and killing a crab from the anterior region. Up to 19 starfish were observed preying upon a single blue crab. Predation intensity increased throughout each winter season as more crabs became available through exposure from the substrate and as declining temperatures must have reduced the ability of crabs to react to starfish attacks. Eighty-one percent of blue crabs observed (n = 21) during a single dive in February 1981 (temperature −1°C) were being preyed upon by starfish.

We assume that all observations of starfish preying on crab remains are the result of active predation and not necrophagy. All crabs examined which were burrowed but not attacked by starfish were alive (n > 200 from 1981–1983). A single observation of a crab removing a starfish attached to its chelae (temperature 5°C) confirms that all attacks are not successful (Fig. 3C).

Winter 1983–1984 Quantitative Surveys

Predation intensity increased throughout the winter, from near zero in early January to 53% of crabs observed during the census on 20 February (Fig. 4). Although sample sizes were small, the relationship between percent predation and cumulative crab number within a census (Fig. 5) indicates that our measures of predation intensity represent a seasonal pattern. There was no significant difference (two-sample t-test, t = −0.81, P = 0.42) in size between crabs being preyed upon by starfish and those that were not (Table 1). Also, there was no difference (two-sample t-test, t = 0.93, P = 0.35) in the lengths of starfish that were collected attached to crabs ($\bar{x}$ = 7.57 cm, SD = 2.93, n = 124) and those collected from the surrounding bottom ($\bar{x}$ = 7.23, SD = 3.07, n = 151). The sex ratio of males:females was 1:2.2 for all crabs collected and there was no preference by starfish for either sex (1:2.1 sex ratio of those preyed upon versus 1:2.4 of those that were not; Chi-square test, χ^2 = 0.09, P > 0.05).

DISCUSSION

These are the first reported observations of significant predation by asteroid starfish on decapod crustacea of which we are aware. The previous literature describes species of the genus *Asterias* as predators of infaunal and epifaunal organisms, primarily bivalves (Galtsoff and Loosanoff 1939, Needler 1941, Hancock 1955, 1958, Seed 1969, Dare 1982). The impacts of starfish predation on crab populations subject to winter torpor have not previously been discussed. Galtsoff and Loosanoff (1939) reported that *A. forbesi* in Long Island Sound fed more actively in summer than in winter but no rates were determined. Experiments conducted in Milford Harbor, Connecticut, by MacKenzie (1969) showed that individual *A. forbesi* fed on *Crassostrea virginica* at rates of 0.12 oysters d^{-1} at average water temperatures of 1°C and 2.3 oysters d^{-1} at 5°C.

Needler (1941) reported that *A. vulgaris*, in eastern Canada, fed mostly in spring and fall and relatively little in winter at temperatures less than 0°C. In contrast, Hancock (1955, 1958) reported that *A. rubens* in English waters fed at a high rate throughout the winter. Asteroid genera from other regions are also reported not to prey heavily on megabenthic decapods. Of 633 *Pisaster ochraceus* observed in Monterey Bay, California, only one was found preying on a *Cancer antennarius* (Feder 1959). Mauzey et al. (1968) found, during 654 observations of *Luidia foliolata*, *Mediaster aequalis*, and *Pycnopodia helianthoides* in Puget Sound, on only nine occasions were these starfish preying upon any crab species. This may indicate greater predation at particular times and places given the large area and time covered by this study.

The study site in the Mystic River Estuary is the only area we know which was consistently used by blue crabs to overwinter on an annual basis. Unfortunately, we have no data to confirm or deny site fidelity for individual crabs. There were no obvious

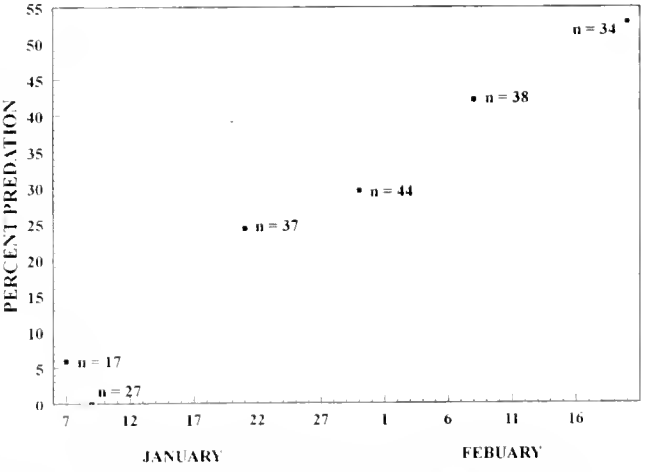

Figure 4. Pattern of predation intensity from 1984 survey dives (n = total number of crabs observed per census).

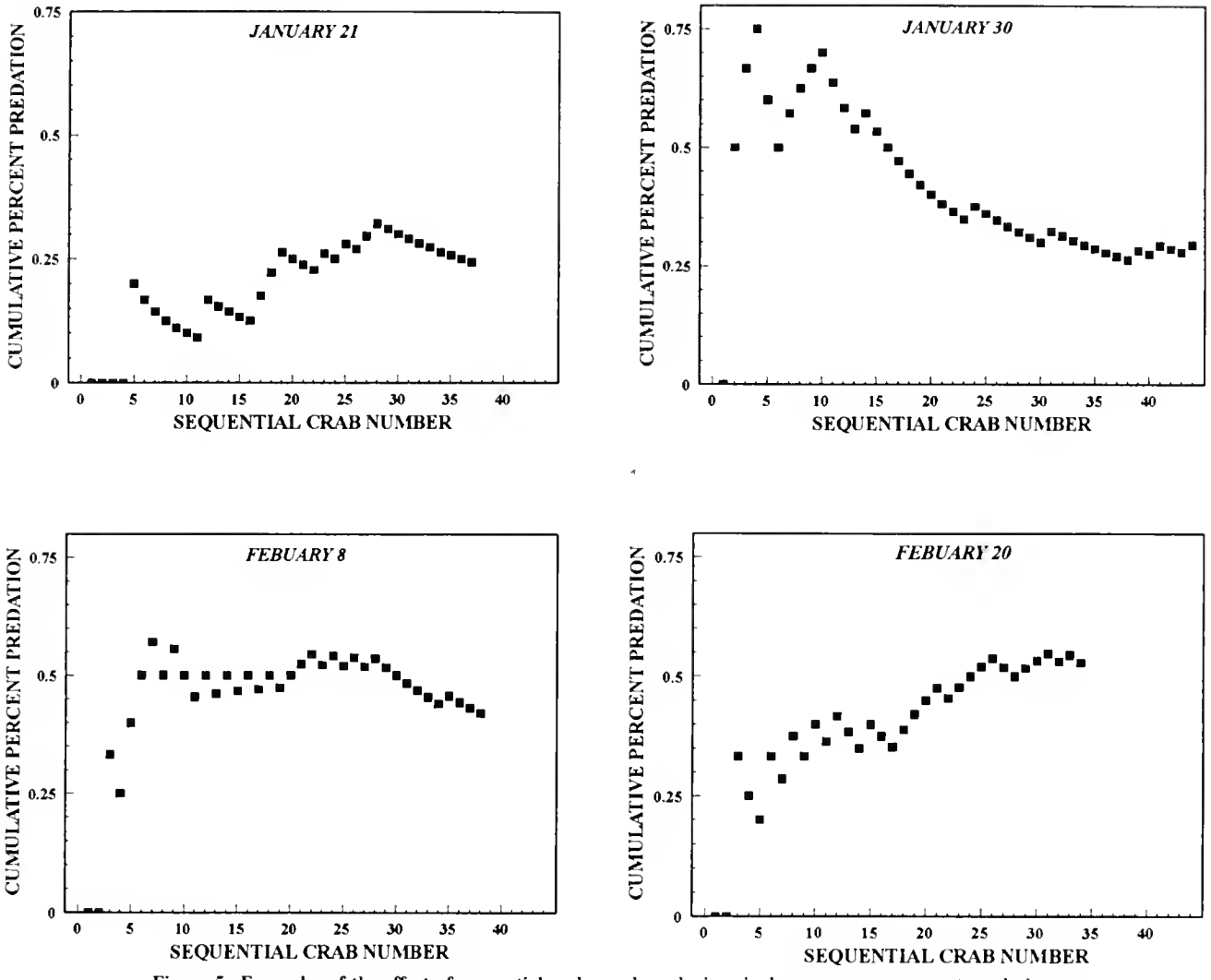

Figure 5. Examples of the effect of sequential crab number, during single censuses, on percent predation.

characteristics of the site which would distinguish this patch of bottom from any other adjacent part of the river. We have found other decapod species which return to other sites year after year for activities such as mating (i.e., spider crab, *Libinia emarginata;* DeGoursey and Auster 1992) and molting (i.e., Atlantic rock crab, *C. irroratus;* DeGoursey and Auster, unpublished data).

Our data showed no difference in the size or sex of blue crabs preyed upon although this pattern could have changed over time during the winter. Also, we found no difference in the size of starfish preying upon crabs. These relationships may change over the course of the winter based on size-dependent physiological responses of both predator and prey species. Predation success in many taxa is known to change based on the size relationship between predator and prey, as well as predator avoidance and escape strategies. Time-series sampling will be required to determine size class relationships between crabs and starfish. We have not found any published studies regarding the dynamics of echinoderm predators on crustacean prey which would allow us to predict the role that temperature and size variation has on predation success.

The importance of winter predation by starfish on torpid decapods has been underestimated. Low temperatures, which affect the physiology of normally motile megafauna, can greatly alter predator-prey dynamics; the interactions appear to be highly temperature dependent (i.e., lower predation at 5°C than −1°C). Additionally, we have observed *A. forbesi* preying on American lobster (*Homarus americanus*), Jonah crab (*Cancer borealis*), Atlantic rock crab (*C. irroratus*), and broad clawed hermit crab (*Pagurus pollicaris*) at temperatures below 5°C. Predator-induced mortality of overwintering decapods should therefore be considered in studies of the dynamics of decapod populations.

Table 1.

Carapace widths (including lateral spines) of blue crabs collected at the Mystic River estuary site on 20 February 1984. Sizes were measured to the nearest 0.1 mm.

	Crabs Preyed Upon By Starfish	Undisturbed Burrowed Crabs	All Crabs
Median	105.9	110.8	108.8
Mean	105.2	107.8	106.3
Standard Deviation	15.7	12.5	14.3
Minimum	65.0	81.8	65.0
Maximum	137.6	131.1	137.6
Number	43	34	77

ACKNOWLEDGMENTS

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SHORT-TERM REDUCTION OF ADULT ZEBRA MUSSELS (*DREISSENA POLYMORPHA*) IN THE HUDSON RIVER NEAR CATSKILL, NEW YORK: AN EFFECT OF JUVENILE BLUE CRAB (*CALLINECTES SAPIDUS*) PREDATION?

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ABSTRACT During the summer of 1992, a loss of 2- to 3-cm zebra mussels, *Dreissena polymorpha*, was recorded in the Hudson River near Catskill, NY. We document this adult population crash and present field and laboratory data suggesting that predation by blue crabs, *Callinectes sapidus*, was responsible. Dredge samples on July 8th indicated that the mean density of the largest zebra mussel size class (length = 2 to 3 cm) in the Hamburg-Catskill-Germantown region was 664 m^{-2} (range of 59 to 2,222 m^{-2}, 14 rocks examined). In sharp contrast, extensive dredging on August 11th did not recover a single mussel of that size class in that area. The largest mussel collected on August 11th was only 0.7 cm long, with over 100 rocks examined. An abundance of shell fragments and detached, live mussels were considered evidence of predation, possibly by a large immigrant blue crab population present in the area. Laboratory trials confirmed that these crabs can aggressively consume zebra mussels, particularly the size class that was virtually eliminated in the Catskill area, and that their feeding does result in piles of shell fragments and removed, yet unconsumed mussels. Further supportive evidence of the blue crab predation hypothesis was gained in the summers of 1993 and 1994, when blue crabs did not migrate into the Catskill area and a massive decline in 2 to 3 cm mussels did not occur.

KEY WORDS: Predation, biological control, zebra mussels, blue crabs

The spread of zebra mussels, *Dreissena polymorpha* (Pallas), throughout North American freshwaters has caused adverse ecological and economic impacts. The first confirmed report of zebra mussels in the Hudson River occurred in May 1991 near Catskill, NY, their introduction probably the result of transient boat/barge traffic. A benthic sampling program was subsequently initiated to monitor their population within the river. During the 1992 sampling period, a sudden and massive reduction in the density of large zebra mussels (length = 2 to 3 cm) was recorded. This article documents this adult population crash and presents field and laboratory data suggesting that predation by blue crabs, *Callinectes sapidus* Rathbun, was responsible. It had been hypothesized (R. Lipcius, Virginia Institute of Marine Science, Glouster Point, VA, personal communication) that, as zebra mussels dispersed into North American estuaries, blue crabs would prey on them, possibly acting to some degree as a population-regulating mechanism. The field and laboratory data presented herein support this hypothesis.

MATERIALS AND METHODS

Fieldwork

Monitoring was conducted in the Catskill, NY, region of the Hudson River, a tidal freshwater habitat 150 km north of the New York City harbor. Rocks were collected at depths of 1 to 15 m using a modified oyster dredge. On May 20, 1992, preliminary dredge samples were collected near the Rip Van Winkle Bridge in Catskill to confirm mussel presence. On July 8th and August 11th, quantitative samples were collected by dredging at the Rip Van Winkle Bridge as well as 0.5 km upriver (Hamburg, NY) and 7 km downriver (Germantown, NY). To calculate mussel density, mussels were counted and the surface area of each rock was approximated from tape measurements. A subsample of attached mussels was removed to determine mean mussel length.

Labwork

The diet of blue crabs varies with crab size (Van Heukelem 1991). Consequently, studies were undertaken to verify that Hudson River blue crabs would eat zebra mussels, particularly in the size range that had been so drastically reduced in the river. Crabs (carapace width point to point: 12.5 to 13.0 cm) were field collected in late summer 1992 from the Catskill area, held in freshwater aquaria (35 L) at ambient room temperatures (16 to 22°C), and used for feeding trials in the fall. For qualitative observations of predatory behavior, rocks containing zebra mussels were placed in aquaria with the crabs. In tests examining feeding size preferences, the rocks in an aquarium were periodically replaced with a tray containing unattached zebra mussels of selected size classes that were randomly mixed together. In each trial, a single crab was allowed to prey on these mussels for 5.5 to 7.5 hours.

RESULTS AND DISCUSSION

Fieldwork

The preliminary dredging on May 20th confirmed the presence of large zebra mussels (2 to 3 cm, densities ca. $\leq$500 m^{-2}) at Catskill's Rip Van Winkle Bridge. The quantitative samples of July 8th indicated that the mean density of this size class in the wider Hamburg-Catskill-Germantown region was 664 m^{-2} (range of 59 to 2,222 m^{-2}, 14 rocks examined). In sharp contrast, the dredging on August 11th in the same area did not recover a single

mussel of that size class, indicating that a severe population reduction had occurred since July 8th. Even with intensive dredging in this area, the largest mussel collected on August 11th was only 0.7 cm long, with over 100 rocks examined.

During the August 11th dredging, we also observed an unusually high abundance of both zebra mussel shell fragments and single, unattached, live zebra mussels. The absence of mussel tissue attached to the shell fragments indicated that these fragments had not resulted from the crushing of live mussels during the dredging process itself, but rather were the remains of mussels that had died before the August 11th sampling.

Further evidence of the disappearance of large zebra mussels was available from a submerged growth-chamber experiment. Rocks containing a total of 23 large mussels (2 to 3 cm) had been collected on July 8th and placed back on the river's bottom in a partially enclosed screened chamber that was opened at the top. When inspected on August 10th, only one live mussel remained in this chamber.

Massive zebra mussel mortality had clearly occurred in the Catskill region between July 8th and August 11th, 1992. Several possible causes for this event were considered, including disease, toxic chemical spill, predation, and "natural" dieoff of older mussels. Although infectious diseases can be a significant element in the reduction of animal populations, parasites were not likely to have caused this population crash. Dissection of adult mussels ($N = 25$; length, 20 to 29 mm) from the July 8th dredging sample showed no signs of parasitic infection; mussel tissues appeared robust and healthy. Furthermore, lethal parasites have not yet been documented from either European or North American dreissenids. The elimination of large mussels was also not likely due to a toxic chemical spill. None had been reported to state regulatory agencies, and a drastic reduction in density was not apparent for any other benthic fauna being monitored in the dredge sampling. A further argument against a chemical spill was the size selectivity of the kill within the zebra mussel population. A chemical spill would likely have eliminated the smaller mussels before the larger ones.

The lack of evidence of mussel parasites and the size/species selectivity of the kill suggested that predation was a plausible explanation. Furthermore, evidence pointed to a benthic, rather than pelagic, predator because the kill appeared to have been limited to bottom-dwelling zebra mussels. Adult mussels attached to substrates near the water surface did not appear to experience the same dramatic population reduction. The density of 2 to 3 cm mussels, for example, on a floating dock in the Catskill area appeared unchanged throughout the July–August period, thus also challenging the "natural" dieoff theory. Bottom-feeding fish, crayfish, and diving ducks have been documented as predators of zebra mussels in North America (French 1993; Love and Savino 1993; Wormington and Leach 1992). Crayfish and diving ducks, however, were eliminated from consideration because they are very rare in the study area. Fish predators such as shortnosed sturgeon (M. Bain, Cornell University, Ithaca, NY, personal communication) and pumpkinseed (L. Boles, Virginia Institute of Marine Science, Glouster Point, VA, personal communication) have been recorded as predators of zebra mussels in the Hudson, but there are no other supportive data to determine what role, if any, they played in the mussel reduction. Freshwater drum, the most documented North American predator of zebra mussels (French and Bur 1992), is rare in the Hudson. A benthic predator, however, that was recorded in large numbers in the study area during the period of mussel decline was the blue crab, *C. sapidus*. Commercial trapping records in July and August 1992 indicated a substantial population of juvenile, male blue crabs (carapace width point to point 11.5 to 13.0 cm). Average catches were 12/trap (total of 15 standard, commercial traps [61 cm square, 4 parlor]). Seine sampling on July 27th also confirmed the presence of large numbers of 7.5 to 14.0 cm crabs in the area.

Juvenile male blue crabs (≈1 to 2 years old) migrate from saline habitats up into freshwater systems in late spring/early summer. Being adept swimmers, they can ride tidal currents upriver during this migration. Observations of exoskeletons on the shoreline and the inadvertent capture of blue crabs in eel traps indicated that, in 1992, juvenile blue crabs began to arrive in the Catskill area in mid-June. If blue crabs were responsible for the mussel kill, it was hypothesized that the two unusual items noted in abundance during the August 11th dredging, i.e., shell fragments and unattached, live mussels, might be artifacts of their feeding activities. Observations made during our laboratory trials supported this theory, as well as provided information on the manner in which crabs feed on zebra mussels.

Labwork

Crabs held in aquaria with zebra mussels exhibited a wide range of behaviors. Periods of active feeding were unpredictable, occurring day or night and lasting from minutes to hours. Although some crabs aggressively and frequently searched out and consumed mussels, others apparently never fed during their entire captivity. Even those crabs that did feed were likely stressed because most did not live for more than a few weeks after the completion of the lab trials.

Crabs that fed did so in open water and did not seek refugia during the actual consumption of mussel tissues. This permitted close observation of their feeding activities, and a pattern of common behavioral traits became evident. Crabs always pulled a mussel off a rock before crushing and consuming it (Fig. 1). Crabs, however, did not eat every mussel they removed; thus, live, detached, undamaged mussels were occasionally found near rocks after crab feeding. Mussels were consumed one at a time; no stockpiling of mussels for subsequent consumption was ever observed. Crabs handled zebra mussels in a delicate manner and with considerable dexterity; whether pulling mussels from a substrate, separating one from a clump, or rotating one inside a claw, their appendage movements were nimble and efficient. After a mussel was crushed in a claw, the shell fragments were pulled apart, passed along to mouthpart appendages for tissue removal, and then discarded. Whereas piles of shell fragments clearly marked the locations where mussels had been consumed, erect tufts of byssal threads marked the position on rocks where the mussels had been removed. Thus, the laboratory feeding trials demonstrated that blue crabs could be aggressive predators of zebra mussels and that their predation could produce artifacts similar to those recovered in the August 11th field samples, i.e., shell fragments and detached, live mussels.

The laboratory feeding trials clearly indicated that, although all mussel sizes offered were preyed upon, larger mussels were significantly preferred ($p < 0.01$ in all trials, Table 1). Pooling the data from trials #2 to 5, crabs ate 14, 32, and 96% of the mussels offered, respectively, in the 4 to 6 mm, 7 to 10 mm, and 11 to 17 mm length categories. Trial #6 included the largest mussels (18 to 25 mm), and again, these were the mussels preferentially consumed. Determining the mussel length that was optimally attrac-

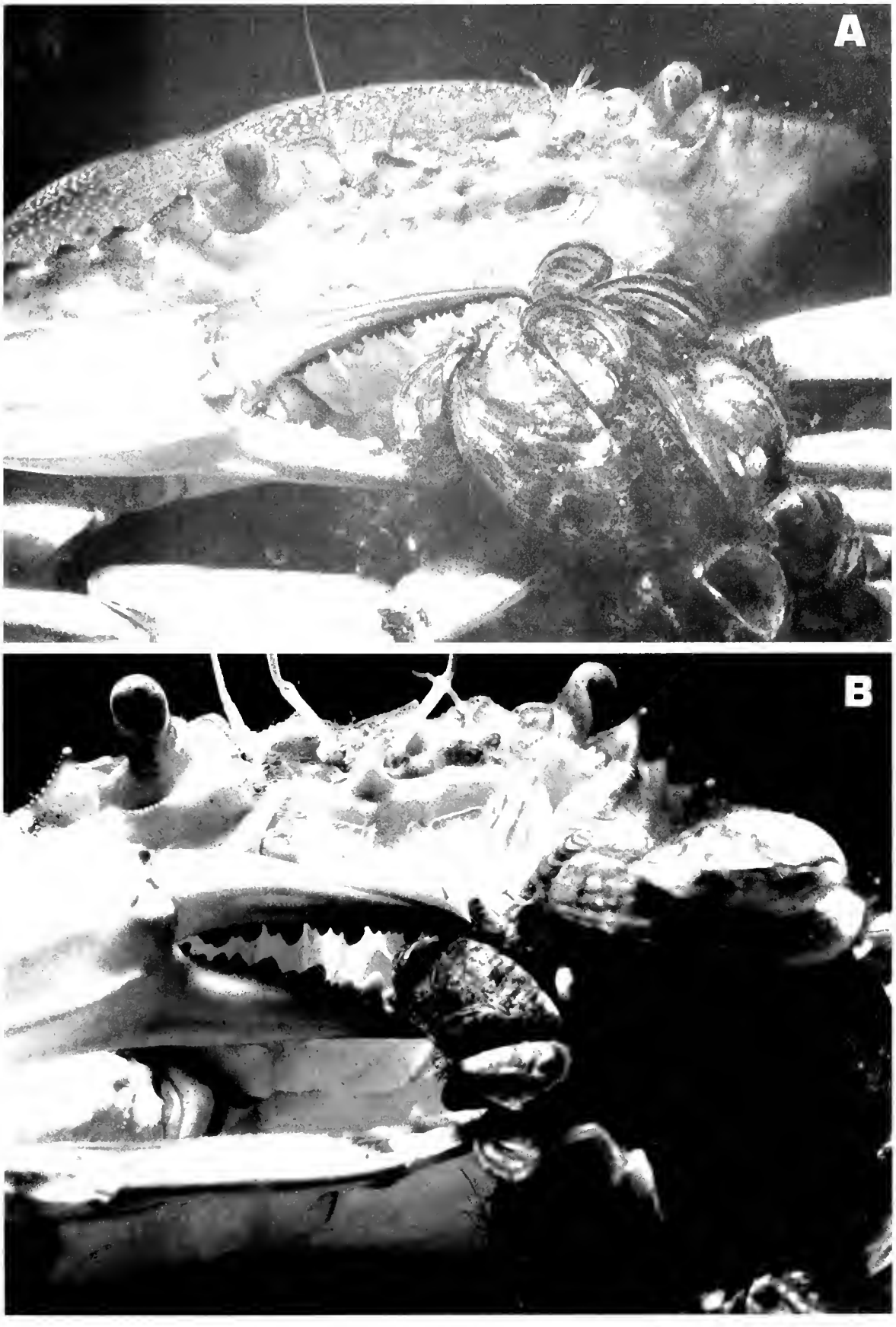

Figure 1. Blue crab examining rock (A) and removing a zebra mussel (B).

TABLE 1.

Laboratory trials examining blue crab (carapace width point to point: 12.5–13.0 cm) predation on zebra mussels of varying size class.

Trial No.	No. of Mussels Offered in Each Size Class	Percentage of Mussels Consumed in Each Size Class				Chi-Square Values[b]
		4–6 mm[a] (5.2 mm)	7–10 mm (8.5 mm)	12–17 mm (14.6 mm)	18–25 mm (21.2 mm)	
1	10	—[c]	50	100	—	6.67
2	10	15[d]	60	90	—	16.36
3	25	24	36	100	—	33.54
4	25	8	0	96	—	62.64
5	25	8	32	96	—	41.75
6	25	4	4	32	52	23.21

[a] Range and mean mussel length of each size class presented.
[b] Statistically significant in each trial ($p < 0.01$), indicating that predation was size selective.
[c] This size class not included in this trial.
[d] Twenty 4- to 6-mm mussels were used in trial 2.

tive for predation was desirable, but unfortunately, mussels >25 mm were not available at the time of the trials.

Although blue crabs are popularly characterized as scavengers, they are omnivores, with a diversity of animals and plant materials eaten (Van Heukelem 1991). Moreover, they are considered opportunistic feeders, using food items that are abundant (Laughlin 1982).

Blue crabs have been documented to be voracious predators, with their feeding activities, on occasion, having a major impact on bivalve populations. Laughlin (1982), Tagatz (1968), and Darnell (1958) reported marine bivalves to be the primary food source for blue crabs, representing 40% (by weight), 39% (by volume), and 34 to 63% (by volume) of items consumed, respectively. Similarly, Alexander (1986) recorded bivalves as the food item present in the highest percentage (i.e., 42%) of blue crab stomachs, and Stoner and Buchanan (1990) recorded bivalves as one of the most abundant dietary items for 12.6- to 15.0-cm blue crabs. Crab predation on zebra mussels, however, had not been previously reported in Europe or North America.

Both our laboratory and field data support the hypothesis that crabs could have been responsible for the dramatic zebra mussel decline recorded in the Hudson River. These data, however, are circumstantial and do not conclusively prove this theory. If, however, one assumes that this hypothesis is true and that blue crabs were responsible for the mussel decline recorded, would this indicate that they are North America's biological control solution to the zebra mussel problem? Clearly not. Because blue crabs are marine and distributed only along the Atlantic and Gulf coasts, their populations will only overlap those of zebra mussels in estuaries along these coast lines. Furthermore, this seasonal overlap, primarily when males migrate up into freshwaters during the summer, will not be a perennial event. Annual blue crab abundance fluctuates greatly in estuaries, the result of numerous biotic and environmental factors (Lipcius and Van Engel 1990). This became very evident in the Hudson River during the two summers subsequent to this study, when the migration of blue crabs into the Catskill area virtually did not occur (a massive decline in 2- to 3-cm mussels was also not observed in either 1993 or 1994, thus providing further circumstantial evidence to support the blue crab predation theory). However, the most overriding reason why blue crabs will only have a sporadic controlling impact, even in estuaries, is the prolific nature of zebra mussel reproduction. Although the rocks collected in the August 11th dredging contained virtually no adults, very high densities (>10,000 m^{-2}) of recently settled juvenile mussels (≤1 mm) were present. Thus, even with the massive decline of adult mussels in the summer of 1992, the overall density of zebra mussels in the Catskill area of the Hudson River actually *increased* during that year.

The European literature indicates that predators have only caused major reductions in mussel densities in isolated situations (e.g., Stempniewicz 1974) and that their overall effect on dreissenid populations in small (Stanczykowska 1977). This will likely be the case in North America also (Hamilton et al. 1994). Although we believe that the potential of blue crabs as a natural control agent of dreissenids is highly limited, we do believe that further investigation, particularly controlled field studies, is a necessity to better understand the dynamic interplay between these two species.

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SENSITIVITY OF ZEBRA MUSSEL (*DREISSENA POLYMORPHA*) LIFE STAGES TO CANDIDATE MOLLUSCICIDES

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ABSTRACT We developed methods for measuring the toxicity of candidate molluscicides to several life stages (preveliger, D-stage veliger, post-D stage veliger, plantigrade, and adult) of the zebra mussel *Dreissena polymorpha*. Veligers were obtained by inducing ripe adults to spawn in the laboratory and by field collection. Plantigrades were collected on slides suspended in Lake Erie. The toxicity of candidate molluscicides on all stages was evaluated in 24 hour static tests in hard standard reference water. The sensitivity of zebra mussel life stages to different chemicals varied. Veliger stages showed similar sensitivity to Bayer 73 and TFM, whereas plantigrades and adults were less sensitive. Sensitivity to Calgon H-130, Clamtrol CT-1, and Sal I decreased with each successive developmental stage: adults were most and preveligers were least sensitive to rotenone. In general, chemical concentrations that kill adults will also be effective against other life stages. However, because chemical minimization strategies require the use of the smallest amount of chemical possible, molluscicides should be tested on the life stage targeted for control.

KEY WORDS: Zebra mussel, veligers, plantigrade, toxicity, molluscicide

INTRODUCTION

A diverse spectrum of chemicals including heavy metals (Dudnikov and Mikheev 1968), oxidants (Van Benschoten et al. 1993), and nonoxidizing organics (McMahon 1993) are toxic to the zebra mussel (*Dreissena polymorpha*), an introduced aquatic bivalve. However, chlorine is still the primary zebra mussel control agent being used in industrial settings. Because chlorine is not selective and can lead to the formation of hazardous byproducts, it is imperative to continue the search for zebra mussel control agents.

Candidate molluscicides for zebra mussel control have been evaluated primarily with adult zebra mussels (Waller et al. 1993, McMahon et al. 1993). However, most industries affected by the zebra mussel work to control the early life stages of the mussel, particularly the veliger stage (Mackie 1993). Unfortunately, the veliger stages are extremely difficult to culture and handle (Nichols 1993). Thus, molluscicidal activity is generally measured for adults. The larval forms are assumed to be more sensitive to molluscicides than are adults. Thus, it is assumed that chemical concentrations that are effective for controlling adults will also control veligers.

Toxicity data from other molluscs suggest that this approach may result in significant misapplication of chemical controls. Roosenburg et al. (1980), for instance, found that straight-hinge veligers of the oyster (*Crassostrea virginica*) were more sensitive to molluscicides than were pediveligers. Similarly, Watling (1982) found that when juvenile and adult specimens of *C. virginica* were exposed to toxins, growth was reduced more severely in juveniles than adults. However, Stromgen and Nielsen (1991) found that larvae and nonreproductive adults of *Mytilus edulis* were equally sensitive to copper intoxication. Limited toxicity data on field-collected zebra mussel veligers suggest that they can be more resistant than adults to some chemicals (Klerks et al. 1993). Thus, it is necessary to evaluate the toxicity of molluscicides to several life stages of the zebra mussel in order to ensure that control efforts are appropriately designed.

The evaluation of molluscicides for control of larval and juvenile zebra mussels has been thwarted by difficulties related to extreme sensitivity to handling and their microscopic size (Nichols 1993). We present methods for conducting toxicity tests with veliger and plantigrade stages of the zebra mussel. In addition, we use these methods to compare the toxicity of six chemicals to different life stages from veliger to adult.

MATERIALS AND METHODS

Chemicals

The six chemicals selected for use (Bayer 73, Calgon H-130, Clamtrol CT-1, Noxfish, Sal I, and TFM) represent several chemical classes and modes of action in aquatic organisms. Physical data for each chemical appear in Table 1. We selected compounds that previously demonstrated toxicity to aquatic nuisance species including adult zebra mussels (Waller et al. 1993).

Organisms

In May 1993, zebra mussels 5 to 25 mm in shell length were collected by SCUBA divers from Lake Erie near Peach Point at Put-in-Bay, OH. Mussels were wrapped in damp paper towels and placed in plastic bags for transport to holding facilities at OSU in Columbus. Animals were maintained in the lab in 210 l glass aquaria containing filtered Lake Erie water. Water temperature was held at 10 ± 1°C to prevent the spawning of ripe animals (Stanyczykowska 1977). Mussels were fed a daily diet of 3.3 g of Tetra Min fish food (Blacksburg, VA) per 1,000 animals. Dead and moribund mussels were removed from the culture daily. The mussels' ability to spawn was checked prior to use in experiments by exposing a subgroup of 10 to 15 mussels to 10^{-3} M serotonin.

We used Nichols' (1993) classification system to separate and

TABLE 1.

Technical data for candidate molluscicides for zebra mussel control.

Compound (Trade Name)	Chemical Name	% Active Ingredient	Source
Bayer 73 (Bayluscide)	2′,5-dichloro-4′-nitro-salicylanilide	70	Mobay Corp.
Calgon H-130	didecyl dimethyl ammonium chloride (polyquarternary ammonium)	50	Calgon Corp.
Clamtrol CT-1	n-alkyl dimethyl benzyl ammonium chloride and dodecylguanidine hydrochloride (polyquarternary ammonium)	13	Betz Chem.
Rotenone (Noxfish)	1,2,12,12a-tetrahydro-2-iso-propenyl 8,9-dimethoxy-[1]benzopyrano-[3, 4] furo [2,3-b] [1] benzo pyran-6 (6aH) one	5	Penick Corp.
Salicylanilide 1 (Sal 1)	2′,5-dichloro-3-*tert*-butyl-6-methyl-4′-nitrosalicylanilide	100	Aldrich Chem. Co.
TFM	3-trifluoromethyl-4-nitrophenol	36	Hoescht

identify early life stages. Veligers were classified as follows: preveliger (no shell or velum), D-stage veliger, post-D veliger (umbonal). Plantigrades were those that had settled from the water column but whose shell length was <0.5 mm with siphons retracted.

Preveligers (1 to 3 days old) were produced by inducing spawning in ripe adult mussels (20 to 25 mm shell length) into filtered lake water. Spawning was induced by placing individuals into 50-ml beakers containing 25 ml of lake water and nomimal concentrations of 10^{-3} M serotonin (Ram et al. 1993, Stoeckel and Garton 1993). Male zebra mussels began releasing sperm within 30 to 60 minutes; females began releasing ova within 60 to 90 minutes. Once spawning had begun, the adults were transferred to 50-ml beakers containing filtered Lake Erie water without serotonin. Animals were held until spawning was complete; sperm and ova were then combined in 1 l glass beakers containing 500 to 800 ml of filtered Lake Erie water. Culture beakers were maintained undisturbed in a Forma Scientific environmental chamber at 17°C for 3 days, on a photoperiod of 14:10 light:dark. Veligers were used for toxicity tests at 3 days of age.

Field-collected veligers (preveliger to umbonal stages) and plantigrades were obtained from Lake Erie. Veligers were collected in 60-μm-mesh phytoplankton nets. This mesh size was sufficient to exclude most zooplankton. However, small numbers of zooplanktors (<10% of total) were obtained along with zebra mussels. These were not removed because of the increased handling stress this would cause. Zebra mussels and associated zooplankton were concentrated and transferred to 3.8 l glass jars containing 2.5 l of Lake Erie water and were maintained in environmental chambers as previously described. Animals were maintained in culture jars at densities of approximately 5 to 10 veligers per milliliter. The only food offered during holding was naturally occurring food that was present in Lake Erie water. Plantigrades were collected from the lake on glass slides (2 cm × 8 cm) that were placed in an acrylic frame and suspended near and perpendicular to the bottom of the lake. Slides were set out in mid-June 1993, retrieved in mid-July 1993, and held between 5,000 and 10,000 plantigrade animals per slide. Plantigrades were maintained in flowthrough tanks at Stone Laboratory, Put-in-Bay, OH, that received Lake Erie water at ambient lake temperature (21 to 23°C) and contained natural suspended materials as a food source.

Toxicity Tests With Early Zebra Mussel Life Stages

Preliminary tests with lab-spawned preveligers showed that preveligers from 48 to 72 hours old were most suitable for use in toxicity tests because they were sufficiently abundant and could survive handling. To determine the density of live preveligers in holding jars, we evenly suspended the animals by agitating the culture water with a plunger with a 3 cm diameter disc in a gentle up-and-down motion. Three 1 ml aliquots were removed, and preveligers were counted with a plankton counting wheel and a dissecting microscope. If the three sample counts did not vary by more than 10%, the density of preveligers was considered to be the mean of the three. If greater than 10% variability occurred, the sample was stirred again, and three additional counts were taken.

Toxicity tests with all veliger stages were conducted in 10 ml glass beakers with hard standard reference water (SRW); pH, 8.4 ± 0.2 pH units; alkalinity 150 mg l^{-1} as $CaCO_3$; hardness 180 mg l^{-1} as $CaCO_3$ at 17 ± 1°C (Committee on Methods for Toxicity Tests With Aquatic Organisms 1975). A minimum of six test concentrations and a control with 10 replicates per concentration were tested for each chemical. The amount of SRW used and the final concentration of toxicant in each beaker were adjusted for the volume of culture water transferred. Generally, a total test volume of 5 to 6 ml per replicate was used, which included 1 to 2 ml of veliger culture water. An estimated number of preveligers were transferred by automatic pipet into the test beaker containing the test chemical; a minimum of 10 preveligers were added to each beaker. Each beaker was examined under a dissecting microscope to assure that all transferred preveligers were alive. Beakers were then placed in an environmental chamber at 17°C on a photoperiod of 14:10 light:dark for 24 hours. Mortality, defined as cessation of ciliary beating, was determined at 24 hours. Toxicity tests for lake-collected veligers were conducted in the manner previously described. Collections were made when only D-stage and umbonal veligers were present, and these were not separated during exposure. However, mortality for each stage was scored separately. In no case did control mortality exceed 10%.

Toxicity tests with plantigrades were conducted in 1 l glass beakers containing 0.5 l of hard SRW (pH 8.4) at 17°C on a photoperiod of 14:10 light:dark. Plantigrade animals were acclimated to the test temperature over a period of 2 to 3 days. A minimum of five test concentrations and a control were tested in triplicate for each chemical. Excess animals were removed from each slide with a razor blade until only 100 to 200 plantigrade mussels remained for addition to each beaker. Plantigrade animals were examined under a dissecting microscope before use in toxicity tests to obtain a ratio of live:dead animals before and after exposure. The number dead before exposure was subtracted from the final mortality count to obtain the number that died during treatment. We scored mortality at 24 hours using the criterion of

failure of mussels with gaping shells to respond to the touch of a probe (Waller et al. 1993). A probe was inserted between the valves of animals with closed shells to determine whether or not they were alive (ciliary beating and adductor muscle activity).

The toxicity of candidate molluscicides to adult zebra mussels was determined for two size classes of mussels (5 to 8 mm shell length; 20 to 25 mm shell length). Adult toxicity tests followed Waller et al. (1993) with modifications, including a 24 hour exposure in hard SRW, pH 8.4, 17°C and with deletion of the post-exposure observation period.

Veliger Survival at Different pH and Temperatures

To examine the suitability of hard SRW, pH 8.4, 17°C for toxicity testing, the sensitivity of laboratory-spawned preveligers to variations in temperature and pH was tested in hard SRW at pH values of 7.0, 7.5, 8.0, and 8.4 and at temperatures of 12, 17, and 22°C. Only preveligers were selected for this evaluation because that was the only stage that was held in SRW. Tests were performed in duplicate. Estimates of survival were made every 24 hours by withdrawing 1 ml samples of the culture water and counting live veligers. We calculated biological half-lives for veligers under each set of culture conditions using the equation:

$$T_{1/2} = \frac{0.693}{k_1}$$

where k_1 is the slope of the veliger survival curve.

Data Analysis

We used the Spearman-Karber probit analysis as modified in Hamilton et al. (1977) to estimate LC_{50} values and 95% confidence limits. The LC_{50} values were considered significantly different when the 95% confidence limits did not overlap. Control mortality was corrected using Abbott's (1925) equation.

RESULTS

Relative Toxicity of Candidate Molluscicides to Zebra Mussel Life Stages

All chemicals evaluated were toxic to zebra mussels, although the LC_{50} values ranged several orders of magnitude from 1.3 μg l^{-1} (Sal 1, preveligers) to >13,000 μg l^{-1} (Clamtrol CT-1, adults).

We noted three general patterns of sensitivity to chemicals among different life stages (Table 2). The first pattern, seen with Calgon H-130, Clamtrol CT-1, and Sal 1, showed a successive decrease in sensitivity for each developmental stage through the adult. The LC_{50} values were greatest for the adult mussels but did not differ significantly between the two adult size classes.

A second pattern, seen with Bayer 73 and TFM (Table 2), showed an equal sensitivity among the three veliger stages. Veligers were significantly more sensitive to these chemicals than plantigrade or adult animals. Adults were least sensitive to TFM, and plantigrades were least sensitive to Bayer 73.

The third pattern was seen with rotenone and was the reverse of the first two patterns (Table 2). Adult zebra mussels were significantly more sensitive to rotenone than were veligers or plantigrade stages.

Veliger Survival Under Different Conditions

The sensitivity of lab-spawned preveligers to changes in pH and temperature in SRW, reported as biological half-lives, is presented in Table 3. The average $T_{1/2}$ of veligers in filtered Lake Erie water was 55.2 hours. Survival was greatest in reconstituted water that approximated the characteristics of Lake Erie with a pH of 8.4, a water hardness of 180 to 200 as $CaCO_3$, and an alkalinity of 90 to 110 as $CaCO_3$. Survival of veligers decreased at lower pHs

TABLE 2.

Toxicity of candidate molluscicides to zebra mussel life stages. Concentrations are based on percent active ingredient of formulation.

	Calgon H-130		Clamtrol CT-1		Bayer 73	
Life Stage	**24 hour LC_{50}[a,b]**	**95% CL**	**24 hour LC_{50}[a,b]**	**95% CL**	**24 hour LC_{50}[a]**	**95% CL**
PreVeliger	39.0^{z}	30.0–50.0	48.0^{z}	38.0–61.0	25.0^{z}	21.0–30.0
D-Stage	89.0^{y}	85.0–95.0	95.0^{y}	79.0–114.0	24.0^{z}	22.0–26.0
Post-D Stage	175.0^{x}	170.0–185.0	179.0^{x}	165.0–195.0	28.0^{z}	27.0–29.0
Planti grade	8.8^{w}*	8.3–9.2	8.8^{w}*	8.6–9.1	92.0^{x}	88.0–95.0
Adult, 5 to 8 mm	$>10^{v}$*		$>13^{v}$*		49.9^{y}	46.1–54.1
Adult, 20 to 25 mm	5.6^{v}*	2.6–12.1	$>13^{v}$*		55.6^{y}	50.4–61.3
	Rotenone		**Sal 1**		**TFM**	
Life Stage	**24 hour LC_{50}[a]**	**95% CL**	**24 hour LC_{50}[a]**	**95% CL**	**24 hour LC_{50}[a,b]**	**95% CL**
PreVeliger	232.0^{xy}	203.0–266.0	1.7^{z}	1.6–1.8	3.7^{zy}*	2.2–6.2
D-Stage	230.0^{y}	221.0– 40.0	1.3^{z}	1.1–1.7	2.3^{z}*	2.1–2.5
Post-D Stage	264.0^{wx}	256.0–272.0	3.2^{y}	3.1–3.4	2.5^{z}*	2.4–2.6
Planti grade	275.0^{w}	270.0–280.0	13.5^{x}	11.4–16.0	4.3^{y}*	4.2–4.5
Adult, 5 to 8 mm	161.0^{z}	137.0–189.0	55.1^{w}	46.2–65.6	10.3^{x}*	7.9–13.5
Adult, 20 to 25 mm	155.0^{z}	139.0–174.0	65.0^{w}	51.8–81.6	11.0^{x}*	7.5–16.2

CL, confidence limit.

[a] For a given compound, LC_{50} values followed by the same letter are not significantly different from each other.

[b] LC_{50} values are reported in μg l^{-1} except where an asterisk appears, in which case the units are mg l^{-1}.

TABLE 3.

Biological half-lives for laboratory-spawned veligers in hard standard reference water as a function of pH and temperature.

pH	Temperature (°C)	$T_{1/2}$[a] (hours)
7.0	17	36.0
7.5	17	31.8
8.0	17	60.0
8.4	17	67.2
8.4	12	62.4
8.4	22	60.0

[a] The $T_{1/2}$ for filtered Lake Erie water was 55.2 hours at pH 8.4, 17°C.

values. Preveligers tolerated changes in temperature when held in hard SRW at pH 8.4 (Table 3).

DISCUSSION

Relative Sensitivity of Zebra Mussel Life Stages to Molluscicides

It is generally assumed that veligers are more sensitive to toxicants than adults because the natural mortality rate at the veliger stage is high, 99.0 to 99.9% (Sprung 1993, Stoeckel and Garton 1993). Moreover, adult zebra mussels can close their valves for days to weeks if they detect an irritating chemical and thus avoid exposure to the toxin (Jenner and Janssen-Mommen 1993, de Kock and Bowmer 1993). Preveligers lack shells and, thus, should be more vulnerable to chemical exposure. We found significant differences in the sensitivity of different zebra mussel life stages, but the pattern of sensitivity among life stages varied among chemicals. Preveligers were the most sensitive stage to all chemicals tested except rotenone (Table 2). However, D-stage and post-D veligers were equally sensitive to Bayer 73, Sal I, and TFM. With the exception of rotenone, adults were significantly less sensitive to each chemical than were veliger stages. The sensitivity of plantigrade zebra mussels was generally intermediate between veligers and adults, except that, for Bayer 73, the plantigrade stage was less sensitive than other stages. These data are consistent with that of other investigators who have reported variation in susceptibility to toxicants among zebra mussel life stages exposed. For instance, McMahon et al. (1993) found that veligers were more sensitive than adults to two cationic polymers; Ram and Walker (1993) reported that zebra mussels of 4 to 11 mm shell length were more sensitive to the toxic effects of deionized water than were adults. However, Klerks et al. (1993) reported that chlorine was less toxic to veligers than to adults.

Differences in sensitivity to molluscicides among life stages have also been observed in studies on related bivalves, and as with the zebra mussel, these also follow different trends (Roosenburg et al. 1980; Watling 1982; Stromgen and Nielsen 1991). However, no other comprehensive data base assessing the toxicity of molluscicides on all identifiable life stages exists for comparison. Thus, it is not possible to evaluate whether trends identified for zebra mussels are unique or representative of other molluscs.

Extrapolation of zebra mussel treatment concentrations from adult toxicity data appears to be appropriate for most of the compounds we tested. Because control efforts are usually aimed at the veliger stages and preveligers are usually most sensitive, concentrations of most toxicants that kill adults should also kill early-stage veligers. This generalization did not apply for rotenone, however. Thus, the testing of candidate molluscicides on early life stages of the zebra mussel should continue until an adequate data base is achieved. In addition, treatment concentrations based on adult sensitivity may use more chemical than is needed for controlling veligers. Strategies to minimize chemicals should be derived from studies of the life stage that is being targeted.

Evaluation of Toxicity Test Methods

Of the three discrete veliger stages (preveliger, D-stage, and post-D) used in our toxicity tests, preveligers about 3 days old were most tolerant of handling and suffered the lowest mortality in culture. Overall, the mortality rate of the veligers in culture was high (Table 3), which limited the number of older veligers available for tests. Thus, it was difficult to obtain sufficient older veligers. Cilia were visible as early as 28 hours postfertilization, suggesting that 1 or 2 day old pre-veligers could be used in testing. However, early-stage veligers were more sensitive to handling than were 3 day old animals and were easily killed during transfer.

Field collection of enough veligers is impractical because densities may be high enough to support testing for only 2 to 3 weeks per year (Garton and Haag 1993). In addition, peaks in adult spawning in natural populations cannot be predicted. Samples of field-collected veligers may also include animals from several local populations, which would include animals that have been exposed to variable temperature and food regimes. Finally, the developmental stage of field-collected veligers cannot be correlated with age because environmental variables, e.g., pH, temperature, dissolved oxygen levels, and calcium concentration, can alter the rate of larval development (Sprung 1993). In contrast, reproductively ripe mussels can be maintained in spawning condition in the lab for up to 6 months, thus expanding the time available for toxicity testing. The age and source of laboratory-spawned veligers are known. Because there was no significant difference in the sensitivity of lab-spawned preveligers vs. field-collected D-stage veligers to rotenone (232 $\mu g\ l^{-1}$, 95% confidence limit = 203 to 266 vs. 219 $\mu g\ l^{-1}$, 95% confidence limit = 200 to 240, respectively), results obtained from testing laboratory-spawned animals should apply to veligers in the field at a similar developmental stage. However, the sensitivity of compounds with diverse modes of action should still be compared in both lab and field populations.

Lake water has been identified as the most suitable growth medium for culturing larval zebra mussels (Stoeckel and Garton 1993). However, reconstituted water, such as SRW, is more suitable for testing animals because water quality characteristics (e.g., hardness, alkalinity, and pH) can be standardized. We found that zebra mussel veligers can be successfully cultured in hard SRW with pH above 8.0 (Table 3). The mortality of the veligers increased dramatically when the pH is <8.0. These data are consistent with those of Sprung (1989), who demonstrated that veligers are sensitive to alterations in pH.

In summary, methods were developed for the accurate assessment of chemical toxicity to six zebra mussel life stages. We used these methods to evaluate the toxicity of six candidate molluscicides to four larval and two adult stages. The data show that preveligers were usually most sensitive to each chemical but that considerable variation exists in the relative susceptibility of the rest of the other life stages tested. Our data show that it is advisable to evaluate the toxicity of any candidate molluscicide against

the life stage for which treatment will be used in order to ensure that appropriate levels of chemicals are used.

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ACCLIMATIZATION OF BLUE MUSSEL, (*MYTILUS EDULIS* LINNAEUS, 1758) TO INTERTIDAL CONDITIONS: EFFECTS ON MORTALITY AND GAPING DURING AIR EXPOSURE

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ABSTRACT Although under most conditions, a short (7 day) acclimatization of cultured mussels to intertidal conditions did not decrease their mortality during air exposure at 4°C, in May and June, this treatment reduced the impact of debyssing on mortality. In contrast, a 35 day intertidal acclimatization halved the mortality of cultured mussels during air exposure. Prevention of air breathing by tightly closing valves increased mortality during air exposure, particularly for wild intertidal mussels. This indicates that air breathing is an important part of the mussel's strategy for surviving air exposure. The 7 day intertidal acclimatization did not alter the reliance of cultured mussels upon air breathing. During air exposure at 4°C, the percentage of living mussels with open valves (gapers) was greater for cultured than for wild mussels. In comparison with the marked effect of prevention of air breathing on wild mussels, this shows that gaping and air breathing are not necessarily correlated. Neither mechanical removal of the byssus nor intertidal acclimatization modified the gaping of wild or cultured mussels. For all mussels, percent gaping and mortality were lower in August than in May, June, and July. Whereas intertidal acclimatization did not alter the anatomical properties of cultured mussels, the adductor muscle of wild mussels represents a greater proportion of the flesh mass than in cultured mussels. The low mortality of wild subtidal mussels during air exposure suggests that acclimatization to the nearshore benthic zone could improve the viability of suspension-cultured mussels.

KEY WORDS: Mytiliculture, mortality, intertidal acclimatization, gaping, air breathing

INTRODUCTION

Because *Mytilus edulis* Linnaeus tolerate extended periods of air exposure, grow rapidly in suspension culture, and are easy to obtain as spat, mussel culture has become a major industry in North America and Europe. However, when mussels are cultured in remote areas, limits on their viability can impose costly transportation methods. Extension of the period during which mussels tolerate air exposure would facilitate their marketing.

The mortality of mussels during air exposure varies among seasons and according to the mussel's habitat. Suspension-cultured mussels have a higher mortality during air exposure than do wild intertidal mussels, particularly during the summer (Wijsman 1976, Poirier and Myrand 1982, Demers and Guderley 1994). Seasonal changes in mortality during air exposure (Slabyj and Hinkle 1976, Poirier and Myrand 1982, Demers and Guderley 1994) suggest that the mussel's tolerance of air exposure is conditioned by the environmental conditions it experienced before air exposure. Also, *Mytilus californianus* from the high intertidal zone have different physiological responses to air exposure than do *M. californianus* from the low intertidal (Moon and Pritchard 1970). These differences may reflect physiological adaptation to specific environmental conditions or selection of different genotypes.

Acclimatization to intertidal conditions modifies the physiological response of subtidal mussels to air exposure (De Vooys 1979) and can decrease the mortality of cultured mussels during air exposure (Poirier and Myrand 1982, Demers and Guderley 1994). These changes are correlated with shifts in the patterns and rates of accumulation of anaerobic endproducts in the adductor muscle and a decreased reliance on air breathing (Demers and Guderley 1994). Reductions in mortality during air exposure could also reflect improvements in the shell closure capacity. Effectively, extended shell gape accelerates loss of fluid in the mantle cavity and desiccation (Loosanoff and Engle 1943). Because a tightly closed mussel probably resists removal of the byssal gland better than a gaping mussel, improvements in shell closure could also reduce the negative impact of debyssing, a mechanical process designed to remove the byssal fibers but that may also damage tissues.

Although a 12 day intertidal acclimatization decreases mortality during air exposure for cultured mussels harvested in June and July (Demers and Guderley 1994), this acclimatization period is likely to be too long to be economically feasible. In this study, we examine whether a shorter (7 day) intertidal acclimatization (1) reduces the mortality of cultured mussels during air exposure, (2) decreases the incidence of "gaping" during air exposure, (3) changes the reliance upon air breathing, and (4) moderates the effect of debyssing on mortality during air exposure. To this end, the responses of intertidally acclimatized cultured mussels were compared with those of subtidally acclimatized cultured mussels and of wild intertidal mussels throughout late spring and summer. To evaluate the reliance upon air breathing, we compared the mortality of mussels that were allowed to open their valves with that of mussels that had their valves forcibly held shut. The impact of debyssing was estimated by comparing the survival of debyssed and undebyssed mussels from a given group. Further, we examined (5) whether increasing the duration of intertidal acclimatization from 7 to 35 days enhances its impact upon the mortality and gaping of cultured mussels and (6) whether the intertidal acclimatization of wild subtidal mussels modifies their mortality during air exposure. Finally, we evaluated whether intertidally and subtidally acclimatized cultured mussels and wild intertidal mussels differed in their anatomical properties.

METHODS

Experimental Animals

Suspension-cultured mussels were obtained from growers in the Baie des Chaleurs, Québec, and declumped mechanically. The

cultured mussels were between 4.5 and 6.0 cm in length (1.5 to 2 years old), whereas the wild mussels were between 3.5 and 5.5 cm (3 to 5 years old). The age of the mussels was determined by their growth lines. As a general evaluation of the condition of the mussels, we measured their meat yield in June, July, and August, by steaming 20 mussels for 10 minutes, draining them, and then weighing the cooked meat and shells. In June, the meat yield was 38% for subtidally acclimatized cultured mussels, 30% for intertidally acclimatized cultured mussels, and 12% for wild intertidal mussels. In July, these values were 27, 36, and 12%, respectively. In August, the meat yields of cultured mussels were systematically lower: 22% for subtidally acclimatized cultured mussels and 24% for intertidally acclimatized cultured mussels, suggesting that spawning had occurred, whereas wild intertidal mussel yields remained at 13%.

Conditions of Intertidal Acclimatization

Cultured mussels were acclimatized either to intertidal or to subtidal conditions. Intertidal acclimatization occurred in a cage (1 × 2 × 0.09 m) placed at a height such that the mussels were exposed to air for approximately 34% of the duration of a tidal cycle. The acclimatization cages were located on a rocky shore at Pointe aux Hayes, Port Daniel, Québec. The cages were made of wood frames and plastic screen (Vexar) with 1 cm mesh. Mussels were acclimatized to intertidal conditions for 7 days. This period was too short to allow fouling of the cages. The subtidally acclimatized group was put in a similar cage placed lower on the shore at Pointe aux Hayes, at a level at which it was submerged, even at the lowest low tides. Wild intertidal mussels were sampled in the intertidal adjacent to the treatment cages and were placed in the same cage as the intertidally acclimatized cultured mussels. Mortality during the acclimatization was very low for the wild mussels (1%), whereas the cultured mussels showed 5 to 13% mortality in the acclimatization cages. Mortality did not differ between the intertidal and subtidal cages.

The acclimatizations occurred from May 27 to June 3, June 21 to 28, July 26 to August 2, and August 23 to 30 in 1989. At the end of the acclimatization period, each group of mussels was divided into two lots: one was mechanically debyssed with a commercial debyssing machine (Island Metal Fabricators), and the other was not. The various groups of mussels were placed in mesh bags on sea water ice and transported to Laval University for determinations of viability and shell gape.

Generally, we assessed the mortality of six groups of mussels: intertidally acclimatized cultured mussels (debyssed and undebyssed), subtidally acclimatized cultured mussels (debyssed and undebyssed), and wild intertidal mussels (debyssed and undebyssed). However, in August, because some of the acclimatized wild intertidal mussels were lost during a storm, we only had enough individuals to study undebyssed wild intertidal mussels.

In addition, in June and July, wild subtidal mussels were harvested by a diver and were acclimatized to intertidal or subtidal conditions. Further, cultured and wild subtidal mussels underwent a 35 day intertidal acclimatization from July 26 to August 30. These additional groups of mussels were not debyssed after the acclimatization.

Mortality and Gaping of Unrestrained Mussels

Mussels (150 per experimental group) were placed in a single layer in plastic trays at 4°C at a relative humidity of approximately 100%. This temperature is routinely used during mussel storage. The high relative humidity should minimize the desiccation of mussels with a small shell gape. The subtidal 35 and cultured 35 groups were composed of 45 mussels. Mussels were considered to be alive when they closed their valves in response to a stimulus or when they resisted forcible valve separation. Determinations were continued until 50% mortality was reached. To evaluate gaping, open mussels were counted before they were moved to determine their mortality. Gaping was scored as the number of mussels with their valves separated by (1) 0 to 3 mm or by (2) >3 mm. Only the gaping of mussels subsequently determined as living was retained for analysis. Because similar tendencies were found for the two extents of valve separation, we analyzed the combined results. For a given group, the percent gaping of living mussels remained fairly constant during the initial 12 days of the viability determinations. Subsequently, this value declined somewhat. As an estimate of the tendency of living mussels in the different treatments to gape, we calculated the mean percent gaping of living mussels throughout the period of air exposure.

Viability of Restrained Mussels: Reliance Upon Air-Breathing

To assess the extent to which the mussels relied upon air breathing, we compared the viability of unrestrained mussels with that of mussels that were prevented from breathing air. For these determinations, 500 mussels per treatment were tightly shut with Parafilm and a rubber band designed to restrain lobster claws. At regular intervals, 60 mussels were sampled to evaluate mortality in the entire group.

Anatomical Differences Between Wild and Cultured Mussels

The cultured and wild mussels collected specifically for these comparisons were of similar size (3.5 to 4.0 cm). The cultured mussels were divided into two groups, one of which was hung in the intertidal in a mesh bag for 7 days, while the other was maintained in the subtidal. This treatment allowed us to evaluate whether intertidal acclimatization modified tissue masses. We compared the length, width, depth, total mass, flesh mass, shell mass, and adductor muscle mass of these groups.

Data Analysis and Statistics

Mortality curves were established for each treatment group. Tests for independence using the G statistic (Sokal and Rohlf 1981) were carried out upon the frequencies of dead and living mussels obtained the last day when data were available for all treatment groups. We obtained identical statistical conclusions when the mortality curves for May were analyzed with the lifetest procedure in SAS.

The values of percent gaping were transformed using the angular transformation (Sokal and Rohlf 1981) before statistical analysis. Untransformed data are shown. Anatomical data did not require transformation. Analyses of variance (ANOVAs) were carried out using the statistical software package SuperANOVA (Abacus Concepts Inc., Berkeley, California). When ANOVAs indicated significant treatment effects, multiple comparison tests were carried out using the Student-Newman-Keuls (SNK) test ($p < 0.05$).

RESULTS

Mortality During Air Exposure

During prolonged air exposure, the time course of mortality varied among the groups as well as among the months (Figs. 1 to

4). However, cultured mussels consistently had a higher mortality than did wild intertidal and wild subtidal mussels.

Specific treatment effects varied from month to month (Table 1). After 13 days of air exposure in May, debyssed subtidally acclimatized cultured mussels had the highest mortality and differed from their undebyssed counterparts and from the intertidally acclimatized cultured mussels ($p < 0.01$, G test), which in turn had higher mortalities than wild intertidal mussels ($p < 0.01$) (Fig. 1a, Table 1). Intertidal acclimatization only decreased the mortality of debyssed mussels.

In June, the mortality curves showed a considerable spread, partly the result of the inclusion of wild subtidal mussels (Fig. 2a). After 12 days of air exposure, wild subtidal mussels had significantly lower mortality than did wild intertidal mussels and all cultured mussels ($p < 0.01$). Wild intertidal mussels had lower mortalities than either intertidally or subtidally acclimatized cultured mussels ($p < 0.01$). Undebyssed intertidally acclimatized cultured mussels had higher mortalities than their subtidally acclimatized counterparts ($p < 0.01$); however, after debyssing, intertidally and subtidally acclimatized cultured mussels had similar mortalities (Table 1).

In July, the mortality curves of wild mussels, both intertidal and subtidal, were clustered, quite separate from those of the cultured mussels (Fig. 3a). After 12 days of air exposure, wild mussels consistently had lower mortalities than did cultured mussels ($p < 0.01$). Intertidal acclimatization did not change the mortality of wild subtidal mussels ($p > 0.05$), but increased the mortality of undebyssed cultured mussels ($p < 0.01$).

Because the mussels took more time to reach 50% mortality during air exposure in August than during the other months (Figs. 1 to 4), mortalities were compared on day 16 (Table 1). Again, wild mussels had lower mortalities than cultured mussels ($p < 0.05$). Cultured mussels acclimatized 35 days to intertidal conditions had lower mortalities during air exposure than did those that were acclimatized to the intertidal for 7 days ($p < 0.05$) (Table 1, Fig. 4).

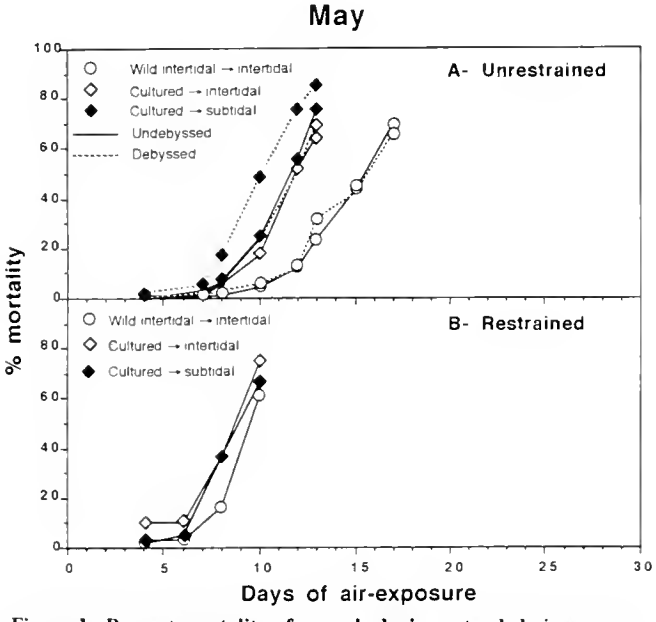

Figure 1. Percent mortality of mussels during extended air exposure at 4°C and ca. 100% relative humidity in May 1989. Cultured and wild mussels were harvested, acclimatized to intertidal or subtidal conditions, and debyssed as explained in Materials and Methods before the determination of their mortality during exposure to air. For the unrestrained mussels, mortality was determined with 150 mussels for each treatment group. For the restrained mussels, mortality was estimated on 60 mussels at each sampling date. Statistical differences among treatments were determined on the last day when data were available for all treatment groups and are shown in Table 1.

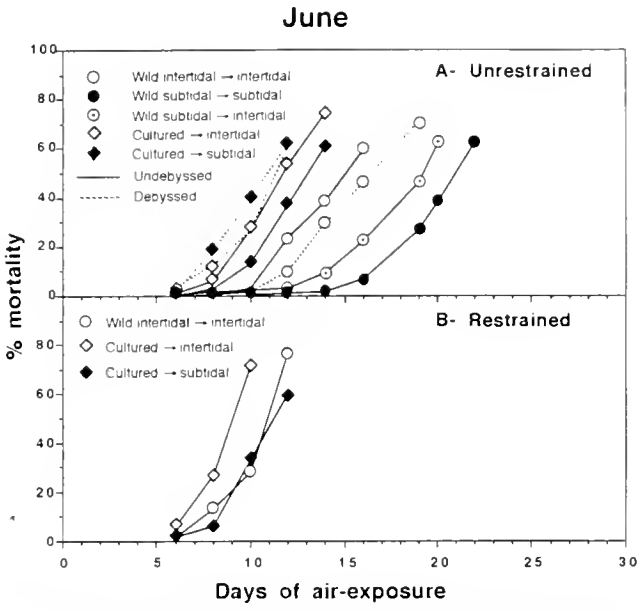

Figure 2. Percent mortality of mussels during extended air exposure in June 1989. Details are as indicated in the legend to Figure 1.

Effects of Debyssing on Mussel Mortality

Generally, debyssing increased the mortality of cultured mussels more than that of wild mussels (Table 1; Figs. 1 to 4). In May,

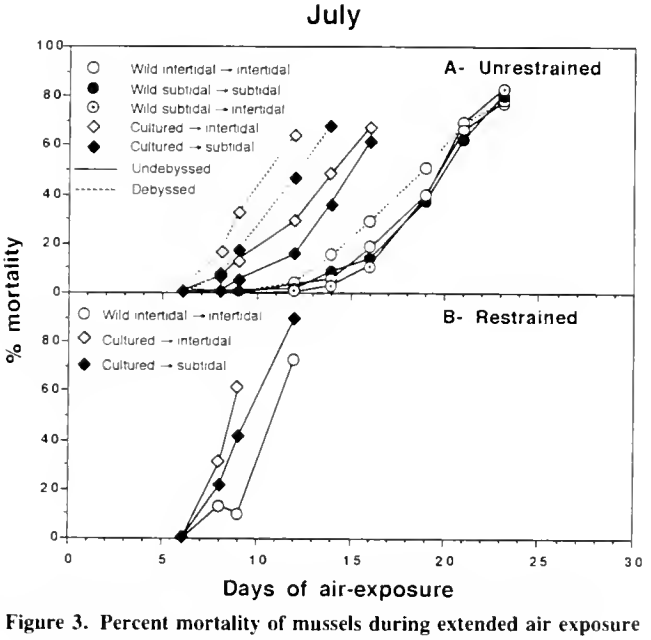

Figure 3. Percent mortality of mussels during extended air exposure in July 1989. Details are as indicated in the legend to Figure 1.

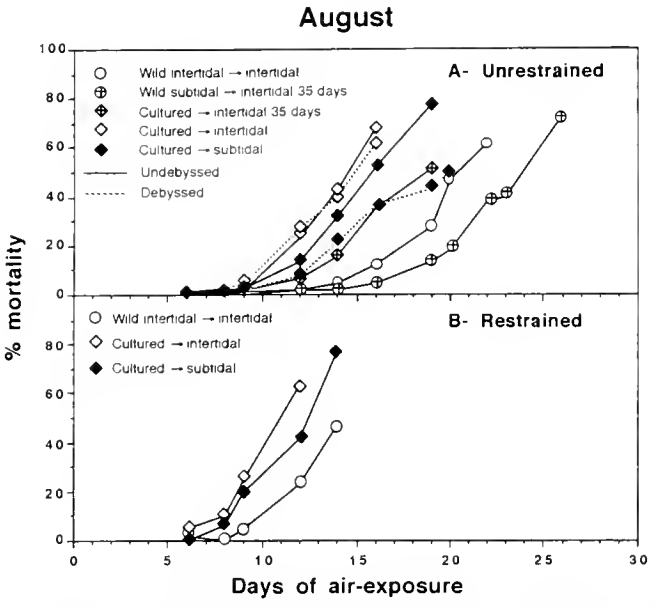

Figure 4. Percent mortality of mussels during extended air exposure in August 1989. Details are as indicated in the legend to Figure 1, except that mortality of the 35 day acclimatized mussels was estimated for 45 individuals.

undebyssed subtidally acclimatized cultured mussels survived better than debyssed ones ($p < 0.01$). By contrast, the mortality of intertidally acclimatized cultured mussels and of wild intertidal mussels did not change with debyssing. In June, debyssing increased the mortality of subtidally acclimatized cultured mussels ($p < 0.01$) but did not increase the already high mortality of intertidally acclimatized cultured mussels. In July, debyssing enhanced the mortality of debyssed intertidally and subtidally acclimatized cultured mussels ($p < 0.05$). In August, debyssed subtidally acclimatized cultured mussels had a lower mortality than their undebyssed counterparts ($p < 0.05$) whereas intertidally acclimatized, cultured mussels were not affected by debyssing.

Reliance Upon Air Breathing

When mussels were prevented from breathing air, mortality increased much earlier during air exposure, particularly for wild mussels (Figs. 1 to 4), and the mortality curves of the cultured and wild mussels became more similar. This response was observed in all months. Prevention of air breathing led to similar shifts of the mortality curves of intertidally and subtidally acclimatized cultured mussels.

Percent Gaping

Generally, between 80 and 100% of mussels with separated valves (gaping) were alive until the sixth day of air exposure. However, by the 12th day, most of the mussels with separated valves were dead (unpublished data).

To analyze whether the mean percent gaping of living mussels during the air exposure period varied among treatments and months, we used a two-factor ANOVA (factors: treatments and months). Treatments and months both affected the gaping of living mussels (treatment: df = 9, F = 15.2, $p < 0.001$; month: df = 3, F = 55.0, $p < 0.001$), as well as interacting to affect gaping (df = 16, F = 3.0, $P < 0.001$). In particular for cultured mussels, the gaping tended to be lower in August than in the other months (Table 2).

To identify the treatment effects on percent gaping in a given month, one-factor ANOVAs were carried out, followed by a posteriori comparisons (SNK) (Table 2). In all months, treatments significantly affected the percent gaping (May: df = 5, F = 9.5, $p < 0.001$; June: df = 7, F = 8.3, $p < 0.001$; July: df = 7, F = 3.8, $P < 0.05$; August: df = 6, F = 4.9, $p < 0.001$). Wild mussels often showed significantly less gaping than cultured mussels ($p < 0.05$, Table 2). By contrast, neither debyssing nor intertidal acclimatization changed the percent gaping of wild or cultured mussels.

Anatomical Differences Between Wild and Cultured Mussels

To examine whether intertidal acclimatization modified the anatomical properties of cultured mussels and to establish whether our wild intertidal and cultured mussels differed in these respects, we sampled wild intertidal and cultured mussels of a restricted size range (35 to 40 mm). Nonetheless, the wild intertidal mussels were significantly longer, wider, higher, and heavier than the cultured mussels (Table 3). By contrast, the total flesh mass of the cultured mussels was greater than that of the wild mussels. Intertidal acclimatization did not modify the tissue masses of cultured mussels. The adductor muscle was heavier and represented a greater proportion of the total flesh mass in wild than in cultured mussels ($p < 0.05$). This suggests that wild mussels invest more in their adductor muscles than do cultured mussels. The ratio of the mass of the adductor muscle to that of the valves is lower in wild than in cultured mussels ($p < 0.05$).

DISCUSSION

If of sufficient duration, intertidal acclimatization can decrease the mortality of cultured mussels during prolonged air exposure. For example, Demers and Guderley (1994) found that, in early summer, a 12 day intertidal acclimatization markedly decreases the mortality of cultured mussels. By contrast, our 7 day treatments only reduced the mortality of debyssed mussels in May. These 7 day treatments did not modify the reliance upon air breathing or the incidence of gaping. In late summer, a 35 day acclimatization decreased mortality, whereas a 7 day treatment increased mortality. Although 35 and 12 day treatments are likely to be impractical for the culture industry, their impact clearly illustrates the malleability of the mussel's response to air exposure. Previous studies also support the concept that the mussel's response to air exposure is correlated with the environment from which it was harvested and can be modified by environmental conditions (De Vooys 1979, Moon and Pritchard 1970, Poirier and Myrand 1982).

The seasonality of mussel mortality during air exposure suggests possible influences of the reproductive cycle, nutrient availability, and environmental conditions. Generally, the lowest mortality during air exposure was found in August, after the cultured mussels had completed spawning. This may reflect a lower maintenance cost of soma relative to gametes. Phytoplankton levels in the Baie des Chaleurs change little between June and August as the phytoplanktonic cells are generally <5 μm and chlorophyll A levels remain near 1 mg m^{-3}. During the major phytoplankton bloom that occurs in late April and lasts until late May, phytoplanktonic cells are >5 μm and chlorophyll A reaches 20 mg m^{-3}. Another bloom occurs in October, with maxima around 5

TABLE 1.

Mortality of wild and cultured mussels during prolonged air exposure: effect of intertidal acclimatization, season, and debyssing.

Origin	Percent Mortality[a]							
	Cultured				Wild Intertidal		Wild Subtidal	
Acclimatization Site	Intertidal		Subtidal		Intertidal		Intertidal	Subtidal
Debyssing	No	Yes	No	Yes	No	Yes	No	No
May (day 13)	66b	67b	71b	85c	18a	21a	—	—
June (day 12)	53e	61e	37d	69e	25c	11b	3a	1a
July (day 12)	29d	52e	16c	47e	0a	5b	1a	3ab
August (day 16)	68e	61e	51d	37c	12b	—	—	—
August (intertidal 35 days)	40c	—	—	—	—	—	2a	—

[a] In a given month, percent mortalities were compared on the last day (indicated in parentheses) that data were available for all groups. For a given month, the treatment groups that do not share the same letter differ statistically ($p < 0.05$).

mg m^{-3} of chlorophyll A (Tamigneau, personal communication). Higher food availability cannot be correlated with decreased mortality during air exposure. Thus, it seems unlikely that differences in food uptake due to intertidal acclimatization lead to the observed differences in mortality during subsequent air exposure. The generalized decrease in mussel mortality during air exposure in late summer may reflect the acclimatization of mussels to the generally higher sea water and air temperatures in this period. Upon transfer to 4°C, a thermally induced decrease in metabolic rate could accentuate the metabolic depression into which mussels enter during air exposure and thus enhance their survival.

Debyssing may damage tissues and thereby enhance mussel mortality. We did not evaluate tissue damage caused by debyssing but found that the effect of debyssing tended to be greater on cultured than on wild mussels. However, the effect of debyssing on the mortality of cultured mussels was variable. In May and June, debyssing generally enhanced the mortality of cultured mussels but in other treatments and months, the debyssed mussels sometimes had lower mortalities than their nondebyssed counterparts. Overall, our data suggest that cultured mussels are more susceptible to debyssing in early summer. In May, intertidal acclimatization increased the resistance of cultured mussels to debyssing.

Shell gape does not seem to be directly linked with air breathing. If the two were correlated, mussels with the highest percent gaping should show the greatest reduction in survival when prevented from breathing air. For example, in May, the percent gaping of cultured mussels is twice that of wild intertidal mussels. However, the prevention of air breathing did not increase the mortality of cultured mussels twice as much as that of the wild mussels. Air breathing does not require a wide shell gape; a small bubble of air is sufficient to support aerobic processes for a certain time (Shick et al. 1988). Other possible benefits of air breathing, such as the removal of volatile anaerobic endproducts, could also occur in fairly rapid pulses that would be difficult to detect with our periodic measurements of shell gape. A wide shell gape certainly would allow oxygen uptake from the air, but also potential desiccation, through loss of fluid in the mantle cavity and evaporation (Loosanoff and Engle 1943). Optimally, mussels should be stored under a high relative humidity because this would allow air breathing while minimizing desiccation, particularly for cultured mussels, which tend to have a high percent gaping of living mussels early during air exposure.

Wild and suspension-cultured mussels differed markedly in their responses to prolonged air exposure. Wild mussels had higher survival, relied more upon air breathing, were less affected by debyssing, and gaped less than their cultured counterparts. The shell of wild mussels was heavier, and the adductor muscle rep-

TABLE 2.

Mean percent gaping during the air exposure period by living wild and cultured *M. edulis* (mean ± SD of the percent gaping (N = the number of observations made during the air exposure period.

	May	June	July	August
Intertidal → intertidal	14.8 ± 8.5 (8) a	8.5 ± 5.0 (6) ab	8.9 ± 4.7 (9) a	5.0 ± 2.7 (9) ab
Intertidal → intertidal (debyssed)	14.2 ± 4.7 (8) ab	8.4 ± 4.2 (9) ab	10.9 ± 5.7 (9) ab	
Culture → intertidal	34.9 ± 5.7 (6) c	17.5 ± 7.8 (5) bcd	18.2 ± 6.9 (6) abc	6.2 ± 3.2 (6) abc
Culture → intertidal (debyssed)	27.5 ± 5.6 (6) c	15.1 ± 6.8 (4) abc	22.3 ± 7.1 (4) c	8.9 ± 3.9 (6) abc
Culture → subtidal	23.4 ± 10.0 (6) bc	26.2 ± 7.8 (5) d	19.4 ± 8.9 (6) bc	9.9 ± 5.0 (7) bc
Culture → subtidal (debyssed)	35.0 ± 11.0 (6) c	24.0 ± 11.0 (4) cd	15.9 ± 10.0 (5) abc	11.4 ± 3.4 (7) c
Subtidal		10.3 ± 4.0 (8) ab	15.1 ± 4.1 (9) abc	
Subtidal → intertidal		6.5 ± 2.2 (7) a	9.8 ± 4.2 (9) ab	
Culture → intertidal 35 days				10.3 ± 5.0 (6) bc
Subtidal → intertidal 35 days				4.4 ± 2.2 (11) a

Statistical comparisons address differences in a given month because treatments and months interacted in their impact upon percent gaping (see text). In a given month, the values followed by different letters are significantly different ($p < 0.05$, one-factor ANOVA; SNK multiple comparisons test, $p < 0.05$).

TABLE 3.

Anatomical properties of wild intertidal and suspension cultured *M. edulis:* effect of acclimatization to intertidal conditions (means ± SD, N = number of individuals measured).

	Intertidal → Intertidal (N = 36)	Culture → Intertidal (N = 35)	Culture → Subtidal (N = 32)
Length (mm)	37.4 ± 1.9 a	36.2 ± 2.3 b	36.4 ± 1.9 b
Width (mm)	20.5 ± 1.5 a	19.7 ± 1.3 b	19.8 ± 1.0 b
Depth (mm)	15.2 ± 1.2 a	14.0 ± 1.3 b	14.1 ± 0.8 b
Total mass (g)	6.35 ± 1.12 a	4.77 ± 1.05 b	4.81 ± 0.92 b
Flesh mass (g)	1.33 ± 0.20 a	1.88 ± 0.62 b	1.93 ± 0.45 b
Adductor muscle (g)	0.097 ± 0.02 a	0.080 ± 0.018 b	0.077 ± 0.013 b
Shell mass (g)	3.28 ± 0.60 a	1.76 ± 0.35 b	1.76 ± 0.35 b
Flesh/Total mass	0.21 ± 0.026 a	0.39 ± 0.09 b	0.40 ± 0.07 b
Adductor muscle/flesh mass	0.073 ± 0.013 a	0.045 ± 0.011 b	0.042 ± 0.011 b
Adductor muscle/shell mass	0.030 ± 0.006 a	0.046 ± 0.007 b	0.045 ± 0.009 b

Suspension-cultured mussels were acclimatized to either intertidal or subtidal conditions as explained in Materials and Methods. When values for a given parameter do not share the same letter, they differ significantly (ANOVA, SNK, $p > 0.05$).

resented a greater proportion of the total flesh mass than the cultured mussels. Because intertidal mussels face air exposure, whereas suspension-cultured mussels are only exposed to air during sock production and harvesting, a relatively larger adductor muscle may facilitate the closure response of intertidal mussels. Furthermore, relatively larger adductor muscles and thicker shells would be adaptive against predation. Relaxation of predation pressure by *Asterias rubens* in the Baltic Sea has been used to explain the smaller adductor muscle, the thinner, less calcified shell, and the higher meat content of Baltic versus North Sea mussels (Kautsky et al. 1990).

Although wild subtidal mussels are not normally exposed to air, they have much the same responses to air exposure as do wild intertidal mussels. Their survival during prolonged air exposure is greater than that of cultured mussels and similar to that of intertidal mussels. The percent gaping of subtidal mussels is lower than that of cultured mussels and similar to that of intertidal mussels. These data suggest that it is not emersion per se that improves the survival of intertidal mussels during air exposure. Perhaps the rigorous physical conditions imposed by breaking waves as well as the presence of predators force benthic mussels to improve their capacity for valve closure and the accompanying metabolic depression. By extension, the mortality of our subtidally acclimatized cultured mussels during air exposure may have been reduced relative to that of freshly harvested cultured mussels.

In summary, if the harvesting of suspension-cultured mussels were facilitated by a period of maintenance in the intertidal, acclimatization to intertidal conditions could be useful for the culture industry because a sufficiently long intertidal acclimatization can improve the survival of mussels and their resistance to debyssing in spring and early summer. A constant positive response to intertidal acclimatization may be possible if the treatment is extended. However, the cost/benefit ratio of this technique may be too small to warrant its implementation. The low mortality of wild subtidal mussels suggests that subtidal acclimatization may also improve the viability of suspension-cultured mussels.

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MOLT-RELATED CHANGES IN HEMOLYMPH CALCIUM OF POSTLARVAL AMERICAN LOBSTERS (*HOMARUS AMERICANUS*)

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ABSTRACT In a laboratory study, postlarval American lobsters experienced changes in hemolymph calcium concentrations over the molt cycle. Values were lowest immediately after molting and increased gradually through postmolt into intermolt and early premolt. Calcium concentration peaked during mid- or late-premolt and usually declined just before the molt to fifth stage. This pattern is similar to that observed in adult American lobsters.

INTRODUCTION

The American lobster, *Homarus americanus* H. Milne-Edwards, 1837, experiences changes in hemolymph calcium concentration over the molt cycle in both the juvenile (Charmantier et al. 1984a) and adult (Donahue 1953, Castell and Budson 1974, Mercaldo-Allen 1991) life stages. This pattern of a premolt increase and postmolt decrease in blood calcium also occurs in the European lobster, *Homarus gammarus*, (Glynn 1968) and the spiny lobster, *Panulirus argus*, (Travis 1955a and b).

No studies have examined the relationship between blood calcium and molting in postlarval (stage IV) lobsters. The postlarval phase is a transition from the planktonic larval form to a more adult-like benthic stage (Charmantier et al. 1984b). Biochemical, morphological, behavioral, and physiological changes occur at this time (Charmantier et al. 1984b, 1991); postlarval osmoregulation becomes similar to that observed in juvenile and adult lobsters (Charmantier et al. 1984b and c, 1988).

Consideration of the molt cycle is important in any study of lobster physiology, particularly blood chemistry (Mercaldo-Allen 1990, 1991). Total protein concentration, an established tool for monitoring physiological condition in lobsters, requires attention to molt stage (Castell and Budson 1974). Hemolymph calcium may provide similar information about lobster health during studies of disease or environmental degradation; however, this requires a basic understanding of calcium blood chemistry under known conditions. The following study describes blood calcium concentrations during the molt cycle of postlarval American lobsters under laboratory conditions.

MATERIALS AND METHODS

Animal Collection and Rearing Conditions

During spring 1992, ovigerous lobsters (67 to 86 mm carapace length [CL]) were collected by lobster pot in mid–Long Island Sound near Milford, Connecticut, and from the far eastern end of the Sound near New London. In 1993, ovigerous females were collected from Milford (68 to 84 mm CL) and the Gulf of Maine (100 to 117 mm CL). Lobsters were held in unfiltered, flowing seawater at ambient temperature and salinity. Hatching larvae were skimmed off the water surface daily or collected in an outflow screen.

Larvae were reared to stage IV in flow-through planktonkreisels containing sand-filtered, ambient temperature and salinity seawater (Van Olst et al. 1980); these kreisels were cleaned three times per week. At each cleaning, the larvae received a 1 minute freshwater dip to rid them of *Vorticella* sp. (a potentially lethal protozoan), which were observed occasionally on the postlarvae. Larvae were fed Mid-Jersey Champlain Lake Saskatchewan-brand frozen brine shrimp three times daily.

Experimental Design

Postlarval lobsters were removed from the planktonkreisels and placed in 10 l static pans. The seawater in each pan was replaced approximately every 4 days. The pans were aerated with airstones and a Sweetwater pump. Temperature was maintained at approximately 15.5 ± 1°C by floating the pans in chilled seawater; salinity was measured daily.

During the 1992 study, seawater for each water change was collected fresh from the flow-through planktonkreisel system to minimize daily fluctuations in ambient seawater salinity and calcium concentration that might occur in the flow-through planktonkreisels as the result of the tidal cycle. Mean pan salinity measured 25.5 ppt (0.04 SE) and ranged from 24.7 to 26.6 ppt.

This procedure was modified in 1993 to compare the effect of ambient versus artificial seawater on hemolymph calcium concentrations. Several carboys of ambient seawater were collected at the beginning of the study and refrigerated until the pan water was changed. Four 10 l pans were maintained, two containing ambient seawater and two consisting of 25 ppt artificial seawater (Instant Ocean). Mean salinity measured 25.2 ppt (0.01 SE) in the ambient pans (range, 24.7 to 25.7 ppt) and 25.0 (0.02 SE) in the Instant Ocean treatments (range, 24.4 to 25.7 ppt).

Postlarvae were placed in individual plastic mesh (1 × 3 mm hole size) cylinders (7.6 cm long with 6.3 cm diameter plastic covers) to prevent cannibalism in the static pans and were not fed. To sample postmolt stage A, some late stage III larvae were placed in the pans so they could be bled upon metamorphosis to fourth stage. Postlarvae were allowed to acclimate in a pan for a minimum of 24 hours before bleeding. When lobsters were removed for bleeding, a seawater sample was collected from each pan and frozen for subsequent calcium analysis.

Molt-staging Criteria and Hemolymph Sampling

Molt stage was determined using the method and staging criteria of Sasaki (1984), which involves examining the uropods

under a dissecting microscope (×70). Stages were classified postmolt (A, B) by the degree of tissue density, intermolt (C, D_0) by the presence of a clear tissue layer beneath the base of the setae, or premolt (D_1, D_{2-3}) by the degree of setal invagination. Because the distinguishing characteristics within each of the three major categories are subtle, hemolymph calcium results were analyzed both by individual molt stage (A, B, C, D_0, D_1, D_{2-3}) and by molt groups (postmolt A + B, intermolt C + D_0, and premolt D_1 + D_{2-3}).

Lobsters were bled under a dissecting microscope (×10) using a Hamilton 10 µl syringe by inserting the end of the needle just posterior and ventral to the heart. The heart and the pericardial cavity were drained, yielding between 2 and 9 µl of hemolymph (Olson 1991). To avoid contamination of the needle, the syringe and needle were rinsed in deionized water and dried after each bleeding. Hemolymph from each lobster was transferred to separate 0.5 ml Eppendorf microtubes. If a sample was cloudy or yellowish in appearance, it was considered contaminated with hepatopancreatic material and discarded (Olson 1991). Samples were frozen at −12°C until analyses were conducted 1 to 2 months later. After thawing, samples were centrifuged for 2 minutes at 12,000 × g with a Brinkman Eppendorf Microcentrifuge 3200.

Chemical Analysis

A Baker CentrifiChem-600 blood analyzer, with automated Pipettor-1000 and Apple II computer, was used to measure calcium levels in the postlarval hemolymph and seawater samples (Baker Instruments Corporation, 1982). After the pipettor dispensed 350 µl of cresolphthalein calcium reagent (Trace Scientific) and 53 µl of diluent (deionized water) into the analyzer disc, a 2 µl hemolymph aliquot was added by hand pipette with a Rainin Pipetman P-20. This sample volume produced values within the linear range of the instrument.

The analyzer was calibrated with the protein-based reference products Standardize 1 (American Monitor Corporation) and Calibrator I (Trace Scientific). Quality control was maintained with Decision (Beckman), a three-level, liquid, comprehensive chemistry control serum. Calcium analyses for seawater samples were conducted by the same procedures.

Statistical Analyses

The data sets were kept distinct by year for statistical analysis, and tests were performed with the SAS System for Elementary Statistical Analysis (SAS Institute Inc. 1985). Analysis of variance (PROC GLM procedure) and Tukey's test were used to determine whether hemolymph calcium values varied significantly between the individual or grouped molt stages.

For 1992, analysis of variance was conducted to determine if the seawater salinity or calcium concentrations in the static pan had a significant effect on hemolymph calcium values at the individual or grouped molt stages. Because salinity had no effect during 1992, the analysis was conducted only for seawater calcium during 1993. Analysis of variance was also performed on the 1993 data to determine whether hemolymph calcium was significantly different between the ambient and Instant Ocean pans.

RESULTS

Hemolymph 1992

Hemolymph calcium measured 29.0 mEq l^{-1} at postmolt A, increased to 32.5 at stage B, and measured 32.0 at intermolt C (Fig. 1). Concentrations measured 31.7 mEq l^{-1} during stage D_0, peaked at 35.2 during premolt D_1, and declined slightly to 32.1 at D_{2-3}. Individual molt stage had a significant effect on hemolymph calcium ($p = 0.0353$); Tukey's test demonstrated a significant difference ($p > 0.05$) between the mean calcium values for premolt D_1 and postmolt A. Hemolymph calcium was significantly higher ($p = 0.0168$) during premolt D_1 + D_{2-3} (34.9 mEq l^{-1}) than during intermolt C + D_0 (31.7 mEq l^{-1}) and postmolt A + B (31.7 mEq l^{-1}) (Fig. 2).

Hemolymph 1993

In the ambient seawater pan, hemolymph calcium measured 30.9 mEq l^{-1} at postmolt A, increased to 35.9 at postmolt B, and leveled off to 35.5 and 35.1 at intermolt stages C and D_0, respectively (Fig. 3). Hemolymph values peaked at 38.5 during premolt D_1 and declined slightly to 37.4 at premolt D_{2-3}. Hemolymph calcium in postlarvae from the ambient seawater pan was significantly lower at individual molt stage A than at stages D_1 and D_{2-3} ($p = 0.0055$). For the grouped molt stages, premolt (38.0 mEq l^{-1}) calcium was significantly higher than postmolt (35.5 mEq l^{-1}) and intermolt (35.4 mEq l^{-1}) ($p = 0.0148$) (Fig. 4).

Postlarval hemolymph in the Instant Ocean treatment contained 30.1 mEq l^{-1} calcium during postmolt stage A and increased to 34.3 in postmolt stage B (Fig. 5). Mean values measured 35.4 at intermolt C and 35.0 at D_0 and increased to 37.4 during premolt D_1. The highest concentration of calcium was measured at 40.4 during D_{2-3}. In the Instant Ocean pan, individual molt stage A was significantly lower than stages D_1 and D_{2-3}. Stage D_{2-3} was significantly higher than A, B, C, and D_0 ($p = 0.0001$). Grouped premolt (38.6 mEq l^{-1}) was significantly higher than postmolt (34.0 mEq l^{-1}) and intermolt (35.3 mEq l^{-1}) ($p = 0.0001$) (Fig. 6). There was no significant difference in hemolymph calcium concentration between the ambient and Instant Ocean treatments.

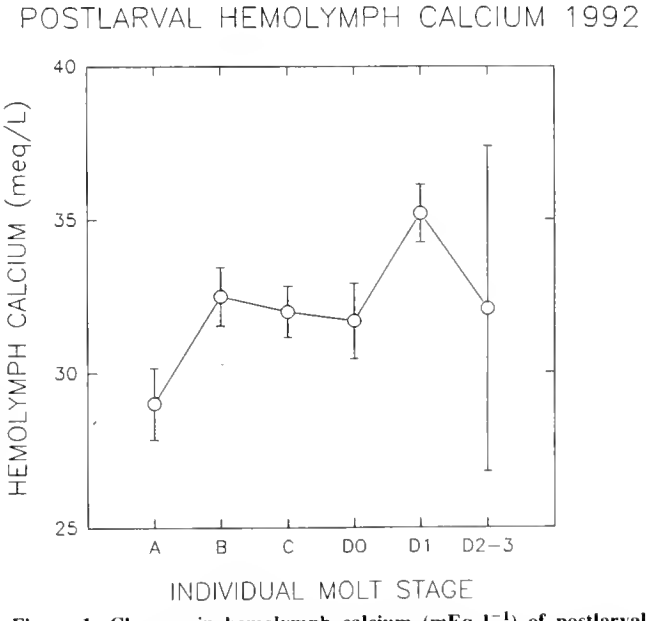

Figure 1. Changes in hemolymph calcium (mEq l^{-1}) of postlarval American lobsters held in ambient seawater at individual stages of the molt cycle during 1992. Each datum point represents an average of 5 (A), 17 (B), 25 (C), 11 (D_0), 18 (D_1), and 2 (D_{2-3}) blood samples. Bars represent standard error.

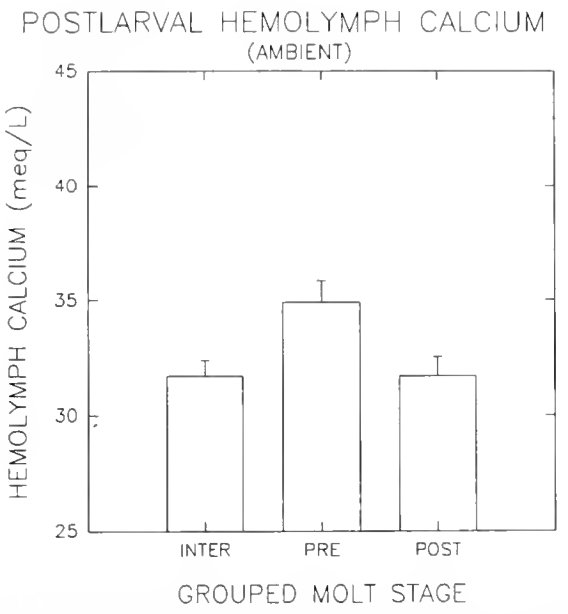

Figure 2. Changes in hemolymph calcium (mEq l^{-1}) of postlarval American lobsters held in ambient seawater at grouped stages of the molt cycle during 1992. Each datum point represents a mean of 36 (intermolt; INTER), 20 (premolt; PRE), and 22 (postmolt; POST) blood samples. Bars represent standard error.

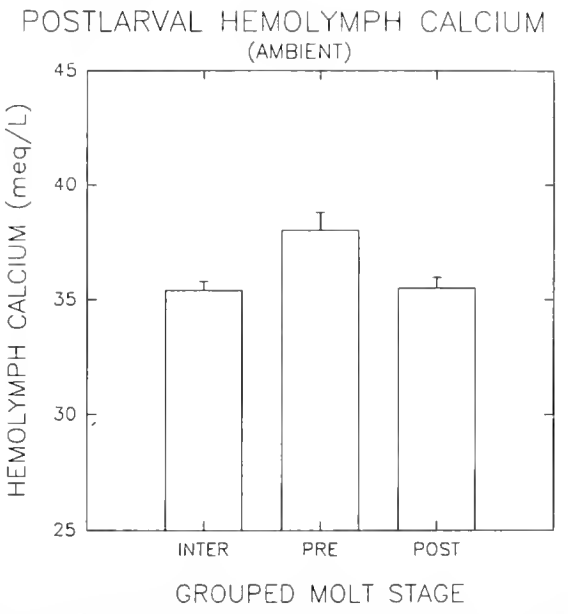

Figure 4. Changes in hemolymph calcium (mEq l^{-1}) of postlarval American lobsters held in ambient seawater at grouped stages of the molt cycle during 1993. Each datum point represents a mean of 127 (intermolt; INTER), 27 (premolt; PRE), and 85 (postmolt; POST) blood samples. Bars represent standard error.

Seawater Calcium/Salinity and Hemolymph Calcium 1992

Mean seawater calcium concentrations were calculated for each individual and grouped molt stage and are shown in Figures 7 and 8. The mean ambient seawater calcium concentration measured 13.8 mEq l^{-1} (0.15 SE) and ranged from 6.8 to 17.4 mEq l^{-1}. Seawater calcium levels significantly affected hemolymph calcium concentrations at individual molt stage B ($p = 0.0211$) but not at stages A, C, D_0, or D_1 (Fig. 7). $D_{2\text{-}3}$ could not be included in the analysis because of a small sample size. Seawater calcium levels affected hemolymph calcium values at grouped postmolt ($p = 0.0040$) but not intermolt or premolt (Fig. 8). Seawater salinity in the pan did not significantly affect hemolymph calcium at any of the individual or grouped molt stages.

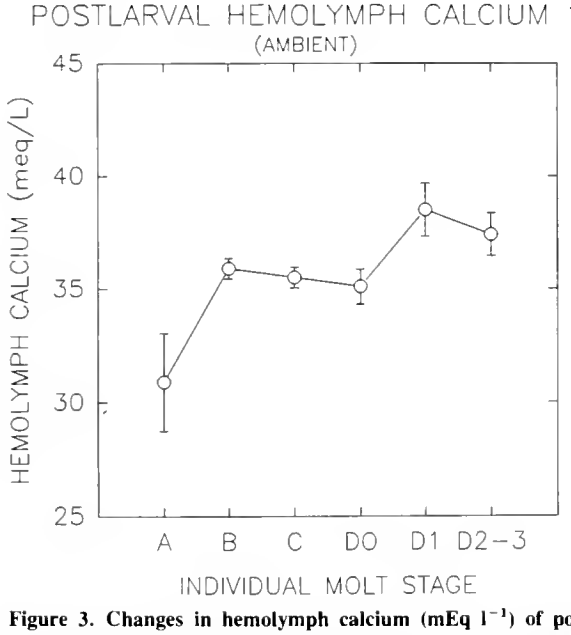

Figure 3. Changes in hemolymph calcium (mEq l^{-1}) of postlarval American lobsters held in ambient seawater at individual stages of the molt cycle during 1993. Each datum point represents a mean of 6 (A), 79 (B), 90 (C), 37 (D_0), 16 (D_1), and 11 ($D_{2\text{-}3}$) blood samples. Bars represent standard error.

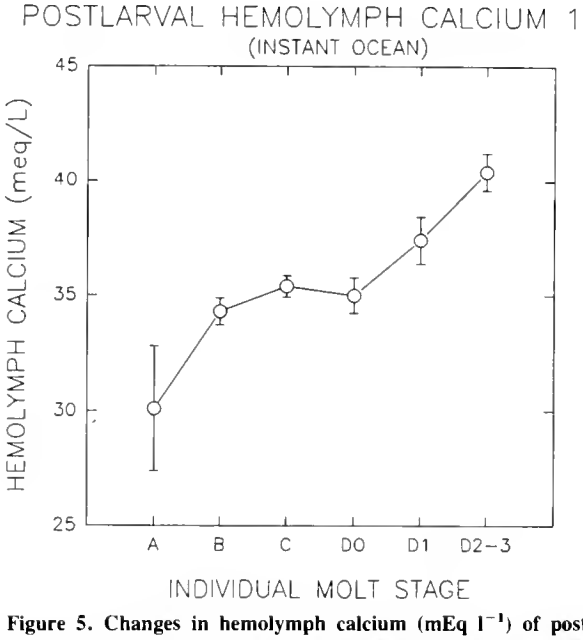

Figure 5. Changes in hemolymph calcium (mEq l^{-1}) of postlarval American lobsters held in artificial seawater (Instant Ocean) at individual stages of the molt cycle. Each datum point represents a mean of 6 (A), 73 (B), 95 (C), 46 (D_0), 20 (D_1), and 12 ($D_{2\text{-}3}$) blood samples. Bars represent standard error.

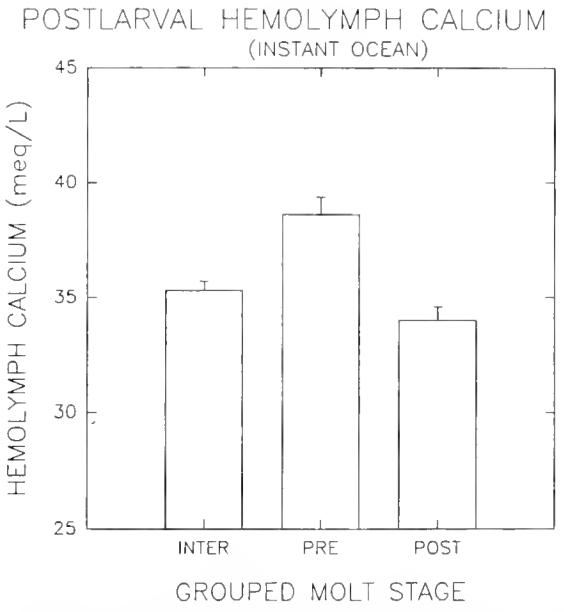

Figure 6. Changes in hemolymph calcium (mEq l^{-1}) of postlarval American lobsters held in artificial seawater (Instant Ocean) at grouped stages of the molt cycle. Each datum point represents a mean of 141 (intermolt; INTER), 32 (premolt; PRE), and 79 (postmolt; POST) blood samples. Bars represent standard error.

Seawater Calcium and Hemolymph Calcium 1993

Mean seawater calcium in the ambient seawater pans measured 19.4 (0.17 SE) mEq l^{-1} and ranged from 11 to 21.8 mEq l^{-1}. Seawater calcium significantly affected hemolymph calcium concentrations at individual molt stages B (p = 0.0003), C (p = 0.0006), D_1 (p = 0.0295), and $D_{2\text{-}3}$ (p = 0.0060) (Fig. 7). All three grouped molt stages—intermolt (p = 0.0002), postmolt (p = 0.0004), and premolt (p = 0.0002)—were also significantly affected (Fig. 8).

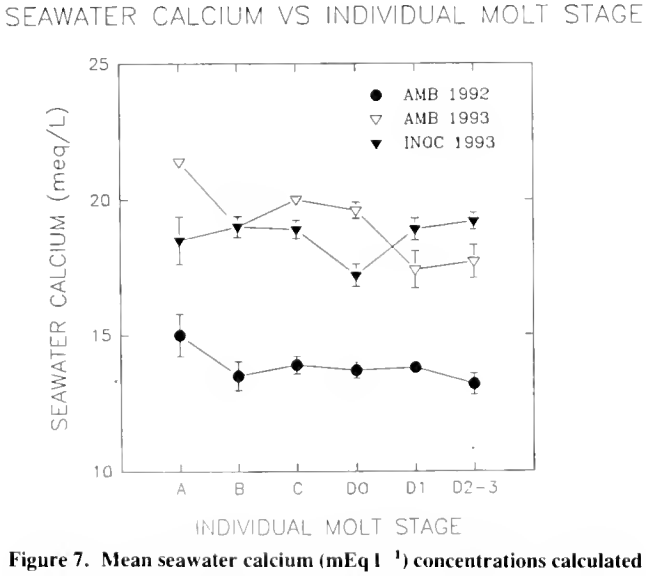

Figure 7. Mean seawater calcium (mEq l^{-1}) concentrations calculated for each individual molt stage. Each datum point represents a mean of 7 to 37 seawater samples for the ambient (AMB) pan during 1992, 6 to 90 for the ambient pan during 1993, and 6 to 95 for the Instant Ocean (INOC) pan during 1993. Bars represent standard error.

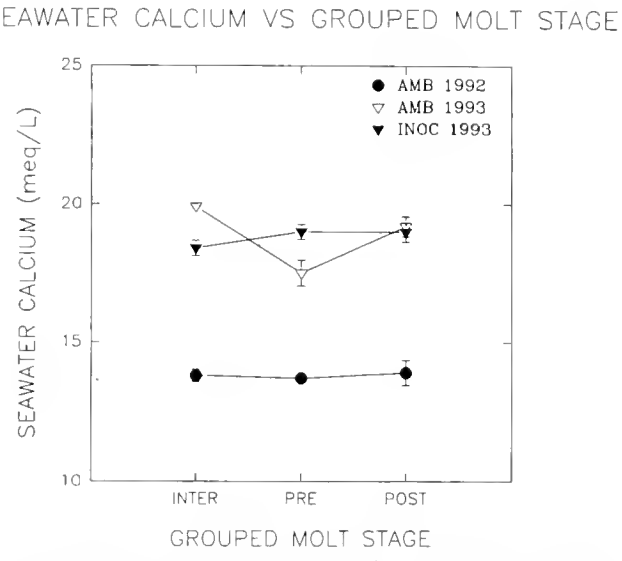

Figure 8. Mean seawater calcium (mEq l^{-1}) concentrations calculated for each grouped molt stage. Each datum point represents a mean of 26 to 49 seawater samples for the ambient (AMB) pan during 1992, 27 to 127 for the ambient pan during 1993, and 32 to 141 for the Instant Ocean (INOC) pan during 1993. Bars represent standard error.

The Instant Ocean pans contained 18.6 mEq l^{-1} (0.19 SE) seawater calcium, with levels ranging from 7.4 to 22.5 mEq l^{-1} over the course of the experiment. Lobster blood calcium was significantly affected by seawater calcium at individual molt stages B (p = 0.0267) and $D_{2\text{-}3}$ (p = 0.0512) (Fig. 7). Grouped stages intermolt (p = 0.0010) and postmolt (p = 0.0052) were also affected (Fig. 8).

DISCUSSION

During this study, postlarval hemolymph calcium concentrations fell within the range of concentrations previously measured for adult American lobsters (Donahue 1953, Castell and Budson 1974, Mercaldo-Allen 1990, 1991). The molt-related changes observed here in postlarvae were similar to those previously observed in adults (Mercaldo-Allen 1991). This was not surprising, because lobsters acquire the juvenile/adult type of osmoregulation (Charmantier et al. 1988) after metamorphosis to the postlarval (fourth) stage.

Hemolymph calcium concentrations were lowest during postmolt A, increased through intermolt C, were highest at premolt D_1, and declined at $D_{2\text{-}3}$ just before molt. However, in the Instant Ocean treatment, calcium remained high through $D_{2\text{-}3}$.

The increase in calcium values from early postmolt through intermolt may reflect decreasing blood volume during the postmolt period (Mykles 1980). Previous studies with adult American lobsters have shown that calcium levels rise steadily into premolt, where peak values are reached just before molting (Donahue 1953, Mercaldo-Allen 1991). The removal of calcium from the old exoskeleton for temporary storage in the hemolymph may account for elevated premolt calcium (Travis 1955b, Mantel and Farmer 1983).

The late premolt decrease in calcium concentration observed in postlarval lobsters during this study has been observed several days before molt in adult European (Glynn 1968) and spiny (Travis 1955a and b) lobster species. In adult American lobsters, concentrations of inorganic phosphorus, glucose, potassium, so-

dium, and total protein in the hemolymph decline during late premolt (Mercaldo-Allen 1991).

Variations in hemolymph ion concentrations reflect changes in hemolymph water content (Charmantier et al. 1984a). Dilution of the hemolymph could explain reduced calcium concentrations observed at premolt D_{2-3} and postmolt A. Juvenile lobsters increase their seawater intake approximately 1 hour before ecdysis (D_4) (Mykles 1980). In some animals, hemolymph volume begins increasing as early as 24 to 36 hours before ecdysis (D_3) and continues into early postmolt (A) (Mykles 1980). Reduced calcium during late premolt and early postmolt may reflect the removal of calcium ions from the hemolymph for deposit into the new exoskeleton (Glynn 1968, Mercaldo-Allen 1991). Calcium ions in the Instant Ocean treatment may have been available in greater concentrations than in the ambient seawater, reducing the amount of blood calcium removed during D_{2-3} for hardening of the new shell.

Earlier studies of adult lobsters have observed considerable variability in the inorganic chemical composition of hemolymph, even in seawater of constant composition (Cole 1940 and 1941, Parker and Cole 1941). During our study, procedures were used to minimize fluctuations in seawater calcium by using stored ambient seawater and artificial seawater. Even so, some variation occurred within each distinct study period and acclimation pan. Our results suggest that hemolymph calcium may be affected by seawater calcium concentrations at certain molt stages.

Premolt hemolymph concentrations were least changed by external calcium levels, perhaps because already high premolt calcium concentrations mask any change that may be due to the surrounding seawater. The postmolt and intermolt stages were usually the most affected. Large quantities of seawater are absorbed by the lobster at the time of ecdysis. Absorption of this water into the new cuticle takes place gradually through postmolt and is completed by intermolt (Mykles 1980). Seawater calcium would be expected to have its greatest impact at this time.

Except under low-salinity conditions, lobsters allow their ionic composition to conform roughly with that of the environment. Although lobsters are not in passive osmotic equilibrium with the environment, their ability to osmoregulate is limited (Dall 1970). However, lobsters must regulate calcium to some extent, because hemolymph contains nearly twice the calcium measured in the surrounding seawater. Lobsters are thought to accumulate calcium from the seawater (Parker and Cole 1941) and to establish and maintain a calcium equilibria between serum and seawater. In full-strength and dilute seawater, lobsters are capable of expending energy against a concentration gradient; the amount of energy is determined by the change in the concentration of the environmental seawater and the duration of exposure (Cole 1940). Although seawater calcium may have some impact on calcium concentrations in the hemolymph, this effect does not obscure the significant variations in hemolymph calcium during the molt cycle.

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POPULATION FIELD STUDIES OF THE GUINEA CHICK LOBSTER (*PANULIRUS GUTTATUS* LATREILLE) AT BERMUDA: ABUNDANCE, CATCHABILITY, AND BEHAVIOR

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ABSTRACT Transect and capture-recapture studies during the summer and autumn infer that the greatest abundance of trappable Guinea chicks in the northeastern reef system of the Bermuda Platform occurs in areas immediately adjacent to the reef crest.

Mean population density of trappable Guinea chick lobsters in the reef crest areas in the summer and autumn periods of 1986/87 was 29 ($\pm$7.6) ha^{-1} (95% confidence limits). The catchability coefficient (q) was 1.3×10^{-3} ($\pm 0.84 \times 10^{-3}$) trap night^{-1}. The mean value for effective area fished (E) was 13×10^{-3} ($\pm 6.7 \times 10^{-3}$) ha trap night^{-1}.

During May to October at the reef crest of the northeastern reef system, both q and E for females were about half of the values for the males, indicating that females there are less catchable than males.

Results from (1) a study of changes in relative abundance and (2) a sequence of three capture-recapture experiments by Bailey's Triple Catch Method (during May–October) infer a peak population size at the reef crest during the months July through September. The size composition (3) of male *Panulirus guttatus* at the northeastern reef crest decreased through the summer (May–Sept). This trend reversed in October. Finally, there are indications (4) of an inward migration of egg-bearing females from the shelf edge and the reef-front terrace to the reef crest during May–August. These females may be accompanied by younger males. The lines of evidence indicate that there was an inward breeding migration of Guinea chicks to the reef crest.

The sex ratio of trappable Guinea chick lobsters at the northeastern reef crest during the summer and autumn was estimated at 7:1 male:female from capture-recapture studies. Different natural mortality rates between the sexes in the juvenile phase is probably the cause. The median sex ratio of trapped Guinea chicks (male:female) was greater for areas seaward of the reef crest (28:1) than for areas landward (11:1), inferring a localization of females to the reef tract.

Data on size/depth, the effect of the lunar cycle, and autumnal off-shore migration are also presented. The combined effect of mortality and emigration resulting from tagging/displacement was 37 to 46%.

KEY WORDS: *Panulirus guttatus*, Bermuda, lobster, abundance, catchability, behavior

INTRODUCTION

The Guinea chick lobster *Panulirus guttatus* (Latreille), commonly known as the spotted lobster elsewhere in the Gulf and Caribbean region, is a by-catch or secondary catch in most areas. Only at Bermuda and at the French West Indies is there a specific fishery. The present study is directed at improving limited knowledge of the species' ecology.

The geography of Bermuda is shown in Figure 1. The reefs and islands form a kind of atoll, and *P. guttatus* lobsters are seldom taken outside of the "narrow band encompassing the outermost reefs or the relatively shallow water immediately adjacent" (Sutcliffe 1953). This area corresponds with the atoll rim and adjacent reefs landward and seaward. The annual fishery catch of Guinea chick lobsters was approximately the same as that for the larger Bermuda spiny lobster *Panulirus argus* (30×10^3 lobsters) during the research period 1986/1987.

MATERIALS AND METHODS

Traps

The principal tool for the present study was the traditional Bermudan arrowhead (or chevron) fish trap (Sutcliffe 1952). Traps were baited with the heads and tails of reef fish and set in "white holes" (sand-floored depressions in the reef).

Study Areas and Experiments

Capture-recapture areas were established on the northeastern reef Guinea chick fishing grounds at St. David's Head, Cooper Island, and Kitchen Shoals (Fig. 1). Carapace length (CL) measurements were also obtained of Guinea chick lobsters from lines of traps at the North Reefs and the associated fore-reef slope and reef-front terrace, East Ledge Flat, St. Catherine's Shoals, and Sea Venture Shoals. The study areas at St. David's Head and Cooper's Island were located on the breaker ledges off the eastern part of the island, adjacent to the reef crest (or breaker line) (Fig. 2), and the first trapping transect across the Bermudan reef system (Transect 1) was also located off Cooper's Island (Fig. 2). A second trapping transect (Transect 2), across the northeastern reef system, was established in a line east-west (magnetic) from the North Lagoon through Kitchen Tower and out to the easternmost edge (Fig. 3).

Field experiments concerned with estimating the combined effects of tag mortality and tag- or displacement-induced emigration were carried out just seaward of the line of boiler reefs off Cooper's Island (Fig. 2) and at Kitchen Shoals (Fig. 3a).

St. David's Head and Cooper's Island study areas were naturally delimited by the reef crest (a line of exposed boiler reefs just landward) and a steep slope to the reef-front terrace (Fig. 2). The East Ledge Flat study area was established by marking out a 0.25 nautical miles (nm) square with four radar buoys and trapping the white holes within it with 16 pots. The St. Catherine's area was established in a similar way. The Kitchen Shoals study area was delimited by trapping the available white holes on the discrete Kitchen Tripod shoal, within about a 0.125 nm radius (shown by radar from Kitchen Tripod), although this "radius" was elongated toward the north (Fig. 3a).

Marking

Marking at St. David's Head was by punching up to a total of four 0.25 inch diameter round holes in the tail fan (i.e., in the

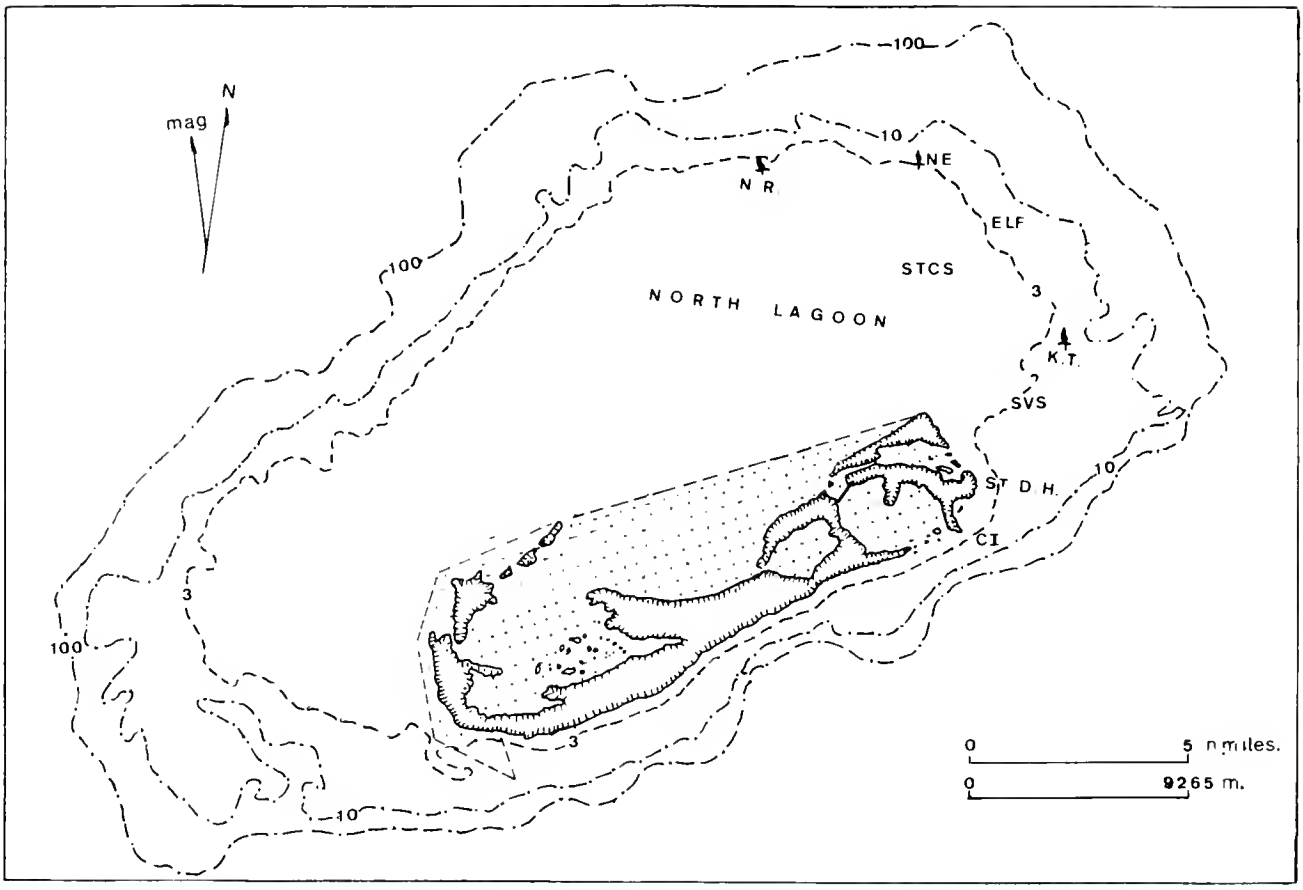


Figure 1. The Bermuda platform and study areas. The Bermuda Reef platform is an atoll system that has become established on the volcanic Bermuda pedestal. The inshore area to the north of the island is marked with a stipple and shows the position of a no-pot zone to protect inshore nursery areas of fish and lobsters. North Rock (N.R.), Kitchen Tripod (K.T.), and Cooper's Island (CI) are the limits of the northeastern or eastern reef system. Depth contours are in fathoms (1 fathom = 1.94 m). Capture-recapture areas comprised the breaker ledges associated with St. David's Head (ST.D H.) and Cooper's Island (CI) and the reef flat on the rim reefs at Kitchen Shoals (near the reef crest at Kitchen Tower). Study areas for sampling for biometrical measurements were located at East Ledge Flat (ELF), patch reef shoals north of St. Catherine's Point (STCS), and Sea Venture Shoals (SVS). The population of *P. guttatus* lobsters located on the fore-reef slope and rim reefs associated with the North Reefs (around Northeast Tower) was also studied by measurement of landings by Guinea chick fishermen.

uropods and/or the telson) in a coded sequence, so that animals could be individually identified. At Kitchen Shoals and along Transect 2, animals were marked with a Floy FD-68BC spaghetti tag with a nylon t-bar, as used in the field experiment that investigated the rate of loss of this tag on juvenile *P. argus* lobsters in Florida (Davis 1978). (Davis's juveniles were comparable in size [34 to 84 mm carapace length] to fishable *P. guttatus* lobsters at Bermuda [48 to 83 mm carapace length].)

Tags were inserted to the right of the middorsal line between the posterior margin of the carapace and the anterior edge of the first abdominal segment. The off-center insertions were made into the abdominal muscle mass (the dorsolateral extensor muscle) and positioned to miss the gut. A Floy FDM-68 gun and a 1.8 mm diameter hollow stainless-steel Floy needle were used. Depth of insertion was ca. 15 mm. These numbered tags were custom made to the same construction as those used in Davis's field evaluation of the tag: the anchor toggles were nylon monofilament 10 mm long on the t-bar, 28 mm long on the shank, and 1.2 mm in diameter, with 25 mm of No. 20 vinyl spaghetti tubing attached to the shank, 13 mm from the t-bar.

Measurement of Carapace Length

CL was measured with an S.P.I. Swiss-made vernier caliper to the nearest 0.1 mm. The accuracies from repetitive measurements were 0.125 mm for animals up to 60 mm CL and 0.25 mm for animals of 60 to 83 mm (CL). CL was the horizontal distance from the anterior margin of the carapace (between the supraorbital horns) to the posterior margin of the carapace at the middorsal line.

RESULTS

Distribution and Historical Trend in Size

All physiographic provinces of the Bermudan atoll reef system were found to be inhabited by the species in Bermuda (Fig. 3b). Guinea chick lobsters were not found in the inshore waters, but on the outer reefs, from the patch reefs landward of the reef crest to the continuous reefs seaward of the reef crest (down to 43 m, i.e., 22 fathoms deep) (Fig. 3b).

The size range and modal CLs for *P. guttatus* lobsters in 1951/52 and 1986 is compared in Table 1, based on data collected by Sutcliffe (1953) and from the present study. The modal sizes observed for each sex were smaller in 1986 (by 16 mm CL for males and by 13 mm CL for females) than they were in 1951/52, and the largest sizes caught in 1986 were also smaller (by 4 mm CL for each sex).

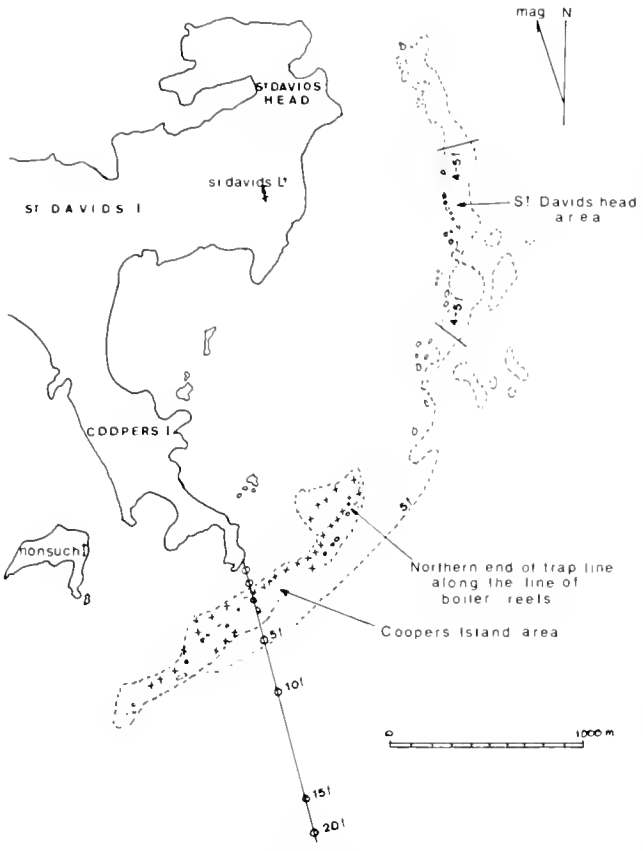


Figure 2. St. David's, Cooper's Island, and Transect 1. St. David's Head. Traps of arrowhead design were reconstructed and restored and baited and set in a line on the reefs just off St. David's Head, at the east end of Bermuda (Fig. 1). An average of 6.9 traps were used here (Table 5A: standard deviation = 1.4; N = 13), over a 6 week period in the autumn of 1986. They were made of traditional Bermudan arrowhead design with chicken wire mesh on a spicewood frame 1.1 m × 1 m × 0.5 m. A 940 m long section of the breaker ledge was selected as the study area (12 ha). The pots were on average 940/5.9 = 159 m apart and were set just seaward of the line of boiler reefs (generally within 10 m, but two to three traps were sometimes further seaward, to take advantage of better "sets"). When water clarity was good, the traps were placed in sand-floored depressions in the reef: "white holes." Hauling of traps took place every 3 to 5 days, and unmarked Guinea chicks were marked with a 0.25 inch diameter hole, by a paper punch, to make up to four holes in the tail fan. Cooper's Island. After the first 6 weeks at St. David's, the traps were removed and a second study area was selected on the breaker ledge off Soldier's Point, Cooper's Island, not far to the south. The traps were set here more closely spaced: 6.7 traps on average (Table 5B: standard deviation = 1.1; N = 13) along a 700 m long section of the breaker ledge, again set just seaward of the line of boiler reefs (all within m). The north end of the trap line was the point at the seaward edge of the boiler reefs where the Bermuda Harbor Radio Building bears 330 degrees mag., and the south end of the line was the Transect 2 line directly off Soldier's point. Trap separation was on average 700/5.7 = 123 m. White holes were set whenever possible; hauling took place every 3 to 6 days from 3 Oct. to 5 Nov., after which, sea conditions worsened and visits became more infrequent. Marking as described at St. David's was carried out again, continuing the sequence in the application of coded marks. Every second animal was also tagged with a Floy 68-BC spaghetti tag. The area of the ledge seaward of the line of boilers (i.e., the breaker ledge) was estimated at 5.7 ha. Transect 1. The location of the Transect 1 trap line is also shown. The boiler reefs are marked by a line of crosses. Circles mark the positions of single traps: two inside the line of boiler reefs, one just seaward of it (on the breaker ledge), and one at each of the 5, 10, 15, and 20 fathom stations (on the reef front).

Animal Size and Depth Location

Results of observations on the relationship between the size of males caught on Transects 1 and 2 across the eastern and northeastern reef systems and their depth location are presented in Figure 4. The combined size composition (added frequencies) for the deeper marine environments (reef front or main terrace and edge of the island shelf) is of smaller animals, chiefly of size 51 to 71 mm CL (median, 61 mm), whereas the combined size composition for the shallower environments (fore reef, reef flat, and patch reef) is of larger animals, chiefly of size 55 to 83 mm CL (median, 69 mm).

A relationship between the size of males caught on the northern reef system and their depth location was discovered (Fig. 5). The smallest individuals are found on the fore-reef slopes, breaker ledges, and inner reef-front terraces at 6 to 9 fathoms (12 to 18 m) deep (i.e., the slope from the reef crest to the main terrace) and are chiefly of 49 to 67 mm CL (median, 58 mm). Medium-sized individuals are found on the main terrace (55 to 67 mm CL; median, 61 mm), and larger individuals are found on the North Reefs (i.e., on the rim reefs from North Rock to Northeast Tower) (57 to 73 mm CL; median, 65 mm).

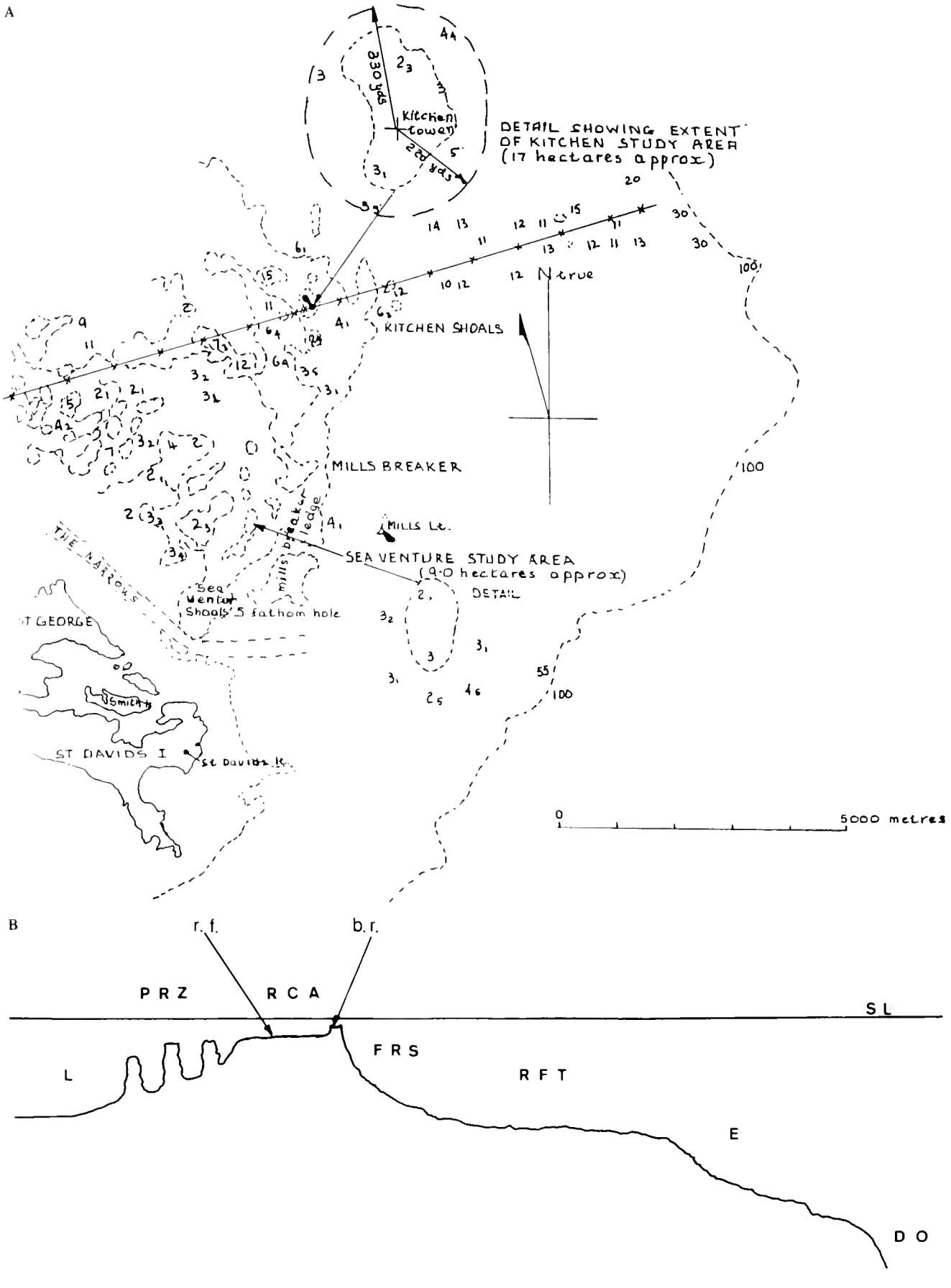
The frequency of females caught on the transects (Fig. 6a) is very much lower than that of males (Fig. 4). Two distinct groups are apparent, one composed of smaller animals of 55 to 57 mm CL, from the outermost reefs, and one of 69 mm CL, from the reef flat. These peaks are also indicated in the size composition of females captured from the northern and eastern reefs (North Rock-Cooper's Island) in 1986 (Fig. 6b).

Boiler reefs are common at the reef crest of the North Reefs and the eastern and southern shores; one such area of "boilers," just off the eastern shore, is the breaker ledge off St. David's Head. The size composition of *P. guttatus* found there during the period 21 August–3 October 1986 is presented in Figure 7. There are three distinct peaks for males, the first at 59 to 65 mm CL, the second at 70 to 77 mm CL, and the third at 77 to 81 mm CL. Males first attain physical maturity at 62.9 to 72.3 mm CL (95% confidence limits) (Evans and Lockwood 1995), so animals of the second and third cohorts comprise mature individuals. The relatively small size and proportion of females in relation to males are readily seen. The 60 to 65 mm size of males is not represented in a reef-crest sample from Cooper's Island Breaker Ledge (3 October to 5 December), and the proportion of females is even smaller (Fig. 8a). The smaller sizes there may have migrated offshore; the change in trap rates along Transect 1 indicates an offshore migration in the last quarter. The two study areas are very similar. This would explain why the smaller sizes found at St. David's were not found at Cooper's Island.

Small sizes of both females and males are found on the fore reef of the North Reefs (Fig. 8b). The male and female size frequency distributions from the study areas and transects illustrate the low proportion of females caught (Fig. 8c).

Transects Across the Reef System

The catch and effort data for *P. guttatus* lobsters on Transect 1 off Cooper's Island are presented in Figure 9. In the last quarter of the year, catch per unit of fishing effort (CPUE) was greatest "inside" the boiler reefs (i.e., on the reef flat or atoll rim). CPUE was second greatest on the inner part of the reef-front terrace at 5 fathoms deep. Monthly CPUE "inside" was relatively high in October and in December, whereas at the 5 fathom station, it was relatively high in November and January. CPUE at the very high


A
DETAIL SHOWING EXTENT OF KITCHEN STUDY AREA (17 hectares approx)
330 yds
220 yds
Kitchen tower
KITCHEN SHOALS
MILLS BREAKER
Mills Breaker ledge
MILLS Lt.
SEA VENTURE STUDY AREA (9·0 hectares approx)
DETAIL
Sea Venture Shoals 5 fathom hole
THE MARROWS
ST GEORGE
Smiths
ST DAVIDS I
St Davids Lt.
N true
0
5000 metres
B
r.f.
b.r.
PRZ
RCA
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L
FRS
RFT
E
DO

TABLE 1.

The Guinea chick *P. gutatus* at Bermuda: range and mode CL for 1951/52 and 1986. Based on data collected by Sutcliffe (1953) and from the present/study.

Sex	1951/52	1986
Male and female		
(N)	152	919
Range (mm)	58–87	48–83
Mode (mm)	79	63
Males		
(N)	137	849
Range (mm)	64–87	50–83
Mode (mm)	79	63
Females		
(N)	15	70
Range (mm)	58–73	48–69
Mode (mm)	70	57
Sex ratio (male:female)	10:1	12:1

The modal size of males changed from 79 to 63 mm, and the modal size of females changed from 70 mm to 57 mm.

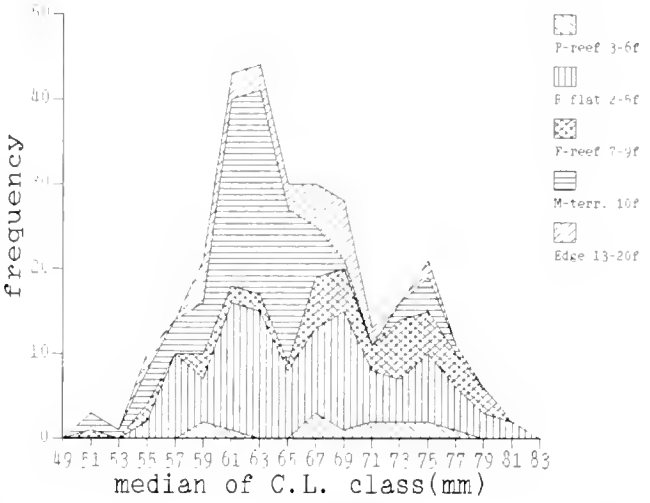

Figure 4. Area graph showing size frequencies of male Guinea chick lobsters (*P. guttatus*) by physiographic province (data from Transects 1 and 2). Legend key: P-reef 3–6f, patch reefs, 3 to 6 fathoms (6 to 12 m); R-flat 2–5f, reef flat, 2 to 5 fathoms (4 to 10 m); F-reef 7–9f, fore reef, 7 to 9 fathoms (14 to 18 m); M-terr 10f, main terrace (i.e., the reef-front terrace), 10 fathoms (19.4 m); Edge 13–20f, edge, 13 to 20 fathoms (25 to 39 m).

energy breaker line station declined continuously from October to January.

On the Transect 2 trap line through Kitchen Shoals, during the months May through September, CPUE was greatest at the rim reefs (on the reef flat) and second greatest on the 10 fathom reef-front terrace (Fig. 10). In a monthly analysis of trap rates, CPUE in the patch reef zone (across Three Hill Shoals) peaked in August. Trap rates on the reef flat increased from May to July, peaked in July, and leveled off in August and September. The highest trap rates on both the fore-reef slope and the 10 fathom (main) terrace occurred in August, but there was a secondary peak on the 10 fathom terrace in June. Trap rates on the edge of the shelf were low but peaked in June. Considering the occurrence of modal trap rates along the transect line (the change in location of modal trap rate during the summer), trap rate was always greatest on the reef flat, but showed an increase on the 10 fathom (main) terrace in June and August, relative to that on the reef flat.

Sex and Depth Found

The results of the study on the relationship between sex and depth are presented in Table 2 (the results from the transects) and Table 3A to C (the results from the study areas). During Transect 1 (October–January), the sex ratio of trapped animals (male:female) was lowest for the reef flat, just inside the reef crest, indicating that females were localized to the atoll rim inside the reef crest during the autumn and early winter, whereas during Transect 2 (May–September), it was lowest on the edge of the island shelf, and on the fore-reef slope and the reef-crest area adjacent, indicating a seasonal localization of females to the rim reefs and outer reefs during the breeding season (Table 2).

The St. Catherine's study area (patch reefs) showed the lowest sex ratio of trapped male:female animals (April–May), followed by the North Reefs (reef flats and patch reefs) (September–December) and the East Ledge Flat (reef flats) (February–March). These areas are all within the 3 fathom contour and suggest a localization of females to the area inside the reef crest (three fathom line) in the autumn, winter, and spring (Table 3A and B). A similar sex ratio to that at East Ledge Flat was found off Soldier's Point, Cooper's Island, around patch reefs just inside the breaker line or reef crest (October–January). On Transect 1 (October–January), this was the only location where females were found (Table 2). Trapping in the high-energy environment (frequently with powerful "surge" on the sea floor) just seaward of the reef crest-boiler reefs off Cooper's Island (October–

Figure 3. The Transect across the northeastern reef system, through Kitchen Shoals, eastern study areas, and section through the reef system. (A) The Transect 2 trap line across the northeastern reef system and the Kitchen Shoals and Sea Venture Shoals study areas. The positions of single traps on the 7 mile long transect line are marked by a cross. Soundings are in fathoms with feet as a subscript (1 fathom = 1.94 m). Sampling was on average twice weekly, from May through October. An average of seven traps per trip were hauled at Kitchen Shoals. The number of stations on the transect was reduced to 13 by the end of the experiment, owing to loss of traps and insufficient replacement. The trap rates of the physiographic provinces traversed by the transect were nevertheless obtained, including that of the reef-crest area at Kitchen Shoals. A capture-recapture experiment was also carried out at the Kitchen Shoals study area, simultaneous to the trapping along the transect line. (b) The reef profile. Sectional diagram showing the major physiographic provinces of the Bermuda reef system. The profile shown is the section from west to east along the line of the transect (Fig. 3a). *Key:* L, lagoon; SL, sea level; PRZ, patch reef zone in the lagoon, landward of the atoll rim, of 6 to 12 metres deep (3 to 6 fathoms); RCA, reef crest area comprising the reef flats (rf) and the algal cup reefs or "boiler reefs" (br) that break the surface, forming the atoll rim; the reef flats are located at ca. 6 to 8 m deep (3 to 4 fathoms); FRS, fore-reef slope, sloping reef surface seaward of the reef crest or "breaker line," ranging from ca. 12 to 18 m in depth (6 to 9 fathoms); RFT, reef-front terrace or "main terrace" at 19 m (10 fathoms); E, edge of the island shelf, or "platform edge," i.e., the terraces and slopes seaward of the reef-front terrace out to the dropoff; in this province, *P. guttatus* was only found at 22 to 45 m deep (11 to 22 fathoms); DO, The "drop off," i.e., the beginning of the steep, precipitous slope to the ocean floor, at ca. 68 to 78 m deep (35 to 40 fathoms).

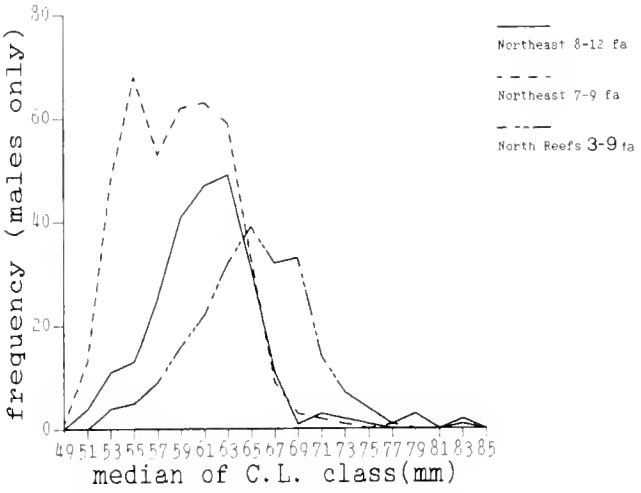

Figure 5. Line graph showing size frequency distributions for male Guinea chick lobsters (*P. guttatus* from the northern reefs by depth. The fore reef associated with Northeast Tower (Northeast 7–9fa) has a pronounced peak (modal class 54 to 56 mm CL) of small lobsters of 50 to 58 mm CL. The same peak is much less apparent in the reef-front terrace, i.e., main terrace (Northeast 8–12fa) size frequency distribution and even less so in that for the reef-crest area and patch reef zone (North Reefs 3–9fa). It should be noted, however, that the other two categories both include the 7 to 9 fathom range. The smallest individuals were on the fore-reef slope at 7 to 9 fathoms (14 to 18 m) in depth; the medium-sized males were on the reef-front terrace at 8 to 12 fathoms (16 to 23 m) in depth; and larger males comprised the "North Reefs" sample, taken from the North Reefs itself at 3 fathoms (6 m) in depth, and the pinnacle patch and table reefs inside of them, at 3 to 9 fathoms (6 to 18 m) in depth.

December) resulted in a high sex ratio of trapped Guinea chicks (Table 3A).

The median sex ratio (male:female) of Guinea chicks caught in the study areas seaward of the reef crest (28:1) was greater than the median sex ratio from the study areas landward of the reef crest (11:1) (see statistical analysis, Table 3C). This inferred a general localization of females to the reef tract.

Change in Size Composition at Kitchen Shoals

The results of a study of the change in size composition of male Guinea chick lobsters over the summer at the northeastern reef crest are presented in Figure 11a to c. Figure 11a and b show the change in size composition for the Kitchen Tripod shoal, and Figure 12c presents the change in the size composition of animals caught under overhanging coral-algal cliffs at the reef crest itself, close to Kitchen Tripod. The three figures show the size composition changing through the summer (May to September), to one of increasingly smaller size, then changing toward its original composition in October.

Relative Abundance of Spawning Females

Along the line of Transect 2 (through Kitchen Shoals), although the trap rates were low, ovigerous *P. guttatus* occurred in a spatial and temporal sequence near the shelf edge in May, at the reef-front terrace in June, at the fore-reef slope in early July, and at the reef-crest area from mid-July to the latter part of August (Table 4), suggesting an inward breeding migration of gravid females.

The relative abundance (CPUE) of spawning females (i.e., females bearing eggs and/or eroded spermatophores) was greatest at the reef crest (along Transect 2 for the months May to September inclusive). CPUE of breeding females was found to peak in a spatial and temporal sequence from deeper waters near the eastern platform edge at 26 to 35 m deep (13 to 18 fathoms) in May to the reef crest in August (Fig. 11d). In September, the reef-crest area was the only location of Transect 2 where breeding females were captured.

Seasonality of Breeding Females

Traps were maintained either on the rim reefs or outside of them for the entire period from August 1986 to March 1987 and were located in the patch reef zone in March–April (St. Catherine's study area). They were located right across the reef system from early May 1987 to late October 1987.

Breeding females with eggs and/or eroded spermatophores were only found in the months of May to September and only on the outermost reefs, comprised by the rim reefs and the fore-reef

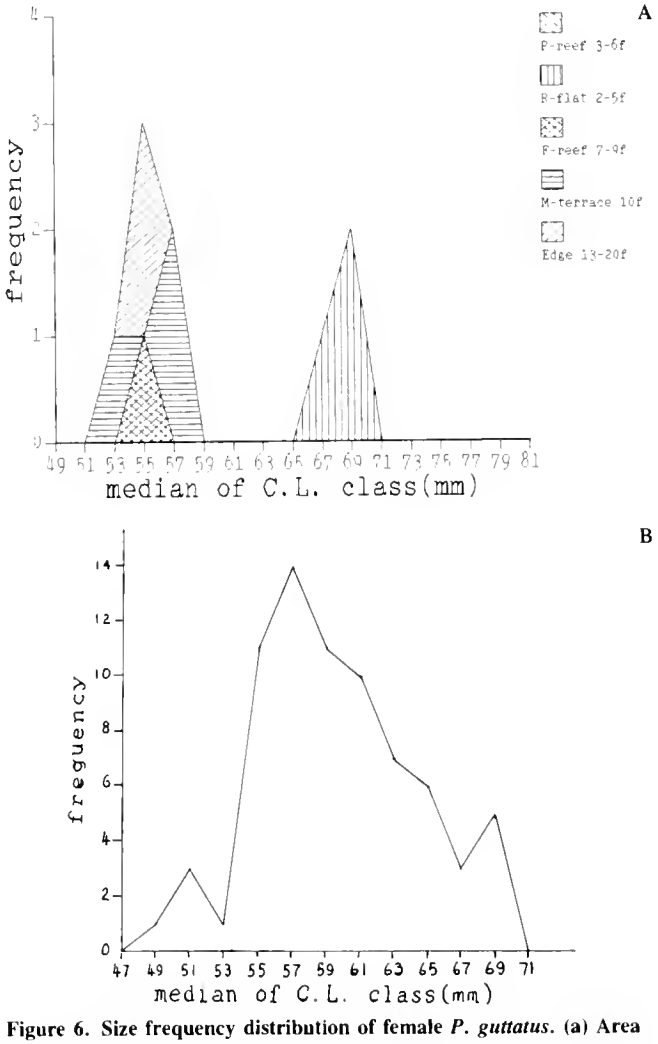

Figure 6. Size frequency distribution of female *P. guttatus*. (a) Area graph showing the size frequencies of females by physiographic Province (Data from Transects 1 and 2). (b) Line graph showing size frequency distribution of females taken from the northern and eastern reef systems (North Rock-Cooper's Island) in 1986.

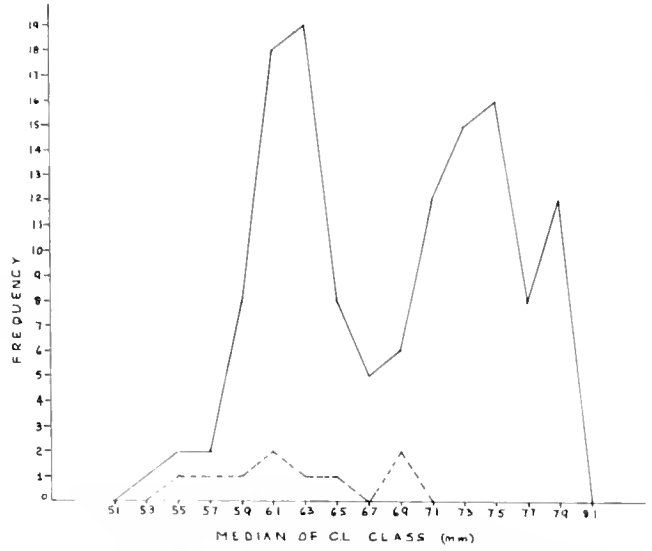

Figure 7. Size frequency distributions of male and female Guinea chick lobsters (*P. guttatus*) from the reef crest (breaker ledge) of St. David's Head, 21 August to 3 October. The curve for males is a solid line, and for females, it is a dashed line.

slope, the reef-front terraces, and the inner shelf edge (the latter ranges from 26 to 39 m in depth (13 to 20 fathoms). They were not found in the patch reef zone or in waters >43 m in depth (22 fathoms). There were always traps hauled from 49 to 55 m in depth (25 to 28 fathoms) during the Transect 2 experiment.

Population Density, Catchability, and Sex Ratio of Trappable Guinea Chick Lobsters

The results of a study of trap rates of males and females at Kitchen Shoals over the period 7 May–21 October 1987 are presented in Figure 12. Catch per trap haul was greatest around the time of the new moon. Sutcliffe (1956) found a similar relationship for *P. argus* at Bermuda.

There was a very strong and wide model peak of trap rates (for male *P. guttatus*) during the period 16 July to 17 August at Kitchen Shoals (Fig. 12).

Results of capture-recapture experiments on the abundance, catchability, and sex ratio of trappable *P. guttatus* in the study areas are presented in Tables 5 to 13, starting with relative abundance (CPUE) (Table 5A to C). Correction factors, which were needed to correct the raw population density estimates, are given in Table 6. Raw density estimates for St. David's Head, Cooper's Island, and Kitchen Shoals made by the Hayne's Index Capture-Recapture Method (Hayne 1949) (Tables 7 to 9) were collated, corrected, and averaged (Table 10A to D); these results indicate a mean population density of 29 (±7.6) ha^{-1} (95% confidence limit).

Separate male and female population density estimates for Kitchen Shoals, real sex ratio, and the difference in catchability between the sexes are presented in Table 11. The estimate for females was derived by subtraction of the estimate for males from the estimate for males and females together. The low number of recaptures of females made it impossible to estimate the female population number separately.

Results of capture-recapture studies for Kitchen Shoals using the Bailey Triple Catch Method (Chalmers and Parker 1989) are

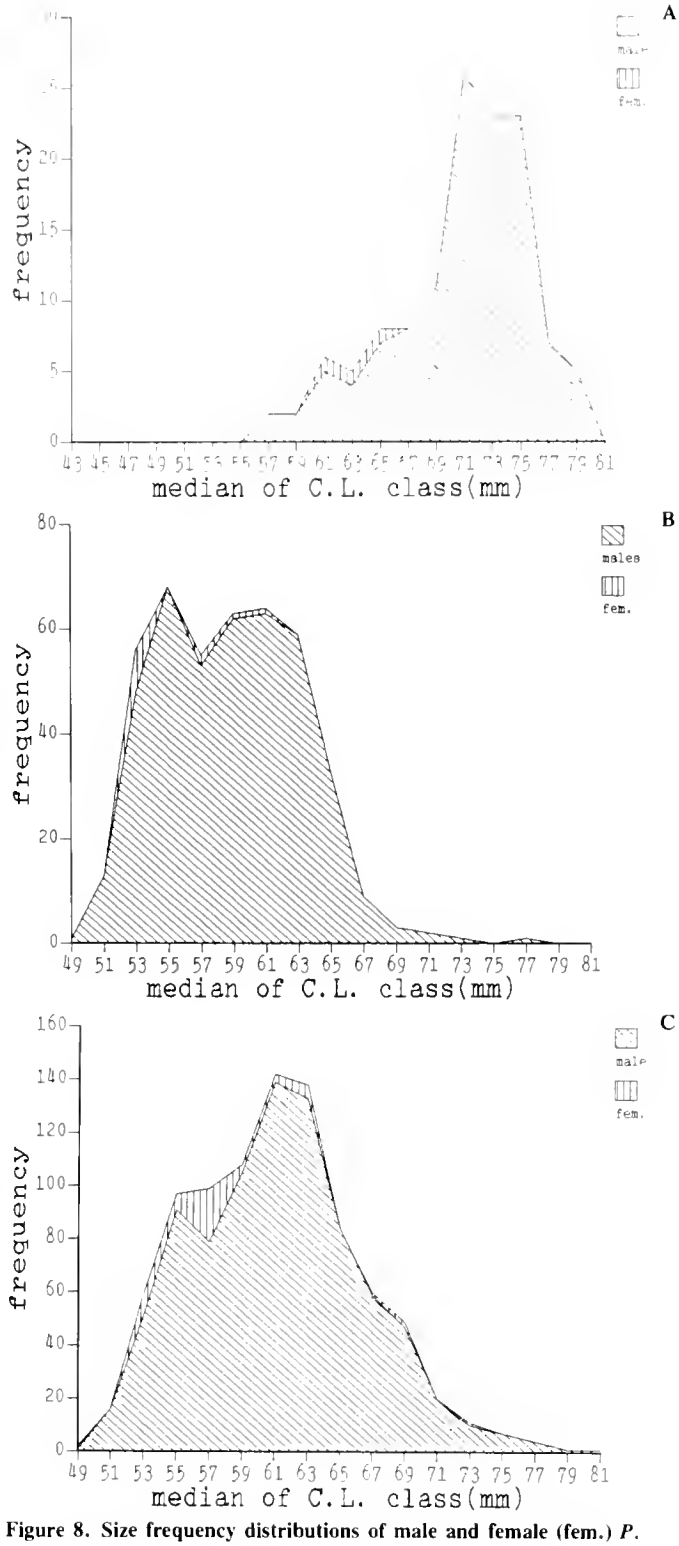

Figure 8. Size frequency distributions of male and female (fem.) *P. guttatus* from the northeastern reef system (North Rock-Cooper's Island). (a) The breaker ledge just seaward of the breaker line (reef crest) of Cooper's Island, October to December. (b) The fore reef associated with the north reefs, around northeast breaker, July and August. (c) Combined data from the study areas and Transects 1 and 2.

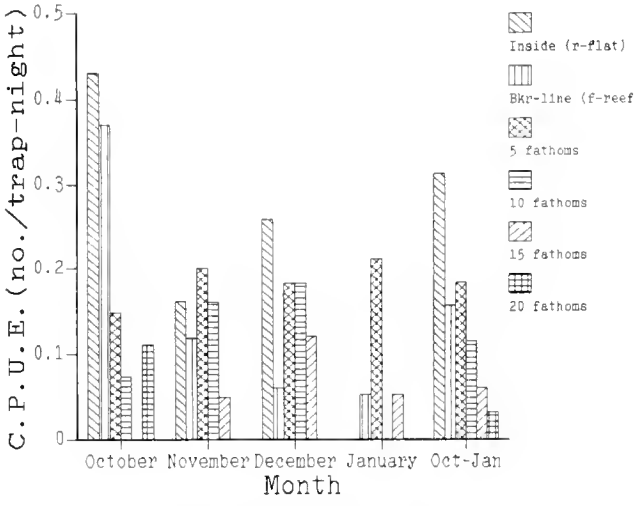

Figure 9. Showing monthly Guinea chick trap rates on Transect 1, off Soldier's Point, Cooper's Island, October–January, and variation with depth. There were two traps inside the breaker line (reef crest), one "at the breaker line" (bkr-line), i.e., just seaward of the boiler reefs, but at 5 fathoms (10 m), and one at each of 5, 10, 15, and 20 fathoms (10, 19, 29, and 39 m). Total effort in trap nights, October through January, was 109 (Inside), 96 (Bkr-line), 104 (5 fathoms), 104 (10 fathoms), 99 (15 fathoms), and 95 (20 fathoms). Surge conditions are a feature of wave energy around the reef crest of the Bermuda atoll system, November through May. For the period October to January, CPUE was greatest "inside" the boiler reefs, i.e., on the reef flat, or atoll rim, and second greatest on the 5 fathom terrace. CPUE was third greatest at the breaker line (i.e., on the breaker ledge just seaward of the coral-algal cliffs forming the boiler reefs). Monthly CPUE "Inside" was relatively high in October and in December, whereas at the 5 fathom station, it was relatively high in November and January. CPUE at the very high-energy breaker line station declined continuously from October to January. There is evidence of a migration away from the breaker line from October through December, chiefly to the reef-front terraces but also to the area inside of the breaker line.

presented in Table 12. Population density of *P. guttatus* in the physiographic provinces is presented as 95% confidence bands in Table 13.

Statistical tests of the four Hayne's Index Method population estimates for the St. David's Head, Cooper's Island, and Kitchen Shoals reef-crest areas showed that these results were conclusive findings (see the test tables in Appendix: Table 14A to D). Statistical tests of population estimates from similar capture-recapture studies at East Ledge Flat and Sea Venture Shoals showed them to be inconclusive owing to low recapture rates.

The mean population density of fishable Guinea chick lobsters in the reef-crest areas in the summer and autumn periods of 1986/87 was 29 (±7.6) Guinea chicks ha^{-1} (95% confidence limits). Similarly, the mean catchability coefficient (q) was 1.3×10^{-3} ($\pm 0.84 \times 10^{-3}$) trap $night^{-1}$, and the mean effective area fished (E) (Miller 1986) was 13×10^{-3} ($\pm 6.7 \times 10^{-3}$) ha. trap $night^{-1}$) (Table 10D).

The average real sex ratio of fishable Guinea chicks at the northeastern reef crest in the period 23 May to 21 October 1987 was estimated at 7:1 (male:female) (Table 11A and B), by the Hayne's Index capture-recapture population estimates for this period for males and females (1) and males only (2), subtracting (2) from (1) to obtain a female population estimate. The sex ratio of trapped animals was 13:1 (male:female). The factor for converting the sex ratios of trapped animals to real sex ratios was estimated at 7/13 = 0.54 (Table 11B). The catchability of females at Kitchen Shoals was lower than that of males both for catchability coefficient (q) and for the effective area fished (E) (Table 11C): female catchability q was 2.6×10^{-4} trap $night^{-1}$, and for males, it was 4.8×10^{-4} trap $night^{-1}$.

Analyses by Bailey's Triple Catch (Table 12) yielded information on population change over summer and autumn at the northeastern reef crest. Although the confidence intervals overlap, the results suggest that the highest population density of fishable Guinea chick lobsters over this period probably occurred at the height of the breeding season, i.e. from 1 July to 30 September (Table 12). There was a large, broad, modal peak in trap rates at the northeastern reef crest during mid-July to mid-August (Fig. 12). This supports the suggestion that density was greatest for the months July through September.

DISCUSSION

Transects Across the Reef System

The results of the Transect 1 experiment (Fig. 9) suggest that, prima facie, there was a migration offshore (i.e., an outward migration) during the period from 3 October to 5 December. There are three possible explanations:

(1) It may have been undertaken to avoid the surge conditions

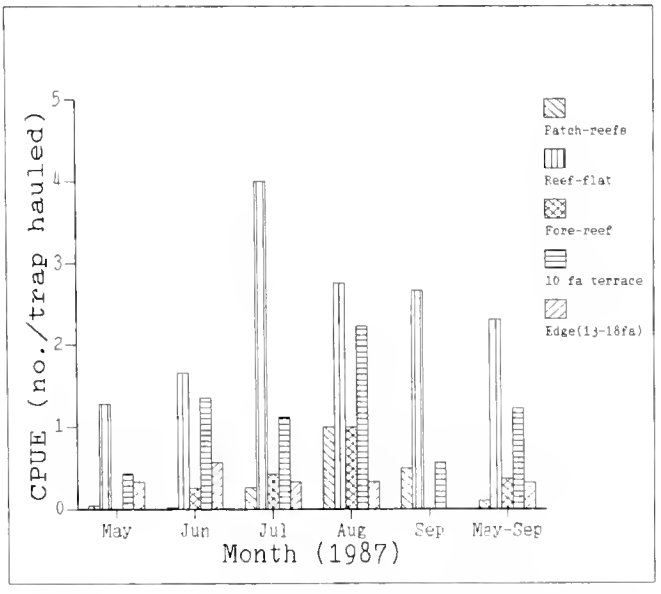

Figure 10. Showing monthly Guinea chick trap rates on Transect 2, east-west across the Northeastern reef system, through Kitchen Shoals, May to September. The reef flat bars refer to the catch and effort data from pot no. 8 in the Kitchen Shoals study area, located close to Kitchen Tower under overhanging cliffs of coral at the reef crest. The total effort for each physiographic province during May through September (in traps hauled) was as follows: patch reefs, 121; reef flat (single pot: pot 8, at Kitchen Tower), 29; fore reef, 30; 10 fathom terrace, 74; and edge, 31. During the summer months, May to September inclusive, CPUE was greatest at the reef flat (i.e., the atoll rim) and second greatest at the 10 fathom (19 m) terrace. CPUE on the reef flat increased from May to July, peaked in July, and then leveled off in August and September, whereas on the 10 fathom terrace, and near the edge of the island shelf, CPUE peaked in June and August.

TABLE 2.

Sex ratio of trapped animals (S.R.) of the Guinea chick lobster *P. guttatus* on Transects 1 and 2 (S.R. = ratio of males caught to females caught).

	Transect 1 (Autumn)			Transect 2 (Summer)		
Province[a]	Male Caught	Female Caught	S.R.	Male Caught	Female Caught	S.R.
PRZ	—[b]	—[b]	—[b]	12	0	12:0[c]
RCA	31	3	10:1	63[d]	4[d]	16:1
FRS	34	0	34:0	10	1	10:1
RFT	12	0	12:0	89	2	45:1
E	9	0	9:0	9	1	9:1

[a] Key; see also Figure 4b. PRZ, patch reef zone; RCA, reef crest area; FRS, fore-reef slope, RFT, reef-front terrace (or main terrace); E, edge of platform (inner shelf edge).

[b] No patch reef zone on Transect 1.

[c] Absence of females in the patch reef zone provides indirect evidence of an outward breeding migration from the patch reef zone to the rim and outer reefs.

[d] From Pot 8 at Kitchen Tower.

at the breaker line (reef crest). These conditions became fully established in early November.

(2) Alternatively, it may have resulted from the migration of newly recruited animals at the breaker ledge (just seaward of the reef crest) into feeding areas on the reef-front terraces and slopes.

(3) Most probably, it was a return migration to the reef-front terrace and outer terraces after a summer breeding migration to the eastern reef-crest areas.

During the period October to January, the greatest abundance of fishable Guinea chick lobsters off the steeper southeastern shore of the island occurred in the somewhat quieter waters of the atoll rim, inside the breaker line, or reef crest. The second greatest abundance occurred on the reef-front terrace (at 10 m or 5 fathoms). Monthly CPUE at the high-energy breaker line station declined continuously from October to January, possibly as a result of animals moving away from the worst areas of surge or a return migration to home foraging grounds on either side of the reef crest.

The results on relative abundance for the Transect 2 experiment support those from the Transect 1 experiment in that, for the period from May to September inclusive, CPUE was greatest on the reef flat adjacent to the reef crest and second greatest on the reef-front terrace (at 20 m or 10 fathoms) (Fig. 10). Thus, the results of both transect experiments indicate that the greatest abundance of Guinea chick lobsters was on the atoll rim and on the reef-front terrace.

Sex and Depth Found

Consideration of the sex ratio of trapped animals at various locations and time sheds further light on the field behavior of Guinea chick lobsters. During the Transect 1 experiment, off the eastern end of the island (October to January), the sex ratio of trapped animals was greatest inside the reef crest and females were only caught at the two stations inside the reef crest.

Whereas, on the Transect 2 trap line during the period from May to September, the sex ratio of trapped animals was greatest at the inner shelf edge and at the fore-reef slope (and the reef crest adjacent to it) (Table 2). Females may thus prefer to be in an area of high reef slope during the breeding season. The cliff-like, labyrinthine structure of such areas of slope may offer protection to the relatively small females at the critical time of larval release, which is generally followed by molting in palinurid lobsters.

Larval release in spiny lobsters (*P. argus*) occurs at the extreme edge of the island shelf (Sutcliffe 1953a). Palinurids (spiny lobsters) "inject" their larvae into the ocean circulation systems by this method and so facilitate genetic mixing and recruitment after lengthy larval drift around the ocean gyre systems (Pollock 1992).

Females may migrate away from areas of powerful surge conditions seaward of the reef crest (on the breaker ledges) and on fore-reef slopes, once these conditions begin in late October/early November, and move to quieter areas such as the reef flats inside the reef crest and the patch reef zone further inside. This is supported by results presented in Tables 2 and 3. Perhaps the larger females are particularly susceptible to the wave action on the outer reefs, and it is chiefly they that migrate to the more comfortable locations inside the reef crest.

There were high (male:female) sex ratios at the Cooper's Island breaker ledge during the stormy autumn/early winter period from October to January (41:1) and at the exposed fore reef (14 to 18 m or 7 to 9 fathoms) between North Rock and Northeast Tower in July and August (35:1) (Table 3A). There are two comparable "reference points" for the latter sample: the progressively lower energy environments of the inner terraces at 16 to 18 m deep (8 to 9 fathoms) and the main terrace (16 to 24 m or 8 to 12 fathoms deep) between North Rock and Northeast Tower, each in the period from July to August (25:1 and 18:1 male:female, respectively). Higher energy environments may thus be avoided by the females in the winter, because females are smaller than males, or females may suffer a higher mortality rate in these environments. This is given support by the observation that the sex ratio (male:female) of Guinea chick lobsters caught at the breaker ledge off St. David's Head increased from autumn through winter to spring, indicating a female flight from this high-energy environment over the winter period (Table 3A).

At the reef flats and patch reefs of the North Reefs, the sex ratio (male:female) of trapped animals was the second lowest observed in the present study: 223 males to 31 females caught (7:1), in the period September to December (Table 3B). Sex ratio was lowest for the patch reefs in the St. Catherine's area: six males to two females caught (3:1) during April–May (Table 3B). The areas landward of the reef crest have the lowest Guinea chick sex ratios (Table 3A to C).

The results in fact infer that there is both a habitat and a seasonal localization of female Guinea chick lobsters on the platform. A general, habitat localization of females was tentatively put forward by Sutcliffe (1953), who was puzzled not only by the low proportion of females caught, by also by the fact that most females had "spent" their eggs and were found at locations well inside of the reef crest.

In the present study, ovigerous females were only found on the rim reefs and outer reefs, during the breeding season (Tables 2 and 4; Figs. 10 and 11d); the median sex ratio of samples from the study areas seaward of the reef crest (male:female) was higher than that for samples from areas landward (Table 3C). Sutcliffe's (1953) hypothesis that there is a localization of female Guinea chicks on the platform is supported by these results, but the hypothesis does not explain the low proportion of females that were caught in both studies.

In summary, the results of the transect studies (Tables 2 and 4; Figs. 10 and 11d) indicate that, in summer, trappable females are

TABLE 3.

P. guttatus sex ratio (S.R.) from the study areas (ratio of males caught to females caught).

Sample Location Depth & Period	Males Caught	Females Caught	S.R.
A. Locations seaward of the reef crest			
St. David's Head, Breaker Ledge, (5–6 fathoms)			
Aug–Oct	132	9	15:1
Dec–Jan	64	4	16:1
April–May	22	1	22:1
Cooper's Island, Breaker Ledge (5–6 fathoms)			
Oct–Jan	123	3	41:1
Eastern Edge			
Aug–Jan (10–28 fathoms)	67	3	22:1
May–Sep (10–20 fathoms)	9	1	9:1
Fore-Reef Slope, North Rock-Northeast Tower (7–9 fathoms)			
Jul–Aug[a]	414	12	35:1
Inner Reef Terraces, North Rock-Northeast Tower-Kitchen Tower (8–9 fathoms)			
July	76	3	25:1
Main Terrace, North Rock Northeast Tower (8–12 fathoms)			
Jul–Aug[a]	229	13	18:1
Fore-Reef Slope off Kitchen Shoals (7–9 fathoms)			
May–Sep	10	1	10:1
Reef-Front Terrace off Kitchen Shoals (10 fathoms)			
May–Sep	89	2	45:1
Median S.R. for samples seaward of reef crest			28:1
B. Locations landward of the reef crest			
*The North Reefs, inside the reef crest (3–9 fathoms)			
Sept–Dec[a]	223	31	7:1
*Off Cooper's Island, inside the boiler reefs (3–5 fathoms)			
Oct–Jan	31	3	10:1
East Ledge Flat, inside the reef crest (3–5 fathoms)			
Feb–Mar	49	5	10:1
St. Catherine's patch reefs, well inside the reef crest (3–6 fathoms)			
Apr–May	6	2	3:1
Kitchen Shoals, on the reef crest (3–5 fathoms)			
23 May–21 Oct)	291	22	13:1
Sea Venture Shoals, inside reef crest (3–5 fathoms)			
Jun–Aug	19	1	19:1
Median S.R. for samples landward of reef-crest			11:1

[a] From landings by Guinea chick fisherman Lynwood Outerbridge.

in breeding condition and are chiefly located on the rim reefs (reef-crest areas) and the reefs seaward, including the outer terraces near the edge of the platform. They probably seasonally migrate there in connection with the release of their larvae from areas having the maximum encounter with the ocean current gyre. Results from the study areas suggest that, in the autumn and winter, trappable females are located in the reef flats and patch reefs of the reef tract (i.e., the outer reefs), extending along the rim of the plateau, inside of the reef crest (Table 3A to C), where they may mate in the spring.

Finally, Sutcliffe (1953) found only larger females of 58 mm CL or greater in a year-round transect across the Bermuda Plateau from rim to rim. These were "well inside of the reef crest." This agrees with the relatively high sex ratios found in the patch reefs of the North Reefs (Table 3B) and correlates with the size at first physical maturity of female Guinea chick lobsters (56.4 to 62.3 mm CL, 95% confidence limits) (Evans and Lockwood 1995).

Size and Depth

The smallest sizes of male Guinea chick lobsters caught in the present study were found on the fore reef slope (14 to 18 m or 7 to 9 fathoms) and reef-front terrace (16 to 24 m or 8 to 12 fathoms) off the North Reefs (Fig. 5).

The smallest females were on the fore-reef slope (Figs. 6a and 8b) and the reef-front terrace (or main terrace) and inner platform edge (Fig. 6a). The smallest female caught was a 42.5 mm CL, dark-colored individual captured on 3 September 1986, with a small mesh trap just seaward of the reef crest associated with St. David's Head (on the breaker ledge, see Fig. 2, close under vertical coral-algal cliffs, representing the fore-reef slope).

The largest males caught in the present study were on the reef-front terrace and reef tract (rim reefs and adjacent patch reefs) between North Rock and Northeast Breaker (the North Reefs; Fig. 5). The largest females were found on the reef flat or atoll rim (Fig. 6).

The results suggest (1) that the fore-reef slope from reef crest to main terrace is an area of recruitment into the fishery and (2) that larger animals are located at, or inside, the reef crest. Subadult *P. argus* lobsters, in contrast with (1), migrate offshore from the inshore waters and recruit into the fishery at the edge of the platform at 28 to 32 fathoms (Evans 1988, 1989); but in agreement with (2) larger *P. argus* animals were found in the reef tract (Evans 1988).

TABLE 3C.

Mann-Whitney U-test on difference in the medians for the areas seaward of the reef crest and the areas landward of it.

Sample A: sex ratios from seaward of reef crest, excluding landings during the breeding season: 9, 10, 15, 16, 22, 22, 25, 41, 45

Sample B: sex ratios from landward of the reef crest (none were from landings in the breeding season). 3, 7, 10, 10, 13, 19

Sample B	No. of Sample A Measurements Smaller Than Sample B Measurement.
3	0
7	0
10	1.5
10	1.5
13	2
19	4
Total	9

Therefore, $U_A = 9$

Repeat in reverse manner

Sample A	No. of Sample B Measurements Smaller Than Sample A Measurement.
9	2
10	3
15	5
16	5
22	6
22	6
25	6
41	6
45	6
Total	45

Therefore, $U_B = 45$.

The test statistic is therefore 9 (the lower of the two values of U_A and U_B). The critical value of U at the 5% level (for samples of 9 and 6) is 10 (Chalmers and Parker 1989, p. 100, Table III (2)). The null hypothesis that there is no difference in the population medians can therefore be rejected at the 5% level.

Three factors suggest that further measures should be taken toward the conservation of the Bermudan female gene pool and the Bermudan breeding stock: (1) size at first physical maturity of female *P. guttatus* at Bermuda is 56.4 to 62.3 mm CL (95% confidence limits) (Evans and Lockwood 1995); (2) the smallest females caught in traps during the present study were 48 to 50 mm CL (Fig. 6b); and (3) low proportions of female Guinea chicks are caught in the northeastern and eastern reef systems (Figs. 4 and 6 to 8). Three possible measures are: (1) a total ban on the taking of females; (2) a minimum size limit of 59 mm C.L. based upon Evans and Lockwood (1995); and (3) a prohibited trap zone in the Guinea chick nursery area.

The latter may be the fore-reef slope and the inner reef-front terraces (the terraces at 12 to 18 m or 6 to 9 fathoms in depth), which are associated with the northern rim reefs, especially the North Reefs (which extend North Rock to the Northeast Breaker) (Figs. 5 and 8b).

It is unlikely that the main Guinea chick lobster nursery areas are located in the coastal and inshore areas enclosed by the established prohibited trapping zone (Fig. 1). Research would need to be undertaken to establish that the fore reef adjacent to the reef crest of the North Reefs is a nursery area.

Change in Size Composition at Kitchen Shoals

One very likely contribution to the change in male size composition over the spring and summer period from May to September at the Kitchen Shoals study area, including the reef crest at Kitchen Shoals Tripod, is recruitment into the fishery. The results suggest that the fore-reef slope, breaker ledge, and inner reef-front terrace at 12 to 18 m (6 to 9 fathoms) seaward of the reef crest are areas of recruitment into the fishery. The change in size composition observed at Kitchen Shoals may therefore be the spring and summer recruitment of animals of size 53 to 61 mm CL into the fishery. Throughout this period, but especially in July and August, many animals on the rim reefs may become subject to commercial trap fishing for the first time, by entering traps and becoming trappable.

A study of the trap rates of male lobsters at the Kitchen Shoals study area (Fig. 12) found that there was a surge of trap rates from mid-July to mid-August and that this resulted in a broad, high, modal peak of trap rate from 27 July to 5 August. These results infer a fundamental change in the population size at the northeastern reef crest mid summer, at the height of the breeding season. The results of the capture-recapture experiments by Bailey's Triple Catch Method at Kitchen Shoals also support this inference (Table 12).

A second highly probable factor in the change of size composition is in-migration of smaller males from the reef-front terraces and slopes. This would have progressively diluted the population of larger animals on the reef flat with smaller individuals. However, there was no evidence of a relatively long migration of males from the trap rates observed on Transect 2. Such a migration should have been apparent unless it was balanced by recruitment on the fore reefs and inner terrace, as suggested, or by a counter-migration of animals from the patch reef zone migrating to the rim reefs, reef-front terraces, and slopes. Sutcliffe (1952, 1953a) recorded such an outward breeding migration for *P. argus*.

There is in fact a persuasive case for the in-migration of males to the reef flat from the reef-front terraces and slopes: there is evidence of a breeding migration of egg-bearing females from these localities to the reef crest and flats of the atoll rim during May–August (Fig. 11d; Table 4). Smaller males would have accompanied these breeding females, as occurs in migrations of egg-bearing *P. argus* females and smaller males from the waters of the lagoon and the inshore waters to the outer reefs (Sutcliffe 1953a). These migrations probably entrain new recruits to the fishery.

In conclusion, the observations perhaps reflect both annual recruitment into the fishery at the reef crest, fore reef, and inner reef-front terrace, and a breeding migration of smaller males (accompanying egg-bearing females) from the broad reef-front terrace to the flats and breaker line (reef crest). This migration may entrain new fishery recruits.

Relative Abundance of Spawning Females

The results of the study of trap and catch rates of breeding females on Transect 2 (Fig. 11d; Table 4) indicate that there was an inward migration of breeding females (bearing eggs) from the reef-front terraces and slopes to the reef-crest area during the period May–August. Some mating of *P. guttatus* lobsters at Ber-

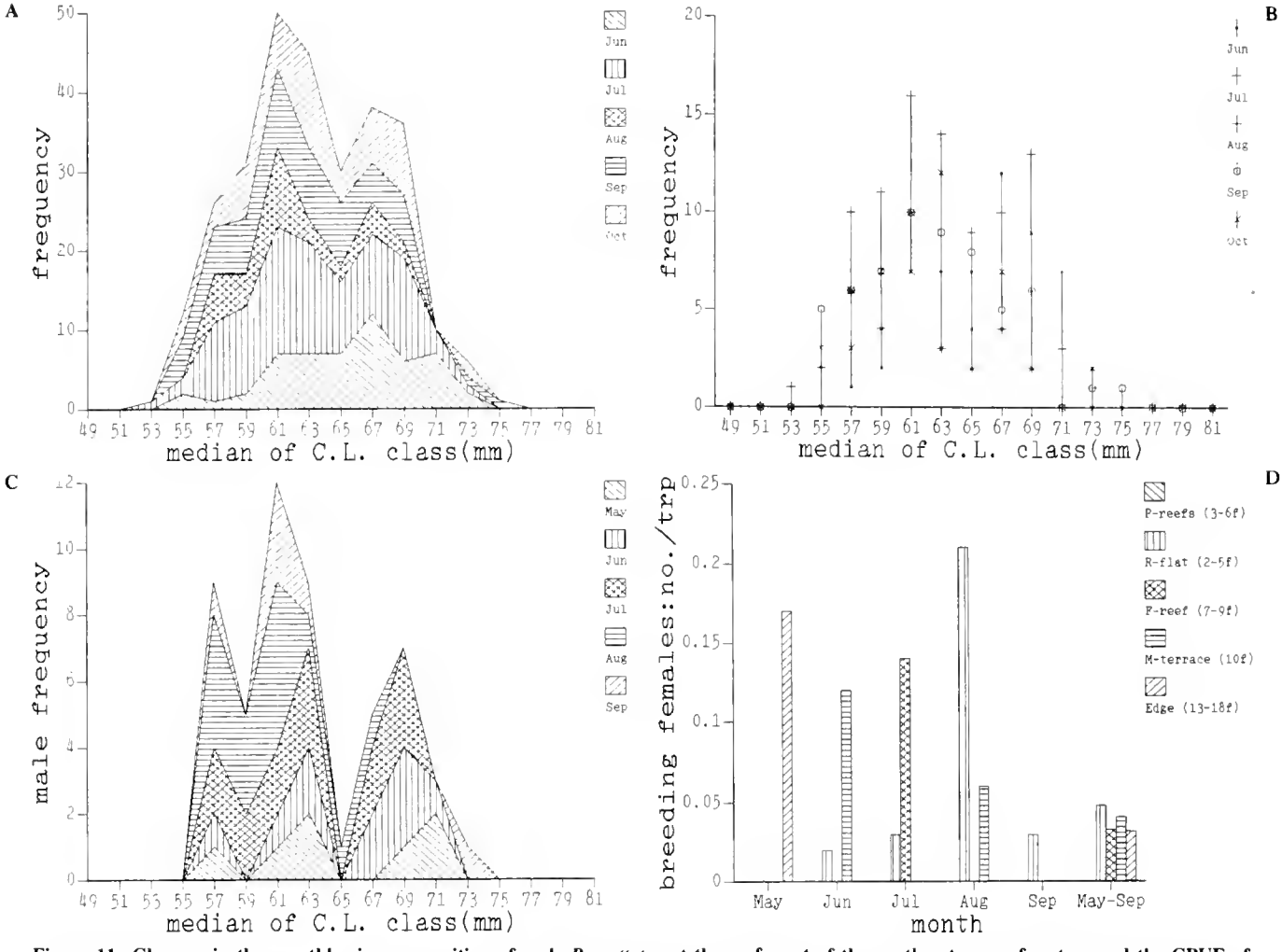

Figure 11. Changes in the monthly size composition of male *P. guttatus* at the reef crest of the northeastern reef system and the CPUE of breeding female *P. guttatus* on Transect 2. (a) Graph showing the monthly size frequency distributions of males at Kitchen Shoals study area. Results were obtained by sampling the population of a discrete shoal (at Kitchen Tower) on the northeastern rim reefs with a small group of traps from May to October. The size composition changed through the summer (June to September), to one of increasingly smaller size. This trend reversed toward the original composition in October. Results are plotted as points in Figure 11b. (b) "Hi-lo" chart showing the monthly size frequency distributions of males at Kitchen Shoals study area with individual plots of frequencies. Size changes of in panel a are quantifiable in panel b. (c) Graph showing monthly size frequency distributions of males caught by Transect Pot 8. Transect pot no. 8 was located close to the Kitchen Shoals Tower and set in a sand-floored depression in the reef close to overhanging coral cliffs. Size composition changed through the summer period (May to August), to one of increasingly smaller size, and the trend began to reverse in September. (d) CPUE for females caught on Transect 2 bearing eggs and/or spermatophores, including data from the Kitchen Shoals study area. Along Transect 2, during the period May to September, breeding females showed the greatest relative abundance (in CPUE) at the Kitchen Shoals reef-crest area. CPUE of breeding females along the Transect was found to peak in a spatial and temporal sequence from the deeper waters of the inner platform edge at 13 to 18 fathoms (25 to 35 m) in May to the rim reefs at Kitchen Shoals in August. Total effort on Transect 2 for the summer was 428 trap hauls. The precise details of catch, dates, reproductive activity, and location are given in Table 4A. The data for the reef flat at Kitchen Shoals in June refer to a single ovigerous female caught that was not handled for measurement, but that was retained for an aquarium experiment on spawning at Bermuda Biological Station for Research.

muda may occur therefore in the quieter waters of the reef-front terraces in April through June. These animals probably migrate to the coral cliffs and flats near the reef crest about a month later to release larvae more effectively in the ocean gyre.

Sutcliffe (1952) concluded, in regard to a study of the breeding and migration in Bermudan *P. argus* lobsters:

> There is tentative evidence of an inward migration (or at least a lack of females) from deeper waters of the shelf and of the slopes since traps in these depths (on the South Shore) have caught only males. This breeding migration to the South Shore apparently follows the first mating and precedes the hatching of eggs.

This information agrees with the evidence of the similar behavior in *P. guttatus* lobsters at Bermuda from the present study.

Furthermore, data simultaneously derived on the CPUE of *P. argus* lobsters along the Transect 2 trap line support Sutcliffe's tentative hypothesis that there is an inward migration of *P. argus* lobsters from the reef-front terraces and slopes to the rim reefs: the CPUE of female lobsters was greatest at the reef flats during the period May to September and least at the lagoon patch reefs and the inner 'edge' at 26 to 39 m (13 to 20 fathoms) (Evans 1988, 1989).

An alternative explanation to a breeding migration is that larger female spiny lobsters frequently spawn earlier than smaller fe-

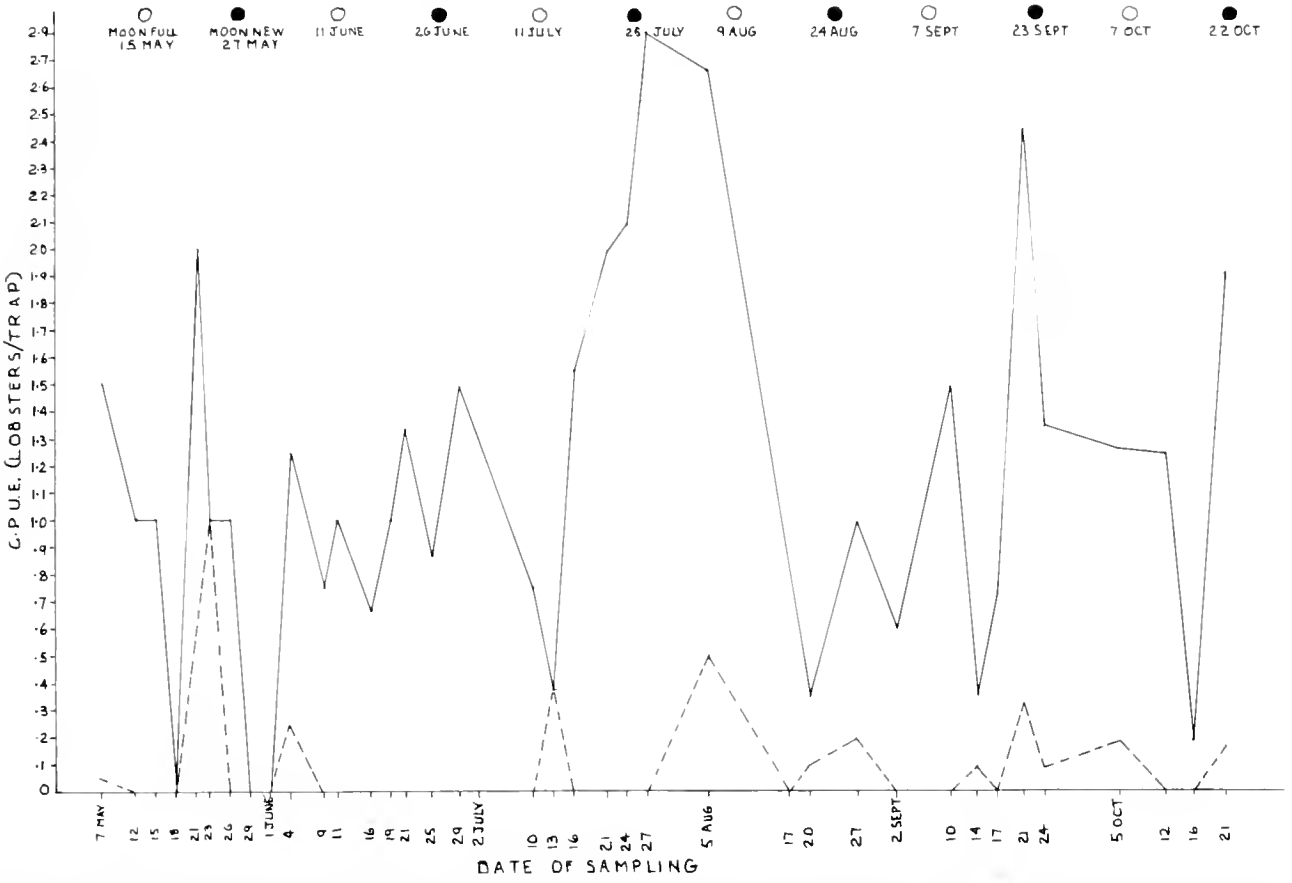

Figure 12. Showing the CPUE for male and female Guinea chicks (*P. guttatus*) at Kitchen Shoals study area, 7 May to 21 October, and the lunar cycle. The CPUE of males is given by the unbroken line, and that of females is given by the broken line. Included in the female trap rates shown, the occurrence of females with eggs was as follows: 4 June, CPUE 0.25; 1 July, CPUE 0.25; 5 August, CPUE 0.50; 20 August, CPUE 0.13. The mean number of traps hauled per visit was seven. The modal trap rate of males occurred on 27 July, whereas for females, it occurred on 23 May. The trapping was characterized by consistent rates of 1 to 1.5 lobsters per trap in June and was dominated by a surge in the trap rates of males beginning in the middle of July. The resulting peak at 27 July did not fully subside until a month later, in the middle of August. The peak coincided with a period of a new moon. The second highest peak for males occurred during 17 to 24 September and also coincided with a new moon. In contrast, the modal trap rates of male and female *P. Argus* lobsters at Kitchen Shoals study area from the same traps and visits both occurred during the period 17 August to 2 September (Evans 1989).

males (Sutcliffe 1953a). This is much less likely because the size of females on the main terrace is only a little larger than those of females from the fore reef at 14 to 18 m (7 to 9 fathoms), just seaward of the crest (Fig. 6).

Seasonality and Location of Breeding Females

Sutcliffe (1953) reported the results of a study of the "seasonal distribution and breeding condition" of *P. guttatus* lobsters. He used 52 traps, but the sample size was low: 158 specimens of which 15 were females, caught between October 1951 and September 1952. He found that the one female with eggs was captured in August and that four females bearing a spermatophore remnant and showing signs of having carried eggs were taken in August and September 1952 and October 1951.

The reason for the discrepancy with the results on seasonality of breeding females from the present study is probably that Sutcliffe's year-round traps were all maintained within the 10 m (5 fathom) contour, with only two traps outside it; of those two, one was visited intermittently and the other only during the summer (Sutcliffe 1952).

In the present study, breeding females on the northeastern rim reefs were only found in the months of May to September and only on the outermost reefs, from the inside edge of the rim reefs (reef flats of about 3 fathoms depth) to the edge of the island shelf. This corresponds in depth from about 6 to 43 m (3 to 22 fathoms). No females showing evidence of bearing eggs and/or eroded spermatophores were found inside the landward edge of the rim reefs in the present study.

Sutcliffe (1953) only found females well inside of the reef crest. He found 15 females. Only one of these bore eggs, but four showed signs of having borne eggs. All of the females he caught were of size 58 mm CL or larger, and these had probably attained the size of first physical maturity—approximately 59 mm CL (Evans and Lockwood 1995). It is therefore reasonable to suggest that some mating also occurs at the patch reef zone inside the rim reefs during the spring, and females move out to the reef crest, fore reef slope, and inner platform edge to release their larvae more effectively into the ocean gyre in the late spring. It would explain the relatively high proportion of females found at these locations on Transect 2, the highest for the summer (Table 2).

Sutcliffe (1953) found ovigerous females in the months of August, September, and October. In the present study, continuous trapping (save for intervals of less than 1 week) in the Bermudan reef system from August 1986 to October 1987 indicated that the

TABLE 4.

Investigation of the breeding and migration of females from trapping Transect 2, across the northeastern reef system, 7 May to 21 October.

Date and P.E. Female Caught	CL (mm)	Category[a] E	*	W	N
7 May (RCA)[b]	58.6				x
12 May (EE)	54.3				x
23 May (EE)	55.5	x			
23 May (RCA)	72.8				x
4 June (RFT)	56.3	x			
9 June (RCA)	72.8				x
16 June (RFT)	53.2	x			
2 July (FRS)	55.7	x			
13 July (RCA)	57.7	x			
13 July (RCA)	57.8				x
13 July (RCA)	62.4	x			
16 July (RCA)	66.6	x			
5 August (RCA)	56.2	x			
5 August (RCA)	61.0	x			
5 August (RCA)	58.3	x			
20 August (RCA)	57.6	x			
27 August (RCA)	63.6		x		
31 August (RFT)	56.0		x		
10 Sept. (RCA)	61.0				x
14 Sept. (RCA)	60.4				x
21 Sept. (RCA)	56.6				x
21 Sept. (RCA)	57.5				x
21 Sept. (RCA)	55.1		x		
24 Sept. (RCA)	63.4		x		
5 October (RCA)	60.0				x
5 October (RCA)	68.3				x
21 October (RCA)	58.2				x
21 October (RCA)	57.9				x

Catch data from the trapping in the study areas at Kitchen Shoals and Sea Venture Shoals and the outer platform edge east of the Transect 2 trapline (Fig. 3a) have been combined with the Transect 2 catch data and presented here. *P. guttatus* females were infrequently caught in traps compared with males: the sex ratio of trapped animals ranged from 49:1 to 3:1 (male:female) in the physiographic provinces that the species inhabits (Tables 2 to 3). The results of this transect and the inshore sampling program found it to be confined to the periphery of the platform, from the patch reefs inside the atoll rim out to 22 fathoms. The data above are a list of the date, size, and location of all females caught on the transect and in the areas mentioned above. An inward migration of egg-bearing females occurred from the inner shelf edge (EE) to the reef-front terrace (RFT) to the fore-reef slope (FRS) to the reef-crest area (RCA); from 11 fathoms (EE) to 3 to 4 fathoms (RCA), at Kitchen Shoals. This was supported by other findings (Figs. 11a–c) on the monthly change in the male size composition at the northeastern reef crest over the summer, from a preponderance of larger animals (which occur in shallower water) to a preponderance of smaller animals (which occur in deeper water).

[a] E, egg bearing (or bearing a remnant of eggs); *, bearing eroded spermatophores; W, bearing "whole" (i.e., intact) spermatophores; N, bearing no sign of reproductive activity.

[b] The reef crest area (RCA) captures listed above refer to the study area at Kitchen Shoals, except the female caught on 16 July, at the Sea Venture Shoals study area.

occurrence of female egg– or spermatophore-bearing *P. guttatus* in the Bermuda reef system is from May to September. It is suggested that the breeding season is therefore May to October in Bermuda.

TABLE 5.

Catch per unit of fishing effort for *P. guttatus*

Sampling,	Catch (c)	Traps ×	Nights =	Effort (f)	CPUE
A. St. David's Head, autumn 1986					
21 August	2	5	3	15	0.133
24 August	7	4	3	12	0.583
27 August	15	5	3	15	1.000
30 August	13	7	3	21	0.867
3 Sept	13	6	4	24	0.583
8 Sept	10	7	5	35	0.286
11 Sept	14	8	3	24	0.583
16 Sept	9	8	5	40	0.225
20 Sept	12	8	4	32	0.375
23 Sept	12	8	3	24	0.500
26 Sept	9	8	3	24	0.375
30 Sept	12	8	4	32	0.375
3 Oct	9	8	3	24	0.375
B. Cooper's Island Breaker Ledge, autumn-early winter 1986					
6 October	16	9	3	27	0.593
9 October	7	6	3	18	0.389
13 October	10	7	4	28	0.357
18 October	6	6	5	30	0.200
21 October	8	7	3	21	0.381
24 October	20	7	3	21	0.952
27 October	2	4	3	12	0.167
31 October	5	7	4	28	0.179
5 November	25	7	5	35	0.714
8 November	3	6	3	18	0.167
17 November	9	7	9	63	0.143
25 November	14	7	8	56	0.250
5 December	12	7	10	70	0.171
C. Kitchen Shoals study area, summer and autumn 1987					
23 May	2	1	2	2	1.00
26 May	1	1	3	3	0.333
29 May	0	1	3	3	0
1 June	0	1	3	3	0
4 June	7	4	3	12	0.583
9 June	4	4	5	20	0.200
11 June	5	5	2	10	0.500
16 June	4	6	5	30	0.133
19 June	10	9	3	27	0.370
21 June	8	6	2	12	0.667
25 June	7	8	4	32	0.219
29 June	12	8	4	32	0.375
2 July	9	7	3	21	0.429
10 July	6	8	8	64	0.094
13 July	6	8	3	24	0.250
16 July	14	9	3	27	0.519
21 July	16	8	5	40	0.400
24 July	19	9	3	27	0.704
27 July	25	9	3	27	0.926
5 August	19	6	9	54	0.352
17 August	7	8	12	96	0.073
20 August	4	8	3	24	0.167
27 August	6	5	7	35	0.171
2 September	3	5	6	30	0.100
10 September	7	4	8	32	0.219
14 September	5	11	4	44	0.114
17 September	8	11	3	33	0.242
21 September	25	9	4	36	0.694
24 September	16	11	3	33	0.485
5 October	16	11	11	121	0.132

continued on next page

TABLE 5.

continued

Sampling,	Catch (c)	Traps × Nights = Effort (f)			CPUE
12 October	15	12	7	84	0.179
16 October	2	12	4	48	0.042
21 October	25	12	5	60	0.417
23 May–21 Oct	313[a]	237		1146	0.273

C, total catch; f, total fishing effort in trap nights; CPUE, catch per unit of fishing effort in Guinea chick lobsters per trap night.

[a] The catch was composed of 291 males and 22 females.

Caillouet et al. (1971) studied the seasonality of ovigerous female *P. guttatus* spiny lobsters by shallow water diver sampling at >3 m depth (1.5 fathoms) on from the shores and jetties of Government Cut, Miami, during the summer period from June to October and found that, in June, 81% of the females were ovigerous. This declined sharply thereafter to 24% in July, 18% in August, 11% in September, and 1% in October. This may be compared with the Transect 2 experiment of the present study, in which all females captured in the period of 13 July–20 August were ovigerous (Table 4).

Caillouet et al. (1971) found that the sex ratio was about unity: of 894 *P. guttatus* lobsters caught, 55% were males and 45% were females; males and females in Miami were almost equally represented in June, August, and October, but males outnumbered females about 2:1 in July and September. Water temperature peaked in July, and salinity was about the same throughout the period; it is therefore unlikely that these factors reduced the mobility of females over the surfaces of the boulders at night. It is more likely that the females were relatively less mobile than males, remaining within the cracks and interstices of the boulders because of post spawning premolt condition.

Population Density and Real Sex Ratio

Linear regression analyses of the Hayne's Index capture-recapture data, i.e., of the "proportion marked in catch" and "number of animals marked previously," showed a significant positive correlation between these variables in each case for the St. David's Head, Cooper's Island, and Kitchen Shoals experiments (Table 14A to D, Appendix). These population estimates (Tables 7 to 9 and 11A) are therefore conclusive findings.

The estimate of population density at St. David's Head breaker ledge is based on an estimate of the area of breaker ledge fished (12 hectares), and the area fished is not accurately known because of the linear nature of the group of pots close to the seaward edge of the boiler reefs. Research to estimate the radius of attraction of baited arrowhead traps would enable a refinement of this estimate. Results from electromagnetic tracking work at Seven Mile Beach

TABLE 6.

Calculation of correction factors for capture-recapture population density estimates—summary of correction factor estimates: A. St. David's Head: raw density estimate × 1.6; B. Cooper's Island: raw density estimate × 1.2; C. Kitchen Shoals (Hayne's Index): (1) Males + females: raw density estimate × 1.1, (2) Males only: raw density estimate × 1.1; D. Kitchen Shoals (Bailey Triple Catch): (1) 1 Jun–31 Aug: raw density estimate × 1.0, (2) 1 Jul–30 Sep: raw density estimate × 1.0, (3) 1 Aug–21 Oct: raw density estimate × 1.0.

A. *St. David's Head (S.D.H.)*

"Trap happiness" may be estimated and taken into account by application of the correction factor applied by Morgan (1974) for *P. cygnus:* raw density estimate × 1.6. It should be noted that the response to recapture in the baited Bermudan arrowhead traps is not precisely known.

Marking was by punching a coded sequence of holes in the tail fan; no tags were applied; therefore, there was no need to take loss of tags into account or to take into account mortality or immigration arising from tagging and/or displacement. Estimate of S.D.H. correction factor: raw density estimate × 1.6.

B. Cooper's Island (C.I): results of Cooper's Island experiment to assess the combined effect of tagging and displacement.

Number tagged along the boiler reefs off Soldier's Point, C.I. (having both tail fan mark and Floy tag) = 38. Number marked with holes in the tail fan only = 35.

Number of marked animals killed by in-trap predation up to 5 November 1986 (midterm of study) with tail fan mark only = 1.

Ratio of only tail fan marked to tagged + tail fan mark animals at large is therefore 34:37 = 1:1.088.

Number of marked animals recaptured with only tail fan mark was 29, but 5 of these had also been tagged, losing the tag from other than emigration, mortality, or trap shyness; these causes were as follows: 1 from molting; 3 from contact with mesh; and 1 from in-trap predation. The "true" number of marked animals we caught with solely tail fan mark is 24.

Number of animals recaptured with tag + tail fan mark = 9, but there were 5 recaught known to have had a tag besides these 9. The "true" number of marked animals recaught with a tag + tail fan mark is 14.

Therefore, because 24 animals with tail fan mark only were recaptured, we should have recaptured about 24 × 1.088 tagged animals = 26 tagged animals.

The apparent reduction in the availability of tags for recapture is given by (26-14) × 100/26% or ca. 46% (probably caused by mortality and emigration).

About half (34/71 or 48%) of the animals that were marked were not tagged, so the estimate of the combined loss of tags from the population from tag- or displacement-induced emigration should be adjusted accordingly (by 48%). The adjusted factor for tag- and displacement-induced mortality and emigration is given by: 0.46 − (0.48 × 0.46) = 0.24; the raw density estimate should therefore be reduced by a factor of 1-0.24 = 0.76, in order to take this into account.

Estimate of tag loss from molting: Tag loss from molting need not be taken into account because tagged animals were double marked by tail fan mark and tag.

Estimate to adjust for "trap happiness". "Trap happiness" may be taking into account by multiplying the raw density estimate by 1.6 (as in the Morgan [1974] experiment). It should be noted that the response to recapture in the baited Bermudan arrowhead traps is not precisely known.

The combined correction factor for tag- and displacement-induced mortality and emigration and "trap happiness' is therefore 0.76 × 1.6 = 1.2.

TABLE 6C.

Kitchen Shoals (Hayne's Index: Studies 1 and 2): results of Kitchen Shoals experiment to assess the combined effect of tagging and displacement.

	Tail Fan Mark (TFM) (single 0.25 inch diameter hole punched in telson)		Spaghetti Tag + TFM	
1987	No. marked	No. recaught	No. marked	No. recaught
7 May	4	0	0	0
12 May	0	1	0	0
15 May	0	1	0	0
21 May	2	0	0	0
23 May	2	0	0	0
26 May	0	0	1	0
29 May	0	0	0	0
1 June	0	0	0	0
4 June	3	0	2	0
9 June	0	2	2	0
11 June	0	0	5	0
16 June	0	0	4	0
19 June	0	1	8	0
21 June	5	0	2	0
25 June	2	0	2	3
29 June	3	0	8	0
2 July	4	1	5	0
10 July	1	0	3	2
13 July	0	0	5	1
16 July	0	1	6	7
21 July	0	0	13	1
24 July	0	2	15	2
27 July	0	2	16	6
5 August	0	0	0	4
Totals	26	11	97	26

Mortality and emigration arising from the combined effect of tagging and displacement would tend to an overestimation of the population estimate, and therefore, a correction factor should be applied to the population estimates from this consideration alone. However, in the course of the census (32 May–21 Oct), 20 out of 202 animals marked were solely tail fan marked = 9.9%

For 97 animals tagged, we should have observed on average 97 × (11/26) recaptures of tagged animals = 41 recaptures. Therefore, the apparent reduction in the availability of tags for recapture is given by:

(41–26) × 100/41 = 36.6%, or about 37%. (Animals were kept in a bucket of fresh water before and after handling; this procedure was not carried out at Cooper's Island).

Final correction factor for this "effect" is given by: 1 − (0.366 − (0.366 × 0.099)) = 0.67.

Estimate of adjustment factor to take into account tag loss from molting: Tag loss from molting was not a problem because tagged animals were double marked with a tail fan mark and a tag.

Estimate to adjust for "trap happiness": "Trap happiness" may be taken into account by multiplying the raw density estimate by 1.6 (as in the Morgan [1974] experiment). It should be noted that the response to recapture in the baited Bermudan arrowhead traps is not precisely known.

The combined correction factor for tag- and displacement-induced mortality and emigration is therefore 0.67 × 1.6 = 1.1.

Table 6D.

Capture-recapture studies by Bailey's Triple Catch Method at Kitchen Shoals (Studies 1–3): Adjustment of the raw population density estimates may be mde by application of the following combined correction factor, composed of three components.

Component effects that must be taken into account

1. "Trap happiness": multiply by 1.6 (estimated "trap happiness" effect, Morgan [1974]).
2. Combined effect of tag- and displacement-induced mortality and emigration: multiply by 0.67 (correction factor estimated in Table 6C above).
3. Tag loss from molting: multiply by 0.90 (from estimate of 10% per molt tag loss from molting[a] (Davis 1978)). It was necessary to take this effect into account because the secondary mark did not permit determination of the time of last capture if a tag had been lost.

Combined correction factor for Bailey's Triple Catch Studies at Kitchen Shoals: combined correction factor taking "trap happiness," tag- and displacement-induced mortality and emigration, and tag loss from molting into account is given by: raw density estimate × 1.6 × 0.67 × 0.90, which is the same as raw density estimate × 1.0.

The combined correction factor for "trap happiness," tag- and displacement-induced mortality and emigration, and tag loss from molting is 1.0.

[a] Other evidence indicated that only one molt occurred (Evans, 1988, 1989).

in Australia on foraging patterns of juvenile *P. cygnus* rock lobsters indicate that a juvenile may be attracted to a baited pot as far away as 80 m from its home reef (Jernakoff and Phillips 1986). The breaker ledge at the St. David's Head study area is 10 to 175 m in width and is about 100 m wide on average. It is therefore unlikely that the entire area of the ledge in the study area was fished. However, the coverage was in fact made greater by the random resetting of pots at their stations.

The combined effect of mortality from tagging and release and of emigration induced by tagging and displacement (the latter was also found to occur in western Australian rock lobsters; Chittle-

TABLE 7.

Population estimate at the Breaker Ledge off St. David's Head, autumn 1986.

Sampling	w	x	y	wx^2	wxy
21 Aug	2	0	0	0	0
24 Aug	7	3	0	63	0
27 Aug	15	10	1/15	1,500	10
30 Aug	13	24	1/13	7,488	24
3 Sept	14	36	5/14	18,144	180
8 Sept	10	45	5/10	20,250	225
11 Sept	14	50	7/14	35,000	350
16 Sept	14	57	2/14	45,486	114
20 Sept	14	65	6/14	59,150	390
23 Sept	12	72	7/12	62,208	504
26 Sept	9	77	2/9	53,361	154
30 Sept	12	82	5/12	80,688	410
3 Oct	10	89	5/10	79,210	445
Totals				462,548	2,796

For each recapture phase: w, total number caught; x, total number previously handled up to, but not including, that phase (i.e., the total marked up to that time); y, the proportion of the catch previously handled (i.e., the proportion of the catch that is marked).

Raw population estimate, St. David's Head breaker ledge: $p = \Sigma wx^2/\Sigma wxy$ (Hayne's Index multiple census).

p = 462,548/2,796 = 165 fishable Guinea chick lobsters. Area of breaker ledge measured by tracing and use of small square graph paper = 12 hectares. Raw estimate of population density = 165/12 = 14 ha^{-1}

TABLE 8.

Population estimate at the Breaker Ledge off Cooper's Island, late autumn/early winter 1986.

Sampling	w	x	y	wx^2	wxy
6 Oct	15	0	0	0	0
9 Oct	7	12	0	1,008	0
13 Oct	10	17	2/10	2,890	34
18 Oct	6	23	1/6	3,174	23
21 Oct	8	29	3/8	6,728	87
24 Oct	20	32	5/20	20,480	160
27 Oct	2	46	1/2	4,232	46
31 Oct	5	46	1/5	10,580	46
5 Nov	24	49	6/24	57,624	294
8 Nov	3	65	2/3	12,675	130
17 Nov	9	66	7/9	39,204	462
25 Nov	14	66	6/14	60,984	396
5 Dec	12	74	4/12	65,712	296
Totals				285,291	1,974

For each recapture phase: w, total number caught; x, total number previously handled up to, but not including, that phase (i.e., the total marked up to that time); y, the proportion of the catch previously handled (i.e., the proportion of the catch that is marked).

Raw population estimate, Cooper's Island breaker ledge: $p = \Sigma wx^2/\Sigma wxy$ (Hayne's Index multiple census). p = 285,291/1,974 = 145 fishable Guinea chick lobsters. Area of breaker ledge measured by tracing and use of small square graph paper = 5.7 hectares. Raw estimate of population density = 145/5.7 = 25 ha^{-1}

borough 1974) was estimated to be about 46% at the Cooper's Island Breaker Ledge, in the late autumn/early winter of 1986 (Table 6B), and to be about 37% at Kitchen Shoals in the summer of 1987 (Table 6C). The loss rate of tags from molting was assumed to be the same as that evaluated by the Davis (1978) study (10% per molt).

There was only one molt of tagged lobsters during the study period June–October (Evans 1988). previous capture-recapture work in Australia from trapping *P. cygnus* rock lobsters in baited lobster pots (Morgan 1974) resulted in an estimate of the effect of previous capture on the probability of recapture. Morgan (1974) found that estimates of density had to be adjusted to correct for a "trap-happiness" effect, by multiplying the estimate by 1.6. The rock lobsters are only slightly larger in size than *P. guttatus* lobsters, and so, the same adjustment factor was used in the present study with some modification. (The correction factors that were calculated and applied to raw population density estimates in the present study were presented in Table 6.)

The estimate of the real sex ratio of fishable Guinea chicks (7:1, male:female) at the northeastern reef crest for the summer and autumn was less than that observed from trapping (13:1), but much more than unity. This does not reflect a higher mortality for trappable females than for trappable males, because the annual total mortality coefficients were estimated at 0.81 for males and 0.77 for females (Evans 1989, p. 122). Prima facie, it points further toward a localization of females on the island shelf, although the transect, area, and capture-recapture studies did not find concentrations elsewhere that were great enough to explain the disparity. Furthermore, an inshore juvenile lobster sampling program by artificial lobster shelters resulted in a nil catch of Guinea chick lobsters, yet attracted 64 early benthic juvenile *P. argus* lobsters into residence (Evans 1988 and 1989, Evans et al. 1994).

A more plausible explanation is that the sex ratio of the early benthic juvenile stage is near unity and that the mortality rate of juvenile female Guinea chick lobsters is significantly higher than that of juvenile males. This would be likely, because the female of the species is smaller: trappable females have a slower growth rate than males. The annual growth coefficient K was estimated to be 0.155 for females, as opposed to 0.22 for males (Evans 1988, 1989).

The mean retention size of the 4.1 m hexagonal mesh traps is 50 mm CL (Munro 1974), and therefore, a proportion of the population of females is precluded from sampling by traps; this may also contribute to the disparity in the sex ratio of Guinea chicks

TABLE 9.

Population estimate at Kitchen Shoals (Kitchen Tripod Shoal) by Hayne's Index (male and female combined) for the summer and autumn of 1987.

Sampling	w	x	y	wx^2	wxy
23 May	2	0	0	0	0
26 May	1	2	0	4	0
1 Jun	0	3	0	0	0
4 Jun	7	3	0	63	0
9 Jun	4	8	2/4	256	16
11 Jun	5	8	0	320	0
16 Jun	4	13	0	676	0
19 Jun	10	17	1/10	2,890	17
21 Jun	8	25	0	5,000	0
25 Jun	7	32	3/7	7,168	96
29 Jun	12	36	0	15,552	0
2 Jul	9	47	1/9	19,881	47
10 Jul	6	55	2/6	18,150	110
13 Jul	6	59	1/6	20,886	59
16 Jul	14	64	8/14	57,344	512
21 Jul	16	70	1/16	78,400	70
24 Jul	19	83	2/19	130,891	166
27 Jul	25	100	6/25	250,000	600
5 August	19	118	4/19	264,556	472
17 August	7	133	3/7	123,823	399
20 August	4	137	2/4	75,076	274
27 August	6	139	2/6	115,926	278
2 Sept	3	143	0	61,347	0
10 Sept	7	144	4/7	145,152	576
14 Sept	5	147	0	108,045	0
17 Sept	8	151	2/8	182,408	302
21 Sept	25	157	6/25	616,225	942
24 Sept	16	176	5/16	495,616	880
5 Oct	16	187	1/16	559,504	187
12 Oct	15	202	10/15	612,060	2,020
16 Oct	2	202	1/2	81,608	202
21 Oct	25	202	5/25	1,020,100	1,010
Totals	313[a]			5,068,927	9,235

For each recapture phase: w, total number caught; x, total number previously handled up to, but not including, that phase (i.e., the total marked up to that time); y, the proportion of the catch previously handled (i.e., the proportion of the catch that is marked).

Raw population estimate, Kitchen Shoals, is given by: $p = \Sigma wx^2/\Sigma wxy$ (Hayne's Index multiple census). p = 5068927/9235 = 549 fishable Guinea chick lobsters. Shoal area measured by small square method = 17 ha. Raw estimate of population density = 549/17 = 32 ha^{-1}

[a] Catch was composed of 291 males and 22 females. In constructing the above table allowances have been made for animals dead from in-trap predation.

TABLE 10.

Calculation and collation of CPUE data, population density estimates and catchability estimates for trappable Guinea chick lobsters in the reef-crest areas of Bermuda, in the summer and autumn periods of 1986–87.

A. S.D.H. (21 August–October 1986)
Raw population estimate = 165 Guinea chicks
Corrected population estimate = 165 × 1.6 = 264 Guinea chicks
Raw density estimate = 14 Guinea chicks ha^{-1}
Corrected density estimate = 14 × 1.6 = 22 Guinea chicks ha^{-1}
CPUE = 137 Guinea chicks/322 trap nights = 0.425 Guinea chicks $trap\ night^{-1}$
$q = 1.6 \times 10^{-3}$ $trap\ night^{-1}$
$E = 19 \times 10^{-3}$ ha. $trap\ night^{-1}$

B. C.I. (3 October–5 December 1986)
Raw population estimate = 145 Guinea chicks
Corrected population estimate = 145 × 1.2 = 174 Guinea chicks
Raw density estimate = 25 Guinea chicks ha^{-1}
Corrected density estimate = 25 × 1.2 = 30 Guinea chicks ha^{-1}
CPUE = 137 Guinea chicks/427 trap nights = 0.321 Guinea chicks $trap\ night^{-1}$
$q = 1.8 \times 10^{-3}$ $trap\ night^{-1}$
$E = 11 \times 10^{-3}$ ha $trap\ night^{-1}$

C. K.S. male and female combined (23 May–21 October 1987)
Raw population estimate = 549 Guinea chicks
Corrected population estimate = 549 × 1.1 = 604 Guinea chicks
Raw density estimate = 32 Guinea chicks ha^{-1}
Corrected density estimate = 32 × 1.1 = 35 Guinea chicks ha^{-1}
CPUE = 313 Guinea chicks/1,146 trap nights = 0.273 Guinea chicks $trap\ night^{-1}$
$q = 0.45 \times 10^{-3}$ trap night
$E = 7.8 \times 10^{-3}$ ha $trap\ night^{-1}$

S.D.H., St. David's Head; C.I., Cooper's Island; K.S., Kitchen Shoals. Catch per unit of effort (CPUE) = q.P, where q is the coefficient of catchability and P is the population number (Nicholson and Bailey 1935, Ricker 1975). CPUE = E.D., where E = effective area fished (Miller 1986) and D = population density.

TABLE 10D.

Summary of results on the density and catchability of Guinea chicks at the reef crest.

Location	Density[a]	Catchability	
		q[b]	E[c]
S.D.H.	22	1.6×10^{-3}	19×10^{-3}
C.I.	30	1.8×10^{-3}	11×10^{-3}
K.S.	35	0.45×10^{-3}	7.8×10^{-3}
Mean	29	1.3×10^{-3}	13×10^{-3}
SD (SD)	6.6	0.73×10^{-3}	5.8×10^{-3}
95% Confidence limits	±7.6	$\pm 0.84 \times 10^{-3}$	$\pm 6.7 \times 10^{-3}$

[a] Guinea chicks ha^{-1}.
[b] Trap $night^{-1}$.
[c] ha. $trap\ night^{-1}$.
[d] 95% confidence limits (2 × standard error) calculated from: standard error = $SD/(N)^{-1/2}$, where SD is the standard deviation and N is the number in the sample (Mathers 1981).

TABLE 11.

Male and female population densities, real sex ratio, and the difference in catchability between the sexes at the Northeastern reef crest summer/autumn.

Sampling	w	x	y	wx^2	wxy
23 May	1	0	0	0	0
26 May	1	1	0	1	0
1 Jun	0	2	0	0	0
4 Jun	5	2	0	20	0
9 Jun	3	7	1/3	147	7
11 Jun	5	7	0	245	0
16 Jun	4	12	0	576	0
19 Jun	10	16	1/10	2,560	16
21 Jun	8	24	0	4,608	0
25 Jun	7	31	3/7	6,727	93
29 Jun	12	35	0	14,700	0
2 Jul	9	46	1/9	19,044	46
10 Jul	6	54	2/6	17,496	108
13 Jul	3	58	1/3	10,092	58
16 Jul	14	60	8/14	50,400	480
21 Jul	16	66	1/16	69,696	66
24 Jul	19	79	2/19	118,579	158
27 Jul	25	96	6/25	230,400	575
5 Aug	16	114	4/16	207,936	456
17 Aug	7	126	3/7	111,132	378
20 Aug	3	130	2/3	50,700	260
27 Aug	5	131	2/5	85,805	262
2 Sept	3	134	0	53,868	0
10 Sept	6	135	4/6	109,350	540
14 Sept	4	137	0	75,076	0
17 Sept	8	141	2/8	159,048	282
21 Sept	22	147	6/22	475,398	882
24 Sept	15	163	5/15	398,535	815
5 Oct	14	173	1/14	419,006	173
12 Oct	15	186	10/15	518,940	1,860
16 Oct	2	186	1/2	69,192	186
21 Oct	23	186	4/23	795,708	744
Totals	291			4,074,985	8,446

For each recapture phase: w, total number caught; x, total number previously handled up to, but not including, that phase (i.e., the total marked up to that time); y, the proportion of the catch previously handled (proportion of catch with a mark).
Raw population estimate, Kitchen Shoals, is given by: $p = \Sigma wx^2/\Sigma wxy$ (Hayne's Index multiple census). p = 4074985/8446 = 482 male fishable Guinea chicks. Shoal area measured by small square method = 17 ha. Raw estimate of population density = 482/17 = 28 ha^{-1}. In constructing the above table, allowances have been made for animals dead from in-trap predation.

caught. This was also suggested by Munro (1974) to explain a similar sex ratio of trapped animals from the population of *P. guttatus* lobsters at Jamaica (77 males to 37 females).

The population density of trappable Guinea chicks at the northeastern reef crest in the period May–October was estimated to be greatest in the middle of this period, July through September (Table 12). This correlates with the modal peak in trap rates that occurred at the reef crest at Kitchen Shoals from mid-July to mid-August (Fig. 12) (which resulted from the very sharp rise in trap rates there during the last 2 weeks of July). It also correlates with the above interpretation of the change in size composition at Kitchen Shoals, viz. recruitment coupled with an inward breeding

TABLE 11B.

Real sex ratio at Kitchen Shoals May–October.

The raw population estimate for females is 549–482 = 67 female fishable Guinea chicks, by subtraction from the male + female population estimate (Table 9).
The real sex ratio is therefore 7:1.
The sex ratio of trapped animals at Kitchen Shoals over the same period was 13:1.
A conversion factor may therefore be computed for transforming the sex ratio of trapped animals to real sex ratio: conversion factor = 7/13 = 0.54.

migration of egg-bearing females and smaller males, to the reef crest (in the period May to August), entraining new fishery recruits.

Catchability

The catchability of spiny lobsters and rock lobsters varies with a whole series of environmental factors and conditions, including the lunar and reproductive cycles. The chief of these are water temperature, water salinity, and percentage of lobsters in a premolt condition (Morgan 1974). Temperature and salinity are positively correlated with catchability, and the percentage in premolt condition is negatively correlated (Morgan 1974).

The catchability of Guinea chick lobsters was higher at St. David's Head and Cooper's Island in the autumn and early winter than at Kitchen Shoals in the summer and autumn (Table 10D). This may reflect the influence of the reproductive cycle and its associated molting cycles, because the period of study at Kitchen Shoals included the height of the breeding season, whereas the other two study periods were after the height of season had passed.

TABLE 11C.

Difference in catchability between the sexes at Kitchen Shoals, May–October.

1. Males
   Raw population estimate = 482 Guinea chicks
   Correct population estimate 482 × 1.1 = 530 chicks
   Raw density estimate = 28 Guinea chicks ha^{-1}
   Corrected density estimate = 28 × 1.1 = 31 chicks ha^{-1}
   C.P.U.E. = 291/1146 = 0.254 Guinea chicks trap $night^{-1}$ or
   C.P.U.E. = 291/237 = 1.23 Guinea chicks trap $haul^{-1}$
   $q = 4.8 \times 10^{-4}$ trap $night^{-1}$
   $E = 8.2 \times 10^{-3}$ ha. trap $night^{-1}$
   $q' = 2.3 \times 10^{-3}$ trap $haul^{-1}$
   $E' = 0.040$ ha. trap $haul^{-1}$
2. Females
   Raw population estimate = 67 Guinea chicks
   Corrected population estimate 67 × 1.1 = 74 chicks
   Raw density estimate = 67/17 = 3.9 Guinea chicks ha^{-1}
   Corrected density estimate = 3.9 × 1.1 = 4.3 chicks ha^{-1}
   C.P.U.E. = 22/1146 = 0.0192 Guinea chicks trap $night^{-1}$ or
   C.P.U.E. = 22/237 = 0.0928 chicks trap $haul^{-1}$
   $q = 2.6 \times 10^{-4}$ trap night
   $E = 4.5 \times 10^{-3}$ ha. trap $night^{-1}$
   $q' = 1.3 \times 10^{-3}$ trap $haul^{-1}$
   $E' = 0.022$ ha. trap $haul^{-1}$
3. Transformation of estimates
   The conversion factor to transform catchability q from units of trap $night^{-1}$ to trap $haul^{-1}$ is given by:
   Multiply by 4.8 for males
   Multiply by 5.0 for females
   Multiply by 4.9 for males + females
   The conversion factor to transform effective area fished E from units of ha. trap $night^{-1}$ ha. trap $haul^{-1}$ is given by:
   Multiply by 4.9 for males
   Multiply by 4.9 for females
   Multiply by 4.9 for males + females

For the period 23 May–21 October 1987, total catch = 291 males and 22 females; total effort = 1,146 trap nights, or 237 trap hauls.
Equations relating to estimates of catchability (q) and effective area fished (E). CPUE, catch per unit of (fishing) effort; q, catchability coefficient; P, population number; D, population density. CPUE, q.P (Nicholson and Bailey 1935); CPUE, E.D (Miller 1986).

TABLE 12.

Capture-recapture population estimates at the Northeastern reef crest (at Kitchen Shoals) for different periods during the summer and autumn of 1987, by Bailey's Triple Catch Method.

A. The Technique: Bailey's Triple Catch Method (Chalmers and Parker, 1989) required a data set as follows:
   (1) The number of individuals that were marked in the first sample and recaptured in the second, $R_{1,2}$.
   (2) The number of individuals that were marked in the second sample and recaptured in the third, $R_{2,3}$.
   (3) The number of individuals that were marked in the first sample and recaptured in the third, $R_{1,3}$.
   (4) The total number of animals caught in the second sample, S_2.
   The procedure carried out was as follows:
   1. The "population of marked individuals" (M) was calculated from the equation:

$$M = ([S_2 \times R_{1,3}]/R_{2,3}) + R_{1,2}.$$

   2. Then, the value of M was used to estimate the total population N from the equation:

$$N = (M \times S_2)/R_{1,2}.$$

   3. The 95% confidence limit of the population estimate were calculated from the equation associated with the Method (Chalmers and Parker 1989):

$$N \pm \sqrt{2N(N - S_2)\left[\left(\frac{(M - R_{1,2}) + S_2}{M}\right)\left(\frac{1}{R_{2,3}} - \frac{1}{S_2}\right) + \left(\frac{1}{R_{1,2}} - \frac{1}{S_2}\right)\right]}$$

TABLE 12B.

Spiny lobsters (*P. guttatus*) in the exploited phase at Kitchen Shoals study area: Table for capture-recapture estimates of population number by Bailey's Triple Catch Method.

	Time of Capture				
	1	2	3	4	5
Time of last capture					
June	4	13	1	1	1
July	—	8	7	10	4
August	—	—	3	3	1
September	—	—	—	3	9
October	—	—	—	—	2
Total marked	4	21	11	17	17
Total unmarked	53	74	25	47	41
Total caught	57	95	36	64	58
Total released	49	92	36	62	16

TABLE 12C.

Estimate for period 1, 1 June–31 August.

$R_{1,2} = 13$

$R_{2,3} = 7$

$R_{1,3} = 1$

$S_2 = 95$, but 92 were released.

$$M = \frac{92 \times 1}{7} + 13 = 26$$

$$N = \frac{26 \times 95}{13} = 190$$

95% confidence limit of the population estimate (from the equation in Part A of Table 12) = 190 ± 171, or 190 ± 90.0%.

TABLE 12D.

Estimate for period 2, 1 July–30 September.

$R_{1,2} = 17$

$R_{2,3} = 3$

$R_{1,3} = 10$

$S_2 = 36$

$$M = \frac{36 \times 10}{3} + 7 = 127$$

$$N = \frac{127 \times 36}{7} = 653$$

95% confidence limit of the population estimate (from the equation in Part A of Table 12) = 653 ± 351, or 653 ± 53.8%.

TABLE 12E.

Estimate for period 3, 1 August–21 October.

$R_{1,2} = 3$

$R_{2,3} = 9$

$R_{1,3} = 1$

$S_2 = 64$, but 62 were released.

$$M = \frac{62 \times 1}{9} + 3 = 10$$

$$N = \frac{10 \times 64}{3} = 213$$

95% confidence limit of the population estimate (from the equation in Part A of Table 12) = 213 ± 251, or 213 ± 118%.

TABLE 12F.

Summary of the population density estimates at Kitchen Shoals using Bailey's Triple Catch Method.

Period 1 (1 June–31 August):	N = 190	Guinea chicks ± 90.0%.
Period 2 (1 July–30 Sept.):	N = 653	Guinea chicks ± 53.8%.
Period 3 (1 August–21 Oct.):	N = 213	Guinea chicks ± 118%.

The correction factor to take into account (1) predilection to reenter traps, (2) the combined effect of tag/displacement-induced mortality and emigration, and (3) tag loss from molting was estimated to be ×1.0, which when applied, left these estimates unchanged (see Table 6D for estimation of components 1 to 3). N, population estimate with 95% confidence limits, calculated from the formula associated with the estimation equation (Table 12A).

At Bermuda, seawater temperatures remain relatively high during the months May to November inclusive (22 to 28°C) but are somewhat lower in the months December to April (17 to 22°C) (Morris et al. 1977).

Catchability can also be affected by intertrap separation if there is overlap between the areas of attraction of each pot. Intertrap separation was on average 159 m at St. David's Head, 123 m at Cooper's Island, and 116 m (1/16 of a nautical mile) at Kitchen Shoals (nine traps in a cross-shaped pattern). It is not known if this influenced the results, because the area of attraction is not accurately known. However, it seems likely that only the traps set at St. David's Head study area were free from interference by overlap, because Western rock lobsters can be attracted 80 m to a baited lobster pot.

The lower catchability of females compared with males (both in terms of q and E) at the reef crest in the period May to October (including the height of the breeding season) indicates that females are less catchable than males in such circumstances. Two possible explanations are (1) that females are smaller and less powerful than males and feed rather more within the labyrinthine reef structure than do males, and/or (2) that breeding females are less catchable than at other times.

The population density estimate for determination of the catchability coefficient is preferably assessed by an independent method (Miller 1986). Morgan (1974) however derived catchability coefficient values from simultaneous observations on the population density, catch, and effort, and from these values, he derived the linear correlations with temperature, salinity, and percentage in premolt, which are referred to above.

In the present study, the trap rate of the special central station (pot 8) of the Transect 2 trapline (in a favored location at the reef crest surrounded on three sides by overhanging labyrinthine coral cliffs) was used for the calibration of the population density estimate (i.e., to determine E for the purpose of the conversion of trap rates: Table 13A to C). This station was a deep, sand-floored

TABLE 13.

Calculation of population density in the different physiographic provinces: Table 13A. Density and catchability in the midsummer period at the northeast reefcrest (Kitchen Shoals.)

From Table 12D, the estimate of population density for period 2 (1 July–30 September) was 653 ± 351 Guinea chicks (95% confidence limits of Bailey's Triple Catch Method).

The upper and lower limits of this estimate are 302 and 1,004 Guinea chicks, respectively. The study area was 17 ha. in area. Thus, the upper and lower limits of the density estimate (D) for this period are 302/17 and 1,004/17 or 18 and 59 Guinea chicks ha^{-1} respectively.

The catch per unit of fishing effort (CPUE) at the reef-crest station on Transect 2 (pot 8) for this period was 43/13 = 3.3 Guinea chicks trap $haul^{-1}$ (see Table 13B below).

An estimate of the effective area fished (E) for this period may be derived from the equation CPUE = E.D. by substitution of the upper and lower limit of D:

Lower limit of E = 3.3/59 = 0.056 ha. trap $haul^{-1}$

Upper limit of E = 3.3/18 = 0.183 ha. trap $haul^{-1}$

These may be used to estimate the 95% confidence upper and lower limits of population density at the other physiographic provinces, from the catch and effort data in Table 13B below. These estimates are presented in Table 13C below.

TABLE 13B.

Spiny lobster *P. guttatus* in Bermuda: catch and effort on Transect 2.

Month in 1987	Patch Reef Zone, Pots 1–7			Reef Flat, Pot 8			Fore Reef, Pot 9			Main Terrace, Pots 10–12			Edge (13–18 fathoms), Pot 13		
	c	f	CPUE	c	f	CPUE	c	f	CPUE	c	f	CPUE	c	f	CPUE
May	2	45	0.044	9	7	1.286	0	5	0	7	16	0.437	2	6	0.333
June	1	46	0.022	15	9	1.667	2	8	0.25	23	17	1.353	4	7	0.571
July	7	27	0.259	24	6	4	3	7	0.429	19	17	1.118	2	6	0.333
August	1	1	1	11	4	2.75	6	6	1	38	17	2.235	2	6	0.333
September	1	2	0.5	8	3	2.667	0	4	0	4	7	0.571	0	6	0

f, no. of traps hauled; c, no. caught.

TABLE 13C.

Population density for the period 1 July–30 September, 1987, in the different physiographic provinces as lower and upper limits of a 95% confidence band (densities in Guinea chicks ha^{-1}).

PRZ	RCA	FRS	RFT	E
1.6–5.4	18–59[a]	2.9–9.5	8.2–27	1.2–3.9

PRZ, patch reef zone; RCA, reef-crest area; FRS, fore-reef slope; RFT, reef-front terrace; E, Edge.

[a] Density of fishable Guinea chicks estimated by the Bailey Triple Catch Method, for Kitchen Shoals area. Example of calculation method (density estimate for the reef-front terrace):

CPUE (Table 13B) = (19 + 38 + 4)/(17 + 17 + 4)
= 61/41
= 1.5 Guinea chicks trap haul^{-1}

Lower limit (LL) of estimate D_{LL} = CPUE E_{UL}
= 1.5/0.183 = 8.2 ha^{-1}

Upper limit (UL) of estimate D_{UL} = CPUE/E_{LL}
= 1.5/0.056 = 27 ha^{-1}.

depression situated close to Kitchen Tower in the center of the shoal on which it stands (the study area). The area's catch rates were not used for the calibration, but the surrounding study area was the site of the capture-recapture experiment. In this way, a degree of independence was attained in the determination of the density.

Robust mean estimates of effective area fished (E) may be used to convert similarly obtained catch rates to density at other times and places (Miller 1986).

Examples of the potential and actual application of conversion are cited below within the context of the present study and the potential for future research.

The estimate of E for the reef crest off Cooper's Island (7.8×10^{-3} ha trap night^{-1}: Table 10D) may be used to transform the trap rates of stations along Transect 1 to population density.

The estimate of E (as a 95% confidence band), which was determined from the Bailey Triple Catch Method for midsummer at Kitchen Shoals, was actually used to transform the simultaneous station trap rates of Transect 2 to population density estimates in

TABLE 14A.

Statistical test on the Hayne's Index population estimate for St. David's Head.

Tabulate the proportion marked in catch (Y data) and the number of animals marked previously (X data):

X	Y
0	0
3	0
10	0.067
24	0.077
36	0.357
45	0.500
50	0.500
57	0.143
65	0.429
72	0.583
77	0.222
82	0.417
89	0.500

Linear regression of above data by programmable calculator for Y = A + BX:

A = 0.04455
B = 0.00527
r = 0.7511

The tabulated value of r in Murdoch and Barnes (2nd ed. 1970) is 0.6835 (p = 0.005) for a significant positive correlation (and there are 11 degrees of freedom). The calculated value of r is greater than this, so there is a significant positive correlation of the "proportion of marked animals in catch" with "number of animals marked previously" at the 0.5% level of significance (p = 0.005, single-tailed test for positive correlation). Furthermore, the tabulated value for r at the 5% level is 0.4762. The result is therefore a conclusive finding.

TABLE 14B.

Statistical test on the Hayne's Index population estimate for St. Cooper's Island.

Tabulate the proportion marked in catch (Y data) and the number of animals marked previously (X data):

X	Y
0	0
12	0
17	0.200
23	0.167
29	0.375
32	0.250
46	0.500
46	0.200
49	0.250
65	0.667
66	0.778
66	0.429
74	0.333

Linear regression of above data by programmable calculator for Y = A + BX:

A = 0.012418
B = 0.007595
r = 0.7728

The tabulated value of r in Murdoch and Barnes (1970) is 0.6835 (p = 0.005) for a significant positive correlation (and there are 11 degrees of freedom). The calculated value of r is greater than this, so there is a significant positive correlation of the "proportion of marked animals in catch" with "number of animals marked previously" at the 0.5% level of significance (p = 0.005, single tail test for positive correlation). Furthermore, the tabulated value of r at the 5% level is 0.4762. The result is therefore a conclusive finding.

TABLE 14C.

Statistical test on the Hayne's Index population estimate for Kitchen Shoals males and females combined.

Tabulate the proportion marked in catch (Y data) and the number of animals marked previously (X data):

X	Y
0	0
2	0
3	0
3	0
8	0.500
8	0
13	0
17	0.100
25	0
32	0.429
36	0
47	0.111
55	0.333
59	0.167
64	0.571
70	0.0625
83	0.105
100	0.240
118	0.211
133	0.429
137	0.500
139	0.333
143	0
144	0.571
147	0
151	0.250
157	0.240
176	0.313
187	0.0625
202	0.667
202	0.500
202	0.200

Linear regression of above data by programmable calculator for $Y = A + BX$:

$A = 0.09234$

$B = 1.38 \times 10^{-3}$

$r = 0.4541$

There are 30 degrees of freedom, and the tabulated value of r in Murdoch and Barnes (1970) is 0.4487 at the 0.5% level (single-tailed test for positive correlation). The calculated value of r is greater than this, and therefore, there is a positive correlation of the "proportion marked in catch" with "number of animals marked previously" at the 0.5% level of significance ($p = 0.005$). Furthermore, the tabulated value for r at the 5% level is 0.2960. The result is therefore a conclusive finding.

TABLE 14D.

Statistical test on the Hayne's Index population estimate for Kitchen Shoals males.

Tabulate the proportion marked in catch (Y data) and the number of animals marked previously (X data):

X	Y
0	0
1	0
2	0
2	0
7	0.333
7	0
12	0
16	0.100
24	0
31	0.429
35	0
46	0.111
54	0.333
58	0.333
60	0.571
66	0.0625
79	0.105
96	0.240
114	0.250
126	0.429
130	0.666
131	0.400
134	0
135	0.666
137	0
141	0.250
147	0.273
163	0.333
173	0.0714
186	0.666
186	0.500
186	0.174

Linear regression calculator for $Y = A + BX$:

$A = 0.08548$

$B = 1.70 \times 10^{-3}$

$r = 0.4917$

There are 30 degrees of freedom, and the tabulated value of r in Murdoch and Barnes (1970) for the 0.5% level of significance is 0.4487. The calculated value of r is greater than this, and therefore, there is a positive correlation of the "proportion marked in catch" with "number of animals marked previously" at the 0.5% level of significance ($p = 0.005$), single-tailed test for positive correlation). Furthermore, the tabulated value for r at the 5% level is 0.2690. The result is therefore a conclusive finding.

the present study (Table 13A to C). (These are two examples of application for different place, same time.)

The mean estimate of E derived for the reef-crest areas (13×10^{-3} ha trap night^{-1}) could be used to estimate density from Guinea chick trap rates at any time interval (in the months June through November) and for other areas of the Bermudan reef system.

Such studies of catchability in terms of effective area fished (E) thus have practical value as a tool for the assessment of spiny lobster resources of tropical islands.

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APPENDICES:

STATISTICAL ANALYSES OF THE POPULATION ESTIMATES MADE BY THE HAYNE's (1949) METHOD.

The Hayne's Index population estimates are based upon Hayne's (1949) equation for population number P:

$$P = \Sigma wx^2/\Sigma wxy$$

Schumacher and Eschmeyer (1943) used this method to compute an estimate of fish population number from records of netting, marking, and releasing.

The formula is the inverted form of the usual expression for slope of a regression line passing through the origin (Hayne 1949).

The linear regression analyses carried out in Tables 14A to D below are "free-standing," i.e., the regression lines are not "forced through the origin."

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SPAWNING CYCLE OF THE RED CLAM *MEGAPITARIA AURANTIACA* (SOWERBY, 1831) (*VENERIDAE*) AT ISLA ESPIRITU SANTO, BAJA CALIFORNIA SUR, MEXICO

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ABSTRACT Adult red clams, *Megapitaria aurantiaca*, were collected at Isla Espíritu Santo, B.C.S., Mexico, from May 1991 to May 1992. Gonadal development was analyzed using histological techniques. Development phases were categorized into five stages: indifferent, developing, ripe, partially spawned, and spent. Spawning occurred all year except in September, with peaks in December and March. A clear relation between temperature and spawning was not observed. Spawning occurs all during the year. Nevertheless, through the year, two distinct periods are observed: one, from May to November, with the mean of the partially spawned population at 8.8, coincides with warmer months of the year; and the second, from December to April, with a mean of 38.9%, coincides with cooler months.

KEY WORDS: Spawning cycle, bivalves, *Megapitaria*, histology

INTRODUCTION

The red clam *Megapitaria aurantiaca* (Sowerby, 1831) is commercially fished along the west coast of Baja California Sur (Holguín 1976). It lives in the sublittoral zone to 25 m depth in medium sand, coarse sand, and coarse sand-pebble areas (Baqueiro 1979). The increasing demand for this species, its high cost, and the decrease of natural stocks make it a prime candidate for commercial culture (Baqueiro 1989).

Studies of reproduction using histological techniques have been made on *Veneridae* clams like *Cyprina islandica* (Loosanoff 1953), *Venerupis japonica* (Holland and Chew 1974), *Ameghinomya antiqua* (Verdinelli and Schuldt 1976), *Venus striatula* (Ansell 1961), *Megapitaria squalida* and *Dosinia ponderosa* (Baqueiro and Stuardo 1977), *Venus antiqua* (Lozada and Bustos 1984), *Callista chione* (Valli et al. 1984), *Mercenaria mercenaria* (Eversole et al. 1980, Manzi et al. 1985), *Chione fluctifraga* (Martínez-Córdova 1988), *C. undatella* (Baqueiro and Masso 1988), *Mercenaria* spp. (Hesselman et al. 1989), and *Chione californiensis* (García-Domínguez et al. 1993). For *M. aurantiaca*, only one study of reproduction, also including some ecological and biological aspects, was made in Bahía Zihuatanejo, Guerrero, Mexico (Baqueiro and Stuardo 1977).

This work was made to study the spawning cycle of a wild adult population of *M. aurantiaca* at Isla Espíritu Santo, B.C.S., Mexico, in relation to temperature and also to condition index (standard weight), because reproductive events have an influence on the weight of an organism. It was expected that the weight would increase during gametogenesis and maturity and decrease during spawning.

MATERIALS AND METHODS

From May 1991 to May 1992, 25 specimens per month of an adult population of clams located at Isla Espíritu Santo, B.C.S. (Fig. 1) were collected by scuba dive at 3 m depth. A total number of 290 organisms were captured. At the time of the collection of biological samples, surface water temperature was recorded.

Before dissection, shell length was measured with a 0.001 mm resolution caliper. Shell and tissue were blotted dry, and total and wet weight without the shell was measured with a balance that read to the nearest 0.1 g. Mantle, adductor muscles, gills, labial palps, and siphons were removed, keeping only the visceral mass (gonad, liver, and gastrointestinal tract) and the foot. These tissues were fixed in buffered 10% formalin and processed using histological techniques (Humason 1979). Paraffin sections 7 to 9 μm thick were stained with hematoxylin and eosin.

Categories of Gonadal Condition

The reproductive process (either spermatogenesis or oogenesis) of *M. aurantiaca* was divided into five stages (indifferent, developing, ripe, partially spawned, and spent) based solely on morphological observations. Categories comparable to those already in use for other species have also been used in this study where appropriate (Brousseau 1981, for *Petricola pholadiformis;* Brousseau 1982, for *Geukensia demissa;* Manzi et al. 1985, for *M. mercenaria;* Brousseau 1987, for *Mya arenaria;* Malachowski 1988, for *Hinnites giganteus;* Hesselman et al. 1989, for *Mercenaria* spp.; Ponurovsky and Yakovlev 1992, for *Tapes phillippinarium;* Jaramillo et al. 1993, for *Chlamys amandi;* and García-Domínguez et al. 1993, for *C. californiensis*).

Developmental Stages of the Male

Indifferent Stage (Fig. 2a)

This was characterized by an absence of gametes; however, residual spermatozoa were occasionally observed in some specimens. Vesicular connective tissue between follicles occupies almost all of the space. Gonoduct secondary ducts were open and empty. These ducts have columnar, ciliated epithelium, with basal nuclei and acidophilic granules in the cytoplasm.

Developing Stage (Fig. 2b)

The spermatogenic cells begin to proliferate around the follicle walls. Inside the follicle, a variable quantity of germinal cells and ripe gametes were observed. Spermatozoa are stored as a dense mass in the lumen of the follicle waiting to spawn. The connective

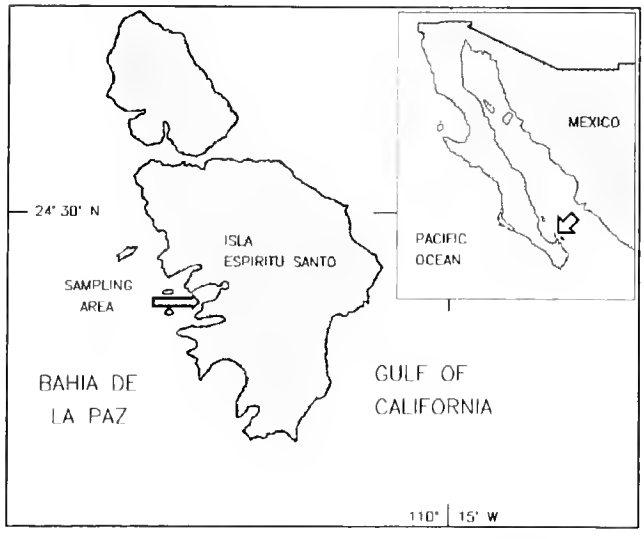


Figure 1. Study area. Isla Espíritu Santo, B.C.S., Mexico.

tissue between follicles decreases when follicles increase because of the accumulation of sperm.

Ripe Stage (Fig. 2c)

The follicles were distended and filled with dense, radiating bands of spermatozoa, the tails of which project into central lumen. Almost all connective tissue between follicles has been substituted by follicles full of spermatozoa.

Partially Spawned Stage (Fig. 2d)

This is the reproductive stage, and spermatozoa are expelled into environment. The follicles were partially empty. There is a marked decrease in the number of spermatozoa filling the lumen. This is the only stage where some secondary ducts are full of spermatozoa.

Spent Stage (Fig. 2e)

The follicles collapsed or had decreased in size and are invaded by amebocytes, which phagocytosed a small amount of unspent spermatozoa. There is no evidence of active spermatogenesis taking place.

Developmental Stages of the Female

Indifferent Stage (Fig. 3a)

This stage is characterized by absence of gametes. The follicles are partially compressed in shape or size and are empty except for occasional residual free oocytes. Vesicular connective tissue occupies the space between follicles.

Developing Stage (Fig. 3b)

This stage is a continuous process, involving a proliferation and growth of the oocytes. As the number of mature ova (free in the lumen) increased, the amount of connective tissue decreased. The developing, oocytes, which begins as hemispherical stalked cells attached to the wall of the follicle, become enlarged spherical cells, 45.9 μm (standard deviation [SD], 5.5) in diameter, as maturity approaches.

Ripe Stage (Fig. 3c)

The ripe ovary is characterized by the presence of distended follicles filled with ripe oocytes, some of which are attached to the follicular wall by slender stalks. Little or no connective tissue was present.

Partially Spawned Stage (Fig. 3d)

Some follicles contain oocytes, whereas others are empty. There is a noticeable reduction in the number of free large oocytes present in the lumen, 52.5 μm (SD, 4.7) in diameter. Little connective tissue was present. Follicular walls are broken.

Spent Stage (Fig. 3e)

The follicles were empty except for a few, large, unspent oocytes free in the lumen that are phagocytosed by amebocytes. Follicular walls are reestablished.

A condition index, the standard weight (SW) (Searcy 1984), was used to relate reproductive events with weight variation. Condition is interpreted as the fatness of the organism. SW was estimated using the equation:

$$SW = a\, l^b$$

where l is a constant length of 95 mm and a and b are parameters estimated with a nonlinear regression analysis using the length and weight data of each monthly sample.

RESULTS

Reproductive Cycle

The annual reproductive cycle of the red clam *M. aurantiaca* from Isla Espiritu Santo is summarized in Figure 4. Ripe clams were found every month, except April 1992. The ripe phase predominated in August 1991 and May 1992 (60 and 55% of the clams). Reproductive individuals (partially spawned) were observed all years except in September 1991. The maximum was in December 1991 and March 1992, when spawning individuals were 50 and 68% of the population and the temperature was 22°C. Spent red clams were observed in May, July, August, November, and December 1991, and January, February, March, and April 1992. Clams in the active phase were encountered throughout the study except February and March 1992. The largest number of active clams occurred in April 1991 (61% of the individuals). Indifferent clams were observed all year except October 1991. Gonadic activity was synchronic for both sexes. The sex ratio was 44.8% males and 55.2% females.

Standard Weight

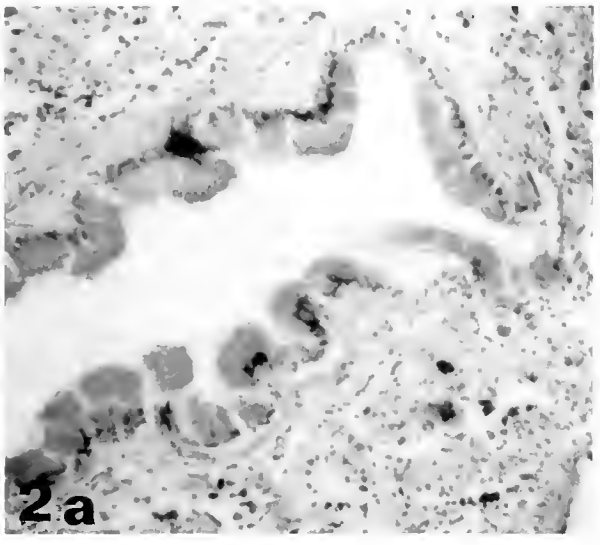
The SW varied between 4.5 g in June 1991 and 13.9 g in April 1992 (Fig. 5). The mean length of the clams was 95.0 mm (SD, 12.01).

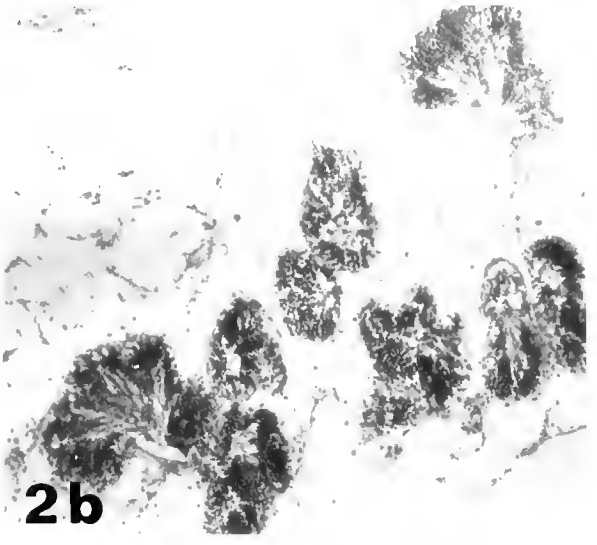
Temperature

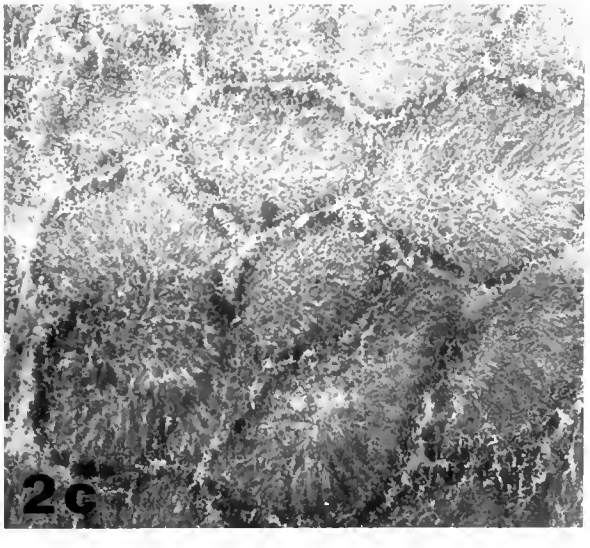
The minimum temperature of 20°C was recorded in January and February, and the maximum of 30°C was in September (Fig. 5).

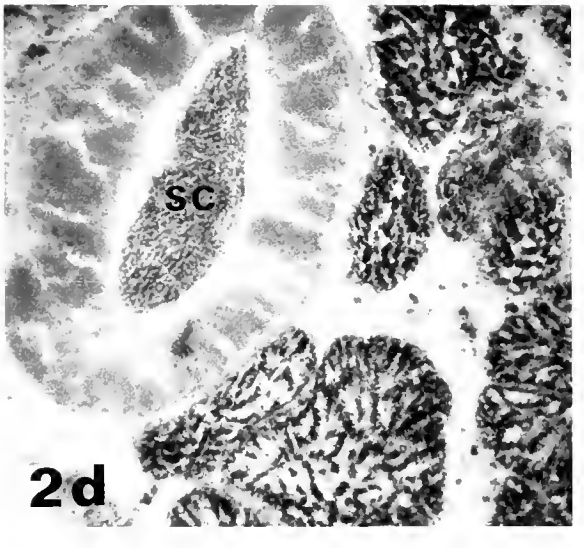
DISCUSSION

The spawning cycle of *M. aurantiaca* shows the same phenomena observed for other venerid clams: growth and development of









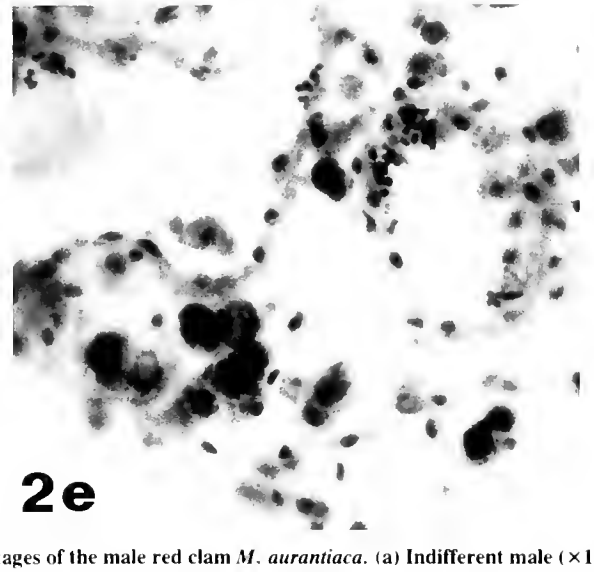


Figure 2. Photomicrographs of gonadal stages of the male red clam *M. aurantiaca*. (a) Indifferent male (×100); (b) developing male (×100); (c) ripe male (×100); (d) partially spawned male (SC, secondary ducts full of spermatozoa) (×100); (e) spent male (×400).

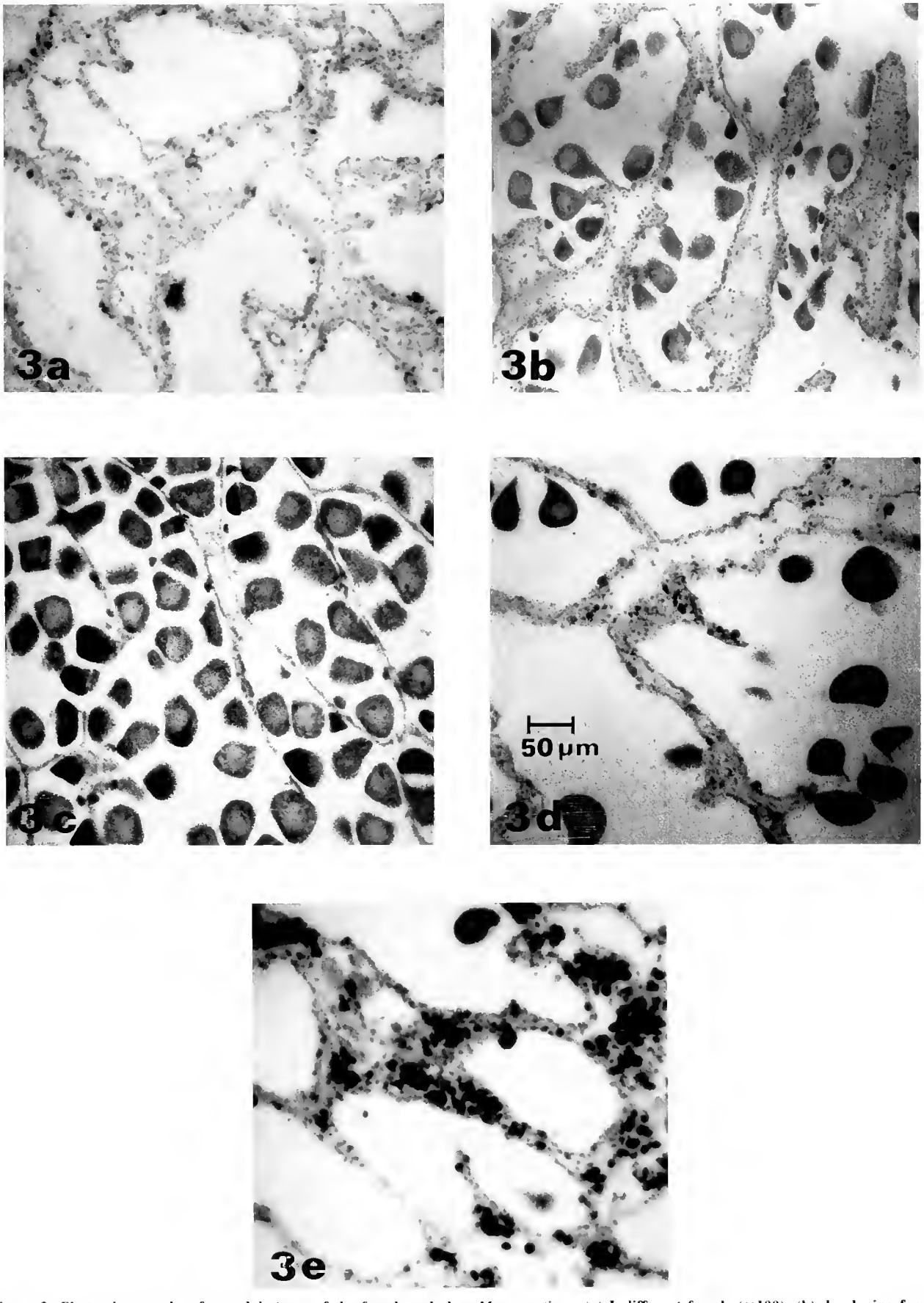


Figure 3. Photomicrographs of gonadal stages of the female red clam *M. aurantiaca*. (a) Indifferent female (×100); (b) developing female (×100); (c) ripe female (×100); (d) partially spawned female (×100); (e) spent female (×100).

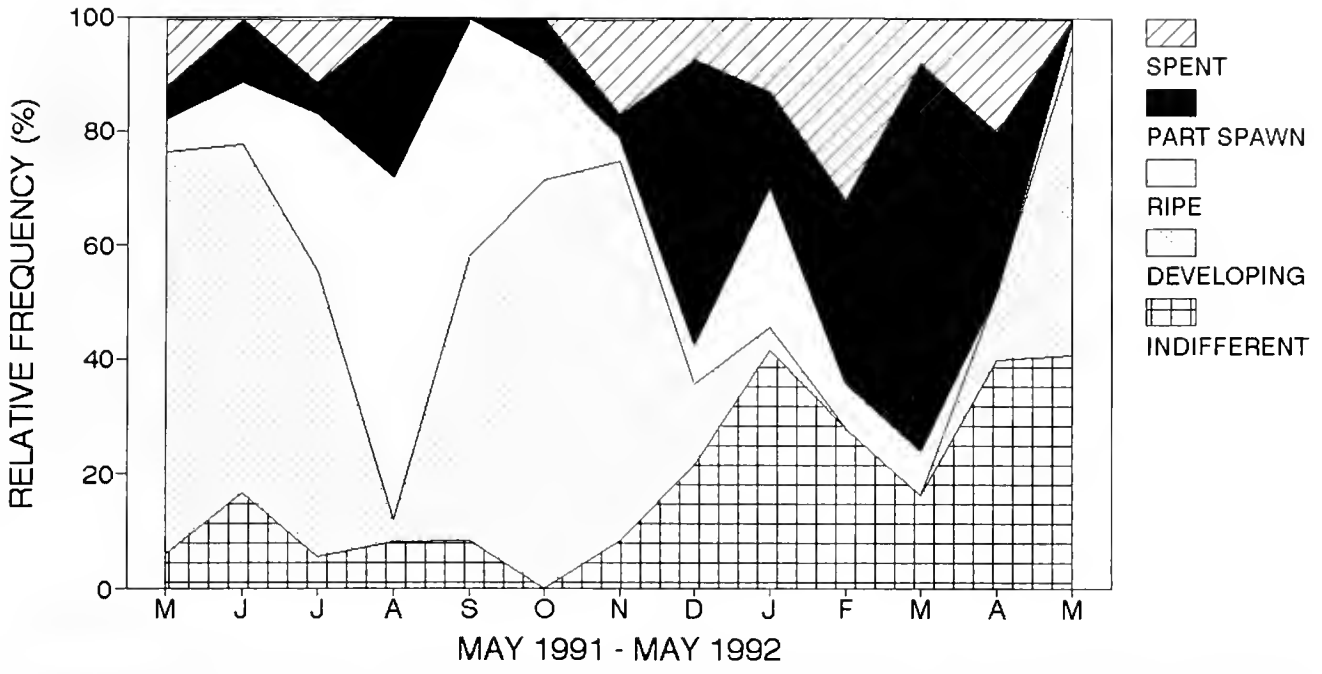


Figure 4. Relative frequency of *M. aurantiaca* population with gonads in each gonadal stage during 1991 to 1992. Observations of males and females are combined.

gametes, ripening, spawning, residual gametes absorbed, and rest or inactivity (Baqueiro and Stuardo 1977, Manzi et al. 1985; Hesselman et al. 1989).

The result of gonadal examination showed no clearly defined seasonal reproductive cycle for *M. aurantiaca* at Isla Espíritu Santo, Mexico. Spawning occurs all during the year as a continuous phenomenon. Nevertheless, through the year, two distinct periods are observed. The first one coincides with the warmer months of the year, from May to November 1991, where the maximum partially spawned population is 28% in August, the minimum is zero in September, and the monthly mean is 8.8%. The second period coincides with cooler months, from December 1991 to April 1992, and has two major peaks: December 1991 (22°C), when partially spawned individuals were 68% of the population, and March 1992 (22°C) with 40% of the population partially spawned. There was a minimum of 16% in January 1992, and the monthly mean was 38.9%.

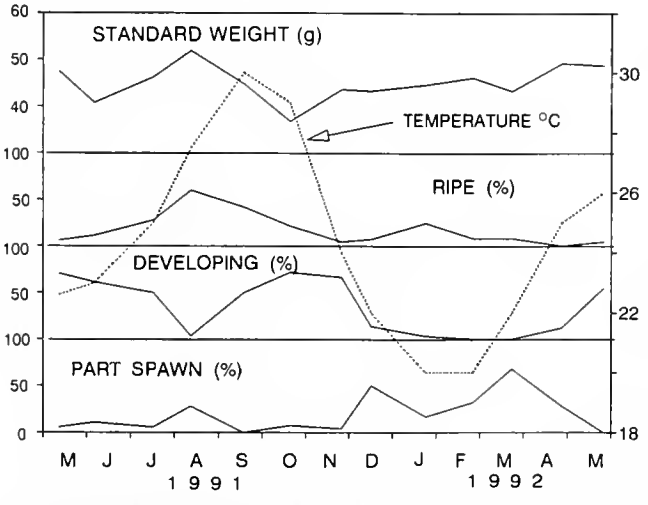


Figure 5. Relation of some gonadal stages (developing, ripe, and partially spawned) with water temperature and SW. Observations of males and females are combined.

Baqueiro and Stuardo (1977) studied a population of *M. aurantiaca* at Bahía Zihuatanejo, Mexico, and found that reproduction extends all year, independent of temperature and with two maximum periods: October (28.8°C), when spawning individuals were 62% of the population, and May (27.8°C), with 40% of the population spawning.

This does not indicate important differences in the reproductive pattern of this species because, per Sastry (1970), the reproductive cycle of a species living in different climatic zones can vary because a species' reproduction is in response to the environment. Differences in the gonadal cycle of different populations of *M. mercenaria* have been suggested as a result of different phenotypic responses to the variation of environmental factors (Porter 1964, Hesselman et al. 1989). In populations of other marine bivalves, regional and local differences of gonadic cycles have been suggested (Ropes and Stickney 1965, Holland and Chew 1974; Thompson et al. 1980, Eversole et al. 1980, García-Domínguez et al. 1993).

In some bivalves, like *Placopecten magellanicus*, reproductive output varies not only between populations from different sites, but also between consecutive years in a given population. This suggested that gamete production is strongly influenced by environmental factors (MacDonald and Thompson 1985), such as temperature and food availability set in a seasonal context, that condition both the reproductive effort and the timing of reproductive events (Bayne and Newell 1983).

In this work, only temperature was considered and we did not observe a very clear relation with spawning. Similarly, Baqueiro and Stuardo (1977) did not observe a clear relation between spawning and temperature. This phenomenon has been observed in other venerid clams like *M. squalida* and *D. ponderosa* (Baqueiro and Stuardo 1977). In *V. japonica* (Holland and Chew 1974), *V. antiqua* (Lozada and Bustos 1984), and *M. mercenaria* (Manzi

et al. 1985), a clear relation between temperature and gonadic activity has been established.

In the first period of the reproductive cycle of *M. aurantiaca*, we observed that changes in gonadic ripeness are reflected in SW variations. From May to August 1991, a gradual increase of the SW is seen until a maximum in August 1991, coinciding with a maximum of ripe and spawning clams. Subsequently, the SW and the frequency of ripe and spawning organisms decreased. Searcy (1984) found that, in some months, variations of SW of *Tivela stultorum*, a *Veneridae* clam, can be associated with spawning because, when SW decreases, the frequency of spawning organisms increases.

In the second period of the cycle of *M. aurantiaca*, corresponding to cooler months, a clear relation between SW and ripe stage, gametogenesis, or spawning is not observed. This indicates that SW is not a good index to quantify reproductive activity. Condition indices, such as SW, are important because of the possibility of using them to make general inferences about the reproductive cycle of a species (Hickman and Illingworth 1980), but they can be modified by other factors like rain (Searcy 1984) and nutritional state (Crosby and Gale 1990), neither of which were considered in this study.

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QUANTIFYING SEASONAL VARIATION IN SOMATIC TISSUE: SURFCLAM *SPISULA SOLIDISSIMA* (DILLWYN, 1817)—A CASE STUDY[1]

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ABSTRACT Condition indexes are commonly derived from bivalve species. Usable meat yields (UMY, in l/bu) from 181 daily landings of Atlantic surfclams, *Spisula solidissima* (Dellwyn, 1817), at a Virginia processing plant in 1974 and 160 landings in 1975 were used as an index in our analysis. The data were fitted to a basic sinusoidal model and a two-compartment sinusoidal model to demonstrate the utility of these models for quantifying cyclic events. The basic model, $x = x_0 + A\cos 2\pi t + B\sin 2\pi t$, is linear in its independent variables and fitted by multiple regression, with x = UMY, t = time in years, where x_0, A, and B are constants determined by the regression procedure (x_0 = mean UMY). Its alternate form is $x = x_0 + r\cos 2\pi(t - t_0)$, with x, x_0, and t as before, r = amplitude of the sinusoidal variation, and t_0 = time when the maximal UMY occurs; r and t_0 are related to A and B as $r = \sqrt{A^2 + B^2}$, and $t_0 = (1/2\pi)\tan^{-1}(B/A)$. The sinusoidal fit to the 1974 data was highly significant ($p < 0.0005$); therefore, the null hypothesis that the data are not a function of time was rejected. The annual mean yield, x_0, was 5.93 l/bu. t_0 was 0.45 (i.e., the maximal UMY occurred about mid-June), and the amplitude r was 0.730; thus, the difference between the lowest and highest yields, $2r$, was almost 1.5 l/bu. Similar estimates were determined from the 1975 data and the combined data. The fit was recalculated for both data sets after excluding *apparent* outliers. As expected, the root-mean-square residual (RMS_{res}) decreased, whereas the coefficient of determination (R^2) increased with the removal of the apparent outliers, but the fitted parameters were inconsequentially affected. A fit of the data to a two-component sinusoidal model, $x = x_0 + A_1\cos 2\pi t + B_1\sin 2\pi t + A_2\cos 4\pi t + B_2\sin 4\pi t$, modeled an annual variation with an asymmetric rise and fall. As a demonstration, the data were also fitted to a parabolic model, $x = a_0 + a_1 t + a_2 t^2$. Although this model produced fits comparably as close as the sinusoidal models, the coefficients are not interpretable in a simple manner, as is the case with the sinusoidal fits, and it does not allow asymmetric behavior.

KEY WORDS: *Spisula solidissima*, condition index, usable meat yields, seasonal variation, maximum, minimum, sinusoidal, parabolic

INTRODUCTION

Condition indexes are commonly derived for bivalve species. Various index models have been used; in general, the condition indexes reflect a relationship between soft tissue weight and the size of the cavity formed by the two valves. The indexes are used primarily to estimate seasonal meat quality or the effects of disease and pollution on meat quality. It has been suggested that a condition index for oysters be used to monitor pollution. Lawrence and Scott (1982) and Crosley and Gale (1990) reviewed and evaluated bivalve condition index methodologies; in each study, the authors recommended that a standardized index be used, although their models were somewhat different. The presentations and literature cited by those authors and references in the index of papers published in the *Journal of Shellfish Resource* (Castagna et al. 1992, Mann et al. 1993) provide an ample introduction to bivalve condition indexes.

Herein, we present methodologies for estimating seasonal indexes, the maximal and minimal annual values, associated confidence intervals, and tests of significance, regardless of the condition index used.

METHODS

Condition Index

To demonstrate the model, we use a condition index defined as usable meat yields (UMY) in liters per bushel of the Atlantic surfclam, *Spisula solidissima* (Dillwyn, 1817).

Source of Data

The UMYs were determined from daily landings of surfclams at the C&D Seafood Co. in Oyster, Virginia—181 landings totaling 167,564 bushels in 1974 and 160 landings totaling 270,170 bushels in 1975. In both years, the surfclams were harvested in an area approximately between 8.5 to 17.5 nautical miles offshore of Cape Henry and south to the North Carolina state line.

Sinusoidal Model

Loesch (1977) reported the relationship between mean monthly water temperature and mean monthly usable meat yield per bushel (mean UMY) for surfclams. The data in terms of *daily* UMYs are resurrected herein to assess parameters not previously considered in order to demonstrate the utility of sinusoidal functions for quantifying cyclic events exhibited in the life history of many marine species.

The basic sinusoidal model used was

$$x = x_0 + A\cos 2\pi t + B\sin 2\pi t$$

where

x = UMY in l/bu

t = time of the year (in years)

and the model parameters determined by regression procedure are

x_0 (annual mean UMY in l/bu), and A and B.

The sample data were fitted to the model by regressing x on $\cos 2\pi t$ and $\sin 2\pi t$. Although $\cos 2\pi t$ and $\sin 2\pi t$ both depend on t, they are *linearly* independent of each other and therefore can be used

[1]Contribution 1896 of The College of William and Mary, Virginia Institute of Marine Science, School of Marine Science, Gloucester Point, Virginia, USA.

as independent variables in a multiple linear regression procedure.

The model is alternatively expressed

$$x = x_0 + r \cos 2\pi(t - t_0)$$

where x_0 is the mean UMY, r is the amplitude of the sinusoidal variation, and t_0 is time when the maximal UMY occurs; r and t_0 are related to A and B as follows:

$$r = (A^2 + B^2)^{1/2}$$

and

$$t_0 = (1/2\pi) \tan^{-1}(B/A).$$ [see footnote 2]

Two-Component Sinusoidal Model

A feature of the basic sinusoidal model is that the rise and fall on either side of the maximum (or minimum) are symmetrical. This could be regarded as an unrealistic constraint to put upon the model. The problem is addressed by including additional terms to account for the additional feature. The appropriate extension of the sinusoidal model is to include *two* additional terms that constitute an additional sinusoidal component with a period of 6 months, i.e., one-half of the period of the basic sinusoid:

$$x = x_0 + A_1 \cos 2\pi t + B_1 \sin 2\pi t + A_2 \cos 4\pi t + B_2 \sin 4\pi t$$

The function is still linear in the parameters, and the fit can again be performed using a standard regression procedure. As with the one-component model, an alternative expression is:

$$x = x_0 + r_1 \cos 2\pi(t - t_0^{(1)}) + r_2 \cos 4\pi(t - t_0^{(2)}),$$

where r_2 is the amplitude of the second component. The interpretation of $t_0^{(1)}$ and $t_0^{(2)}$ in terms of time of maximum is, however, now more complex.

Alternative Quadratic (Parabolic) Models

For method comparison purposes, in addition to fitting the data to a sinusoid, we consider the quadratic function:

$$x = a_0 + a_1 t + a_2 t^2$$

as an alternative model. This function describes a parabola, containing a single maximum (when $a_2 < 0$) or minimum (when $a_2 > 0$). The position of the maximum (or minimum) is given in terms of the model parameters by the following expressions:

$$t_{max} = -\frac{a_1}{2a_2}; x_{max} = a_0 - \frac{a_1^2}{4a_2}$$

In order to treat the feature of asymmetry, a term in t^3 can be added to the quadratic model to give a cubic model:

[2]There are two angles in the range 0–2π radians whose tangent is B/A. The appropriate one lies in the quadrant where its *cosine* has the same sign as A and its *sine* has the same sign as B.

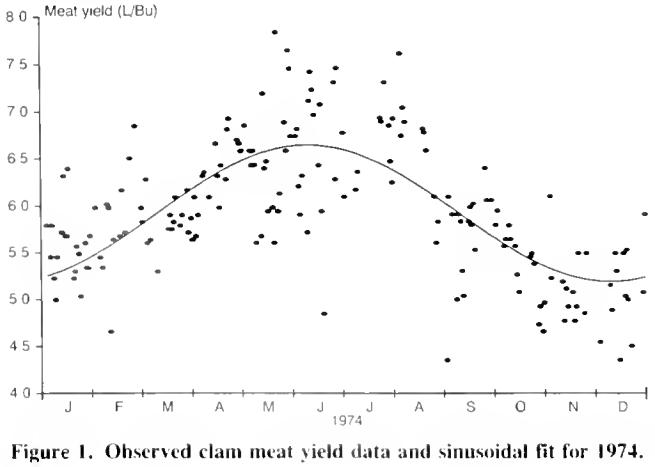

Figure 1. Observed clam meat yield data and sinusoidal fit for 1974.

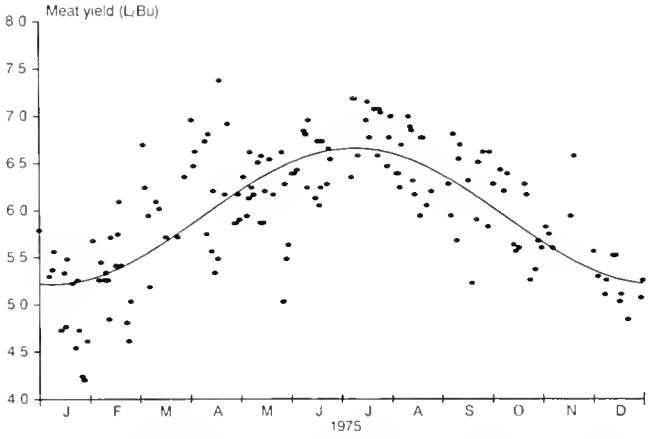

Figure 2. Observed clam meat yield data and sinusoidal fit for 1975.

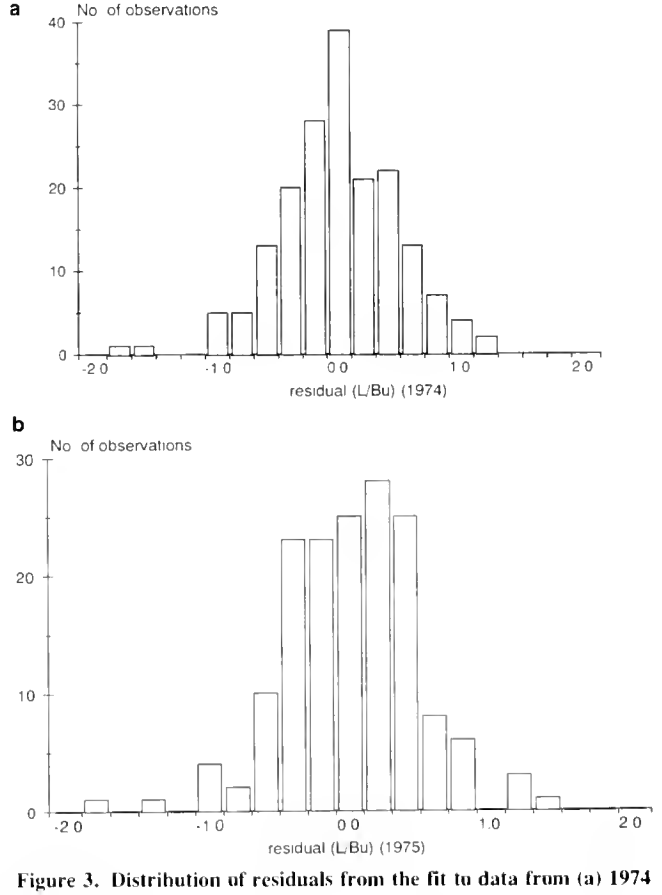

Figure 3. Distribution of residuals from the fit to data from (a) 1974 and (b) 1975.

TABLE 1.

Results from fitting a sinusoidal model $x = x_0 + A\cos 2\pi t + B\sin 2\pi t$ to the clam meat yield data. The model is alternatively expressed as $x = x_0 + r\cos 2\pi(t - t_0)$.

Year/Cut	N	x_0	A	B	r	t_0	R^2	RMS_{res}	p
1974, all	182	5.93	−0.682	0.261	0.730	0.45	0.53	0.496	<0.0005
1.96σ cut ("5%")	172	5.91	−0.720	0.232	0.756	0.45	0.63	0.416	
1.65σ cut ("10%")	162	5.93	−0.709	0.233	0.746	0.45	0.68	0.367	
1975, all	159	5.94	−0.718	−0.094	0.724	0.52	0.54	0.479	<0.0005
1.96σ cut ("5%")	150	5.96	−0.715	−0.134	0.727	0.53	0.66	0.375	
1.65σ ("10%")	146	5.96	−0.712	−0.141	0.726	0.53	0.69	0.354	
Both years	341	5.93	−0.706	0.097	0.713	0.48	0.50	0.502	<0.0005

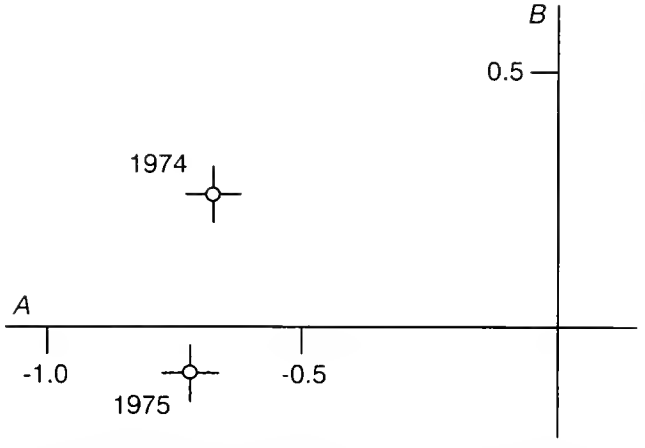

Figure 4. Geometrical representation of the sinusoidal fit parameters for the 2 years. The error bars represent the standard error in the estimation of the parameters.

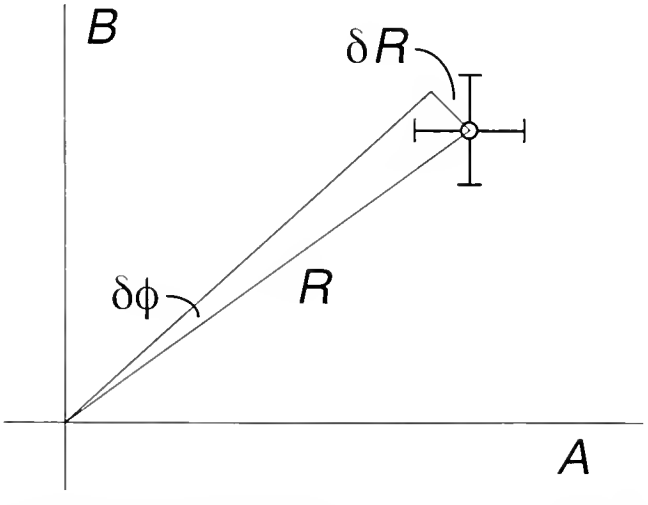

Figure 5. Geometrical representation of the derivation of the approximate error in the phase angle.

$$x = a_0 + a_1 t + a_2 t^2 + a_3 t^3.$$

The addition of this extra term usually results in completely different values for the coefficients obtained from the quadratic fit; furthermore, interpretation of the coefficients becomes obscure. In the data under consideration, another problem occurs. Over the range of t, the value of t^3 is very nearly linearly dependent upon t and t^2, the normal equations are ill-conditioned, and thus, the coefficients are very poorly determined. The cubic expression $x = a_0 + a_1 t + a_3 t^3$ can be fitted to the data and gives an asymmetrical curve about a maximum. It is, however, logically inadmissible because an attempt is being made to characterize an additional feature (the asymmetry of the slopes) *with the same number of parameters as was used in the quadratic fit*. The three parameters in the quadratic can be associated with three defining features of a parabola: the location of the maximum (or minimum) requires two parameters; the "shallowness" of the curve is the third. An additional parameter is therefore required to explain any asymmetry. Consequently, a cubic expression with the term in t^2 suppressed cannot characterize *independently* the asymmetry and location of the maximum. For this set of data, the quadratic fit is as far as one can go with simple polynomial models and this model does not incorporate any asymmetry.

RESULTS

Basic Sinusoidal Model: 1974 and 1975

The seasonal variation in UMY is obvious, as is the variation about the fitted curve (Figs. 1 and 2). The basic sinusoidal fit to

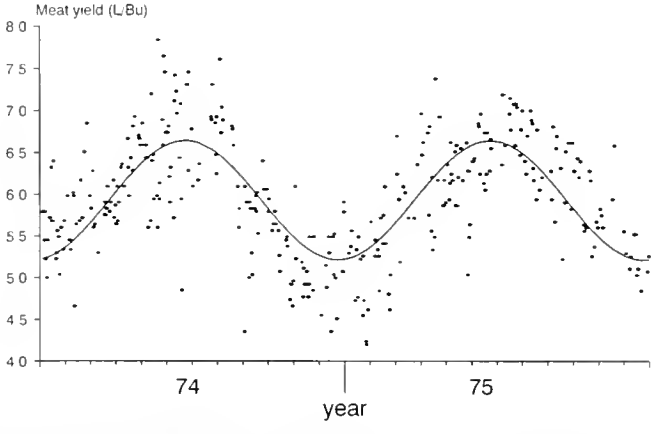

Figure 6. Observed clam meat yield data and sinusoidal fit for the 2-year period 1974 (74) to 1975 (75).

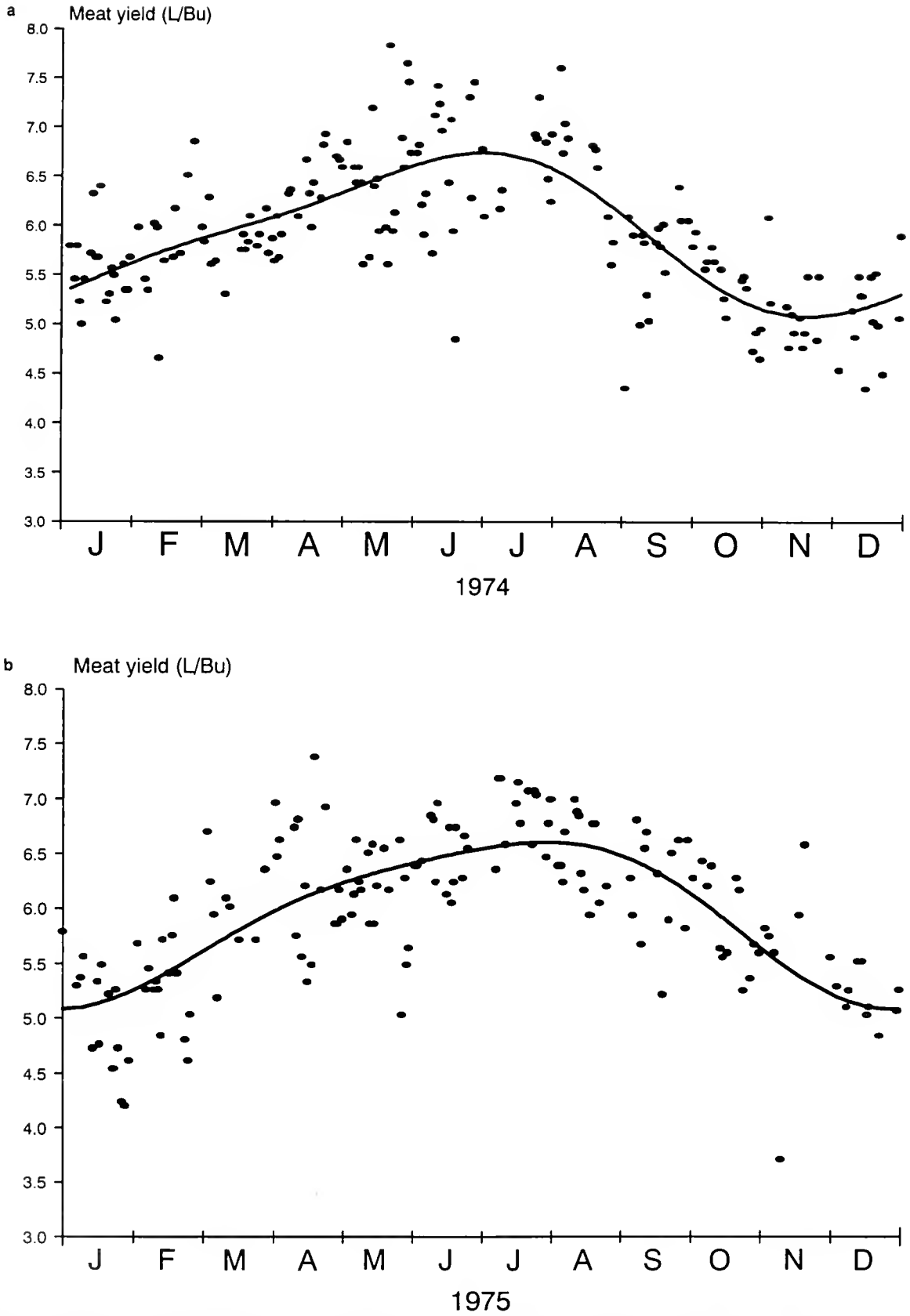

Figure 7. Two-component sinusoidal fit for (a) 1974 data and (b) 1975 data. The additional component (with a 6-month period) allows asymmetry to appear in the fitted function.

TABLE 2.

Coefficients for fit of a two-component sinusoidal function to the clam yield data. The fitted function is: $x = x_0 + A_1 \cos 2\pi t + B_1 \sin 2\pi t + A_2 \cos 4\pi t + B_2 \sin 4\pi t$.

Year	A_0	A_1	B_1	A_2	B_2	R^2	p	RMS_{res}
1974	5.927	−0.712	0.264	0.107	0.141	0.558	<0.0005	0.483
1975	5.937	−0.731	−0.082	−0.119	0.063	0.557	<0.0005	0.473

the 1974 data was highly significant ($p < 0.0005$); therefore, the null hypothesis that the data are not a function of time was rejected. The mean yield, x_0, was 5.93 l/bu, and t_0 was 0.45; thus, the maximal UMY occurred about mid-June. The amplitude r was 0.730, giving a difference between the lowest and highest yields ($2r$) of almost 1.5 l/bu.

For 1975 the sinusoidal fit was also highly significant ($p < 0.0005$); the mean yield, x_0, was 5.94 l/bu, and t_0 was 0.52; thus, the maximal UMY occurred about early to mid-July. The amplitude (r) was 0.724, and the difference between the lowest and highest yields was again almost 1.5 l/bu.

Sensitivity to "Outliers"

The fit was recalculated for both data sets after excluding *apparent* outliers that lay more than $1.96 \times RMS_{res}$ from the initial curves. The distributions of residuals from the fit to all of the 1974 and 1975 data are shown in Figure 3a and b. Assuming the residuals are normally distributed about the curve with a common standard deviation equal to the RMS_{res}, one expects about 5% of the datum points to be excluded. In fact, of the 182 observations in 1974, 10 were excluded by this criterion; in 1975, 9 observations from a total of 159 were excluded. Thus, the rate of occurrence of outliers is consistent with normality. As expected, the RMS_{res} decreased and R^2 increased with the rejection of apparent outliers (Table 1). The point estimates of the parameters, however, were inconsequently affected. The procedure was repeated for a cutoff of $\pm 1.65 \times RMS_{res}$, corresponding to an expected 10% rejection rate. Similar results were obtained and are presented in Table 1. All subsequent analyses use the whole set of observations.

Comparison Between Years

The fit coefficients for a single year can be represented by a point whose coordinates are A and B. The distance of this point from the origin is equal to the amplitude r, and the angular position of the point, measured counterclockwise from the x-axis, is an angle $\phi = 2\pi t_0$. Figure 4 shows the two points corresponding to the two years. The cross arms represent the standard errors in the estimation of A and B. They are all approximately equal to 0.053. As is discussed later, these errors are uncorrelated so that the standard error in r for each year is also approximately 0.053. The difference between the two amplitudes is 0.007; the standard error in this quantity is approximately $\sqrt{2} \times 0.053 = 0.075$. This gives a t-statistic of 0.09. There is, therefore, no evidence of a difference in amplitude between the years.

The treatment of the phase angles is different. Because the error in r is much smaller than r itself, one may say that, approximately:

$$\delta\phi = \textit{error in phase (in radians)} \approx \textit{(error in r)}/r$$

This result is demonstrated in Figure 5. For each of the years, the error in the phase is approximately $0.053/0.73 = 0.073$ radians. This, in turn, corresponds to $0.073/2\pi = 0.012$ years for the error in t_0. The error in the difference of the t_0 values is $0.012 \times \sqrt{2} = 0.017$. The observations shows:

$$t_0(1975) - t_0(1974) = 0.52 - 0.45 = 0.07$$

Thus, the t-statistic[3] is $0.07/0.017 = 4.1$. The appropriate degrees of freedom are very large (the total number of observations less two sets of three parameters), so that the Student's t-distribution is very nearly normal. The observed value is therefore highly significant. It can be concluded that 1975 was a year in which the maximum yield occurred later than in 1974. Qualitatively, a glance at Figure 4 indicates that the angle between the two dotted radii is much larger than can be accounted for by the size of the standard error.

Combined Data

Although it has been demonstrated that the best-fit sinusoidal variation is different in each of the two years, the whole two-year sequence of data can be fitted to the model. The fitted function

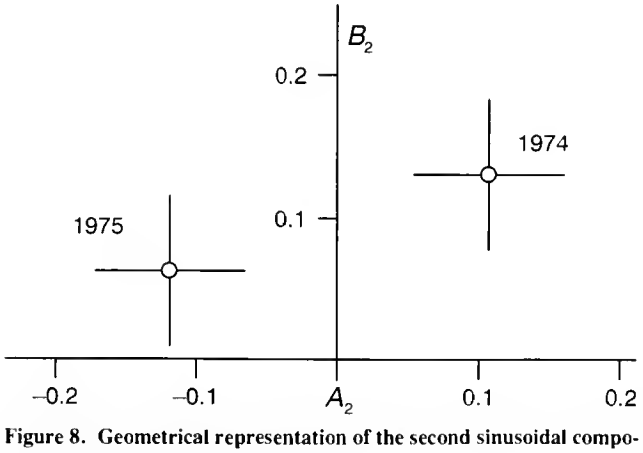

Figure 8. Geometrical representation of the second sinusoidal component for each of the years. The error bars represent the standard error in the estimation of the parameters.

[3]An unfortunate ambiguity of notation occurs here: the Student's t statistic is not to be confused with the use of t for time and the parameter t_0.

TABLE 3.

Fitted coefficients from the parabolic function $x = a_0 + a_1 t + a_2 t^2$ for the clam yield data for the calendar years 1974 and 1975. Also shown are R^2, the significance of the fit, and the root-mean-square deviation for the fit.

Year	a_0	a_1	a_2	R^2	p	RMS_{res}
1974	5.257	5.420	−6.151	0.475	<0.0005	0.524
1975	4.709	7.005	−6.800	0.532	<0.0005	0.483

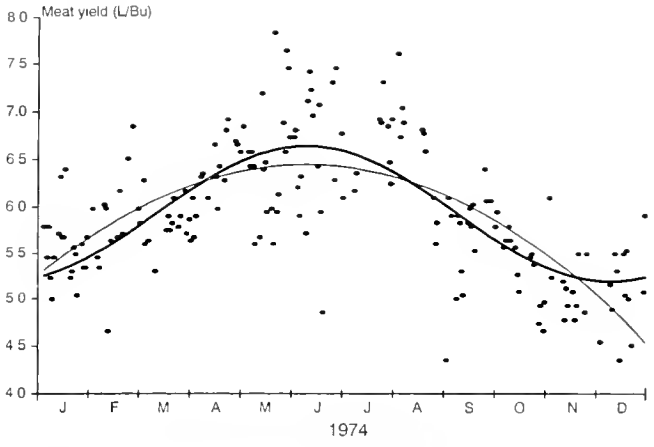

Figure 9. Quadratic and sinusoid fits for 1974 data. The heavy curve is the sinusoid.

together with the data is shown in Figure 6, and the fitted parameters are given in Table 1. The same annual variation is now imposed upon each year and thus represents a compromise between the two differing years. This is reflected in a reduced value of R^2 and an increased value of RMS_{res} compared with the single-year fits. For a data set that spanned several years, the overall fit would be useful in establishing a "typical" annual variation. This would then enable a classification of each year by comparing, in some suitable manner, the fit of one year's data with the "typical" year.

Two-Component Sinusoidal Model

The results of this fit for the 1974 and 1975 data are shown in Figure 7a and b. The coefficients for each year are given in Table 2. For both years, the incorporation of the extra component is statistically significant: in 1974, $p(A_2) = 0.039$ and $p(B_2) = 0.007$, and in 1975, $p(A_2) = 0.032$ and $p(B_2) = 0.237$. Note that the significant presence of the component is indicated by rejecting the null hypothesis, H_0:$r_2 = 0$. This is fully justified for only one of the parameters, A_2, B_2 being significantly different from zero. It is seen that, in each year, the fit reproduces an asymmetric rise

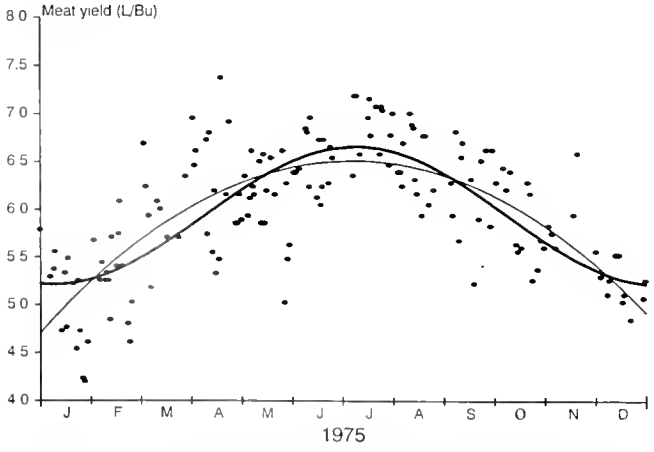

Figure 10. Quadratic and sinusoid fits for 1975 data. The heavy curve is the sinusoid.

TABLE 4.

Comparison of the parabolic and sinusoidal fits for the clam yield data for the 12 months July 1974 to June 1975.

Parabola	a_0	a_1	a_2	R^2	p	RMS_{res}
	5.216	0.058	6.999	0.488	<0.0005	0.520
Sinusoid	x_0	r	t_0	R^2	p	RMS_{res}
	5.787	0.752	5.646	0.546	<0.0005	0.490

and fall. The details of this feature appear to differ markedly between the two years. Qualitatively, the peak occurrence in 1974 is relatively sharp compared with that in 1975, where the persistence of larger values into August and September is quite marked. The difference between the years is statistically significant, as may be judged from Figure 8, which is a plot displaying the coefficients of the second component in a manner similar to that in Figure 4 for the first component.

Alternative Quadratic (Parabolic) Models

Table 3 shows the fitted values for the three parameters a_0, a_1, and a_2 for each of the years. Figures 9 and 10 show the datum points, the fitted parabola, and the fitted sinusoid for each of the years. Although the tightnesses of the fits, characterized by the values of R^2 and RMS_{res}, are of the same order as the sinusoids, the interpretation of the model parameters is less clear. An inverted parabola represents quite well the occurrence of the maximum yield approximately halfway through the year. The estimates of maximal UMY are $t_{max} = 0.441$ for 1974 and $t_{max} = 0.515$ for 1975, in quite good agreement with the sinusoidal fit. If, however, the data for the 12 months from July 1974 through June 1975 are fitted to a quadratic function, a totally different set of coefficients is obtained (Table 4), with in particular, $a_2 > 0$, i.e., a concave-up parabola with a single *minimum*. On the other hand, fitting the sinusoidal function to the July 1974 to June 1975 data gives values for the model parameters r and t_0 (Table 4) that are very similar to those from the two fits for the data from January through December in 1974 and 1975. These two fitted functions and the data are shown in Figure 11.

DISCUSSION

It is seen that the sinusoidal model is superior to the quadratic: the parameters are interpretable in terms of meaningful quantities such as annual mean value, the amplitude of the annual variation, and the phasing of the sinusoid, which relates to the time of occurrence of the maximum and minimum. The values of the parameters are relatively insensitive to where the year's data begin, whereas the quadratic fits give totally different descriptions. The values of the sinusoidal parameters for successive years can be compared in a meaningful way by considering the changes in the overall mean, the amplitude, and the phasing.

A further advantage of the sinusoidal characterization of the data is that the year-to-year comparison of amplitude and phase can be made quite simply with a graphical presentation. The parameters in the quadratic fit do not lend themselves to a similar simple geometric interpretation. Calculation of the errors in functions of the parameters (such as differences) is more complicated because the errors of determination in the parameters are corre-

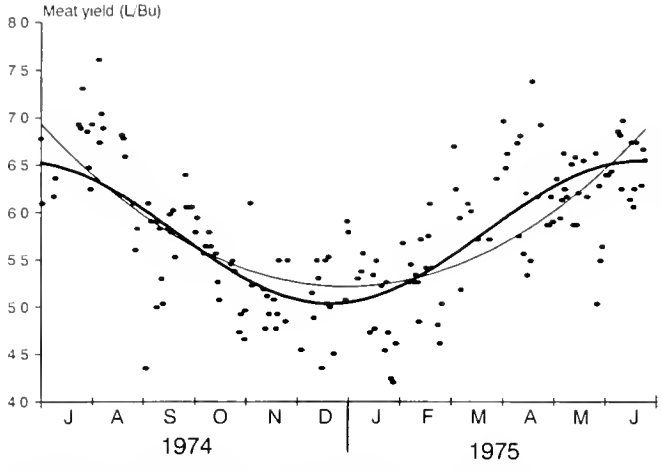

Figure 11. Quadratic and sinusoid fits for the July 1974 to June 1975 sequence. The heavy curve is the sinusoid.

lated. It is a feature of the sinusoidal functions that, if the observations are evenly spaced over a complete period (or multiple of periods), the determinations of the parameters are perfectly uncorrelated. For a relatively large number of points that are distributed approximately uniformly over a complete period, as is the case here, the correlation is negligible. The addition to the model of a second sinusoidal component with a 6-month period allows the characterization of an asymmetric rise and fall. The coefficients of the basic sinusoid are little affected by the addition of these extra terms to the model. This is because the determinations of the parameters are uncorrelated with each other as noted above.

The quadratic fit has no intrinsic merit and is merely an arbitrary parametrization of the observations. The model is suggested because the data for a complete year from January to December show the presence of an apparent maximum and the quadratic function can reproduce this feature. However, 12 months of data from July through June exhibit the opposite appearance, with the presence of a minimum, which gives rise to a totally different fit.

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AGE, GROWTH RATE, AND SIZE OF THE SOUTHERN SURFCLAM, *SPISULA SOLIDISSIMA SIMILIS* (SAY, 1822)

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ABSTRACT The age, growth rate, and size of the southern surfclam, *Spisula solidissima similis*, were determined by shell-sectioning techniques for clams collected from beach drift off of Wassaw Island, Georgia (Atlantic coast), and Cape San Blas, St. Joseph Bay, Florida (Gulf of Mexico coast). The shell-sectioning results for the Georgia population were validated by analysis of monthly size-frequency data for a field population collected from St. Catherines Sound, Georgia. The southern surfclam deposited a single age band within the shell matrix during the summer months at both sites. A distinct alternating pattern of translucent to opaque to translucent zones in the outer shell was evident for clams from both sites. The translucent zone is formed from May to October, whereas the opaque zone is formed from November to April. The annual band occurs within the translucent zone. According to the von Bertalanffy growth regressions, maximum size estimates of 76 and 122 mm for Georgia and Florida surfclam populations, respectively, are predicted. In Georgia, surfclams obtained a maximum shell length of 74 mm and were aged to a maximum of 4 years, compared with 106 mm in shell length and 5.5 years for clams from Florida. In Georgia, the majority of surfclams (92%) collected alive or from beach drift lived to a mean age of 1.5 years. Clams from Florida tended to survive to a mean age of 3.5 years. Clam cohorts collected from St. Catherines Sound grew to a mean shell length of 48 mm in 1990 and 47 mm in 1991 in 1.5 years before dying. Southern surfclams from Georgia were found to differ in age, growth rate, and size from a population from the Gulf coast of Florida, whereas both southern groups contrasted greatly with the Atlantic surfclam, *Spisula solidissima*, which has been shown to grow to 226 mm and has a lifespan of 37 years.

KEY WORDS: *Spisula solidissima similis*, surfclam, growth, size, longevity

INTRODUCTION

Because of the commercial importance of the Atlantic surfclam, *Spisula solidissima* (Dillwyn, 1817), much is known about its life history. Although the clam ranges from Nova Scotia to South Carolina (Abbott 1974), the fishery is centered between Virginia and Massachusetts. The clam obtains an average shell length of 100 to 130 mm (Abbott 1974), but has been reported to reach 226 mm in shell length (Ropes and Ward 1977) and lives to 37 years of age (Sephton and Bryan 1990). See reviews of the life history (Fay et al. 1983, Ropes 1980) of the Atlantic surfclam and its fishery (Ropes 1980 and 1982; Ropes et al. 1969, Yancey and Welch 1968) for further information.

Unlike the Atlantic surfclam, little other than taxonomic range and size is known about the life history of the southern surfclam, *Spisula solidissima similis* (Say 1822). This subspecies ranges from Cape Cod, Massachusetts, to both coasts of Florida and to Texas (Abbott 1974) and grows to 127 mm (Andrews 1977). Because of the dearth of life history information pertaining to this subspecies, we investigated its aquacultural potential by studying its relative growth rate, size, and longevity patterns from the coastal waters of Georgia and the Gulf coast of Florida.

METHODOLOGY

Articulated shells from beach drift were collected from two sites (Fig. 1): the beaches at the northeastern end of Wassaw Island, Georgia (Atlantic coast), and Cape San Blas, St. Joseph Bay, Florida (Gulf of Mexico coast). Articulated shells only were collected by hand from beach drift at low tide. Each shell was numbered and labeled as to collection site and date. Shells were collected from Wassaw Island on March 25, April 6, July 23, September 21 and 23, October 25, November 2 and 27, December 28, 1988, and January 6, 1989. Because of the distance factor, Cape San Blas, Florida, shells were collected only on March 9, June 15, and December 6, 1988.

Clams were returned to the laboratory where they were measured for shell length (i.e., anterior-posterior measurement), and aged by shell-sectioning techniques (see Rhoads and Lutz 1980, Rhoads and Pannella 1970). Growth curves for each clam were constructed by measuring each summer (translucent) and winter ring increment (opaque zone) per clam (Fig. 2). Population growth curves were constructed by averaging the shell length measure per ring per clam for each population. Mean shell length data per ring were fitted to a von Bertalanffy growth equation for each population using an IBM PC computer with the Fishery Science Applications System developed by the University of Rhode Island (Saila et al. 1988). The resulting initial parameters of the von Bertalanffy growth model generated from mean shell length data were then used as starting parameters for a SAS program (SAS Institute Inc., 1989) designed to determine the parameters of the growth model using all of the growth data.

To validate the results of the shell-sectioning technique for the Georgia sample, monthly samples of live clams were collected via trawling with a 12.2 m otter-trawl net or a 1 m planning dredge pulled by the 10.6 m R/V SEA DAWG in St. Catherines Sound, Georgia. Clams occurred in sand ridges at the mouth of the Sound in approximately 8 m depth. An otter trawl and planning dredge were used as collection devices, because they would knock off the tops of the ridges, momentarily suspending the surfclams within the water column, while the net or bag was being pulled through

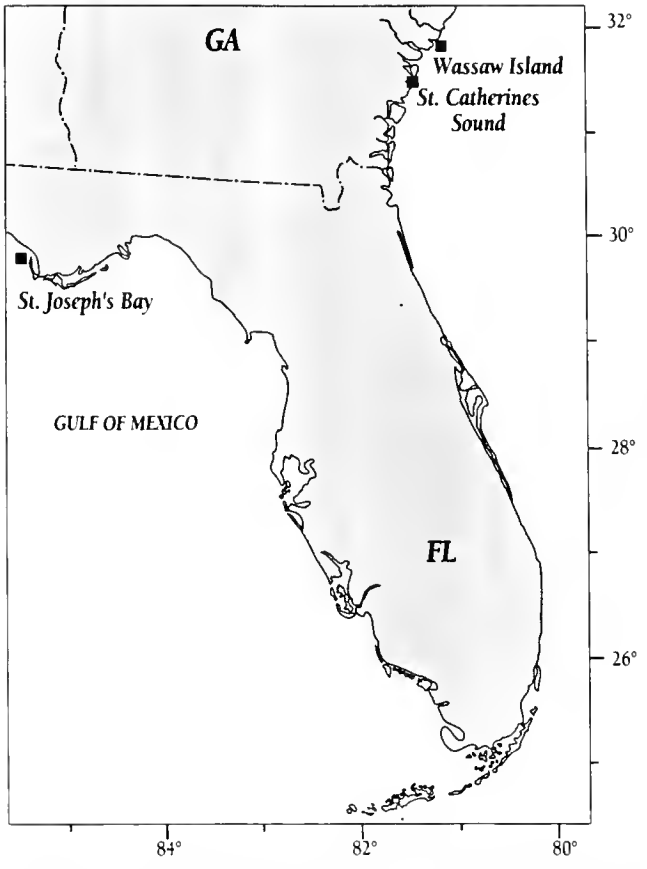

Figure 1. The sampling sites for *S. s. similis* shells collected from beach drift from Wassaw Island, Georgia, and Cape San Blas, St. Joseph Bay, Florida, and live animals collected from St. Catherines Sound, Georgia.

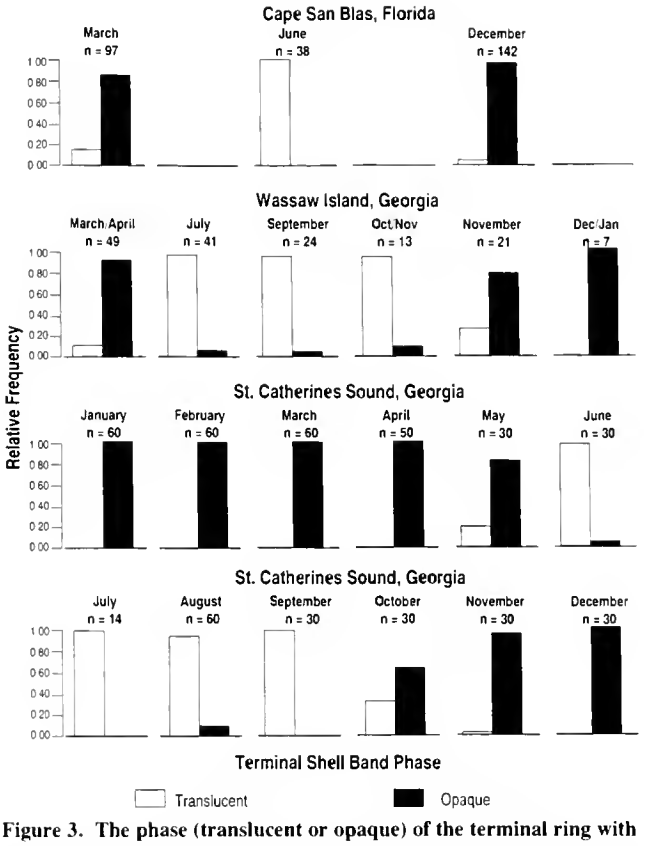

Figure 3. The phase (translucent or opaque) of the terminal ring with time of samples of *S. s. similis* collected from Wassaw Island, Georgia, Cape San Blas, St. Joseph Bay, Florida, and St. Catherines Sound, Georgia. Note that a lag time may occur between time of dominance between one phase and other for live animals and those shells from beach assemblages. See text for Discussion.

the impact area. Other sampling devices (i.e., benthic dredges, box corers, oyster dredges, Van Veen grab samplers) proved unsuccessful at sampling for surfclams. Trawling continued until at least 30 clams were obtained. Clams were measured for shell length.

RESULTS

It became apparent during the course of this study that the southern surfclam can be aged, even without sectioning, by holding the shell up to a strong light source. A distinct alternating pattern of translucent, then opaque, then translucent zones is observed in the outer shell (Fig. 2). Each of these phases represent 0.5 years' growth (Fig. 3). Within the shell matrix, the annual bands are laid down within the translucent zone. The first year's annual band is generally absent from within the shell matrix. Further year bands are quite distinct within the shell matrix.

A single annual band is laid down during the summer months

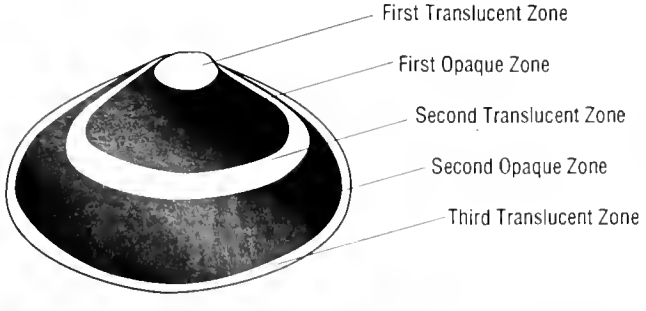

Figure 2. A graphic illustration of the translucent and opaque zonation observed in the shells of *S. s. similis*.

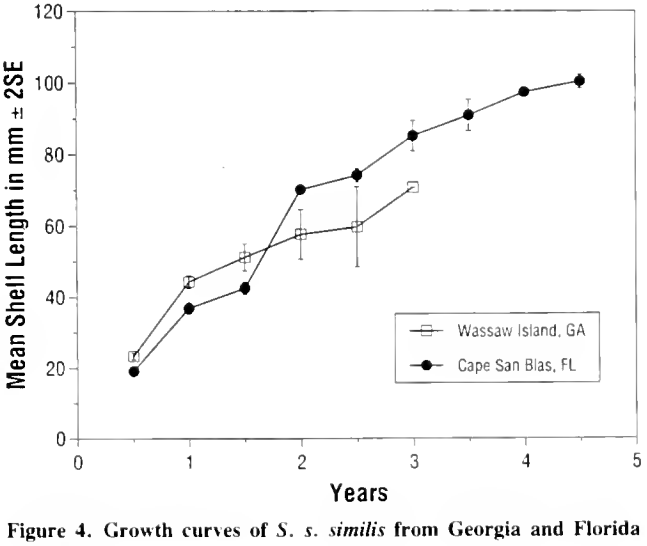

Figure 4. Growth curves of *S. s. similis* from Georgia and Florida populations.

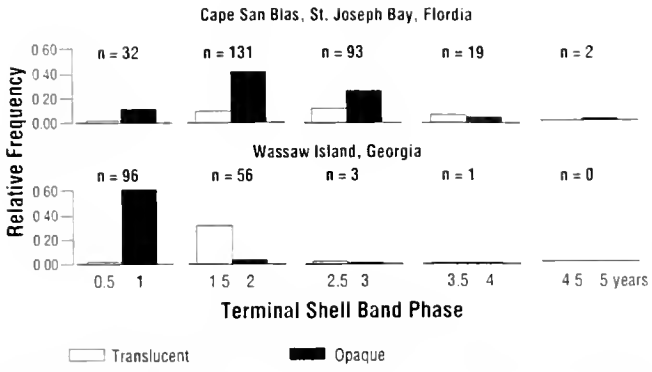

Figure 5. The percentage of *S. s. similis* that died in each 0.5 year increment.

for the southern surfclam. March and April surfclam samples from Wassaw Island showed 90% of the clams were in the opaque phase at the terminal ring (Fig. 3). By July, 95% of clam terminal rings were in the translucent phase. With fall, the dominance shifts back to the opaque phase. November (76%), December, and January (both 100%) samples showed the majority of terminal rings were in the opaque phase (Fig. 3). The same pattern was observed for clams from Florida (Fig. 3). For live clams collected from St. Catherines Sound, Georgia, the terminal ring is in the opaque phase January through April, before changing from a dominance of opaque phases in May to a dominance of translucent phases in June. Dominance of shells in the opaque phase occurs again by October (Fig. 3).

The growth curves of the two surfclam populations are given in Figure 4. Up to age 2, the surfclams from Georgia show growth similar to those from Florida. By age 2, most Georgia clams have died (see Fig. 5), and among those surviving, growth rate has slowed; however, growth continues in the Florida population. Shell length data per ring (translucent and opaque per year) were fitted to a von Bertalanffy equation, and the results are given in Table 1.

Figure 5 shows the stage of the terminal ring at time of death for both populations of surfclams. Maximum age for the Georgia surfclam was 4 years, with the majority (92%) dying before 2 years of age. In the Florida surfclam population, maximum age was 5.5 years with only 19.5% of the clams dying before 2 years. Only 3% of the Georgia clams lived longer than 3 years compared with 41% in the Florida population.

The shell length size-frequency data for surfclams from the two populations are given in Figure 6. Surfclams from Florida ranged in shell length from 21.1 to 105.5 mm, with a mean of 60.0 ± 1.21 (standard error [SE]) mm. Clams from Georgia ranged in size from 24.4 to 73.9 mm, with a mean of 43.9 ± 0.79 (SE) mm. The von Bertalanffy growth equation yielded maximum size estimates (L_{max}) of 76 and 122 mm for the Georgia and Florida clam populations, respectively (Table 1). Surfclams obtained a greater size in the Florida population because of their longer life span.

The monthly size-frequency data for the live clams collected from St. Catherines Sound are given in Figure 7. Beginning in January 1990, a single cohort at a mean shell length size of 33.5 mm occurred. This cohort grew to a mean size of 42.3 mm by April, when the occurrence of the new 0+ cohort emerged at a mean size of 20.2 mm. By September, the 0+ cohort had a mean size of 26.7 mm and the remaining 1+ cohort had a mean size of 47.9 mm. By September, the majority of the 1+ cohort, then approximately 1.5 years old, had died. No 1+ cohort clams were collected after September. The 0+ cohort grew little from August 8 ($\bar{x}$ = 27.3 mm) to December 5 ($\bar{x}$ = 30.7 mm), after which, rapid growth occurred from December ($\bar{x}$ = 30.7 mm) to February ($\bar{x}$ = 41 mm). From February to April ($\bar{x}$ = 43.5 mm), little growth occurred. At that time, clams were approximately 1 year old. The mean shell length of the cohort increased, reaching a maximum of 46.6 mm by July before dying off. By June, the new 0+ cohort emerged and obtained a mean shell length of 16.3 mm. By July, they reached 22.3 mm.

For the cohort that was monitored from emergence in April 1990 to death in July 1991, growth rate was 3.6 mm/month for the first year and 1.0 mm/month for the next 3 months, assuming that clams were spawned in March to April of 1990 (Kanti et al. 1993). The growth of the cohort over its lifespan (1.25 years) is 3.1 mm/month.

DISCUSSION

The southern surfclam occurs in a unique habitat within the sounds of coastal Georgia. Clams inhabit the sand ridges in areas at the mouth of sounds where a major creek or river, draining from the marsh system of a barrier island, enters into the sound. Because of the relatively high current speed present in these areas, the bottom tends to be sandy, forming a series of ridges along the bottom. Clams generally occur at the top of these ridges. Furthermore, with help from the Florida Department of Natural Resources, attempts at acquiring reproductively active surfclams from the west coast of Florida revealed that the only clams located (<10 mm in shell length) by scuba occurred in the top of sand ridges in Tampa Bay, at a site previously described with abundant clams (Godcharles 1971).

TABLE 1.

The results of a von Bertalanffy growth function for the Georgia and Florida *S. s. similis* populations. The von Bertalanffy growth equation is $L_t = L_{max}(1 - e^{-k(t-to)})$.

Population	L_{max} ± SE in mm	K ± SE	t_0 ± SE	R^2
Death assemblage For t = 0.5 year increments				
Georgia	75.77 ± 12.12	0.74 ± 0.26	−0.04 ± 0.10	0.9719
Florida	121.50 ± 11.85	0.46 ± 0.08	0.156 ± 0.04	0.9563

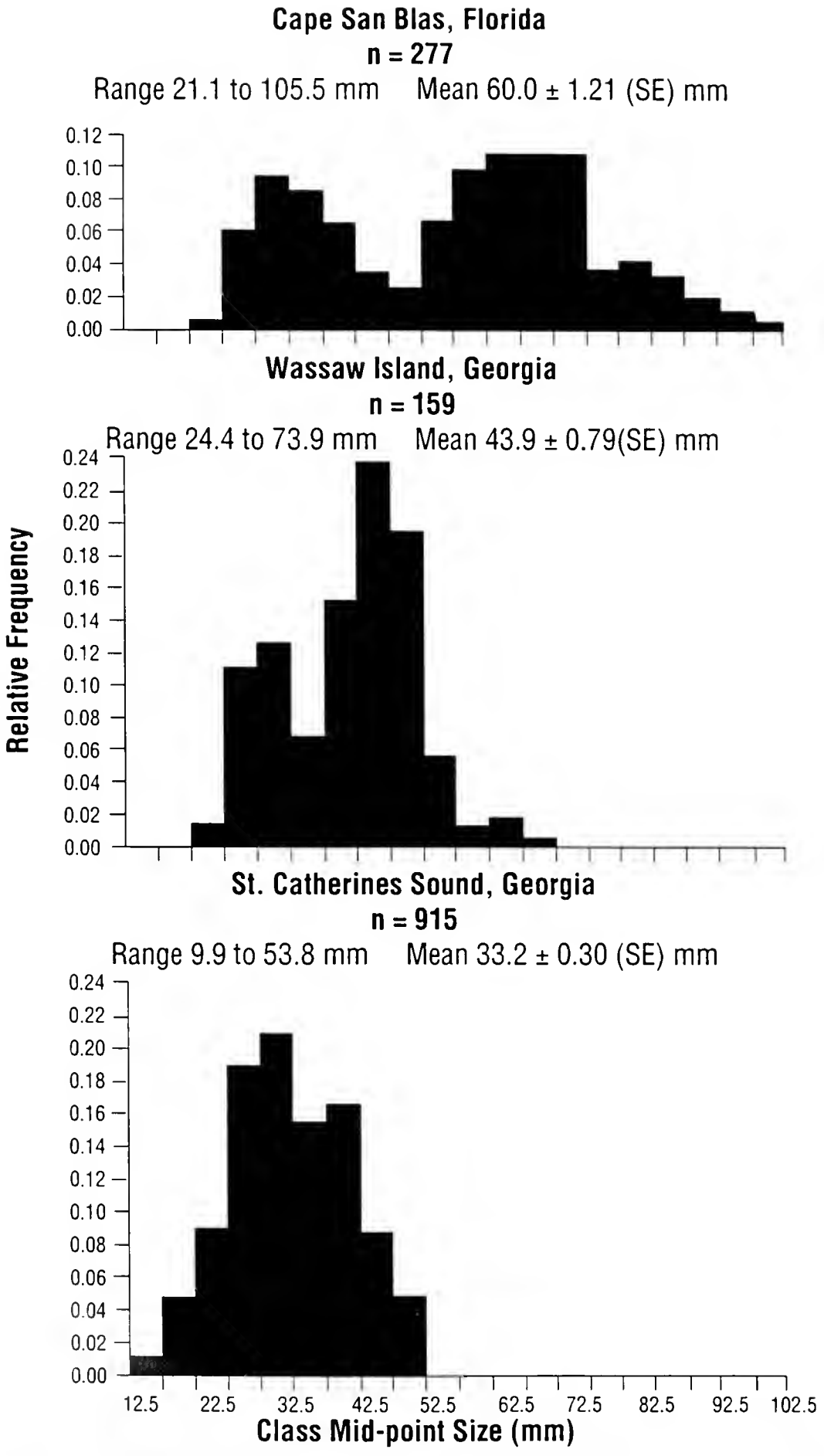

Figure 6. The size distribution of *S. s. similis* collected from Wassaw Island, Georgia, Cape San Blas, Florida, and St. Catherines Sound, Georgia.

The southern surfclam is easily aged, and shell-sectioning techniques are not necessary to achieve good estimates of age and size for this species from either Florida or Georgia coastal waters. The alternating pattern of translucent to opaque to translucent zones within the shell precludes the necessity of using expensive and time-consuming shell-sectioning techniques to determine age. However, for exact size measurements, the shell-sectioning technique may be required, because growth slows and the translucent zone becomes less obvious with increased age (>4 years). In the case of the Georgia clams from the sounds, shell sectioning is not required, because the first visible annual band is not laid down in the shell matrix until year 2, by which time the cohort has died out. This method of aging bivalves according to the alternating patterns of transparency in the shell has been used for *Spisula sachalinensis* in Japan and Korea (Sasaki 1981, Kang and Kim 1983, respectively), for *Meretrix meretrix* in China (Zhang and Fuxue 1988), and for the mussel *Mytilus galloprovincialis* in Japan (Hosomi 1983).

Although this method is reliable for determining the age and size of the southern surfclam, caution is needed when determining the exact timing of the transitional phases between translucent to opaque zones for specimens collected from death assemblages. For shells collected from the beach drift at Wassaw Island in October 1989, the majority (92%) were still in the translucent phases. By contrast, live specimens collected in October 1989 were all in the opaque phase. It is to be expected that a lag time occurred between the death of the clam and the time that the shell is washed ashore.

Southern surfclams are smaller and shorter lived than their northern counterpart. The results of both the shell-sectioning analysis of beach-death assemblages and size-frequency data for live cohorts for the Georgia surfclam population show that clams generally live less than 2 years. No live clams older than 1.5 years were collected, although a few clams older than 2 years of age of unknown origin were found in the beach drift samples from Georgia (Fig. 5).

For the surfclam population within the sounds of coastal Georgia, the 1+ year cohort dies during the August to September period. By August, ambient water temperatures may reach as high as 31°C (Winker et al. 1985). Sustained water temperatures of 30°C occur from June well into September in most years. Georgia also possesses roughly 30% of the total area of salt marshes along the Atlantic coast, so coastal waters experience heavy organic loading (Odum and de la Cruz 1967). The heavy organic loading, high ambient water temperatures, and consequent low dissolved oxygen levels (Winker et al. 1985) that occur during summer may be responsible for the dieoff of 1+ year class. Physiological testing on the southern surfclam is required to test this hypothesis.

The Atlantic surfclam requires an environment with ample dissolved oxygen. Thurberg and Goodlet (1979) found that oxygen levels of 1.4 mg l^{-1} at 10°C were lethal to 50% of adult clams exposed for up to 10 weeks. Major surfclam mortalities were recorded off of New Jersey in 1976, in which an estimated 62% of the standing stocks of surfclams were killed (Ropes 1980). Approximately 25% of the ocean quahog, *Arctica islandica*, population occurring within this area of anoxic conditions also died. Anoxic water combined with hydrographic water conditions, organic loading of coastal waters, and a massive bloom and dieoff of a dinoflagellate were responsible (Steimle and Sinderman 1978). Low oxygen levels adversely affected surfclam burrowing by lowering the rate of activity (Savage 1976). Furthermore, water temperature, which is inversely proportional to dissolved oxygen levels, may play a role. Northern surfclams tolerate water temperatures from 14 to 30°C (Loosanoff and Davis 1963), with juvenile clams surviving higher temperatures better than adults (Saila and Pratt 1973). Experimental field grow-out studies, using *S. solidissima* stocks from Connecticut and Delaware cultured in coastal Georgia, showed excellent growth and survival of clams from October to May, but total mortality if clams are not harvested in May (Goldberg and Walker 1990, Walker and Heffernan 1990). Ambient water temperatures may reach 28 to 30°C (lethal levels) by the end of May in Georgia.

From the monthly size-frequency data for the St. Catherines Sound population (Fig. 7), an accurate record of growth for the southern surfclam can be constructed. In January 1990, a single cohort exists. This cohort grew little from January ($\bar{x}$ = 33.5 mm) to February ($\bar{x}$ = 33.7 mm) but grew rapidly from February to May, followed by a slowing phase from May ($\bar{x}$ = 43.6 mm) through August ($\bar{x}$ = 44.3 mm). By August, the 1+ cohort was dying off and was completely gone by October 1991. This cyclic form of growth was closely matched by its gametogenic cycle (Kanti et al. 1993). In early winter, surfclams have entered the late active stage of their maturation process (Kanti et al. 1993). Thus, a large portion of their energy budget probably was devoted to gametogenesis. During March and April, clams have reached their final maturation phase and food is again available for growth. By April, the clams have begun spawning and food presumably is being devoted primarily to the build-up of somatic reserves with shell growth, consequently, slowed.

The 0+ cohort was detected in samples during May ($\bar{x}$ = 20.9 mm) and grew rapidly until August ($\bar{x}$ = 27.3 mm), when growth rate slowed down. Growth remained slow until November before it accelerated again. This growth slowdown during summer presumably is the result of the physiological stress of high water temperatures, low dissolved oxygen levels, and high organic loading (Steimle and Sinderman 1978), which apparently kill the more susceptible adults (Saila and Pratt 1973). The 0+ cohorts growth slowed during winter, when sexual maturation probably required the majority of the energy available.

Growth rates of Georgia and Florida surfclam populations were similar during 0+ year groups. Overall, the Florida population continues to grow at a relatively good rate during the second year before the rate begins to decline. For the Georgia population, the growth rate decreases significantly after the first year.

Jones et al. (1978), studying an inshore-and-offshore population of surfclams off of New Jersey, found a similar growth pattern between the two sites for the first 3 years, after which, the growth in the inshore site was markedly reduced. In addition, clams from the inshore site obtained a lower maximum size, grew slower, and had a shorter lifespan (Chang et al. 1976, Jones et al. 1978). No explanation was presented to explain the differences observed. Cerrato and Keith (1992) also found that a surfclam population from an estuary in New York was dominated by two age classes and had an apparent life span of 10 years, whereas, in an inshore population, clams ranged in age from 2 to 22 years with no age class dominance. Adults from the estuarine environment grew slower and obtained a smaller maximum size than did clams from the inshore population. They hypothesized that physiological stresses due to reduced salinity and more extreme temperatures found under estuarine conditions were probably responsible for the observed differences in population parameters.

A substantial body of literature shows that, with decreases in latitude, greater growth rates, earlier age at maturity, and shorter

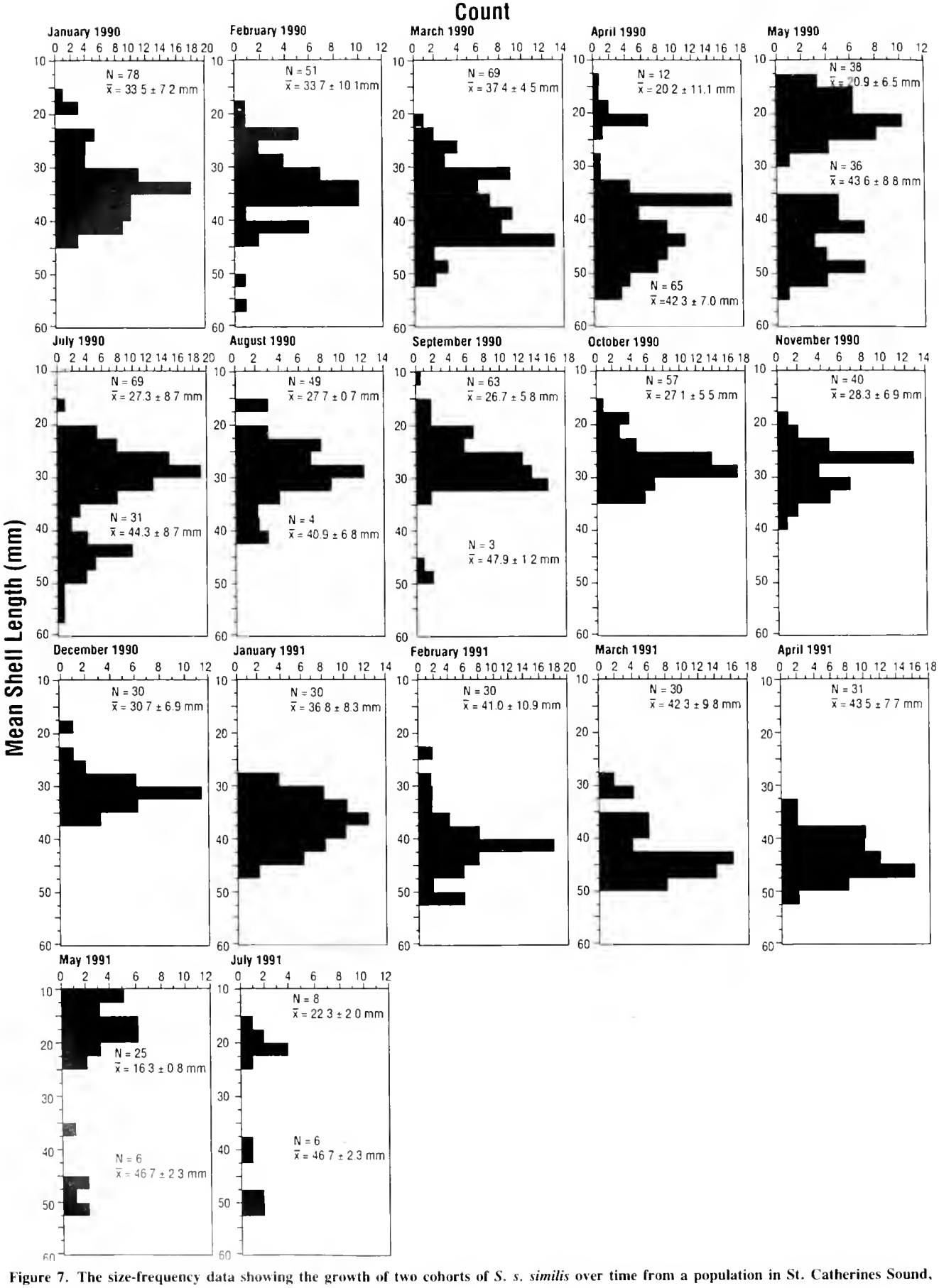

Figure 7. The size-frequency data showing the growth of two cohorts of *S. s. similis* over time from a population in St. Catherines Sound, Georgia.

TABLE 2.

Annual growth increments of the northern surfclams versus the southern surfclams for the first 3 years of life from natural populations along the Atlantic coast of the United States. (Based on Table 4 of Ropes 1980).

Location	Shell Length at: 1 year	2 years (in mm)	3 years	Source
S. solidissima				
P.E.I. Canada[a]	15.9	34.9	53.2	Kerswill 1944
Canada	15.0	31.0	49.0	Caddy and Billard 1976
Cardigan Bay	12.0	46.2	72.8	Sephton and Bryan 1990
Northumberland Strait	32.2	57.9	77.6	Sephton and Bryan 1990
Gulf of St. Lawrence	23.5	46.0	64.2	Sephton and Bryan 1990
Monomoy Point, MA	25.4	40.6	81.3	Belding 1910
Long Island, NY	34.0	60.0	79.0	Ropes 1980
Pt. Pleasant, NJ				Jones et al. 1978
Inshore	38.0	62.0	79.0	
Offshore'	29.0	55.0	81.0	
Barnegat Bay, NJ	34.0	56.0	73.0	Chang et al. 1976
Offshore, NJ	47.2	79.2	100.8	Loesch and Ropes 1977
Central, NJ	45.7	78.7	99.1	Ropes 1980
Ocean City, MD	39.0	57.0	94.0	Chang et al. 1976
Chincoteague Bay, VA	42.2	68.6	90.5	Ropes et al. 1969
S. s. similis				
Wassaw Island, GA	44.3	57.7	70.8	This study
St. Joseph Bay, FL[b]	37.1	78.8	92.3	This study
St. Catherines Sound, GA	43.5			This study

[a] P.E.I., Prince Edward Island.

[b] Gulf of Mexico coast.

lifespans occur for intraspecific comparisons of marine bivalve species (Newell 1964). The growth rate of the southern surfclam is comparable to that of the Atlantic surfclam in its southern distributional range (Table 2). An increase in growth rate for the Atlantic surfclam with a decrease in latitude does appear evident (Table 2 and Ropes 1980). On the basis of the mean size achieved at 1 year, the southern surfclam achieves size comparable to that of the northern species at its lower distributional limit for both the Georgia and the Florida populations. At 2 years, only the Florida population is comparable, because the majority of the Georgia clams have died.

In terms of achieving sexual maturity at an earlier age, the southern surfclam in Georgia behaves more like a semelparous species rather than an iteroparous species, like the northern species. The 0+ cohort begins sexual development within its first year and spawns at approximately age 1 at a size of 40 mm (Kanti et al. 1993). After spawning, clams survived for another 5 to 6 months before the cohort (1+ year) died off. The gametogenic cycle of the Florida population was not studied, but it is likely that it would be similar in terms of age and size at sexual maturity to that found in the Georgia population. For the Atlantic surfclam, Sephton and Bryan (1990) stated that sexual maturity occurred at 80 mm in shell length at an age of 4 years for a population of clams from Prince Edward Island, Canada, whereas, Ropes et al. (1969) observed that sexual maturity occurred at 45 mm and 1 year of age for clams from an inshore population in Chincoteague Inlet, Virginia. Belding (1910) in Massachusetts found that sexual maturity could occur in 1 year olds at a size of 39 mm, but that the majority of clams matured at 2 years of age and at 67 mm in shell length.

The Atlantic surfclam in offshore populations commonly attains ages of 25 and occasionally 30 years (Jones et al. 1978). Southern surfclams in Georgia from inshore populations rarely survive 2 years. By contrast, Florida clams reach a mean age of 3.5 years, with some individuals surviving to 5 years. Thus, there is a definite decrease in lifespan with a decrease in latitude when comparing the southern and the Atlantic surfclams.

In Georgia, the southern surfclam resembles the life history traits of *Spisula subtruncata* and *Notospisula trigonella*. It is interesting to note that *N. trigonella*, *S. subtruncata*, and the Georgia surfclam are all basically annual species and occur within the estuary, whereas, commercial *Spisula* species (*S. polynyma*, *S. sachalinensis*, and *S. solidissima*) are long-lived species that occur primarily offshore. For the three estuarine species, a single recruitment period occurs: *N. trigonella* recruits from August to November in Australia (Jones et al. 1988), *S. s. similis* recruits from April to June in Georgia (Kanti et al. 1993), and *S. subtruncata* recruits over the summer in Italy (Ambrogi and Ambrogi 1987). These estuarine species also achieve the smallest maximum size of the *Spisula* species; *N. trigonella* reaches 21 mm (Jones et al. 1988), *S. s. similis* in inshore areas of Georgia reaches 57 mm (this study), and *S. subtruncata* grows to 22 mm (Ambrogi and Ambrogi 1987). The stressful environmental parameters common to estuarine conditions may well regulate the lifespan of these *Spisula* species.

The feasibility of establishing a fishery for this animal is poor, but some potential may exist at least for the development of an aquacultural product in Georgia. The southern surfclam occurs in a rather unique habitat in Georgia. These habitats are generally small in overall area and occur in only half of the sounds of coastal Georgia. Although no density estimates have been determined for surfclams in these habitats (because of the inefficiency of most bottom-sampling devices within these sand ridges), it is our opin-

ion that overall densities are low and insufficient to support even a small natural fishery. The distribution pattern of surfclams in offshore areas is unknown, but past natural resource surveys by the R/V SILVER BAY during 1959 and 1960 failed to report any findings of surfclam beds in offshore areas of Georgia (Cummins 1966); however, some surfclams were found at one site off of North Carolina (Cummins et al. 1962). The sampling devices used in these surveys were geared toward harvesting larger sized clams (R. Cummins, Jr., 1992, personal communication), so small-sized southern surfclams may have been missed during those surveys.

There is some potential for the development of an aquaculture product for the southern surfclam. Clams attained a mean size of 40 to 50 mm in shell length, which is about the ideal size for a raw or steamer product. In previous studies evaluating the aquaculture potential of growing the Atlantic surfclam over the winter in Georgia, we had targeted 50 mm as an ideal size for a steamer product (Goldberg and Walker 1990). In those studies, 10 to 15 mm seed planted in October grew to mean sizes of 45 to 60 mm by May, depending on location, initial planting size, tidal-planting height, cage-mesh size, and substrate type (Goldberg and Walker 1990, Walker and Heffernan 1990). To develop southern surfclam aquaculture, hatchery and nursery-rearing protocols will need to be developed. Increasing the growth rate of the southern surfclam by selective breeding programs or by triploidy induction may yield larger clams for production purposes than are currently found in natural inshore populations in coastal Georgia. These avenues of research are being investigated.

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EXPOSURE OF THE SANDY-BEACH BIVALVE *DONAX SERRA* RÖDING TO A HEATED AND CHLORINATED EFFLUENT 1. EFFECTS OF TEMPERATURE ON BURROWING AND SURVIVAL

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ABSTRACT The potential impact of the thermal plume from a nuclear power station on burrowing and survival of the bivalve *Donax serra*, which inhabits a nearby sandy beach, was investigated. Median lethal time and median lethal temperature were used to define the size-related upper thermal tolerance of *D. serra*. Small individuals would best tolerate heated effluent from the power station. Temperatures above 32°C were lethal to all sizes of *D. serra*. After extended exposure to temperatures between 24 and 29°C, 50% of the animals no longer remained buried. Because these temperatures occur in the thermal plume, such displacement from the sand can result in exposure to predation as well as possible stranding on the beach.

KEY WORDS: Bivalve, burrowing, *Donax serra*, effluent, sandy beach, survival, temperature

INTRODUCTION

The burrowing bivalve *Donax serra* Röding inhabits the intertidal zone of high-energy sandy beaches along the southern African coastline, where it often comprises 80 to 90% of the total macrofaunal biomass (McLachlan 1977a and b, Bally 1981, Cook and Birkett 1984). It is the largest member of its genus, attaining a shell length of 80 mm and a dry tissue mass of 5 to 6 g. South Africa's first nuclear power station was constructed in the immediate vicinity of an expansive sandy beach densely populated by *D. serra*. The power station uses sea water as a coolant, the heated effluent being discharged directly into the surf zone at temperatures approximately 10°C warmer than ambient. Inshore thermal plumes are thereby created that have the potential to range in temperature from 18 to 27°C. As part of a larger ecological impact assessment (Cook and Birkett 1984 and 1986), a study of the possible effects of the plume on the physiological fitness of *D. serra* was undertaken.

The extensive research on the thermal tolerances of marine organisms has focused strongly on the survival of fish larvae and juveniles near power plants using sea water as a coolant (reviewed by Schubel et al. 1978) and, from a different perspective, the ways in which intertidal animals survive desiccation and increases in temperature during aerial exposure (reviewed by Newell 1979). The thermal tolerance of an organism is a product of an interaction between exposure temperature and the duration of that exposure and it is imperative to recognize both of these factors in any experiments designed to determine upper lethal limits. Earlier data were obtained by slowly heating the water in which animals were contained at an arbitrary rate of 1°C in 5 to 10 minutes (Huntsman and Sparks 1924, Henderson 1929, Broekhuysen 1940, Evans 1948, Southward 1958, McLachlan and Erasmus 1974). This method resulted in the estimation of upper lethal limits higher than conditions experienced in nature (Newell 1979).

The importance of the interaction between the duration of exposure and temperature was recognized by researchers working on fish (see Schubel et al. 1978) and the methods they established are now widely used to measure temperature tolerances of marine organisms in terms of both natural and human-induced changes. In this approach, the time taken to reach 50% mortality is determined, or alternatively, the temperature at which 50% mortality occurs can be used as the criterion of upper temperature tolerances (Kennedy and Mihursky 1971). In this study, both median lethal time and median lethal temperature are used to define survival limits and burrowing responses in *D. serra*. This is the first in a series of three papers, the subsequent two considering the effects of chlorine on burrowing and survival and the effects of temperature and chlorine on the heart rate of *D. serra*.

MATERIALS AND METHODS

Collection and Maintenance

Bivalves were collected from Ouskip beach 1.5 km south of the power station, an area unaffected by the thermal plume. Animals were kept in flowing sea water in 25 l tanks fitted with air-lift pumps to facilitate the circulation of water at 15°C through a bed of sand and gravel and fed mixtures of natural detritus and the alga *Tetraselmis suecica*. Experiments were designed to investigate the effect of acute and stepwise changes in temperature on survival (percent mortality) and burrowing.

Acute Temperature Exposure Experiments

These experiments simulated acute temperature changes as may arise when the prevailing wind direction shifts to an onshore northwesterly, resulting in the concentration of the thermal plume and a rapid increase in temperature in the surf zone (Rattey and Potgieter 1987). Experimental temperature changes used in the laboratory exceeded natural swift variations in ambient temperatures to which *D. serra* may be acclimatized.

The temperature at which 50% of test animals died after acute exposure for 4 days was defined as the median lethal temperature (LT_{50}) (see Kennedy and Mihursky 1971). Median lethal time, that is, the time to 50% mortality at a particular temperature (Newell 1979) was used to further define mortality in terms of thermal resistance lines.

D. serra were grouped into three size classes on the basis of shell width (in millimeters), measured as the greatest distance between the dorsal and ventral shell extremities. Juveniles were divided into two groups, namely <7 mm and between 7 and 35

mm, whereas breeding individuals were taken as >35 mm. This grouping was chosen because it corresponds closely to the three major growth cohorts in the population (Cook and Birkett 1986) and the size-related intertidal distribution of *D. serra*. The large animals occur subtidally, whereas the smallest individuals are most abundant at midtide levels.

Forty bivalves from each size group were placed in 20 l tanks at temperatures between 20 and 38°C at 2°C intervals for exposure times of 1, 3, 6, 12, 24, 48, 72, and 96 hours. The tanks were fitted with air-lift pumps that circulated sea water through a bed of sand, thus ensuring an even temperature throughout. Percent mortality was assessed together with the upper temperature limit at which 50% of the bivalves retained an ability to burrow (BT_{50}) (see Ansell et al. 1980a). Throughout the experiment, controls were maintained at 15°C and any incidental deaths in this group were used to correct data. During each observation, the numbers of dead individuals, individuals that were buried, and the number that were not buried but in a state of stress were noted. Stress was evident from shell gaping and flaccid siphons and foot. The absence of reaction to mechanical stimulation in the foot and siphons and the mantle edge and adductor muscles were taken as criteria for death in the same manner as used by Ansell et al. (1980a) and Ansell and McLachlan (1980). The absence of valve closure on tactile stimulation of the cruciform muscle also proved a useful criterion. If death was uncertain, possible recovery was monitored by placing the individual in fresh, circulating sea water at 15°C for 2 days.

Four individuals from each exposure temperature were transferred daily to 15°C in order to monitor recovery. The criterion for recovery was the ability of 50% of the test individuals to burrow and ventilate after 4 days at 15°C.

Stepwise Temperature Exposure Experiments

These experiments were designed to establish whether stepwise exposure to the temperatures above would improve survival rate. Bivalves, approximately 120 within each size group, were progressively exposed to temperatures from 14 to 36°C at 2°C increments per day over 16 days. Sea water, at the appropriate experimental temperature, was replaced every second day. Recovery was determined by transferring an individual to 15°C after 24 hours of exposure to a test temperature after stepwise introduction to that temperature. The criteria for recovery were the same as used before.

In all experiments, the LT_{50} was determined graphically. Numbers dead, expressed as percentages of the original number, were plotted for each temperature. Time interval and LT_{50} were determined from the resulting plots. The temperature at which 50% of the bivalves originally present had burrowed, BT_{50}, was determined in a similar manner. The same method has been used by a number of researchers (Bodoy and Massé 1977 and 1978, Ansell et al. 1980a and b, Ansell and McLachlan 1980). Probit analysis (Finney 1964) enables a statistical measure of 50% lethal limits, and this was applied to some of the data. This method provided similar or identical results, as found by Lent (1968), and because it is a long and tedious procedure, the graphical method described was used throughout.

Median lethal time was also determined graphically by plotting percent mortality for each exposure temperature against exposure time on a logarithmic scale. In turn, median lethal times were plotted as a function of exposure temperature on a semilogarithmic scale to obtain thermal resistance lines for each size group; these lines define the zone of resistance of an organism (Newell 1979).

RESULTS

Acute Temperature Exposure Experiments

The rapid decline in LT_{50} values within the first 24 hours (Fig. 1) indicates the extreme sensitivity of the bivalves to temperatures >31°C, where a 2°C increase sometimes meant the difference between 0 and 100% mortality. Between 24 and 96 hours, tolerance limits were size dependent, a stable LT_{50} of 30°C being reached after 48 hours for the small ones, after 72 hours at 28°C for individuals >7 but <35 mm, and at 27°C for large *D. serra* (Fig. 1). Thus, the smallest showed marginally better survival of acute temperature exposure, resulting in a steady LT_{50} value 3°C higher than that for the largest animals after 4 days.

It is evident from thermal resistance lines (Fig. 2) that the survival time for all *D. serra* shows an exponential decline as exposure temperature increases. Size-related differences in temperature tolerance were only marginal because there were no significant differences between the slopes and elevations of the three resistance lines in Fig. 2 (slopes: $F = 0.42$, $df = 18$, $p > 0.25$; elevations: $F = 1.89$, $df = 20$, $0.25 > p > 0.10$). Data were therefore combined to produce a single zone of tolerance where the resistance line $(Y) = 42.02 - 7.72(\log X)$. With extrapolation, this line defines the upper lethal temperature as 42°C and the incipient temperature as 25°C (highest temperature to which an organism can be continuously exposed for an indefinite period without increasing mortality).

BT_{50} values indicated that the smallest individuals burrowed immediately on transfer to temperatures near the lethal limits (36 to 38°C), whereas large ones displayed initial thermal shock by lying on the surface for about 1 hour before burrowing (Fig. 3). Longer exposure (>1 hour) to these high temperatures resulted in the reemergence of *D. serra* <35 mm, followed by thermal paralysis and death. BT_{50} values increased for large *D. serra* as a consequence of many individuals on the sand surface burying themselves after initial thermal shock.

Between 12 and 48 hours, BT_{50} values stabilized for all sizes, 50% of the larger animals remaining buried at temperatures 2 to 3°C higher than individuals <35 mm. Over the last 2 days of the experiment, values remained steady for the smallest bivalves but declined to 26°C for the larger ones as they gradually emerged from the sand to lie on the surface with their shells gaping. At this

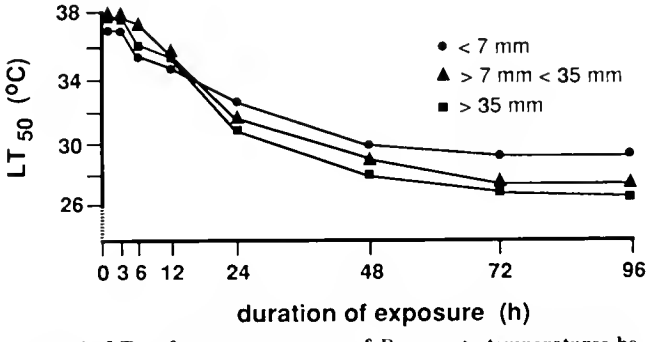

Figure 1. LT_{50} after acute exposure of *D. serra* to temperatures between 20 and 38°C for periods of 0, 3, 6, 12, 24, 48, 72, and 96 hours. Three size groups were used with shell widths <7 mm, >7 but <35 mm, and >35 mm.

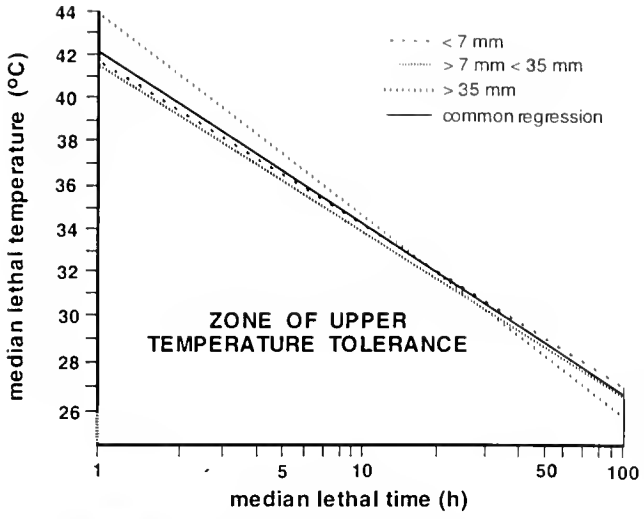

Figure 2. Median lethal times plotted against upper lethal temperatures; data were regressed onto semilogarithmic axes to produce thermal resistance lines, the common regression (solid line) designating the zone of upper temperature tolerance (shaded area).

point, BT_{50} and LT_{50} values were nearly equal for each respective size group, emphasizing the close association between emergence from the sand and death due to thermal stress (Figs. 1 and 3).

In Figure 4, recovery of test individuals is defined as the ability of 50% of animals to burrow and ventilate after being returned to 15°C for 4 days from temperatures between 20 and 38°C. On the basis of these criteria, all sizes recovered after 4 days' exposure to temperatures between 20 and 28°C and after 1 day at 30°C. Only large individuals recovered after 2 days at 30°C, whereas more than 50% of medium and small sizes lay on the sand surface with their shells gaping and eventually died. No further recovery was observed for any size beyond 2 days' exposure to 30°C, and at 32°C and above, there was no recovery, even after only 1 day of exposure.

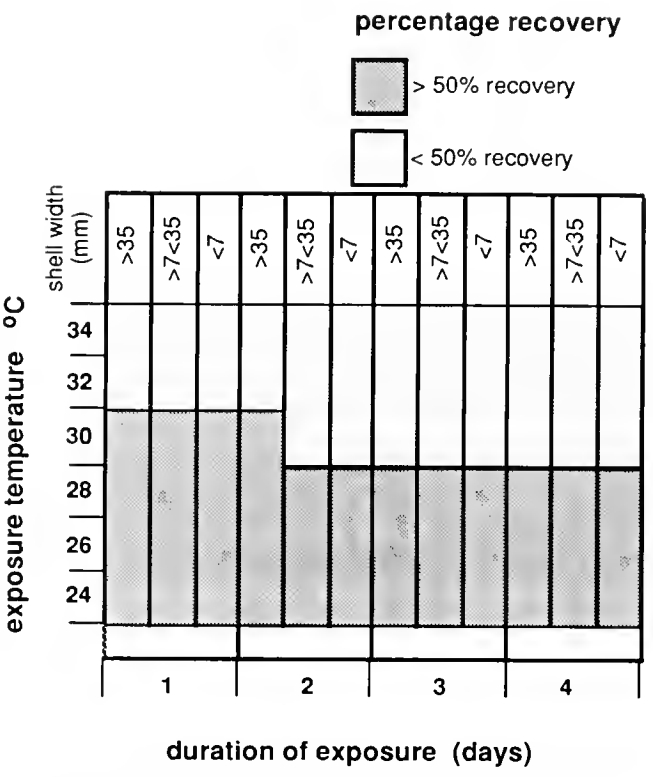

Figure 4. Recovery rate of three size groups of *D. serra* over 4 days at 15°C after acute exposure for 4 days to temperatures ranging from 20 to 38°C.

Stepwise Temperature Exposure Experiments

For any one particular temperature, the percent mortality was plotted for the number of days exposed to that temperature *after* the temperature was reached by stepwise daily increments of 2°C from 14°C. All bivalves <7 mm held at 36°C for 1 day (after stepwise introduction) died, whereas for individuals >7 mm, this occurred at 32°C, resulting in corresponding LT_{50} values of 33°C for the small size and 30°C for the larger ones (Fig. 5). The decline in LT_{50} values from these values over 4 days was similar to that for acute exposure for the same duration (Fig. 1), values being only slightly higher by 1 to 2°C; thus, stepwise exposure to increasing temperatures did little to enhance survival.

As exposure increased to 12 days, LT_{50} values remained fairly constant between 27 and 30°C for all sizes. For small *D. serra*, longer exposure resulted in LT_{50} declining rapidly to 20°C and then stabilizing from 14 to 16 days of exposure. LT_{50} values for larger bivalves followed the same trend, but stability was reached at higher LT_{50} values—24°C for individuals >7 but <35 mm and

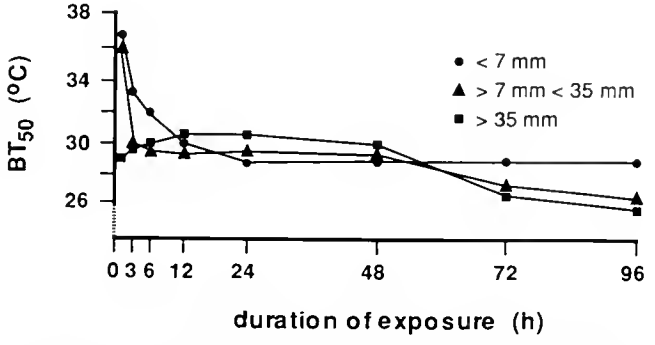

Figure 3. BT_{50} after acute exposure of *D. serra* to temperatures between 20 and 38°C for periods of 0, 3, 6, 12, 24, 48, 72, and 96 hours. Three size groups were used with shell widths <7 mm, >7 but <35 mm, and >35 mm.

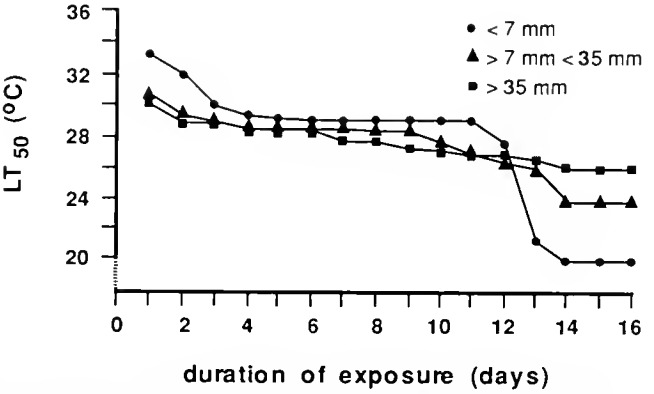

Figure 5. LT_{50} values for *D. serra* gradually exposed to temperatures increased daily by 2°C from 14 to 38°C over 16 days.

26°C for those >35 mm. Unlike acute exposure, stepwise exposure up to 16 days resulted in large bivalves displaying the better thermal tolerance.

BT_{50} values followed LT_{50} values for the first 8 days of exposure (Fig. 6), when individuals died as soon as they emerged from the sand. Once again, a slight increase in BT_{50} for large sizes during the first 2 days of exposure represents an initial delay in burrowing, even after gradual introduction to increasing temperatures. Beyond 8 days, BT_{50} values lagged behind LT_{50} values as more individuals emerged from the sand before dying. By the end of the experiment $BT_{50} = LT_{50}$ for small animals (20°C), but for larger animals, BT_{50} = 23°C, whereas LT_{50} = 26°C. This difference demonstrates that large animals surface at a temperature below the 50% lethal limit and lie gaping for some time before dying.

More than 50% of all sizes recovered after 16 days' exposure to temperatures less than and equal to 26°C, after 13 days at 28°C, and after 1 day exposure to 30°C (Fig. 7). After 14 and 15 days at 28°C, only large individuals recovered, and after 16 days, more than 50% of all sizes showed no signs of recovery. At 30°C, after 2 days' exposure, medium and small sizes recovered, but after 3 and 4 days, only small ones fulfilled the recovery criteria; beyond 4 days, no recovery was noted. At 32°C, large *D. serra* recovered after 1 day, but beyond this time and at higher temperatures, all sizes lay on the surface until death.

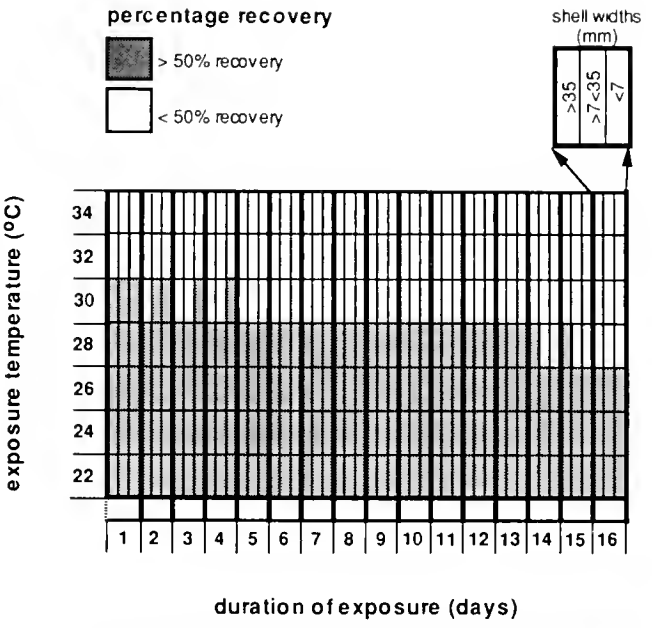

Figure 7. Recovery rate for three size groups of *D. serra* after 4 days at 15°C after stepwise exposure at 2°C per day to temperatures ranging from 14 to 38°C.

DISCUSSION

Intertidal distribution of *D. serra* at Ouskip is distinctly size related, with the smallest animals at midtide, middle sizes between mid- and low tide, and the largest in the surf zone. This implies different temperature regimens, the smallest individuals being exposed to the highest and most fluctuating temperatures. The temperatures to which marine bivalves are exposed in their natural environments are a function of microhabitat plus latitudinal position (Henderson 1929, Dickie 1958, Kennedy and Mihursky 1971, Ansell et al. 1980a and b, Ansell and McLachlan 1980). These factors interact with characteristics such as body shape and size, growth, and reproductive condition to influence the upper temperature tolerances of a species (Bayne 1976). Thus, size-related differences in *D. serra* (Figs. 1 and 5) may be ecologically important, even though such differences, after acute exposure at least, proved nonsignificant according to median lethal times (Fig. 2).

During low-water spring tides, temperatures in the sand at depths of 10 to 15 cm at midtide, where small *D. serra* are found, are usually warmer than in the surf zone by 1 to 3°C. Physiological adjustment to such temperature changes is reflected in LT_{50} values for small *D. serra* being 2 to 3°C higher than those for the larger subtidal individuals after 4 to 11 days' exposure to near-lethal temperatures (Figs. 1 and 5). The smaller size would therefore best tolerate heat loading from the power station, largely by virtue of intermittent exposure to the thermal plume at high tide. It has been shown in *Mytilus edulis* that exposure to a high temperature in 6 hour cycles, rather than continuously, improved survival time by 83% (Pearce 1969).

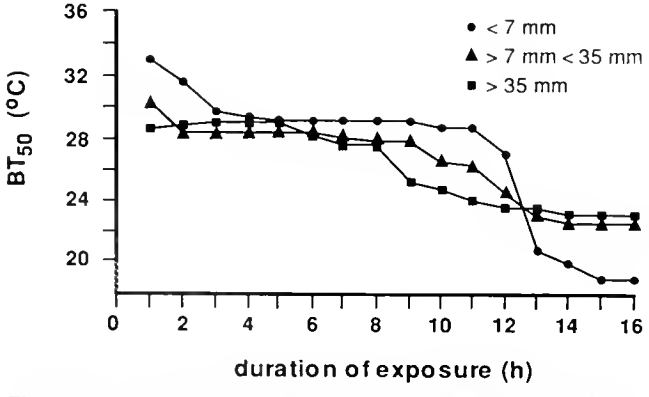

Figure 6. BT_{50} values for *D. serra* gradually exposed to temperatures increased daily by 2°C from 14 to 38°C over 16 days.

The greater temperature tolerance of small individuals is further demonstrated by the fact that they burrowed more rapidly than adults at near-lethal temperatures. However, even though the speed of burrowing declined with size, animals of large size still retained the ability to burrow. This is of ecological significance, because burrowing in nature, sometimes to a depth of 30 cm, enables individuals to escape predation, dislodgment, and unfavorable temperatures. Although it is unlikely that the *D. serra* population near the power station would ever be exposed to lethal temperatures (ca. >32°C), sublethal effects, such as reemergence from the sand, could be of major importance. The ability to maintain position is especially important in the immediate vicinity of the outfall, where the scouring effect of the effluent water, which can reach a velocity of 80 $m^3\ s^{-1}$, is most concentrated.

After about 12 days of stepwise temperature exposure in the laboratory, BT_{50} values were between 20 and 24°C, depending on size (Fig. 6). These temperatures are found in the thermal plume at the outfall, and long-term exposure is possible during extended periods of onshore northwesterly winds, which trap the plume in the surf zone (Rattey and Potgieter 1987). If such conditions prevailed, individuals, especially the subtidal adults, would lose their position in the sand to be swept ashore or preyed on by sandsharks, crabs, and seabirds.

D. serra has an extensive distribution in southern Africa, from Walvis Bay on the west coast to just north of East London on the

TABLE 1.

Comparison of LT_{50} values meaned over 48 and 72 hours of exposure when LT_{50} values had stabilized for small (juvenile) and large (adult) *Donax* species from the southern temperate (South Africa), European warm-temperate (Mediterranean), and Mediterranean-boreal (North Atlantic) (after Ansell and McLachlan 1980).

Locality	Species	Mean 48–72 Hour LT_{50} (°C) Size	
		Small	Large
S. Africa (south coast)	*D. sordidus*		30.6
	D. serra	27.1	29.0
S. Africa (west coast)	*D. serra*	29.2	28.5
Mediterranean	*D. trunculus*	32.9	32.6
	D. semistriatus		29.9
N. Atlantic	*D. vittatus*	28.2	24.8

east. Intertidal distribution in Algoa Bay, on the south coast, is also size related but is the reverse of the west coast pattern, with juveniles in the swash zone at low tide and the adults in the intertidal. A number of reasons, including differences in interspecific competition, food supply, and temperature regimens (Donn 1986), have been proposed for this reversal. On the west coast, sea temperatures range from 8 to 14°C in summer and from 11 to 17°C in winter (Walker et al. 1984), and food supply is mainly detritus and nearshore phytoplankton, which bloom in response to upwelling (Stenton-Dozey 1989). In Algoa Bay, the summer maximum is 26°C with an annual mean maximum of 21 to 22°C (Ansell and McLachlan 1980), and in winter, temperatures drop to 15 to 17°C (Hanekom 1975). *D. serra* and another smaller species, *Donax sordidus*, which is absent in the west, feed mostly on diatoms sustained within the surf zone in a semienclosed ecosystem (McLachlan and Bate 1984).

LT_{50} data presented by Ansell and McLachlan (1980) for *D. serra* in Algoa Bay are directly comparable with data presented here. After 48 and 72 hours of acute exposure, large south coast individuals showed a higher tolerance than small ones (Table 1). The burial response also differed in that, on exposure to high temperatures, south coast adults burrowed immediately with a BT_{50} after 1 hour of 34.5°C, compared with 29.5°C for adults from the west coast, which also showed a marked delay in burrowing (Fig. 4). Small individuals from both areas burrowed immediately, but after 1 hour, those near the power station displayed a BT_{50} of 37°C, compared with only 31°C for a similar size from Algoa Bay. These differences suggest that upper temperature tolerances within the two populations are most strongly influenced by differences between their respective microhabitats.

A characteristic stability of LT_{50} values after 48 to 72 hours' exposure allows direct comparison of *Donax* species from South Africa with those from European waters (Table 1). Such a comparison provides an insight into the interaction between upper thermal tolerances and latitudinal and bathymetric distributions. Ansell et al. (1980b) attribute differences between the European species to differences in zonational position on the shore and zoogeographic locality where higher LT_{50} values correspond to greater tidal exposure and a more southerly distribution. *Donax trunculus*, followed by *Donax semistriatus*, has the greater thermal tolerances, reflecting the distribution of the former species in shallower water and the more southern range of both compared with *Donax vittatus* from the North Atlantic. The two South African

TABLE 2.

LT_{50} values after 24 hours exposure to near-lethal temperatures for small (juvenile) and large (adult) burrowing bivalves acclimated between 15 and 20°C from intertidal and subtidal habitats in the northern and southern hemisphere.

Species	24 Hour LT_{50} (°C) Size		Reference
	Small	Large	
Northern hemisphere			
Subtidal			
Placopecten magillanicus	—	22.5	Dickie (1958)
T. fabula (European N. Atlantic)	—	27.0	Ansell et al. (1980a)
T. fabula (Mediterranean)	—	29.0	Ansell et al. (1980a)
T. tenuis (Mediterranean)	—	33.5	Ansell et al. (1980a)
D. semistriatus	—	30.0	Ansell et al. (1980b)
D. trunculus	—	33.5	Ansell et al. (1980b)
Intertidal			
M. arenaria	31.6	30.5	Kennedy and Mihursky (1971)
G. gemma	—	37.2	Kennedy and Mihursky (1971)
M. balthica	32.0	31.5	Kennedy and Mihursky (1971)
Modiolus demissus	—	38.4	Waugh and Garside (1971)
T. tenuis (European N. Atlantic)	—	31.5	Ansell et al. (1980a)
D. vittatus	—	29.0	Ansell et al. (1980b)
Southern hemisphere			
Intertidal			
D. sordidus	—	33.0	Ansell and McLachlan (1980)
D. serra	29.0	31.0	Ansell and McLachlan (1980)
D. serra	32.0	31.0	This study

species compare most closely with *D. semistriatus* and *D. vittatus:* the size-related difference in *D. vittatus* does not reflect different microhabitats but is believed to be an influence of age. In South Africa, *D. sordidus* has the highest thermal tolerance, which is indicative of its truly intertidal habitat where it migrates with the tides. However, there is little difference in tolerance between the west and south coast *D. serra* at the same tidal levels and this probably reflects their similar latitudinal positions between 33 and 34°S.

Data for burrowing bivalves of other genera allow for a broader comparison of LT_{50} values, but only after acute exposure to near-lethal temperatures for 24 hours. Table 2 compares bivalves from different shoreline distributions and latitudes in both northern and southern hemispheres. The high LT_{50} values among the intertidal group, which compare well with values for South African *Donax*, support the general maxim that molluscs experiencing tidal exposure have the greater thermal tolerances (Henderson 1929, Southward 1958, Kennedy and Mihursky 1971). Indeed, like *D. serra*, the greater tolerance of small-sized *Mya arenaria* and *Macoma balthica* is directly related to a difference in microhabitat, where juveniles are more exposed to the warming of mud flats on receding tide.

The thermal response of species compared within one study do reflect zoogeographic influences. Between *M. arenaria, M. balthica,* and *Gemma gemma*, the last species, followed by *M. balthica*, have the greatest tolerances, coinciding with their wider distribution range on the east coast of the United States. European *Tellina fabula* and *Tellina tenuis* from the Mediterranean have higher LT_{50} values than do populations in the North Atlantic, even though *T. tenuis* is subtidal in its southern distribution.

Gradual exposure to increasing temperatures slightly increased the upper temperature tolerances of *D. serra* (Fig. 5). This suggests an ability to adjust upper limits and can be compared with geographically separated *Tellina* species (Table 2), as well as to seasonally acclimated *Mya, Gemma, Macoma, Tellina,* and European *Donax* species, in which shifts in upper tolerances corresponded to seasonal changes in temperature. Thus, it should be borne in mind that LT_{50} values determined in the laboratory do not represent a finite limit but rather an average upper tolerance, which can assist in understanding not only an individual species but also the thermal load that its biotic environment can tolerate. Furthermore, it should be recognized that many factors, including salinity, pO_2, the thermal limits of protein stability (Stenton-Dozey 1989), and the thermal history of an organism (Newell 1979) interact to influence upper temperature tolerances, and each combination of factors can be specific to a species and in some cases to individuals within a species.

In conclusion, the upper temperature tolerances estimated for *D. serra* in terms of median mortality indicate that the population near the outfall of the power station would experience no adverse effects from the thermal plume. However, Schubel et al. (1978) have criticized the use of only 50% lethal limits and suggest that a family of mortality curves be used ranging from 10 to 90% in intervals of 10%. Such an approach would cover the eventuality of unmeasured sublethal effects and provide a broader estimate of thermal tolerances. In this study, estimates of burrowing success provided a median sublethal measure that showed that *D. serra* surfaced when exposed to temperatures that were not necessarily lethal. Such a response must be considered when assessing effects of a warmed effluent, because the loss of anchorage may mean eventual death.

ACKNOWLEDGMENT

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EXPOSURE OF THE SANDY-BEACH BIVALVE *DONAX SERRA* RÖDING TO A HEATED AND CHLORINATED EFFLUENT II. EFFECTS OF CHLORINE ON BURROWING AND SURVIVAL

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ABSTRACT The effect of chlorine as free residuals in the range of 0.1 to 1.2 ppm on the survival and burrowing ability of the sandy-beach bivalve *Donax serra* was investigated. Median lethal times were calculated from daily observations over 2 weeks of the number of dead and buried *D. serra*. Recovery in nonchlorinated sea water was monitored for 12 days after the transference of some individuals every 24 hours. The addition of chlorine resulted in immediate valve closure, a position maintained for 6 hours at concentrations <0.3 ppm and up to 8 days at >0.6 ppm. No median lethal times were measurable at <0.6 ppm, but above this concentration, the median lethal times approximated 10 days. After 14 days' exposure to between 0.6 and 1.2 ppm, 90 to 100% of the bivalves died. Full recovery in fresh sea water occurred after 6 days' exposure to all chlorine concentrations. Longer exposure to >0.6 ppm resulted in 50% mortality during the recovery test, whereas below this concentration, >50% recovered but burrowing was delayed. Chemoreceptors on the siphons and mantle edge of *D. serra* probably enable the rapid detection of chlorine and hence immediate valve closure. Possible modes of action of chlorine on physiological fitness are discussed. Long-term (>14 days) survival and retention of burrowing ability were invariable at chlorine concentrations <0.6 ppm.

KEY WORDS: Bivalve, burrow, chlorine, power station effluent, sandy beach, survival

INTRODUCTION

A nuclear power station on the west coast of South Africa, 35 km north of Cape Town, discharges a heated and chlorinated effluent directly into the surf zone of a sandy beach densely inhabited by the burrowing bivalve *Donax serra* Röding. Thermal effects from the plume were considered in a previous article in terms of survival (% mortality) and burrowing ability (Stenton-Dozey and Brown 1994). Temperatures >32°C were lethal to all sizes of *D. serra*, whereas sublethal effects were noted after extended exposure (4 to 11 days) to between 24 and 29°C, when 50% of the animals could no longer burrow. The loss of this ability in the surf zone near the outfall would mean displacement from the sand and eventual death by predation or stranding on the beach.

At the inlet of the power station, chlorine (NaOCl) is added to prevent fouling of the condenser cooling system's pipes. In water, chlorine reacts immediately to form a mixture of hypochlorous acid (HOCl) plus hypochlorite ion (OCl^-), depending on pH, temperature, and dissolved solids (Sugam and Helz 1977). This mixture, referred to as free available chlorine (FAC), has strong oxidizing properties and, in sea water, reacts rapidly with bromide to produce hypobromous acid and hypobromite (Sugam and Helz 1977, Morgan and Carpenter 1978), which in turn combine readily with organics in sea water (Goldman et al. 1978). Thus, the water discharged at the outlet contains not only FAC, but also varying amounts of bromine halites and halogens. Because chlorine chemistry in sea water is complex and because of the restrictive nature of analytical techniques (Johnson 1978), most researchers elect to measure chlorine concentrations as FAC or combined chlorine, with the fate of the derivatives remaining unknown.

We selected to measure FAC; at the power station outfall, these concentrations do not normally exceed 0.5 ppm but can reach 2.0 ppm if shock dosing is undertaken. Once the discharged water reaches the surf zone, however, FAC is rapidly dispersed (Rattey and Potgieter 1987), so that spatially, the possible impact on the population of *D. serra* would be more limited than that of the thermal plume (Stenton-Dozey and Brown 1994). The aim of this article is to investigate the effect of FAC at concentrations present at the discharge point on the survival and burrowing ability of *D. serra*.

MATERIALS AND METHODS

Collection and Maintenance

Bivalves were collected 1.5 km south of the power station, an area unaffected by the discharge plume. Animals were kept in flowing sea water in 25 l tanks that were fitted with air-lift pumps to facilitate the circulation of water at 15°C over a sand bed. Because water in the aquarium was replaced regularly, natural detritus was available as food but this was supplemented by periodic additions of cultured algae (*Tetraselmis suecica*).

Effect of Chlorine on Survival

Stock solutions of 2 g of calcium hypochlorite per l of double-distilled water were made up daily in drip bags fitted with adjustable wheels whereby 24 hour dosages could be controlled by setting the number of drops per minute. The addition of chlorine in distilled water did not reduce salinity levels, which were maintained between 34 and 35‰. Because dosing with chlorine in this manner did not allow precise control over concentration, it proved more practical to work within chlorine ranges between 0.1 and 1.2 ppm.

The concentration of FAC in sea water was determined by the colorimetric, stabilized, neutral orthotolodine method (Johnson and Overby 1969). This procedure minimizes stoichiometrical interference from chloramines as well as iron, nitrite, alkalinity, and acidity by using sulfosuccinate as a stabilizer so that a neutral phosphate buffer could be applied at pH 7.0.

Because chlorine toxicity is affected by salinity, pH, and ammonium-nitrogen (Burton 1977), these parameters were measured every day. Salinity was adjusted to 34‰ by dilution with double-distilled water; pH was maintained at 7, and by adding fresh sea water, ammonium-nitrogen was kept below 0.4 mg l^{-1}, the

threshold concentration for the production of toxic, slow-decaying chloramines (Inman and Johnson 1978).

Five batches of 30 adult bivalves each were placed in separate 20 l tanks with slow-flowing, aerated sea water at 15°C, which was circulated through a bed of sand via air-lift pumps. Four batches were exposed to chlorine concentrations ranging from 0.1 to 0.3, 0.3 to 0.6, 0.6 to 0.9, and 0.9 to 1.2 ppm for 2 weeks, whereas the fifth batch was a control without chlorine. Median lethal times were calculated from daily observation of dead and buried *D. serra*, the numbers being corrected by deaths in controls. Recovery was followed by transferring one individual from each chlorine level to a tank with fresh, flowing, nonchlorinated sea water every 24 hours and noting the time taken to reburrow or die over 12 days.

RESULTS

Effect of chlorine in the range from 0.1 to 1.2 ppm on percent mortality and burial response in adult *D. serra* at 15°C is illustrated in Figure 1. On dosing at all concentrations, *D. serra* immediately retracted the siphons and foot and closed the valves while buried in the sand. At the lowest chlorine concentration, this position was maintained intermittently for approximately 3 to 6 hours; thereafter, the valves and siphons remained open. After 14 days' exposure, no adverse effects were apparent; mortality was zero, and all individuals remained completely buried with their siphons fully or partially open on the surface.

After the initial dosing between 0.3 and 0.6 ppm, animals remained retracted for 1 to 2 days and then periodically opened and closed their valves and siphons to ventilate over the next 12 days; a low percent mortality (<10%) was preceded by the emergence of approximately 30% of individuals. No median lethal times were measurable below 0.6 ppm.

At concentrations above 0.6 ppm, animals remained withdrawn with their siphons and valves tightly closed for 7 to 8 days, during which time the tissues were effectively isolated and protected from the external medium. Thereafter, shell gape increased and the siphons became limp; eventually, all emerged from the sand to lie on the surface with their shells wide open. The median lethal time for the range from 0.6 to 0.9 ppm was 10.5 days, and between 0.9 and 1.2 ppm, it was 10 days. By the end of the 14 day experiment, 90% of those exposed to 0.6 to 0.9 ppm were dead, as were 100% at 0.9 to 1.2 ppm.

On transference to fresh, nonchlorinated sea water, there was full recovery after 6 days' exposure to all concentrations of chlorine, although some delay in reburrowing was observed in the range from 0.6 to 1.2 ppm (Fig. 2). However, beyond the sixth exposure day, >50% of individuals subjected to >0.6 ppm never reburied in fresh sea water. Time on the surface before dying ranged from 5 days, after 7 days' exposure, to less than 1 day by the end of the experiment. On the other hand, at concentrations of <0.6 ppm, >50% of transferred animals recovered fully, although reburial was slow after 12 days' exposure.

DISCUSSION

The immediate withdrawal of the siphons and foot, as well as valve closure, by *D. serra* on dosing with chlorine is a common escape response among burrowing bivalves suddenly exposed to a chemical pollutant, as well as to drastic changes in salinity (Block 1977, Akberali and Black 1980, Trueman and Akberali 1981, Akberali and Davenport 1982, Trueman 1983). At low FAC concentrations (<0.3 ppm), *D. serra* reemerged and resumed pump-

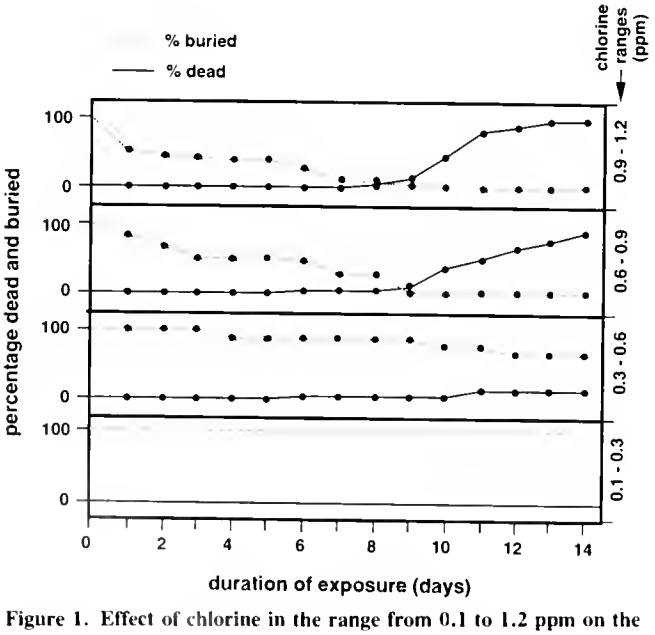

Figure 1. Effect of chlorine in the range from 0.1 to 1.2 ppm on the mortality rate and burial response of *D. serra* at 15°C.

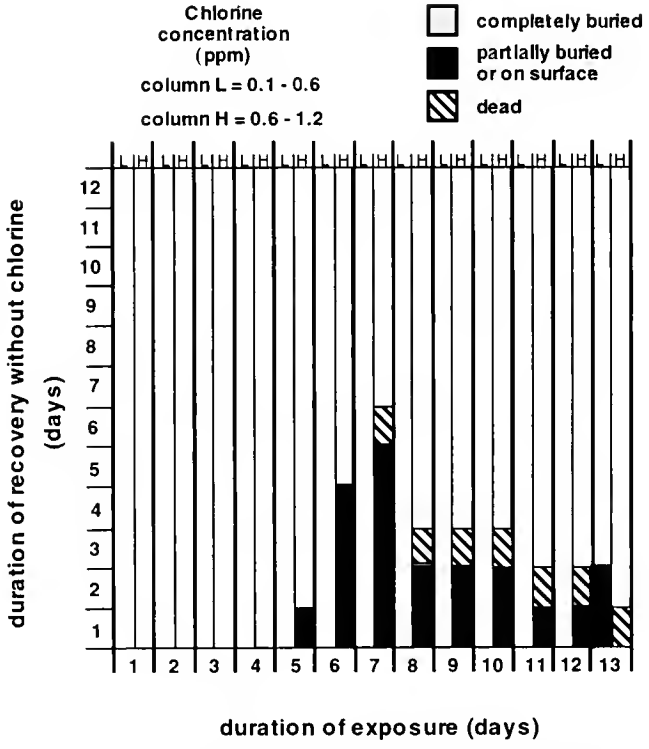

Figure 2. Recovery of adult *D. serra* at 15°C with no chlorine after exposure to chlorine in the lower range (column L) of 0.1 to 0.6 ppm and the higher range (column H) of 0.6 to 1.2 ppm. Total recovery was recognized by an ability to burrow (speckled area), partial recovery by an inability to burrow (black area), and no recovery by ultimate death (striped area). Recovery criteria were set by the relevant behavior of more than 50% of the bivalves.

ing within 3 to 6 hours of chlorine addition. At levels approaching lethal limits (>0.6 ppm), the valves remained closed for up to 8 days, although full recovery in nonchlorinated sea water only occurred after 6 days' exposure. During this time, the mantle margins often protruded, but because they were always held tightly together, it seems likely they afforded the same protection as sealed valves.

The estuarine bivalve *Scrobicularia plana* can remain closed for a similar period, 5 to 7 days, in the presence of copper and low salinities (Trueman and Akberali 1981, Trueman 1983), and *Mytilus edulis* responded to salinity decline by closing for 4 days (Davenport, 1981). The duration of valve closure must be a critical period because eventually depletion of energy resources (Stenton-Dozey 1989), build-up of an oxygen debt (van Wijk et al. 1989), or the accumulation of metabolites would force the animals to open their valves and interact with the environment (Akberali and Black 1980). Valve closure is thus effective in isolating tissues from unfavorable conditions, provided that these conditions are of a transient or recurrent short-term nature. Such avoidance behavior is not restricted to bivalves but also occurs in other valved organisms, for example, barnacles and cirripede cyprids. It is for this reason that power stations usually chlorinate continuously, because intermittent addition is ineffective in removing such animals.

At moderate FAC levels, 0.3 to 0.6 ppm, *D. serra* periodically opened and closed valves and siphons to draw in water, and this action is probably a way of sampling external conditions. Hodgson and Fielden (1984) have shown the existence of ciliated sensory receptors on the inner and outer side of both siphons, as well as on the lobes, of the tentacles and mantle edge of *D. serra*, and there are strong indications that these are chemoreceptors. The cruciform muscle complex could be another site of mechano- and chemoreception, as found in other burrowing bivalves (Odiete 1978, Pichon et al. 1980). Siphonal receptors have also been observed in *M. edulis* (Davenport 1981), on the mantle tentacles of the giant scallop *Placopecten magellanicus* (Moir 1977), and on *Lima hians* (Owen and McCrae 1979). The sensitivity of isolated siphonal preparations to low levels of chemical stimuli (Akberali et al. 1981, Hodgson 1982) is further evidence of the efficiency of chemoreception in bivalves.

The percentage of *D. serra* dying on exposure to FAC was not gradual but rather displayed a stepwise response, with the threshold between chronic and acute toxicity at around 0.6 ppm. Above 0.6 ppm, median lethal time approximated 10 days, but below this concentration, percent mortality never reached 50% over a period of 14 days. The American oyster *Crassostrea virginica* shows even greater tolerance than *D. serra* with <10% mortality when exposed to chlorine in the range from 0.35 to 0.85 ppm for 15 days (Scott and Middaugh 1978). Such resilience to chlorination was not evident in a model suggested by Mattice and Zittel (1976) that predicted the threshold between acute and chronic toxicity for a heterogeneous array of organisms to be only 0.02 ppm. However, this model could be biased toward a low threshold, because a high proportion of fish that are extremely sensitive to chlorine (Morgan and Carpenter 1978, Jolly et al. 1978, Hocutt et al. 1980) were included.

The effect on marine organisms of the variety of halogenated organics that are rapidly formed when chlorine is added to sea water is, as yet, not well understood. There is some evidence that they may be more toxic than chlorine itself (Waugh 1964, Morgan and Carpenter 1978, Hileman 1982, Helz and Kosak-Channing 1984). However, irrespective of the effective pollutant being chlorine or its derivatives, it is not known which aspect of the organism's physiology is most impaired. Interference with chemosenory ability in *C. virginica* has been documented (Hillman 1980), with secondary consequences such as lack of detection of food and predators. Research on juvenile fish and zooplankton (Capuzzo 1977, Capuzzo et al. 1977a and b) suggests that the mode of action of chlorine appears to be some form of metabolic inhibition, although the actual mechanisms remained unknown. Those authors also found that respiration rates in fish declined by 50% at standard threshold concentrations, possibly indicating physical damage to the gills. Furthermore, decreases in the respiration rates of zooplankton larvae occurred at levels of residual chlorine that were virtually undetectable. Such results negate the value of the median lethal chlorine concentration and highlight the necessity to set threshold dosage below concentrations that have sublethal effects. This aspect is addressed in the final article in this series in terms of effects on the heart rate of *D. serra*.

CONCLUSIONS

Laboratory studies have shown that *D. serra* is able to protect itself against a wide range of chlorine levels as long as exposure does not exceed 6 days. During this period, the animal remains buried and effectively isolated from the environment by closing the valves but still retaining an ability to detect and respond to external changes via chemoreceptors on the mantle margin. Nevertheless, on a wave-swept beach, such a response could result in dislodgment from the sand if the animal is not buried deeply, with lethal consequences. However, this danger is far less at chlorine concentrations of <0.6 ppm, because *D. serra* is able to resume burrowing and pumping within 2 days after the initial closure response.

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EXPOSURE OF THE SANDY-BEACH BIVALVE *DONAX SERRA* RÖDING TO A HEATED AND CHLORINATED EFFLUENT III. EFFECTS OF TEMPERATURE AND CHLORINE ON HEART RATE

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ABSTRACT The singular and synergistic effects of temperature and free available chlorine in the range of 0.1 to 1.2 ppm on the heart rate of the sandy-beach bivalve *Donax serra* were investigated. The aim was to identify the possible sublethal impact of a heated and chlorinated effluent on a dense bivalve population near the outfall. The heart rate of adults, in the presence or absence of chlorine, was recorded at 15, 20, 25, and 30°C after daily increments of 5°C from 15°C. One individual from each exposure temperature was transferred daily to fresh sea water at 15°C to monitor recovery. Heart rate reached a maximum of 44 beats min^{-1} at 25°C from a basal rate of 15 beats min^{-1} at 15°C. The heart rate of those exposed to 30°C did not return to basal frequency in fresh sea water at 15°C. On dosing with chlorine, beat frequency immediately dropped to half the basal rate as the valves closed and ventilation stopped. Below 0.6 ppm and <25°C, the basal rate returned within 24 hours, and full recovery occurred in fresh sea water at 15°C. Results indicated that plume conditions would not be lethal to *D. serra* but that sublethal effects relating to burrowing activity and heart rate can be expected.

KEY WORDS: Bivalve, chlorine, effluent, heart rate, temperature, sandy beach

INTRODUCTION

This article is the final in a series of three concerned with the effects of a heated and chlorinated effluent from a nuclear power station on the physiological fitness of *Donax serra* Röding in South Africa. Dense populations of this intertidal sandy-beach bivalve are directly exposed to a thermal plume 10°C above the ambient seasonal range of 8 to 17°C and chlorine concentrations between 0.1 and 0.4 ppm. Initial laboratory studies on the effects of temperature and chlorine on survival and burrowing (Stenton-Dozey and Brown 1994a and b) showed that the discharge plume was unlikely to have a lethal effect. However, *D. serra* did display distinct postural changes, such as increased valve adduction and siphonal closure, which indicated possible sublethal effects. This necessitated the selection of a physiological parameter to quantify these behavioral responses.

A common physiological measure of the response of bivalves to raised temperatures, as well as to the presence of chemical pollutants, is change in heart activity (Coleman 1974, Lowe 1974, Earll 1975, Trueman and Akberali 1981, Trueman 1983). Heart rate is easy to monitor using impedance techniques (Trueman et al. 1973), which in addition, provide evidence of gross changes in behavior, such as valve movements, burrowing, and periods of inactivity or heightened activity. These responses in turn correspond closely to changes in the physical and chemical environment (Earll 1975). In this article, heart rate is used as an indicator of the sublethal effects on *D. serra* of raised temperatures both in the presence and in the absence of chlorine.

MATERIALS AND METHODS

Effect of Temperature on Heart Rate

The electronic recording technique developed by Trueman (1967) was used to monitor heart rate. Two small holes were drilled in each valve over the heart. Fine silver-wire electrodes were inserted through these holes to lie on opposite sides of the pericardium. The electrodes were sealed in place with dental wax and connected in series to an impedance pneumograph (Alternate Current coupling) and oscillograph by a lightly screened cable. Changes in impedance between electrodes resulting from pulsatile changes in heart volume or from movements of the animal within its shell were recorded on the oscillograph.

Adult bivalves with implanted electrodes, plus others without electrodes, which served to demonstrate any detrimental effect of the implant on postural behavior, were kept in 20 l tanks and allowed to equilibrate overnight at 15°C. Because starvation can markedly depress heart rate in bivalves (Bayne 1976), all animals were fed cultured algae during the equilibration period. Heart rate was initially monitored by transferring individuals directly from 15°C to either 20, 25, or 30°C for 1 to 2 days. However, this involved long periods of reequilibration before recording could commence, so as an alternative, the temperature was raised, without disturbing the animals, by 5°C every 24 hours from 15 to 30°C. Both methods of increasing temperature resulted in similar heart activity.

Preliminary experiments involving continuous recordings indicated that no unpredictable short-term variations in heart activity occurred. The heart rate of each individual was, therefore, monitored for 15 minutes in every hour for periods up to 18 hours. The experiment was repeated six times at each temperature, and five individuals were used per run, four with implants and one as a control.

One individual from each exposure temperature was transferred daily to fresh sea water at 15°C to monitor recovery over 24 hours. An equilibration period of 6 hours was allowed before the heart rate of these animals was measured.

Combined Effect of Temperature and Chlorine on Heart Rate

Adult bivalves were simultaneously exposed to the temperature regimen described above and free available chlorine (FAC) concentrations ranging from 0.1 to 0.3, 0.3 to 0.6, 0.6 to 0.9, and 0.9 to 1.2 ppm. Chlorine was administered as calcium hypochlorite

and measured as FAC, as described in Stenton-Dozey and Brown (1994b). Experiments were repeated three times for each chlorine range and temperature, using five individuals per run in which one served as a control without implanted electrodes. Some individuals were removed daily after 24 hours' exposure to each treatment, placed in nonchlorinated sea water at 15°C, and allowed to equilibrate for 6 hours before the recovery heart rate was monitored for 24 hours.

RESULTS

Effect of Temperature

While buried and ventilating at 15°C, heart activity steadied at 13 ± 2 beats min^{-1}; experiments commenced once this rate was maintained for 24 hours (Fig. 1A). When the temperature was raised to 20°C, heat frequency increased immediately, the maximum rate of 26 beats min^{-1} being reached after 5 hours. After 24 hours at 20°C and at the beginning of exposure to 25°C, heart activity was less stable, fluctuating between means of 18 and 33 beats min^{-1}, with standard deviations much higher than for data at 15 or 20°C. Just before the temperature was raised from 25 to 30°C, heart rate had risen to 44 beats min^{-1}, but at 30°C, it became irregular, followed most often by death after 12 hours. Because controls showed stress but did not die at 30°C, death must to some degree be attributed to implanted electrodes. At maximum heart rate, Q_{10} values equaled 4.0 between 15 and 20°C and 2.9 from 20 to 25°C.

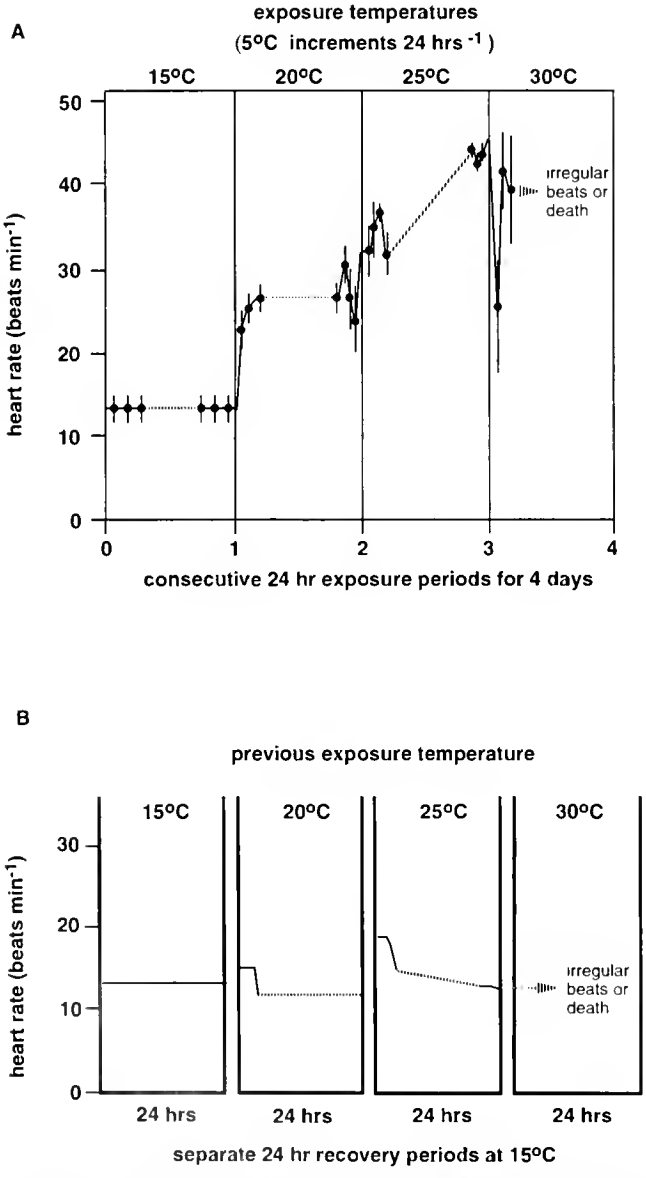

Figure 1. (A) Effect of temperature on the heart rate of adult *D. serra* when raised 5°C per day from 15 to 30°C. Vertical bars represent one standard deviation around the mean. (B) Recovery heart rates at 15°C after exposure to 20, 25, and 30°C for 24 hours.

Bivalves exposed to 20 and 25°C recovered completely when returned to 15°C, as shown by return to a normal heart rate of ±13 beats min^{-1} (Fig. 1B). Those from 30°C displayed erratic heart rates on transference to 15°C and, after monitoring for 24 hours, either retained an irregular beat while lying on the surface gaping or were dead. No controls died during recovery, but some individuals did emerge to lie on the sand surface, thereby indicating stress related to temperature alone rather than to electrode implants.

On increasing the temperature to 20°C, a rapid increase in beat frequency was apparent from 15 to 22 beats min^{-1} in 5 minutes (Fig. 2B); adductions were less frequent, more protracted, and often followed by cessation of beats for 30 seconds when the valves closed (Fig. 2C). At 25°C, regular beating often gave way to extensive contraction and retardation or suppression (Fig. 2D). Suppression often alternated with a reduction in beat amplitude, followed by rapid reextension and commencement of ventilation (Fig. 2E). Throughout exposure to 25°C, the shell gaped and the siphons were, most often, fully extended.

Shell gape was maximal at 30°C, and both siphons remained fully extended but limp. Beat frequency, although rapid and often steady, was of low and irregular amplitude, with infrequent adductions, followed by an extended suppression of heart activity (Fig. 2F). At death, the shell gaped, the siphons collapsed, and beats became more indistinct, fading away or ceasing after a period of regular, strong beats (Fig. 2G).

Synergistic Effect of Chlorine and Temperature

On dosing between 0.1 and 0.3 ppm at 15°C, the valves closed, resulting in a fall in heart rate from 13 to around 8 beats min^{-1} (Fig. 3). Over the next 24 hours, heart rate increased to 10 beats min^{-1} as *D. serra* reemerged. A rise to 20°C and then 25°C over the next 48 hours resulted in beats increasing to 11 and 14 beats min^{-1}, respectively. This frequency was nearly four times lower than that reached at 25°C without chlorine (see Fig. 1). At 30°C, 13 to 14 beats min^{-1} were maintained for 6 hours, and then, heart activity became indistinct as animals began dying. In the range from 0.3 to 0.6 ppm at 15°C, heart rate dropped to 6 and only rose to 10 at 20°C when the valves began gaping. At 25 and 30°C, the heart responded as for the dosage of <0.3 ppm (Fig. 3).

On dosing with chlorine between 0.6 and 0.9 ppm at 15°C, heartbeat dropped immediately to 5 beats min^{-1}, only increasing to 7 over the next 2 days with an increase from 20 to 25°C (Fig. 3). After 18 hours at 25°C, the heartbeat showed no regular pattern and bivalves began dying at 30°C. A similar response occurred in the range from 0.9 to 1.2 ppm, when heart rate never rose above 6 beats min^{-1}, becoming indistinct after 3 hours at 25°C; individuals began dying before the temperature was raised to 30°C.

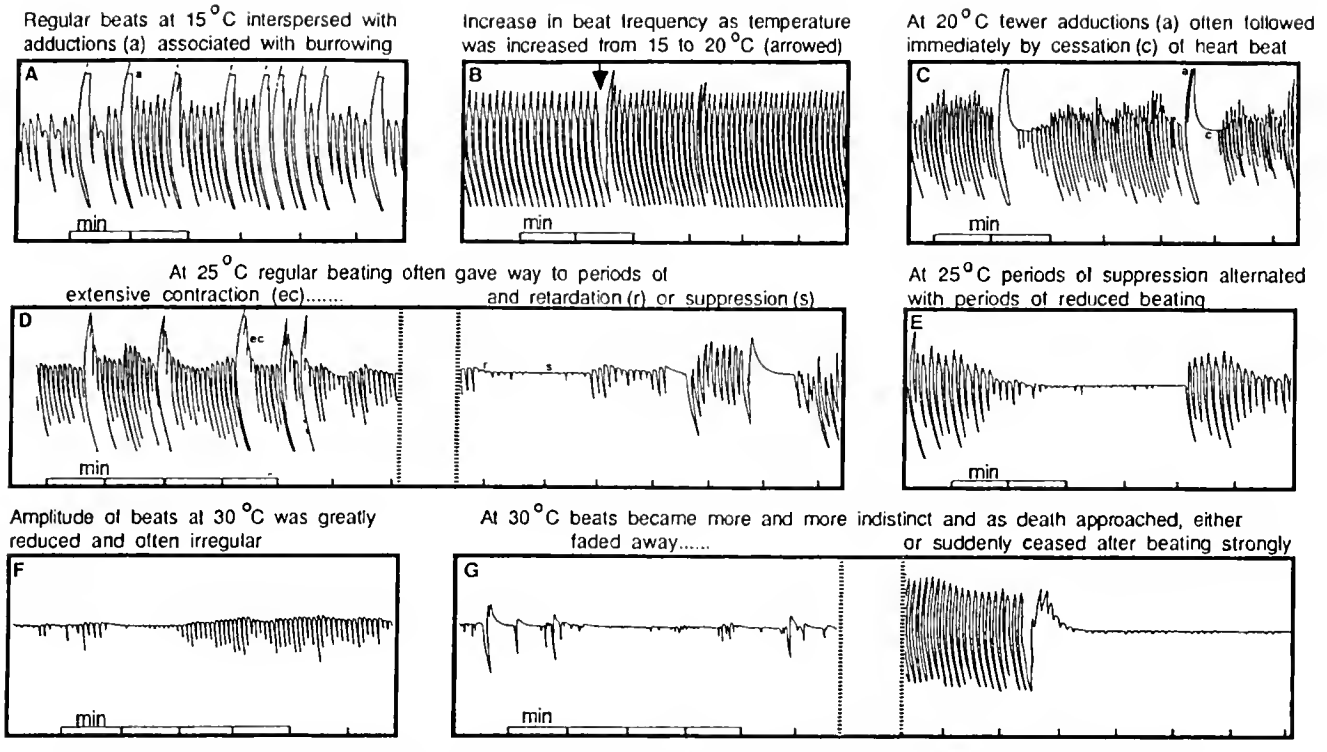

Figure 2. Traces of heart activity showing the effect of temperature (15 to 30°C) on the heart rate of adult *D. serra*.

Q_{10} was negative above 0.6 ppm, demonstrating the predominance of the chlorine effect, so that heart rate was no longer positively temperature dependent. Furthermore, in this chlorine range, heart activity was suppressed to a steady, slow beat, with small standard deviations from means, irrespective of temperature, whereas at concentrations below 0.6 ppm, deviations were higher and more variable as temperature and chlorine interacted to raise and suppress beat frequency (Fig. 3).

On transference to nonchlorinated sea water at 15°C, all individuals exposed to FAC <0.6 ppm and 25°C or less recovered fully, as indicated by a return to the normal basal rate of 13 beats min^{-1} (Fig. 3). However, individuals from 30°C either died or continued to display irregular heart activity. In the range from 0.6 to 1.2 ppm, a return to normal beat frequency was only observed in individuals from 15°C (Fig. 3). Those from 20°C displayed a suppressed heart rate (10 beats min^{-1}) during the recovery test, whereas those exposed to 25°C retained a disrupted beat pattern.

Traces of heart activity showed that at FAC concentrations <0.6 ppm, heartbeats became greatly protracted (Fig. 4A), but once the temperature was raised to 20°C, the normal pattern returned (Fig. 4B). At 25°C, beats were once again protracted but weaker, with periods of total suppression (Fig. 4C), and at 30°C, they were even weaker and hence lower in amplitude and indistinct (Fig. 4D). Above 0.6 ppm, beat frequency became irregular on dosing at 15°C and this pattern persisted at 20°C (Fig. 4E). At 25°C, regular protracted beats disintegrated into an indiscreet pattern as animals neared death (Fig. 4F).

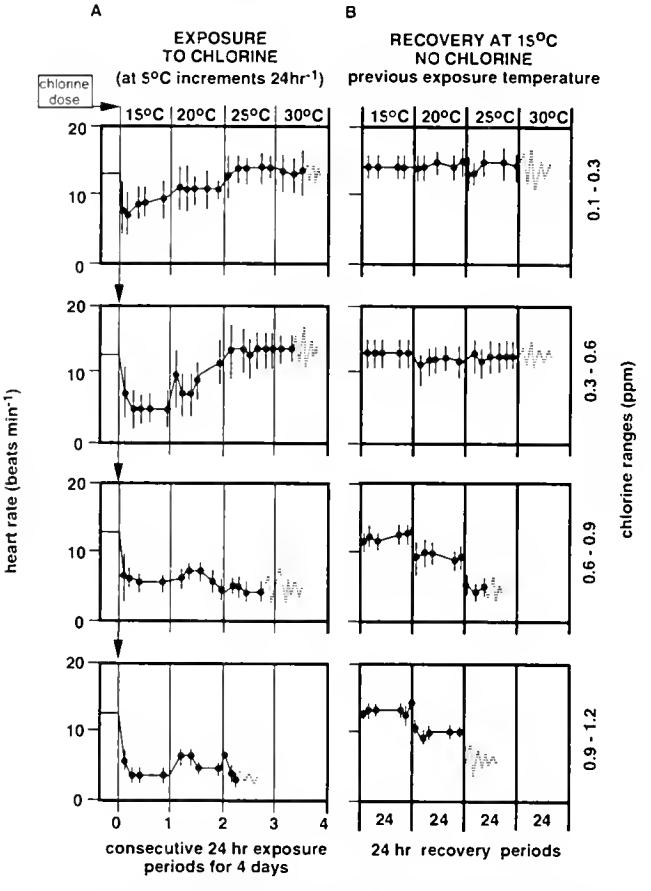

Figure 3. (A) Combined effect of temperature and chlorine on the heart rate of adult *D. serra*. Temperature was increased by 5°C daily for 4 days from 15 to 30°C within the FAC range from 0.1 and 1.2 ppm. (B) Recovery heart rates at 15°C without chlorine after exposure to 15, 20, 25, and 30°C with chlorine.

DISCUSSION

With an increase in temperature, the heart rate of *D. serra* increased immediately and did not acclimate over the range from 15 to 30°C, two responses that make this physiological parameter a sensitive and hence convincing measure of sublethal effects.

Nonacclimation is common among bivalves, for example, *Isognomon alatus* (Trueman and Lowe 1971), *Mya arenaria* (Lowe and Trueman 1972), *Mytilus edulis* and *Mytilus californianus* (Pickens 1965, Widdows 1973), *Crassostrea gigas* (Lowe 1974) and *Perna perna* (Bayne 1976). Those species, like *D. serra*, displayed Q_{10} values >2 between 10 and 25/27°C, beyond which, beat frequencies generally became erratic, thereby reflecting the critical temperature at which homeostatic mechanisms begin to break down. For *D. serra*, this coincided with failure to return to a normal heartbeat at 15°C after exposure to 30°C.

Also common among bivalves is an immediate increase in heart rate on increasing temperature. It has been suggested that thermoreceptors, possibly in the mantle tissue, play an important role in this response (Trueman and Lowe 1971, Lowe 1974). Because there is an abundance of sensory cells on the siphons and mantle edge of *D. serra*, even though most appear adapted to detect chemical or mechanical changes in the external medium (Hodgson and Fieldin 1984), it seems reasonable to assume that at least some of these cells function as thermoreceptors with a possible neural connection to the heart.

The addition of chlorine between 0.1 and 1.2 ppm to sea water at 15°C led to immediate bradycardia in *D. serra;* beat frequency drastically declined as the siphons and foot withdrew and the valves closed tightly. This is a common stress response among bivalves to a pollutant, a salinity drop, or aerial exposure, and in all of these instances, valve closure is accompanied by a decline in heart rate (Trueman 1967, Coleman and Trueman 1971, Earll 1975, Akberali and Black 1980, Trueman and Akberali 1981). Valve closure leads to a drastic drop in pO_2 and an increase in pCO_2 levels of the mantle cavity water in *M. edulis* (Bayne 1971) and *Scrobicularia plana* (Akberali and Trueman 1979), and it is this drop in oxygen tension, rather than any mechanical effect of closed valves, that is believed to attenuate beat frequency (Bayne 1976).

D. serra became acclimatized to continuous exposure to low FAC concentrations at 15°C, as demonstrated by a return to normal heart rate, paralleled by increasing shell gape and pedal and siphonal extension, during exposure to <0.3 ppm. This is supported by the low mortality rate of *D. serra* exposed to 0.1 to 0.3 ppm at 15°C for 2 months in experiments after biochemical changes in the body tissues (Stenton-Dozey 1989). Above 0.3 ppm, a suppression of heart activity and valve closure persisted for the 24 hours of the experiment. Because *D. serra* is able to maintain valve closure for 7 to 8 days when continuously exposed to this FAC range (Stenton-Dozey 1989), the resultant oxygen debt (Bayne 1976) would prevent heart activity from returning to normal and anaerobic respiration would probably sustain basal metabolism (De Zwaan 1977). The accumulated endproducts of

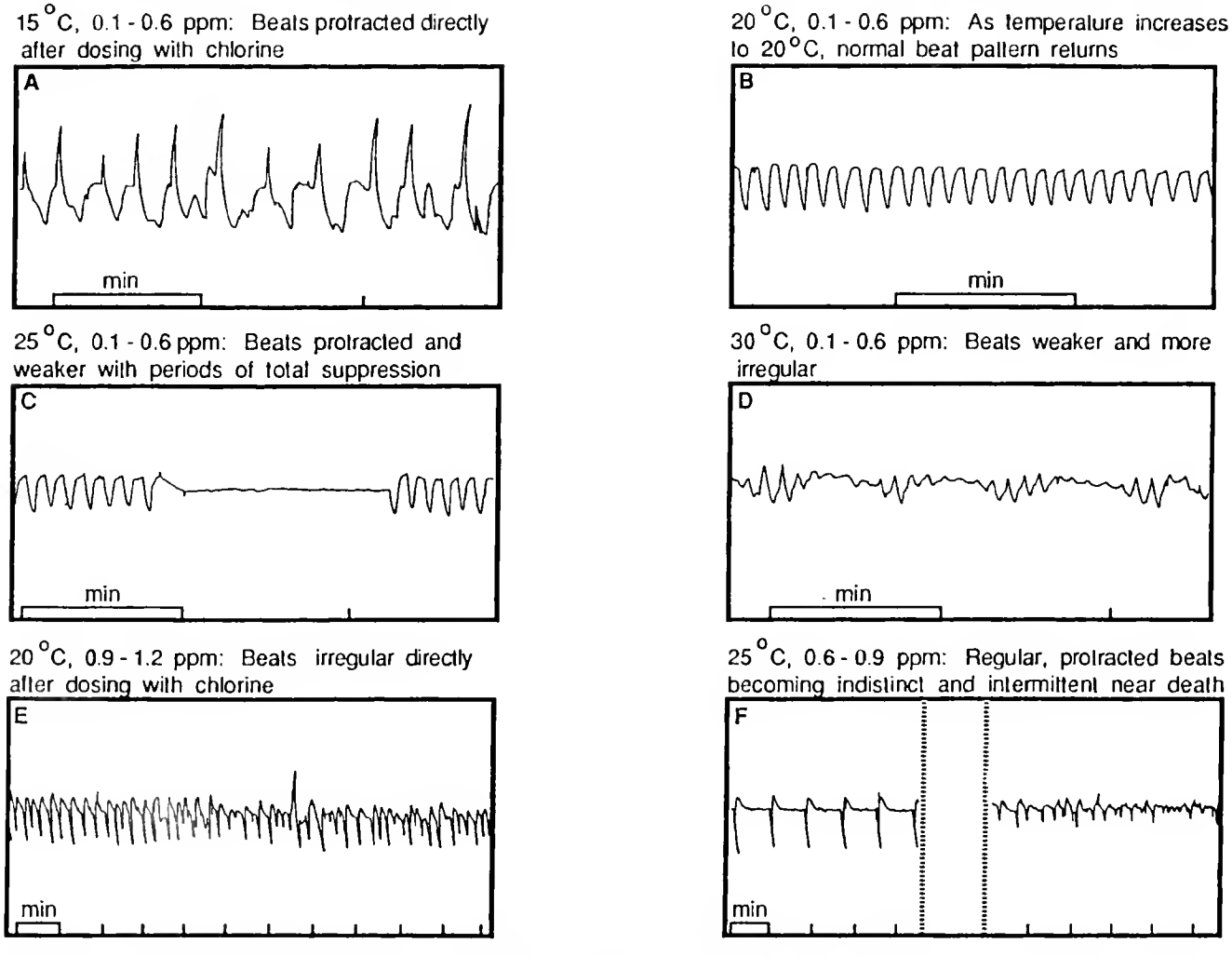

Figure 4. Traces of heart activity showing the combined effect of temperature (15 to 39°C) and FAC (0.1 to 1.2 ppm) on the heart rate of adult *D. serra*.

anaerobiosis could be buffered by calcium mobilized from the calcareous shell, as occurs in *S. plana* (Akberali 1980, Akberali et al. 1977). Once the valves open after 8 days and if the chlorine dose is maintained indefinitely >0.3 ppm, eventual death would be inevitable.

A combination of high temperatures and chlorine resulted in an increase in the number of valve adductions and thereby an increase in ventilation, so that heart activity was raised above levels observed in solely chlorine treatments. Lacking the protection afforded by continuous closure of the valves, internal organs were intermittently exposed to FAC. Below 0.3 ppm at temperatures <25°C, *D. serra* would survive prolonged exposure (8 days or more), but at higher concentrations (especially >0.6 ppm), passive shell gaping, as a result of weakened and/or paralyzed adductor muscles (Trueman and Lowe 1971), would lead to eventual death.

Recovery after short-term exposure (±24 hours) followed by transference to 15°C without FAC was rapid for individuals from the range 0.1 to 1.2 ppm at 15°C and <0.6 ppm at 20 and 25°C. In the first group, continual valve closure at high concentrations assured protection, whereas in the second, intermittent valve opening and closing prevented permanent internal damage during the 24 hour experiment. During recovery in fresh sea water, valve adductions were accompanied by an overshoot in heart rate, indicative of oxygen deprivation. This is consistent with the fact that *D. serra* is an oxyconformer, incurring an oxygen debt with a decline in oxygen tensions (Van Wijk et al. 1989). Other bivalves have shown a similar recovery response not only after exposure to a pollutant (Akberali and Black 1980, Trueman and Akberali 1981) but also on reimmersion after aerial exposure (Trueman 1967, Coleman and Trueman 1971).

CONCLUSION

A discharge plume with FAC concentrations >0.6 ppm in combination with temperatures >25°C for longer than 24 hours will have a sublethal effect on *D. serra* from which recovery is unlikely. In most instances, however, chlorination within the plume is unlikely to exceed 0.3 ppm (Rattey and Potgieter 1987), and at this concentration, *D. serra* was able to tolerate temperatures up to 25°C for at least 24 hours. However, longer exposure results in a slow burrowing response (Stenton-Dozey and Brown 1994a and b), so that even though heart rate may normalize at 25°C and <0.6 ppm, the danger of dislodgment from the sand near the outfall, with possible lethal consequences, still persists. Conservative dosing with FAC levels at <0.3 ppm and temperatures ±20°C would result in invariable tolerance by *D. serra*.

ACKNOWLEDGMENT

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THE *IN VITRO* LIFE CYCLE OF A *PERKINSUS* SPECIES (APICOMPLEXA, PERKINSIDAE) ISOLATED FROM *MACOMA BALTHICA* (LINNEAUS, 1758)

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ABSTRACT Using standard sterile techniques and a single medium previously described (Kleinschuster and Swink 1993), the *in vitro* culture of a *Perkinsus* species isolated from *Macoma balthica* was possible. Zoosporulation, the release of zoospores, and the reestablishment of secondary cultures from the zoospores completed an *in vitro* life cycle.

KEY WORDS: *Perkinsus* species, *Macoma balthica*, cell culture

INTRODUCTION

Species of the apicomplexan protozoan *Perkinsus* have been reported to cause major mortalities in bivalve populations and cross-transmission of *Perkinsus* species infections between certain bivalve species is possible (Goggin et al. 1989). Although a simple *in vitro* culture technique for the propagation of *Perkinsus marinus* Levine 1978 has been established (Kleinschuster and Swink 1993), techniques for the routine induction of zoosporulation and release of zoospores in quantity have not been available in recent years either *in vivo* or *in vitro*. Consequently, the role of the zoospore in the infection/invasion process has not been fully investigated.

We report herein simple methodology for the *in vitro* culture of a *Perkinsus* species isolated from *Macoma balthica* including vegetative propagation of the isolate, zoosporulation, and release of zoospores followed by the reestablishment of secondary cultures from the zoospores, thereby completing an *in vitro* life cycle.

MATERIALS AND METHODS

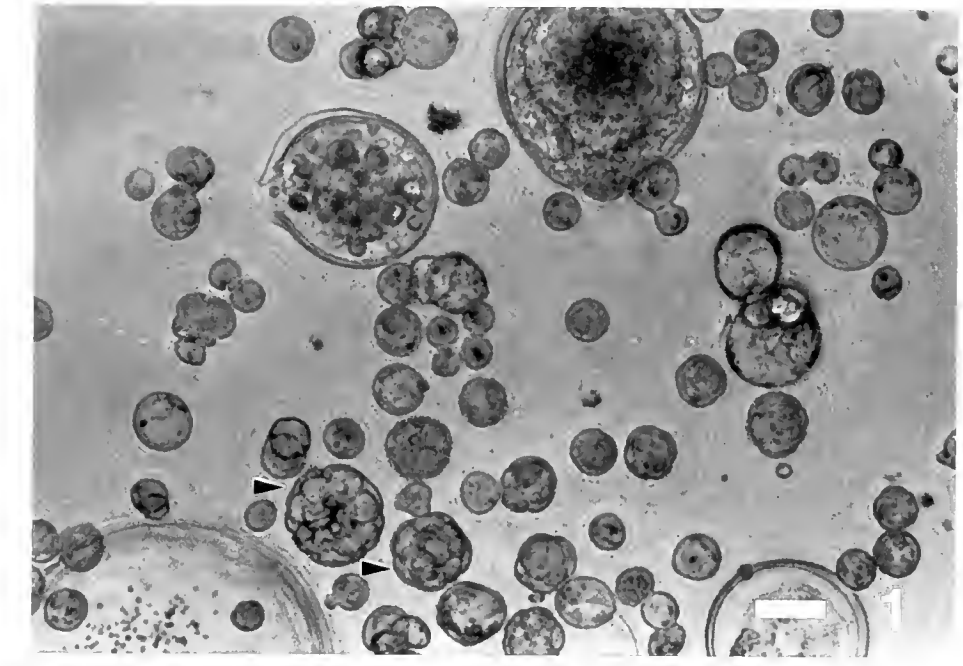
Infected clams were obtained from King's Creek, (14 ppt salinity), a tributary of the York River in Virginia, and maintained in aquaria at the Virginia Institute of Marine Science. Clams used as a source of *Perkinsus* species were transported to the Haskin Shellfish Research Lab and maintained in recirculating sea water at 12 to 15°C. Cells of the parasite were obtained from hemolymph aspirated from the blood sinuses of the adductor muscles. Immediately after aspiration, hemolymph and cellular components were transferred to 25 cm^2 T-flasks containing 1 to 2 ml of sterile sea water and appropriate antimicrobics (streptomycin, 0.2 mg ml^{-1}, penicillin, 200 U ml^{-1}, and amphotericin B 0.25 μg ml^{-1}) and held at room temperature for 3 hours. After this treatment, the isolation medium was replaced by a medium used for the *in vitro* culture of *P. marinus* (Kleinschuster and Swink 1993), and the cultures were kept at 28°C under ambient air. The medium was exchanged (50%) every 3 days. Phase contrast microscopy was used to obtain photomicrographs.

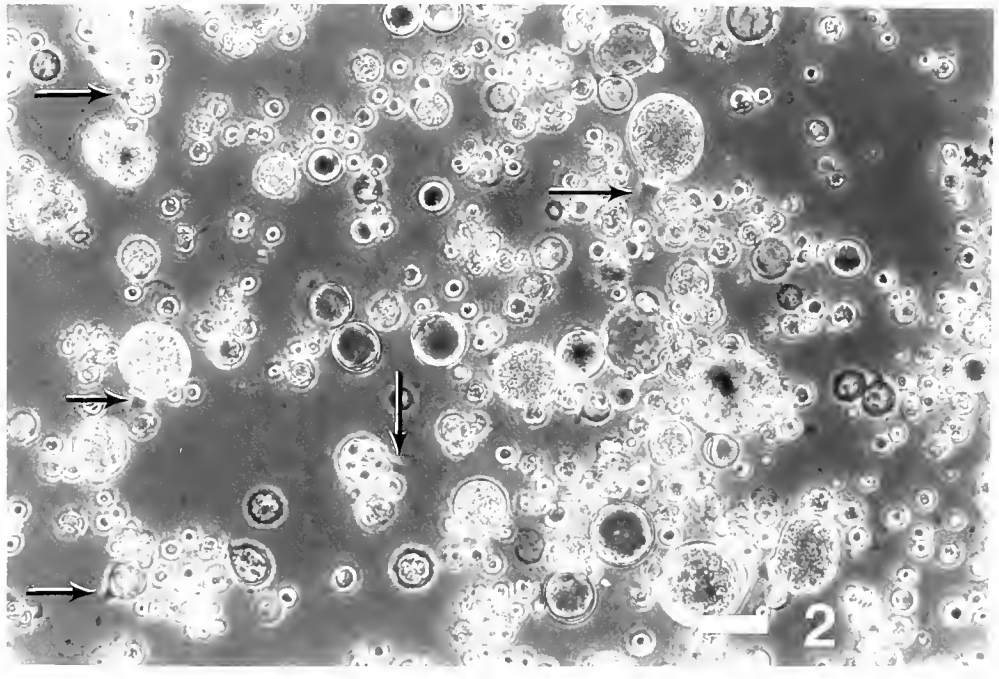
Routine roller bottle technique and subculturing were used to upscale the production of cultured cells. Aliquots of these cultures were centrifuged at low speed to pellet the organisms, which were then washed two times with sterile sea water and resuspended in T-flasks with sterile sea water to induce zoospore formation.

For ultrastructural analysis, prezoosporangia, zoosporangia, and zoospores in sterile sea water were sent to N.C. State University in T-flasks. Contents of the flasks were decanted into a centrifuge tube, and after the bottom of the flask was scraped with a rubber policeman, the cells were pelleted at 1000 × *g* for 5 minutes. The medium was gently pipetted from the tube and replaced with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.8 (Azevedo 1989). After 1 hour of primary fixation at room temperature, the cells were rinsed in fresh buffer several times. The cells were pelleted after each rinse. After the final rinse, the cells were pelleted, the medium was removed, and the cells were resuspended in molten (approximately 50°C) 4% water agar and quickly centrifuged at 1000 × *g* for 30 seconds. Once the agar had solidified, the agar containing the cells was sliced into 1 mm thin slices with a razor blade and placed into 1% osmium tetroxide in the same buffer at room temperature. After 1 hour, the samples were rinsed three times in distilled water and subsequently dehydrated in a graded ethanol series, passed through 100% acetone, and infiltrated with Spurr resin (Dykstra 1993). Ultrathin sections were obtained, stained with methanolic uranyl acetate and lead citrate, and evaluated with a transmission electron microscope (TEM).

RESULTS

Low-density *in vitro* cultures of the *Perkinsus* isolate are represented in Figures 1 and 2. In nutrient-rich medium, vegetative



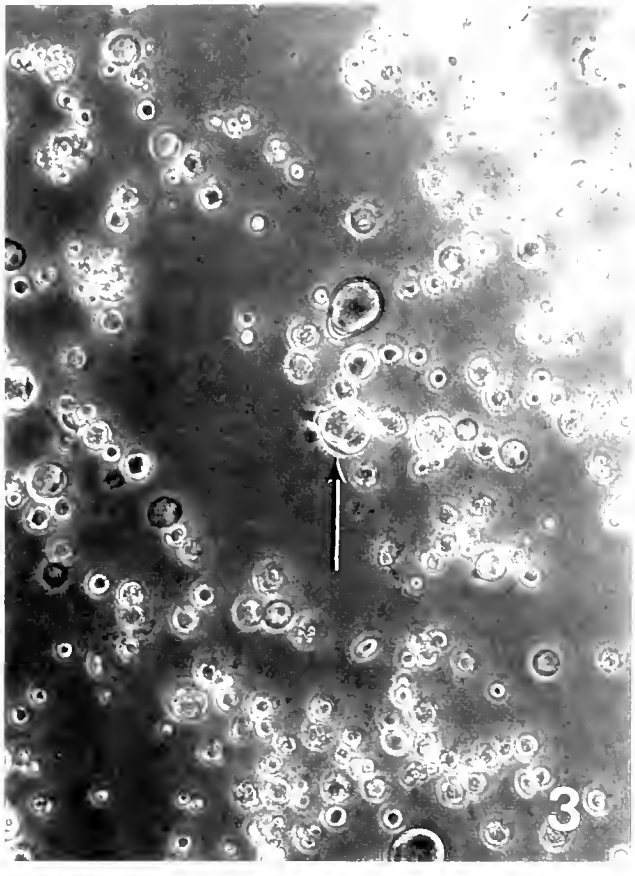


Figures 1 and 2. Photomicrographs of low-density *in vitro* cultures of a *Perkinsus* species isolated from *M. balthica*. Note schizonts typical of *Perkinsus* species (arrowheads) in nutrient-enriched medium and the discharge tubes of zoosporangia present in sea water cultures (arrows). Scale bar, 0.05 mm (Fig. 1) and 0.1 mm (Fig. 2).

reproduction was vigorous and typically consisted of schizonts in various stages of merogeny; meronts and merozoites were evident (Fig. 1). Generally, vegetative stages of this isolate were larger and more ovoid than *P. marinus* under similar conditions; however, merogeny appeared to be similar. A typical low-density sea water culture is represented by Figure 2. Various presporulation stages were evident, including prezoosporangia and zoosporangia with discharge tubes.

Several developmental stages of induced zoosporulation are seen in Figures 3 to 7. Typical two-, four-, and eight-cell stages of developments are seen in Figures 3 to 5, respectively. Discharge tubes were routinely evident at the two-cell stage (Fig. 3),

Figures 3 to 6. Photomicrographs of a low-density sea water culture of a *Perkinsus* species isolated from *M. balthica*. Represented are typical two-, four-, and eight-cell stages of zoosporulation (arrows). Figure 6 exhibits a sporangium with two discharges tubes (arrows). Blurred background images are motile zoospores. Scale bar, 0.1 mm.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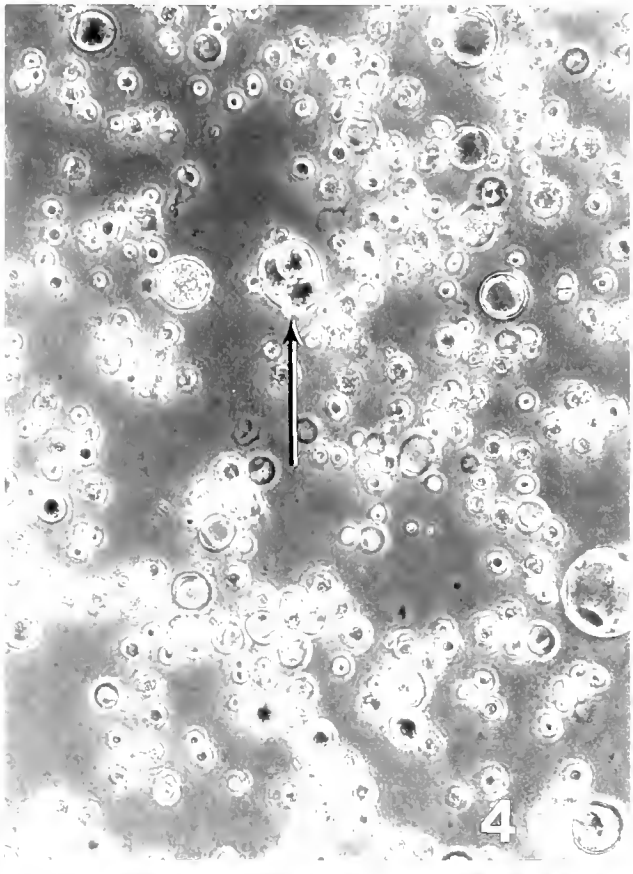
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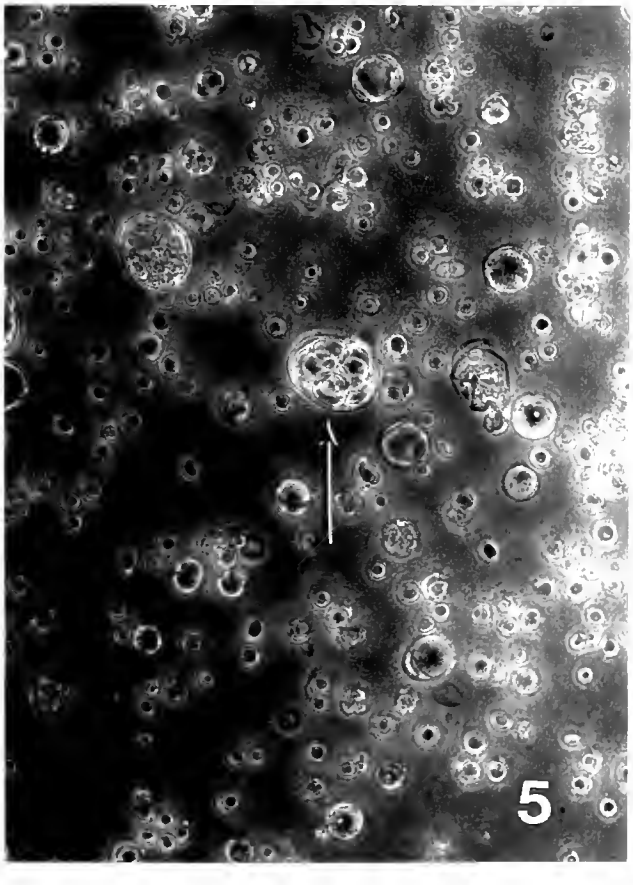
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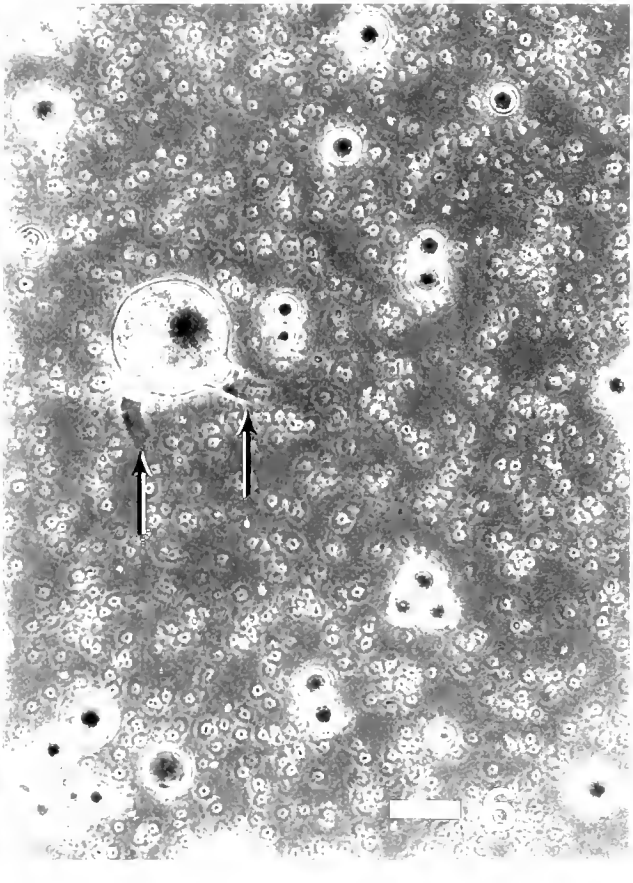

and multiple discharge tubes were not uncommon (Fig. 6). Successive karyokineses and cytokineses resulted in the formation and release of motile zoospores (Fig. 7). The blurred and dotted backgrounds of Figures 6 and 7 represent discharged and motile zoospores.

A representative TEM photomicrograph of a mature prezoosporangium with typical thickened cell wall, discharge tube, and multiple prezoospores is seen in Figure 8.

A TEM photomicrograph of a zoospore displaying apicomplexan structures, including rhoptries, a conoid, and subpellicular microtubular and microneme-like organelles is seen in Figure 9. The kinetosome substructure was the same as in *P. marinus* (Per-

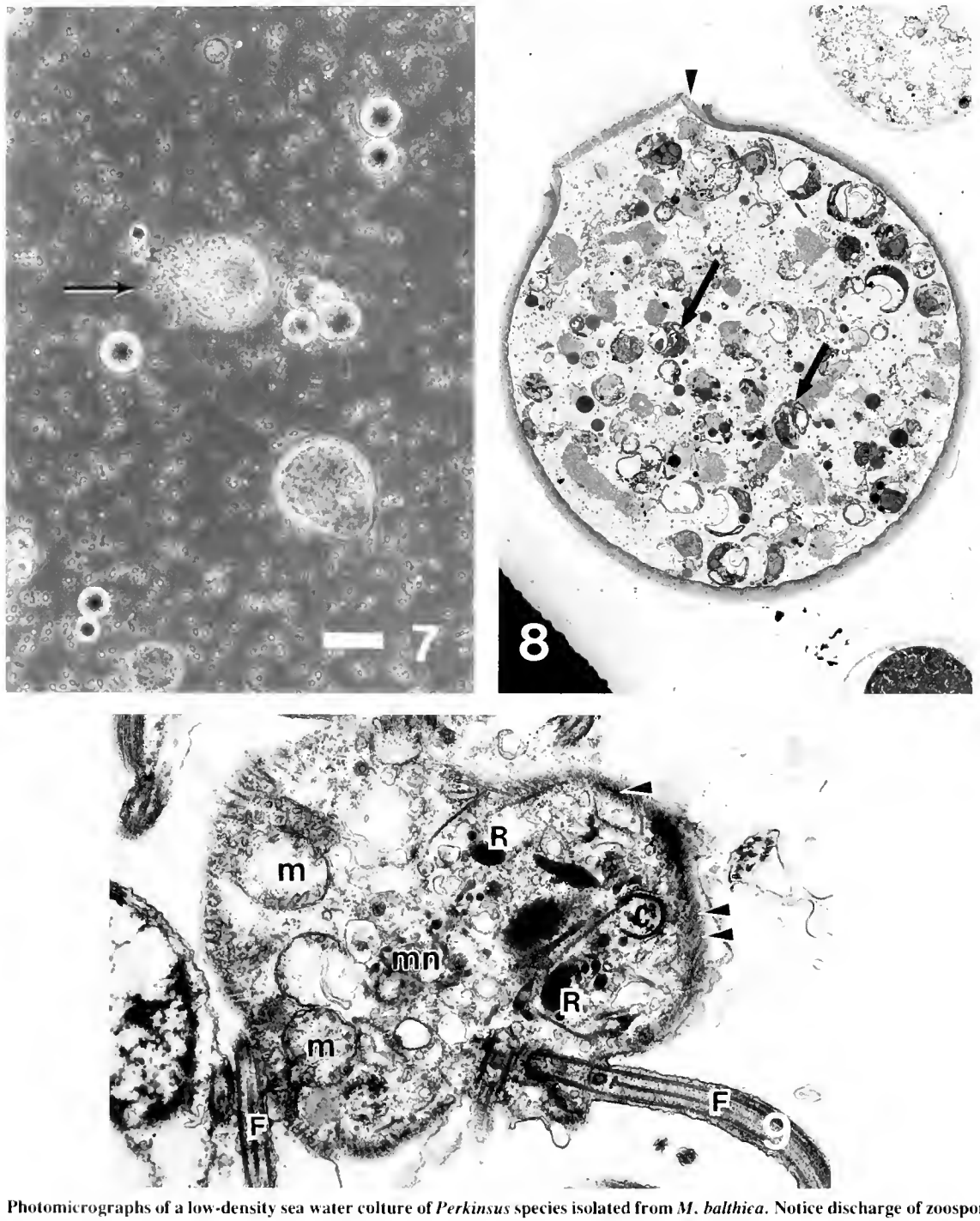

Figure 7. Photomicrographs of a low-density sea water culture of *Perkinsus* species isolated from *M. balthica*. Notice discharge of zoospores from zoosporangium (arrow). Blurred background images are motile zoospores. Scale bar, 0.1 mm.

Figure 8. Zoosporangium of a *Perkinsus* species containing zoospores (arrows). Note the thick zoosporangial wall and the discharge tube and plug of wall material derived from the inner layer of the zoosporangial wall (arrowhead). Original magnification, ×2,240.

Figure 9. Zoospore of a *Perkinsus* species showing apicomplexan structure: Conoid (C), rhoptries (R), subpellicular microtubules (arrowheads), microneme-like organelles (mn), flagella (F), and mitochondrion (m). Original magnification, ×22,800.

kins 1988). Whole mounts of zoospores negatively stained in 0.5% aqueous uranyl acetate exhibited filamentous mastigonemes and spur-like structures identical to those of *P. marinus* (Perkins 1991).

In general, this *Perkinsus* species exhibited more rigorous growth and reproduction than *P. marinus* under similar culture conditions and had a very short doubling time (approximately 20 hours in log phase). Additionally, sea water–induced zoospores were readily returned to the vegetative-propagation state by substitution of the sea water with nutrient-enriched medium and cultured as described above.

DISCUSSION

Isolation and *in vitro* propagation of a vigorous *Perkinsus* species will facilitate studies of the basic biology of this parasite. The ability to induce zoospores and their subsequent release directly from vegetative cultures without the use of the Ray thioglycolate technique (1952) together with the reestablishment of vegetative cultures from zoospores may provide an impetus toward our understanding of this organism's parasitic profile through experimental manipulation. Although species identification was not an objective of this study, in consideration of the morphologial characteristics of this isolate as seen in the host, as well as the possibility of cross-species infection, it is suggested that this organism may be *Perkinsus atlanticus*, which has been described from *Ruditapes decussatres*, a Portuguese clam (Azevedo 1989). Because of the ease of culturing this parasite through an *in vitro* life cycle, it may be appropriate to develop this system as a model for the study of *Perkinsus* species/host interactions.

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A COMPARATIVE STUDY OF THE GAMETOGENIC CYCLES OF THE CLAMS *TAPES PHILIPPINARUM* (A. ADAMS & REEVE 1850) AND *TAPES DECUSSATUS* (LINNAEUS) ON THE SOUTH COAST OF IRELAND

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ABSTRACT A comparative study was carried out on the gametogenic cycles of the introduced Manila clam *Tapes philippinarum* and the native carpet-shell clam *Tapes decussatus* during the period from September 1990 to September 1991. Examination of histological preparations showed that obvious gonadal development began in March for the Manila clam, and in April for the carpet-shell clam. Histological evidence showed that, in the Manila clam, ripe specimens appeared in May with major spawning starting in September. Spawning of this exotic species was not previously recorded in Ireland. In the carpet-shell clam, first ripe specimens were observed in June and major spawning commenced in August. Both species had a unimodal gametogenic cycle in southern Irish waters. Measurement of oocyte size was facilitated by application of an image analysis system, and the resultant data were used to determine the various stages of gonadal development. Mean diameter of free oocytes was 43.2 μm for the Manila clam and 42.6 μm for the carpet-shell clam.

KEY WORDS: gametogenic cycle, oocyte size, image analysis, *Tapes philippinarum*, *Tapes decussatus*

INTRODUCTION

Manila clams, *Tapes philippinarum*, are indigenous to the Indo-Pacific region. They were introduced into Ireland in 1982 because of faster growth rate and the ability to tolerate a wider range of environmental conditions than the native carpet-shell clam *Tapes decussatus*. Despite their commercial importance, there are no published records on the gametogenic cycles of these two species in Irish waters. It is well documented that gametogenic cycles in marine invertebrates are influenced by exogenous as well as endogenous factors (Giese 1959, Sastry 1975, Sastry 1979). The most important exogenous factor, temperature, is closely associated with geographic location. Studies carried out in Japan, North America, and Europe have shown geographic variations in the gametogenic cycles of these two species (Ohba 1959, Holland and Chew 1974, Breber 1980, Beninger and Lucas 1984, Sarasquete et al. 1990, Shafee and Daoudi 1991). It is therefore likely that the gametogenic cycles of the Manila clam and the carpet-shell clam in Ireland are somewhat different from those in other areas. It was observed that the carpet-shell clam occurs only sporadically along the Irish coast (Partridge 1977a, Partridge 1977b). This may be attributed to biological as well as hydrographic factors; for example, abnormality in gametogenesis, asynchronous spawning, poor fecundity, and disruption of metamorphosis may all contribute to the patchy distribution of the carpet-shell clam in Irish water. The main objective of this study was to compare gametogenic cycles of these two species in Ireland and correlate spawning time with environmental conditions in Irish waters.

MATERIALS AND METHODS

Approximately 9 month old juveniles of both species were purchased from a commercial hatchery in May 1990. They were held in trays (1 mm mesh) at a density of 5,000 m^{-2} over a muddy bed in a sheltered inlet at the Atlantic Shellfish Ltd. in Cork Harbour, in the south of Ireland (51°50′N, 8°17′W). Average air exposure was approximately 15%. Density was reduced to 2,500 m^{-2}, and mesh size was increased to 6 mm in July 1990 to facilitate water flow and reduce siltation.

Water temperature and salinity were measured during high tide about every 14 days between May 1990 and March 1992. From September 1990 to September 1991, 12 to 20 clams of each species were used for histological examination of gonadal development and gametogenesis. The visceral mass was fixed in Helly's fixative for 12 to 24 hours. Tissue was then rinsed in running tap water to remove excessive fixative before being stored in 70% ethyl alcohol. Whole visceral mass or, for large specimens, the portion between the labial palps and the foot, was dehydrated in ethyl alcohol and then embedded in paraffin wax. Cross sections of 7 μm were then made in the middle region of the visceral mass. These thin sections were stained with Harris's hemotoxylin and eosin and were examined under a light microscope to determine the stages of the gametogenic cycle.

Changes in cytological characteristics were used to categorize specimens into one of six arbitrary gonadal stages. The five stages used by Holland and Chew (1974) for the Manila clam were adopted, but the criteria were modified, and one extra stage, the inactive stage, was added for sexually undifferentiated specimens (Mann 1979a). The criteria used were as follows.

Inactive

Gonad was predominantly composed of connective tissue; sex was not distinguishable.

Female

Early Active

Gonad proliferation had started, as seen by increasing numbers of discernible oocytes; oocytes were still small; no free oocytes were present in the lumen; mean oocyte diameter was <20 μm; no oocytes were >30 μm; follicle boundaries were not easy to distinguish.

Late Active

Connective tissue was decreasing; free oocytes were present in the lumen but accounted for less than half of the total oocytes in

the follicles; many young oocytes of various sizes were attached to the follicle wall; the mean oocyte diameter was between 20 and 35 μm; more than half of the oocytes had a diameter greater than 20 μm; most of the follicle walls were thick; individual follicles were relatively small but easily discernible.

Ripe

Half or more than half of the oocytes were free in the lumen and had a polygonal configuration; half or more than half of the oocytes had a diameter of ≥35 μm and the mean oocyte diameter was ≥35 μm; follicle size increased; follicle wall was thin.

Partially Spent

Number of free oocytes per follicle had declined; empty follicles appeared; some oocytes had undergone cytolysis.

Spent

Half or more than half of the follicles were empty; follicles became shrunk, fused, or scattered; follicle wall was broken; few residual free oocytes remained; in some specimens, only oogonia and small oocytes remained among the connective tissue and phagocytes.

Male

Early Active

Gonad proliferation started; spermatogonia and spermatocytes were present in the follicles; in more developed specimens, spermatids were also found; no spermatozoa were present.

Late Active

Spermatogonia, spermatocytes, spermatids, and spermatozoa coexisted in the follicles; in less developed specimens, there was no dominant cell type, but in more developed specimens, spermatids and spermatozoa were the major cell types; spermatozoa formed centric or elongate bands in the follicles; spermatozoa mass had a radius of less than half of that of the follicle.

Ripe

Gonad was mainly composed of mature spermatozoa, which formed centric or elongate bands or "plugs" in the follicles; the spermatozoa mass had a radius of more than half of that of the follicle; in very ripe specimens, the spermatozoa bands were close to the follicle wall.

Partially Spent

Mature spermatozoa started to release; an empty space appeared in the center in over 20% of the follicles.

Spent

Follicles were shrunk, fused, scattered, and disorganized; spermatozoa mass occupied approximately 20% or less of the follicle space; in completely spent specimens, only residual spermatozoa could be found in some follicles; connective tissue and phagocytes became increasingly prominent.

For the March and April 1991 samples, 50 oocytes per specimen were measured by the use of ArcImage 5 image analysis software on an ACORN computer. This was increased to 100 oocytes per specimen for May to September 1991 samples as the variance of oocyte size increased. Video images of gonads were digitized and displayed on a color monitor. The boundaries of oocytes were defined by hand, and the areas were then calculated automatically by the computer. Calibration was made with a stage micrometer. For each field, all of the oocytes were sectioned through the nucleus, or for small oocytes, those with nucleolus clearly visible were measured. Several sequential fields were required to achieve the predefined number of oocytes. The "spent" specimens had few measurable oocytes and therefore were excluded from measurement (Grant and Tyler 1983). The area of each oocyte was then converted to the diameter of a circle with equal area. Measuring oocyte size by the image analysis system is considered to be more accurate and less tedious than the traditional method, which used an eyepiece graticule to measure the long axis only (Barber and Blake 1983) or both long and short axes (Brown 1984). These traditional methods are likely to overestimate oocyte size, particularly the near-ripe oocytes with slender stalk. The mean oocyte size and the oocyte size distribution were used in some of the staging criteria to minimize the subjectiveness of a qualitative description.

The size of oocytes lying free in the lumen was measured by the same image analysis system but with a different technique: oocytes were highlighted and only those sectioned through the nucleus were selected for measurement. For each species, 50 free oocytes from each of the 10 randomly selected ripe specimens (2 from June, 4 from July, and 4 from August) were measured.

RESULTS

Data for surface and depth samples were averaged, and the seasonal variations in temperature and salinity are illustrated in Figure 1. Figure 1 shows a cyclical change in temperature with a minimum temperature of 5.6°C in December 1990 and a maximum temperature of 20.2°C in July 1990. Temperature rose to above 8°C, a reported lower temperature limit for gonad activation in the Manila clam (Mann 1979b, Bourne 1982), at the end of February. Each year, there were over 4 months when the temperature was above 14°C, the reported lower temperature limit for spawning in the Manila clam (Mann 1979b). Salinity fluctuated between 24.6 to 34.9‰. Salinity was generally lower during winter and spring than during the summer months.

No hermaphrodites were found in this study in either species. Of the 248 Manila clam specimens examined, 34 were sexually undifferentiated, 109 were female, and 105 were male (Table 1). Of the 250 carpet-shell clam specimens examined, 99 were sexually undifferentiated, 75 were female, and 76 were male in the

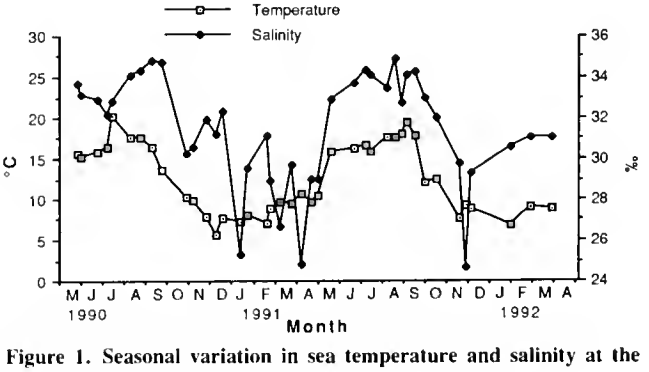

Figure 1. Seasonal variation in sea temperature and salinity at the sampling site (51°51′N, 8°71′W).

TABLE 1.

Seasonal changes in frequency of various gonadal stages in *T. philippinarum* in the period from September 1990 to September 1991.

Date	No. of Clams Examined	Male[a]						Female[a]						Inactive
		EA	LA	R	PS	SP	Total	EA	LA	R	PS	SP	Total	
20 Sept. 90	12	0	2	2	0	0	4	0	0	6	0	1	7	1
19 Oct. 90	18	0	0	1	7	1	9	0	0	0	1	8	9	0
4 Nov. 90	20	0	0	0	1	4	5	0	0	1	4	10	15	0
4 Dec. 90	20	0	0	0	0	3	3	0	0	0	0	10	10	7
31 Jan. 91	20	0	0	0	0	4	4	0	0	0	0	6	6	10
14 Feb. 91	20	0	0	0	0	3	3	0	0	0	0	8	8	9
19 Mar. 91	20	9	1	0	0	0	10	5	0	0	0	0	5	5
15 Apr. 91	19	4	6	0	0	0	10	4	4	0	0	0	8	1
14 May 91	20	0	10	0	0	0	10	1	2	6	0	0	9	1
26 June 91	20	0	7	6	0	0	13	0	1	6	0	0	7	0
15 July 91	20	0	0	12	0	0	12	0	0	8	0	0	8	0
13 Aug. 91	19	0	0	8	0	0	8	0	0	11	0	0	11	0
10 Sept. 91	20	0	0	11	3	0	14	0	0	2	2	2	6	0

[a] EA, early active; LA, late active; R, ripe; PS, partially spent; SP, spent.

same period (Table 2). The sex ratio was not significantly different from a 1:1 ratio for either species ($p > 0.05$, chi-square test, Zar 1984). The seasonal changes in the frequency of various gonadal stages for the two species are listed in Table 1 for the Manila clam and in Table 2 for the carpet-shell clam.

Manila Clams

Gametogenesis began in March. This was seen by the increasing numbers of discernible oogonia and small oocytes in females and the occurrence of spermatocytes or even spermatids in males. Active gamete proliferation took place in April and May. In April, 10 of 19 samples were in the late active stage. The first ripe specimens appeared in May, and all specimens were in the ripe stage in July and August (Table 1). Although minor release of spermatozoa from more developed follicles might occur as early as May, mature oocytes appeared to be retained in the follicles whereas young oocytes grew to full size. This was reflected by the declining percentage of oocytes of <35 μm between May and August (Fig. 2a). From March to August, mean oocyte size steadily increased (Fig. 3) as a result of oocyte growth and the associated decline in the number of young oocytes. During vitellogenesis, both follicle size and number of mature oocytes per follicle appeared to increase significantly. In August, very few young oocytes were present. As the pressure in the follicles increased, the mature oocytes acquired a polygonal configuration. Major spawning did not commence until September, when partially spent and spent specimens were found. Development of a new batch of oocytes was observed in some specimens in this month; however, these young oocytes failed to grow because water temperature was decreasing. Mean oocyte size decreased in September as a result of spawning and redevelopment (Fig. 3). Most of the specimens were either in the partially spent stage or in the spent stage in October. Although occasional ripe specimens could still be found in November, most of the clams were in the spent stage by this time. Beginning in December, the gonads entered a resting period during which there was no sign of gonadal activity. This resting period ended in March when gametogenesis started once again.

TABLE 2.

Seasonal changes in frequency of various gonadal stages in *T. decussatus* in the period from September 1990 to September 1991.

Date	No. of Clams Examined	Male[a]						Female[a]						Inactive
		EA	LA	R	PS	SP	Total	EA	LA	R	PS	SP	Total	
20 Sept. 90	14	0	0	0	1	5	6	0	0	0	1	6	7	1
19 Oct. 90	19	0	0	0	0	5	5	0	0	0	0	3	3	11
4 Nov. 90	19	0	0	0	0	4	4	0	0	0	0	2	2	13
4 Dec. 90	20	0	0	0	0	0	0	0	0	0	0	1	1	19
31 Jan. 91	20	0	0	0	0	0	0	0	0	0	0	0	0	20
14 Feb. 91	20	0	0	0	0	0	0	0	0	0	0	0	0	20
19 Mar. 91	20	0	0	0	0	0	0	12	0	0	0	0	12	8
15 Apr. 91	20	7	0	0	0	0	7	6	0	0	0	0	6	7
14 May 91	20	5	3	0	0	0	8	3	9	0	0	0	12	0
26 June 91	19	0	7	3	0	0	10	0	7	2	0	0	9	0
15 July 91	20	0	4	10	0	0	14	0	1	5	0	0	6	0
13 Aug. 91	19	0	0	10	1	0	11	0	0	4	3	1	8	0
10 Sept. 91	20	0	0	1	7	3	11	0	0	0	8	1	9	20

[a] EA, early active; LA, late active; R, ripe; PS, partially spent; SP, spent.

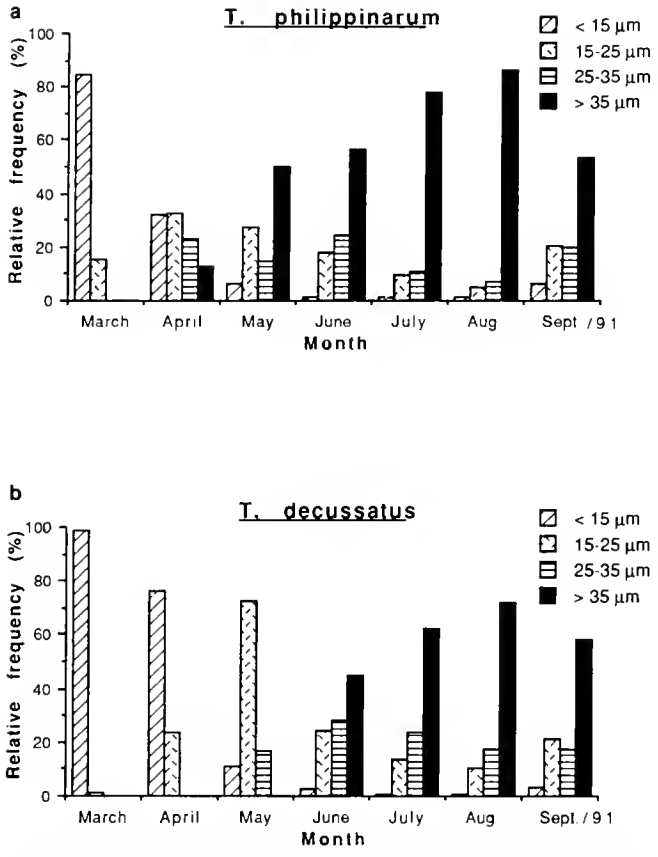

Figure 2. Changes in the relative frequency distribution (%) of oocyte size in the period from March 1991 to September 1991 for *T. philippinarum* (a) and *T. decussatus* (b). N = 250 to 1,200.

Carpet-Shell Clams

Although female gonads showed early signs of gonadal activity in March, male gonads were still in the resting stage (Table 2). Obvious gonadal development was not observed for either sex until April. The first late active specimens were observed in May, and the first ripe clams were seen in June. The highest frequency of ripe stages was observed in July and August. Major spawning activity began in August when 1 of 11 of males was partially spent, 3 of 8 of females were partially spent, and 1 of 8 of females was spent. Spawning appeared to be completed by the end of September or early October. There was a higher percentage of oocytes <35 µm in the follicles each month between May and August in the carpet-shell clam than in the Manila clam (Fig. 2a and b). Follicle size and number of mature oocytes per follicle also appeared to be smaller than those of the Manila clam. Mean oocyte size steadily increased from March to July and remained more or less unchanged in August and September (Fig. 3). Gonadal redevelopment may also have occurred in August and September; however, the persistent presence of small oocytes made it difficult to state with confidence whether it truly happened. After spawning was completed, residual gametes were absorbed and the gonad of the carpet-shell clam entered a resting period beginning in September (Table 2).

The size of free oocytes from morphologically ripe females ranged between 20.2 and 58.7 µm for the Manila clam and between 23.0 and 56.3 µm for the carpet-shell clam. The mean diameter of 500 free oocytes was 43.2 ± 6.2 µm (standard deviation [SD]) for the Manila clam and 42.6 ± 6.1 µm (SD) for the carpet-shell clam. There was no significant difference in mean oocyte diameter between the two species when all of the data were pooled (N = 500) ($p > 0.05$, *t*-test, Zar 1984). However, a statistically significant difference existed among individuals of the same species sampled in the same month ($p < 0.05$, analysis of variance, Zar 1984) in July and August for the Manila clam and in August for the carpet-shell clam. Most of the free oocytes (78.6% for the Manila clam and 77.8% for the carpet-shell clam) had a diameter between 35 and 50 µm. Oocytes that were larger than 50 µm only accounted for 13.0% of the total number in the Manila clam and 9.6% in the carpet-shell clam (Fig. 4).

DISCUSSION

It is well established that temperature is one of the most important environmental factors that influence the gametogenic cycles of molluscs (Giese 1959, Mann 1979a and b, Sastry 1975). The effect of temperature on the gametogenic cycles of *T. philippinarum* and *T. decussatus* was also evident when these results were compared with those obtained from other areas.

The Manila clam has been reported to have one spawning period in Washington state (Holland and Chew 1974), two spawning periods in Japan (Ohba 1959), and three spawning periods in southwest Spain (Sarasquete et al. 1990). From these records and others (Mann 1979b), it appeared that, for the Manila clam, the lower temperature limit for gonadal activity is approximately 8°C, 12°C for gamete ripening, and 14°C for spawning. Low temperature affects gametogenesis as well as spawning (Mann 1979b, Bourne 1982). In the south of Ireland, the temperature require-

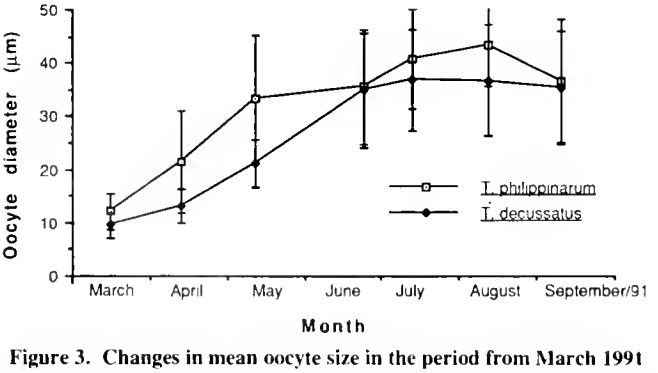

Figure 3. Changes in mean oocyte size in the period from March 1991 to September 1991 for *T. philippinarum* and *T. decussatus*, with vertical bars representing SD. N = 250 to 1,200.

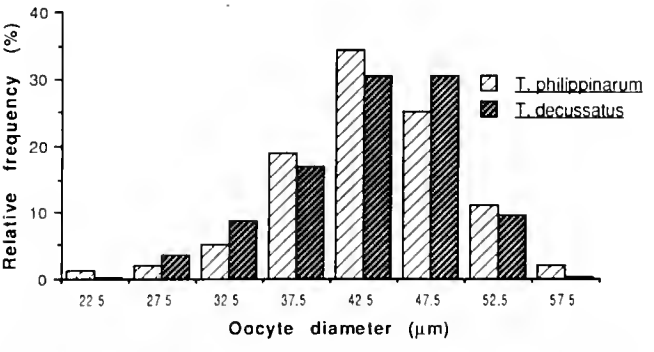

Figure 4. The relative frequency distribution (%) of free oocyte size (N = 500) of *T. philippinarum* and *T. decussatus*.

ments for various stages of the gametogenic cycle were generally met (Fig. 1). Histological evidence obtained in this study showed that most Manila clams completed their gametogenic cycle. However, the relatively low temperature encountered by this population in the south of Ireland seemed to delay gonadal development and spawning when compared with populations in warmer climates (Ohba 1959, Sarasquete et al. 1990). This time lag could be explained by a time-temperature effect (Mann 1979b). Nevertheless, these results were generally consistent with findings from areas with similar temperature ranges (Beninger and Lucas 1984, Coleman 1989). Although there is little doubt that the temperature in Irish waters is sufficient for adult Manila clams to reach the ripe stage, questions have been raised as to whether the ripe gametes were actually released by spawning or resorbed (Coleman 1989). This study showed that, in September 1991, 4 of 6 females were either partially spent or spent and 3 of 11 males were partially spent. Temperature at that time was ≈18°C, well above the lower temperature limit (14°C) for spawning. This would therefore appear to be the first recorded case of the Manila clam spawning in Irish waters. There were approximately 2 more weeks before the water temperature decreased below the 14°C limit. During this period, further spawning could have occurred. Although only 1 of 12 clams was in the spent stage on 20 September 1990, most of specimens were in the partially spent or spent stages on 19 October (Table 1). Between 20 September 1990 and 19 October 1990, water temperature was above 14°C for approximately 7 days, during which spawning could have taken place. Resorption of these ripe gametes during this period was not very likely because no massive cytolysis was observed. One of the 20 specimens was in the ripe stage in November, the rest were either in the partially spent or spent stage. Mature gametes remaining in the follicles in this month were believed to be resorbed because the temperature was too low to stimulate spawning. The delay in spawning, which appeared to occur in the apparently ripe clams sampled in the summer of 1991, suggested a degree of synchronization in spawning activity among individuals in the population.

Since its introduction to Ireland in 1982, natural recruitment of the Manila clam has not been recorded. Late spawning is believed to be one of the major reasons for no recruitment because the water temperature in the months after spawning would not be conducive for larval development.

These results of the gametogenic cycle of the carpet-shell clam showed that this species had only one spawning period. Gametogenesis began in late spring (March/April), and the first ripe clams appeared in June. Spawning commenced in August and ceased in September. These findings compared well with previous observations of this species from temperate regions (Breber 1980, Beninger and Lucas 1984). In warmer years, gonadal development and spawning began earlier in the year, as observed by Partridge (1977b) in populations from the west of Ireland. The timing of the major events in the reproductive cycle was also clearly associated with latitude, with clams from more southerly waters of the northern hemisphere tending to reach the ripe stage and spawn earlier in the year. For example, spawning started in early May in the Atlantic coast of Morocco and there were two major spawning periods instead of one (Shafee and Daoudi 1991).

Although there is natural recruitment, the carpet-shell clams are believed to be less successful in Irish waters than in southern European waters (Partridge 1977a and b). Examination of the histological preparations alone did not provide conclusive evidence to suggest that gametogenesis in the carpet-shell clam was abnormal. However, visual assessment of the gonads of the two species showed that follicle size, number of free oocytes per follicle, and overall density of free oocytes appeared to be markedly smaller in the carpet-shell clam than in the Manila clam. Whether this is an interspecific difference or a sign of poor fecundity is not clear. The late spawning of both species in Ireland, as observed in this study, is believed to reduce the chance of larval survival, which is optimal at 23 to 26°C for *T. philippinarum* (Helm and Pellizzato 1990) and at 25°C for *T. decussatus* (Partridge 1977b).

The wide range of free oocyte size is accounted for by the presence of some small oocytes and also by the fact that some specimens had significantly different mean free oocyte size. There is no direct evidence that the small oocytes (<35 μm) were fully mature. An artificial fertilization is required to test if these small oocytes are in fact physiologically ripe.

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IN SITU GROWTH RATES OF THE OCEAN QUAHOG, *ARCTICA ISLANDICA* (LINNAEUS, 1767), IN THE MIDDLE ATLANTIC BIGHT

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ABSTRACT Shell morphometric measurements of laboratory-spawned and Gulf of Maine ocean quahogs (*Arctica islandica*) transplanted at a site of commercially important shellfish beds off Cape May, New Jersey, indicate slow adult growth rates. Clams greater than 50 mm in length grew very slowly or not at all for extensive periods of time after transplantation. The mean increase in the shell length of adult clams from the Gulf of Maine amounted to 0.35, 0.39, −0.02, 0.40, and −0.10 mm when measured 213, 307, 368, 520, and 606 days after transplantation, respectively. Younger, laboratory-spawned clams exhibited greater growth rates than adults, with a mean increase in shell length of 1.87, 1.34, 5.28, and 0.54 mm being recorded 75, 120, 288, and 374 days after transplantation, respectively. These findings support those of other investigations, which have shown that the growth of *A. islandica* is among the slowest of all continental-shelf bivalves.

KEY WORDS: ocean quahogs, *Arctica islandica*, transplantation experiments, growth rates

INTRODUCTION

The ocean quahog *Arctica* (=*Cyprina*) *islandica* Linne, a sea clam of great commercial value, is currently harvested in large quantities off the East Coast of the United States. In 1991, U.S. commerical landings of *A. islandica* totaled 22,275 mt of meats, with most landings derived from the Exclusive Economic Zone off of New Jersey and the Delmarva Peninsula (Mid-Atlantic Fishery Management Council 1992). Although the Delmarva Peninsula had the greatest landings and fishing effort between 1982 and 1989, New Jersey is now the principal area for commercial exploitation of ocean quahogs. Concentrated fishing activity off New Jersey reduced the catch per unit effort (CPUE) by 29% between 1986 and 1992, and it now appears to be depleting the stock (Weinberg 1993). According to Weinberg (1993), the ocean quahog resource off New Jersey may be exhausted within 10 years at the current removal rates.

Because of the increasing importance of the ocean quahog to the sea clam industry during the 1970s, the Mid-Atlantic Regional Fisheries Council initiated a management plan in 1977 that included research on the biology and ecology of the species (Ropes 1979). As a result, detailed studies have been conducted on the distribution, abundance, growth, and reproduction of *A. islandica* during the past 17 years (Fogarty 1981, Jones 1981, Mann 1982, Murawski et al. 1982 and 1989, Ropes 1984, Ropes et al. 1984a, Rowell et al. 1990, Fritz 1991, Kraus et al. 1991). Despite these studies, considerable uncertainty still exists with regard to the size of the newly recruited resources in the Mid-Atlantic Bight area and whether these resources can continue to adequately support the fishery in the near and distant future. Early management of the stock was based on the concept of a very limited productivity potential of the resource, ascribable to slow growth and poor recruitment rates. Hence, harvest rates greater than a few percent of the extant stock were predicted to rapidly deplete the accumulated stock (Murawski et al. 1989). Presently, annual landings represent about 2 to 6% of the total harvestable stocks.

Proper management of sea clam stocks is clearly dependent on accurate age and growth rate data as well as reliable recruitment estimates of natural populations. Although there has been a gradually accumulating data base on the growth rates and age structure of ocean quahogs along the East Coast of the United States, there is a dearth of information on recruitment of the clams in this region. Several studies have shown that growth rates are extremely slow and longevity very long in the species (Jones 1980a and 1980b, Thompson et al. 1980a and 1980b, Forster 1981, Ropes et al. 1981 and 1984b, Ropes and Pyoas 1982, Murawski et al. 1982, Turekian et al. 1982, Ropes and Murawski 1983, Murawski and Serchuk 1989). Age estimates as high as 157 and 225 years have been reported for clams dredged off central New Jersey and southern Massachusetts, respectively, on the basis of analyses of "annual" growth patterns within the organism's shell (Ropes and Murawski 1983). Murawski and Serchuk (1983), using data on shell growth, have projected that 17% of the New Jersey resource and 16% of the Delmarva resource is in excess of 100 years old. In addition, the median age of these exploited stocks may be more than 70 years. These sea clams may take 20 years or more to reach commercial size, with depleted stocks requiring 50 to 100 years to replenish themselves (Weinberg 1993). These data, if correct, have serious implications for the prudent management of this valuable resource.

National Oceanic and Atmospheric Administration (NOAA) surveys of ocean quahogs along the East Coast of the United States

since 1965 have not detected any new recruitment in the fishery, although only two surveys (1989 and 1992) have been conducted since 1986 (Mid-Atlantic Fishery Management Council 1992). Estimates of the resource distribution are as follows: southern Virginia–North Carolina = 1%, Delmarva Peninsula = 8%, New Jersey = 21%, Long Island = 28%, southern New England = 28%, Georges Bank = 22%. Although the proportion of the resource off southern New England (28%) is substantial, it does not appear to be economically harvestable because of problems with bottom topography. In addition, the resource on the Georges Bank (22%) cannot be harvested because the area is closed as the result of paralytic shellfish poisoning. Hence, at least 50% of the ocean quahog resource is currently unharvestable. Current management strategies for the ocean quahog resource are predicated on the stock as essentially a mine, with limited harvest rates relative to standing stock, but no known frequency of recruitment. That no new recruitment has been observed anywhere in the Mid-Atlantic Bight raises concern regarding the long-term management plan for the resource (Mid-Atlantic Fishery Management Council 1992). At present, there is little interannual variability in population size or structure of ocean quahogs in the Mid-Atlantic Bight, owing to the absence of recruitment, long generation time of the species, slow adult growth rates, and low mortality rates (Weinberg 1993).

Data on recruitment of ocean quahogs in exploited populations of the Mid-Atlantic Bight are critically important to decision makers in the sea clam industry and government fisheries programs who must provide effective long-term management of this valuable resource. The paucity of recruitment data presently available on this species places a significant constraint on effective management of the fishery. Recruitment data are especially needed in the deeper coastal waters (i.e., 40 to 65 m), where the greatest densities of the clam are found. Plans are underway to conduct initial recruitment studies of ocean quahogs along the continental shelf off of New Jersey.

MATERIALS AND METHODS

Between June 1987 and June 1992, investigations of *in situ* growth rates of *A. islandica* were conducted at a continental-shelf site located 65 km off of Cape May, New Jersey (39°00′45″N, 74°04′32″W) at a depth of 45 m (Fig. 1). This site was selected for several reasons: (1) the area is representative of the most actively fished locations off the coasts of New Jersey and the Delmarva Peninsula, (2) the presence of dense populations of clams suggests a most suitable habitat for survival of transplanted clams, and (3) the wreck of a Norwegian vessel, the *Varanger*, provides an ideal location for transplantation experiments. These investigations served as a precursor to recruitment studies being planned at this location. The principal objective of the study was to monitor the growth of ocean quahogs (some of known age) transplanted in experimental predator-exclusion cages at the coastal site. To obtain growth rates on the clams, SCUBA divers deployed and recovered the animals on regularly scheduled cruises to the site, enabling the acquisition of a time series of size measurements. The experimental cages were initially used to mollify the effect of sea star predation, which was a major problem early in the study.

Investigators followed several procedural steps when transplanting the clams:

1. Numbered specimens were first placed within 0.64 cm mesh, polyethylene bags (one to five individuals per bag; bag dimensions measured 20.32 × 20.32 × 7.62 cm).
2. The bags were subsequently sealed onboard the ship and transported to the bottom by SCUBA divers, where the bags were placed within 1.27 cm mesh, polyvinylchloride (PVC)-coated galvanized wire cages (one to four bags per cage; cage dimensions measured 60.96 × 60.96 × 17.78 cm).
3. The bags were then nestled within the wreckage of the forward, starboard section of the stern of the military vessel (MV) *Varanger*, a vessel that was torpedoed and sunk in 1942 during World War II.

The use of polyethylene bags decreased the handling time of individual clams, thereby easing deployment operations. The *cage-within-a-cage* system not only effectively eliminated sea star predation but also facilitated rapid recovery and measurement of the clams. Placement of the cages directly in the wreck of the *Varanger* was intended to mitigate dredge/weather interference, thus increasing the probability of achieving the goals of the project.

Early in the research program, 96 sea clams were transplanted in four cages at the study site, including 67 specimens (shell length = 48.5 to 63.3 mm) transferred from a Gulf of Maine population in November 1989 and 29 known-age, laboratory-spawned sea clams (shell length = 9.2 to 19.9 mm) placed at the site in July 1990. These latter 29 clams were spawned in laboratories of the Virginia Institute of Marine Science at Wachapreague, Virginia, under the direction of Michael Castagna. Sequential numbers were etched into the shells of the clams transplanted from the Gulf of Maine, allowing the growth rate of each individual to be carefully tracked. Because of the small size of the laboratory-spawned clams at the time of transplantation and concerns with potentially adverse effects of the etching process, the shells of these clams were not numbered.

During the 5-year study period, 16 cruises were successfully completed, with the last cruise to the site being June 1, 1992. At that time, it was discovered that nearly all experimental animals had been lost, probably when two major coastal storms (''nor'easters'') passed through the area, one in October 1991 and the other in January 1992. On the final trip to the study site, SCUBA divers found that the clam cages had been extensively damaged (i.e., they were broken apart and/or buried deep in sediment), and only two live clams were recovered.

RESULTS

Laboratory-Spawned Clams

Laboratory-spawned clams (N = 29) were transplanted to the study site on July 1, 1990. Standard shell morphometric measurements (i.e., shell length, height, and width) of these clams were made on September 16 and November 16, 1990, and April 19 and July 12, 1991. These dates represent *in situ* terms of 75, 120, 288, and 374 days, respectively, for the known-age specimens. As noted previously, the clams were also recovered on June 1, 1992, but extensive damage to the cages and poor survivorship of the transplants resulted in a limited number of growth measurements. The total length of time that the laboratory-spawned clams were transplanted at the experimental site is estimated to be between 485 and 577 days.

Table 1 lists the mean incremental growth rate of the laboratory-spawned clams during the period of transplantation. On the basis of length measurements, the clams exhibited steady, albeit slow growth over the year. Growth was generally variable between sampling periods, with the greatest increase in the axial measurements occurring during the colder months between November

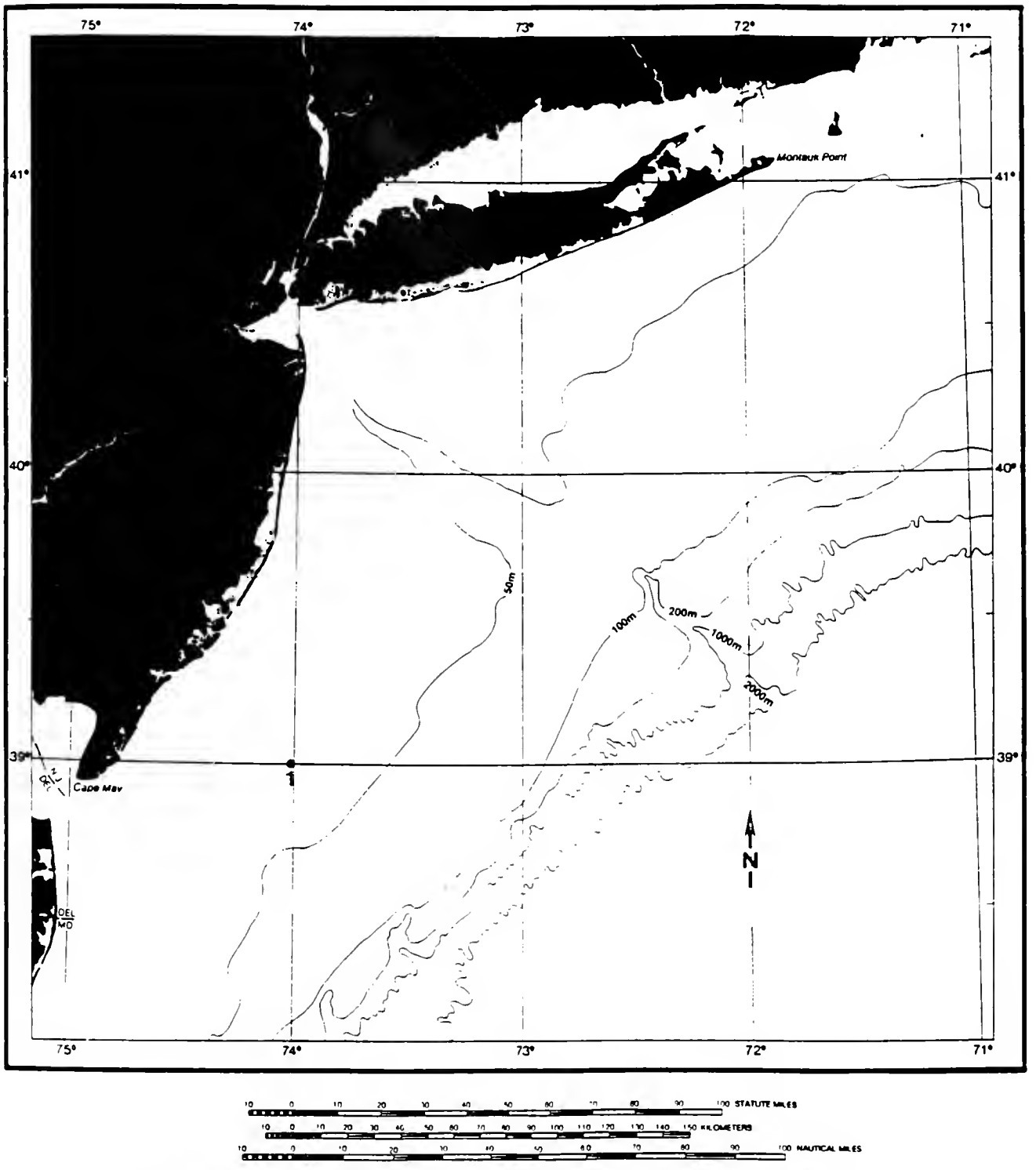

Figure 1. Map of research area showing site of *in situ* growth studies of *A. islandica* off Cape May, New Jersey.

1990 (120 days) and April 1991 (288 days). Mean increases in length, height, and width during this period amounted to 4.55, 3.53, and 2.73 mm, respectively (Fig. 2). Growth was relatively uniform during the other sampling periods, except in the final year, when the least amount of growth was recorded for each axial measurement.

Difficulties of measuring precisely with calipers along the same plane of growth (i.e., length, height, and width planes) each time, together with operator error with the calipers, resulted in spurious growth measurements on some clams. In most cases, when discrepancies were noted during the recording process, the clams were remeasured for verification. In cases when the discrepancies were not uncovered during the recording process, an effort was made to resolve the spurious measurement during the subsequent cruise.

Until the last cruise to the study site on June 1, 1992, mortality

TABLE 1.

In situ mean incremental growth rate (in millimeters) of laboratory-spawned transplants.

Days	T_0[a]	75	120	288	374	485/577
Length	0	1.87	1.34	5.28	0.54	0.16
Height	0	1.91	1.03	4.24	1.37	0.16
Width	0	1.09	0.98	2.81	0.46	0.36

[a] T_0, time of initial planting.

TABLE 2.

In situ mean incremental growth rate (in millimeters) of Gulf of Maine transplants.

Days	T_0[a]	213	307	368	520	606	717/809
Length	0	0.35	0.39	−0.02	0.40	−0.10	0.51
Height	0	0.40	0.38	−1.45	−1.07	0.80	0.94
Width	0	0.44	0.41	0.16	0.36	0.30	0.80

[a] T_0, time of initial planting.

of the known-age clams was low. No individuals died between July 1 and November 1990. On both April 19, 1991, and July 12, 1991, four dead clams were found. On June 1, 1992, SCUBA divers recovered the cages, but they were severely damaged. The polyethylene bags containing the clams were also missing. Two of the bags were located more than 15 m from the project site, and all but two of the clams in these bags were dead.

Gulf of Maine Transplants

In November 1989, 67 clams were transplanted from the Gulf of Maine to the study site after being measured and numbered. The length of these clams ranged from 48.5 to 63.3 mm. Size measurements on the clams were registered on November 13, 1989, June 14, 1990, September 16, 1990, April 17, 1991, July 12, 1991, and June 1, 1992. Not all of the clams were measured on September 16, 1990, because only three of the cages were recovered. SCUBA divers were unable to locate one cage because of poor visibility and because the cage had been displaced from the study site. It was recovered on the subsequent cruise date and relocated to the site of the *Varanger*. All of the cages containing the Gulf of Maine transplants incurred extensive damage before their recovery on June 1, 1992, and mortality of the experimental population was substantial. The total length of time that the Gulf of Maine clams were transplanted at the experimental site is estimated to be between 717 and 809 days.

The mean incremental growth rate of the Gulf of Maine transplants during the period of transplantation is presented in Table 2. Negative growth in shell height was recorded in November 1990 (368 days) and April 1991 (520 days), amounting to −1.45 and −1.07 mm, respectively. This negative growth may have been due to inaccuracies in measuring procedures or actual shell dissolution. Overall, the population surviving through July 1991 (606 days) had a small positive mean growth increment along each axis of the shell. The total increase in axial measurements for the entire period of transplantation was as follows: length, 0.51 mm; height, 0.94 mm; and width, 0.80 mm. The greatest increase in growth occurred during the final year of transplantation.

The growth rate of the Gulf of Maine transplants was significantly lower than that of the smaller laboratory-reared stock. On the first two cruises after transplantation, June and September 1990, the mean increase in the shell length of the Gulf of Maine transplants was 0.35 and 0.39 mm, respectively. These measurements represent *in situ* time periods of 213 and 307 days.

Mortality of the Gulf of Maine transplants was low before the major coastal storms of November 1991 and January 1992. During

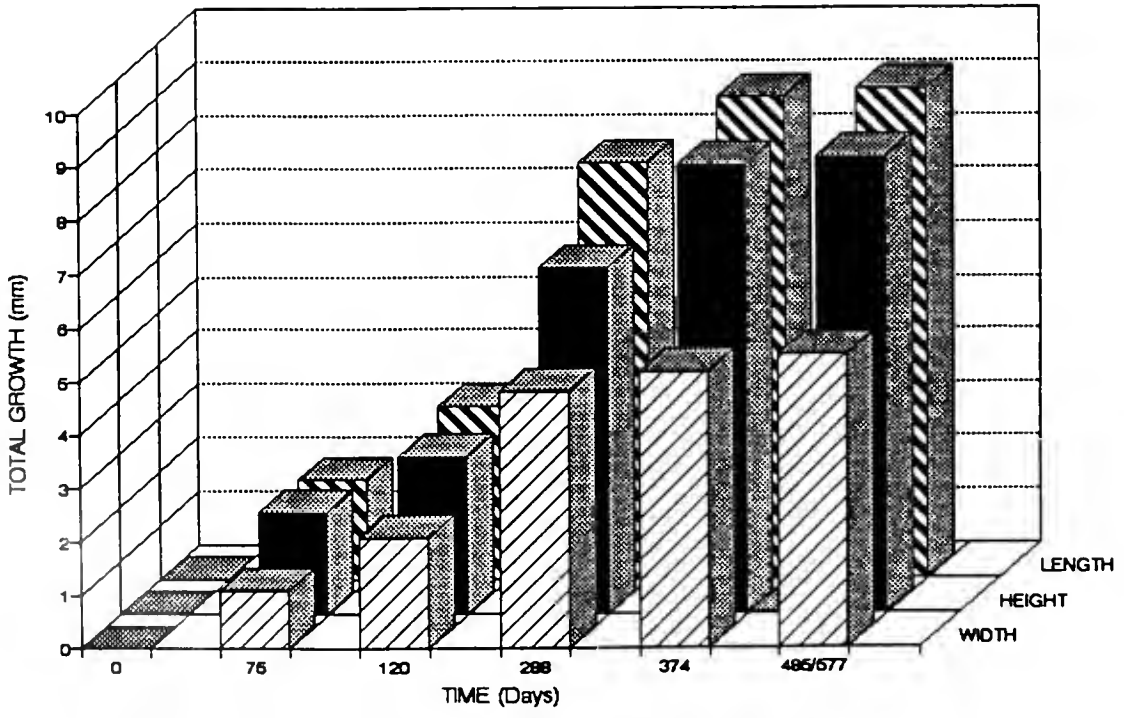

Figure 2. *In situ* mean cumulative growth rate (length, height, width) of laboratory-spawned transplants.

the 2-year period before these storms, only 29 of the original 67 transplants had died. Seventeen clams died before the redundant caging technique was implemented, presumably from sea star predation. Twelve clams died for unexplained reasons after being placed in the redundant cages. Several of the clams had broken or damaged shells, possibly the result of being tossed about in the cages by shear stresses along the bottom, associated with storm surges.

DISCUSSION

On the basis of the transplantation experiments described above, a general pattern of growth is beginning to emerge for *A. islandica* in the New York Bight off of Cape May, New Jersey. The *in situ* data suggest that, although growth may be variable, it is relatively rapid in small, juvenile clams. Smaller clams (<25 mm in length) may grow at rates of 10 to 12 mm per year. Larger clams (>50 mm in length) appear to have substantially reduced growth rates and may not grow at all for long periods of time. These findings support the results of other investigators (e.g., Jones 1980b, Thompson et al. 1980a, Murawski et al. 1980 and 1982, Ropes et al. 1984b), who reported extremely slow (*in situ*) growth of sea clam populations (at least in clams >20 mm).

Murawski et al. (1982) conducted one of the most comprehensive studies of ocean quahog growth to date by planting about 42,000 marked clams at a deep-water (53 m) site located 48 km SSE of Shinnecock Inlet, Long Island, New York (40°25.1′N, 72°23.7′W), in 1978 and recovering individuals annually thereafter. By examining annuli formed in the shells of these recovered specimens, Murawski et al. (1982) recorded annual increases in shell length of the experimental animals amounting to 6.3% at age 10, 0.5% at age 50, and 0.2% at an estimated age of 100 years. These investigators also developed a growth rate relationship for the marked clams (59 to 104 mm in shell length [SL]) recaptured 1 year after their release ($SL_{t+1} = 2.0811 + 0.9802\ SL_t$) and an age/growth relationship [$SL = 75.68–81.31\ (0.9056)^t$] for younger, smaller specimens sampled from a natural population of unmarked clams in the vicinity of the experimental site. These results reveal that ocean quahogs are among the slowest growing bivalves inhabiting continental-shelf waters, except perhaps during the first 20 years of their life. They are also one of the longest lived bivalve species, with a potential lifespan of about 225 years (Ropes and Murawski 1983).

Growth rates of the ocean quahog vary along its geographic range, and considerable variability may occur in the size/age relationships and longevity of the species. Ropes and Pyoas (1982), comparing the annuli in the shells of ocean quahogs from sites off Long Island, New York, off Sable Island, Canada, and from Georges Bank, found significant variation in growth rates of the clams. Specimens from Georges Bank exhibited substantially greater growth rates than those from off Long Island, New York, and off Sable Island, Canada. However, as noted by Murawski et al. (1980), data are not comprehensive enough to state conclusively that a latitudinal cline in ocean quahog growth exists.

It is unclear what effect the artificial environment created by the cages had on the growth or mortality of the experimental clams. On all dives to the seafloor, SCUBA divers observed sediment accumulation in the cages that buried the clams. Hence, the conditions for the clams in the cages may not have been substantially different from those along the neighboring sea floor. The artificial environment of the cages could have contributed to the observed mortality, but it has not been possible to determine how or why this is the case.

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TEMPORAL AND SPATIAL EFFECTS OF TIDAL EXPOSURE ON THE GAMETOGENIC CYCLE OF THE NORTHERN QUAHOG, *MERCENARIA MERCENARIA* (LINNAEUS, 1758), IN COASTAL GEORGIA

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ABSTRACT Experimental reproductive studies of the gametogenic cycle of the northern quahog, *Mercenaria mercenaria* (Linnaeus, 1758), were performed on clams planted at various tidal heights within their natural tidal distribution. Quahogs from a homogenous natural population were planted at the subtidal, mean low-water mark, oyster zone at the 2 hours above mean low-water mark, and within the salt marsh for a 12 month acclimation period before sampling. Clams sampled starting 12 months after being moved to the marsh were significantly ($p < 0.0001$) smaller in size than were clams from the other three tidal heights. There were no significant ($p = 0.5458$) differences in ages of transplanted quahogs at the four tidal heights. Qualitative results show a similar timing of oogenic cycles at all four sites. Females from the marsh site had consistently lower gonadal index values, delayed peak gonadal index values, and a delay of 1 month in spawning. The pattern for males from the marsh is dissimilar to that for males found at other sites, with no peak values occurring and spawning occurring a month later. For quantitative results, quahogs from the marsh site had significantly lower values (% male gonadal area, % gonadal area occupied by spermatozoa, % female gonadal area, % gonadal area occupied by ova, mean egg number per field of view, and mean egg diameter) than quahogs from the other sites. Although reproductive parameters for the three lower tidal sites were not significantly different, there was a consistent decrease in all reproductive parameters measured with increases in tidal exposure. Cage fouling appeared to alter the gametogenic pattern for clams planted subtidally, as observed in decreases in percent gonadal area and percent area occupied by gametes, followed a month later by decreases in mean egg size and numbers of eggs present. Subtidal clams had a more intense spawn compared with clams from the other sites. Temporal alterations in the reproductive pattern of quahogs occurred according to tidal exposure.

KEY WORDS: Clam, gametogenesis, intertidal, *Mercenaria*, oocyte, reproduction, spawning

INTRODUCTION

The northern quahog, *Mercenaria mercenaria* (Linnaeus, 1758), primarily inhabits intertidal areas within coastal Georgia (Walker and Tenore 1984, Walker 1987), but it occurs principally in subtidal areas throughout the remainder of its natural range. In the salt-marsh ecosystem of coastal Georgia, quahogs inhabit both subtidal and intertidal areas (creek bottoms and banks, river banks, tidal flats), up into the lower edge of the salt marsh itself. There were no significant differences in survival of quahogs planted from the spring low-water mark up to 3 hours above mean-low water; however, growth decreased with increases in aerial exposure time (Walker and Heffernan 1990). Because gametogenesis is directly related to size, it is assumed that reproductive effort may also be adversely affected by increases in intertidal exposure time. Increased intertidal exposure can result in the reduction in the amount of gametes produced by bivalve populations (Seed 1969, Harvey and Vincent 1989), alternation in spawning patterns (Hunter 1949, Boyden 1971, Griffiths 1981, Borrero 1987), or a combination of both effects (Seed and Brown 1977).

The northern quahog represents an important commercial species for the eastern U.S. coastal fisherman. The gametogenic cycle of the northern quahog has been studied throughout its natural range (reviewed by Eversole 1989). However, spatial effects on reproduction studies within a single locale are lacking for this species with two exceptions. Keck et al. (1975) determined that no differences in spawning patterns occurred between two quahog beds located within the Delaware Bay; however, differences in the maturation process were detected. Yet, these beds occurred in typical subtidal habitats where large differences in gametogenesis are less likely to occur. Eversole et al. (1980), using standard histological analysis techniques, found no differences in reproductive parameters between quahogs grown at different densities or between intertidal and subtidal areas in South Carolina. However, gonadal-somatic indices (GSI) analysis revealed that clams grown at lower densities and at the subtidal site had higher GSI values than did those grown at high densities or at the intertidal site (Eversole et al. 1984). The intertidal zone represents a harsh environment for quahogs, with the degree of stress increasing with increases in tidal exposure time. This study uses quantitative histological techniques to determine spatial (increased tidal exposure) effects on quahog gametogenesis in coastal Georgia.

MATERIALS AND METHODS

In August 1990, approximately 1,200 quahogs ranging in size from 30 to 65 mm in shell length (i.e., longest possible measurement: anterior-posterior) were collected from House Creek, Little Tybee Island, Wassaw Sound, Georgia. Quahogs were divided into four equal groups of 300 clams. A set of quahogs was placed within a 12.7 mm mesh, vinyl-coated wire cage, $1 \times 1 \times 0.5$ m that was placed just below the spring low-water mark, at the mean-low water, at 2 hours above mean-low water (the oyster zone), and just within the salt marsh adjacent to the Shellfish Research Laboratory on Skidaway Island, Georgia. Quahogs were allowed to grow at these sites until October 1991, before sampling began, to

allow clams to fully acclimate to their respective intertidal heights. By allowing a 12 month delay in sampling, the quahogs would have presumably gone through at least two natural reproductive episodes (Heffernan et al. 1989) before the start of this experiment.

Beginning October 1991, monthly samples of 10 to 20 quahogs were collected per tidal site. Each quahog was measured for shell length with Vernier calipers, aged according to shell band analysis (Walker 1987), and dissected along a standard axis from umbo-to-foot with a gonadal sample removed for histological analysis (Howard and Smith 1983). The visceral mass section containing the gonadal sample was preserved in Davidson's solution under refrigeration for 48 hours. After 48 hours, the samples were washed with 50% ethanol and replaced with 70% ethanol for storage until samples were processed. Tissue samples were dehydrated in an alcohol series, cleared in toluene, and embedded in paraplast. Sections were cut 7 to 10 μm in thickness with a rotary microtome. Sections were stained with Harris hematoxylin and counterstained with eosin (Howard and Smith 1983).

Qualitative Reproductive Analysis

Prepared histological slides were examined with a Zeiss Axiovert 10 microscope (×20), sexed, and assigned to a development stage as described in Heffernan et al. (1989) where sexually undifferentiated = 0; male and female active = 2; male and female ripe = 3; and male and female ripe and spawning = 1. Male and female monthly gonadal index (GI) values were computed for each of the four intertidal sites. The monthly GI for both sexes was determined by multiplying the number of specimens ascribed to each stage, summing all those values, and dividing this figure by the total number of clams analyzed.

Quantitative Reproductive Analysis

Quantitative analysis of gonadal preparations was performed with Color Image Analyzed Densitometry Microscopy housed at Skidaway Institute of Oceanography, Savannah, Georgia (Kanti et al. 1993). Photomicroscope images (10 × 1.25 optivar) were viewed and captured by a Hitachi Model DK-7000 SU-3 Chip CCD color camera. The images were then viewed on a Trinitron color-video monitor. The image analyzer is capable of performing detailed area measurements and statistical analysis on features detected within the blue thresholds (operator controlled). Two microscopic fields of view (×10) per specimen were analyzed to ensure detection of variations in gametogenic development within specimen.

An operator-controlled marker was used to edit nongonadal tissue (e.g., intestines and digestive diverticula) in the evaluation of percent gonadal area per field. Females were analyzed for percent gonadal area, percent gonadal area occupied by oocytes, oocyte number per field of view, and mean oocyte diameter. Egg number was manually counted from the Trinitron screen, and oocyte diameter of nucleolated oocytes was measured directly on the screen. Males were analyzed for percent gonadal area and percent gonadal area occupied by spermatozoa.

Mean individual values for each data category were analyzed. Mean monthly values were then computed and used in the quantitative assessment of gametogenesis. Sex ratios were tested against a 1:1 ratio with a Chi-square statistic (Elliott 1977).

Statistical analysis of data was performed by analysis of variance (ANOVA) and Tukey's multiple range tests (MRT) with SAS for a personal computer (SAS Institute, Inc. 1989). All percentage data were arcsine transformed before statistical analysis. Mean quahog reproductive values for all qualitative and quantitative parameters analyzed were compared statistically (t-test) on a monthly basis during periods of suspected spawning activity.

Water temperature and salinity data were recorded daily at 0800 hours from September 1991 to October 1992 from the dock of the Marine Extension Service, Skidaway River, directly adjacent to the grow-out site.

RESULTS

The results of the ANOVA reveal that no significant differences ($p = 0.5458$) occurred in the age (range, 2 to 11 years; $\bar{x} = 4.6$ years) of quahogs collected from the four tidal heights; however, significant ($p < 0.0001$) differences occurred in size (range, 39 to 81 mm). Tukey's MRT shows that quahogs from the marsh ($\bar{x} = 55.3$ mm) were smaller than clams from the other three sites ($\bar{x} = 60.4$ mm for quahogs at the oyster site and 63 mm for clams at the two lower sites).

A total of 406 quahogs were sexed during this experiment. The ratio of male to females was 1.00:0.91 and did not significantly differ from unity (Chi-square = 0.9852, $p > 0.05$). Sex ratios were equal for quahogs collected from each tidal level (Table 1).

Qualitative Results

A significantly higher ($p = 0.0052$) percentage of quahogs from the marsh site (21.2%) were sexually undifferentiated compared with clams (Table 1) from the oyster (6.5%), mean low-water (7.2%), and subtidal sites (4.5%). Sexually undifferentiated clams occurred from October to February, November to January, and April to June for the oyster zone, mean low-water, and subtidal sites, respectively, but occurred from October to June in the marsh site (Fig. 1).

GI for male and female quahogs planted at the various tidal heights are given in Fig. 2. The results show similar timing of maturation for both males and females from the three lower tidal

TABLE 1.

Sex ratios and number of quahogs, *M. mercenaria* (Linnaeus, 1758), sexually undifferentiated at each tidal level

Tidal Level	No. of Males	No. of Females	No. Undifferentiated	Percent Undifferentiated	Chi-Square Value
Marsh	33	34	18	21.2	0.0149
Oyster	65	51	8	6.5	1.6897
Mean low water	55	61	9	7.2	0.3103
Subtidal	60	47	5	4.5	1.5790
Total	213	193	40	9.0	0.9852

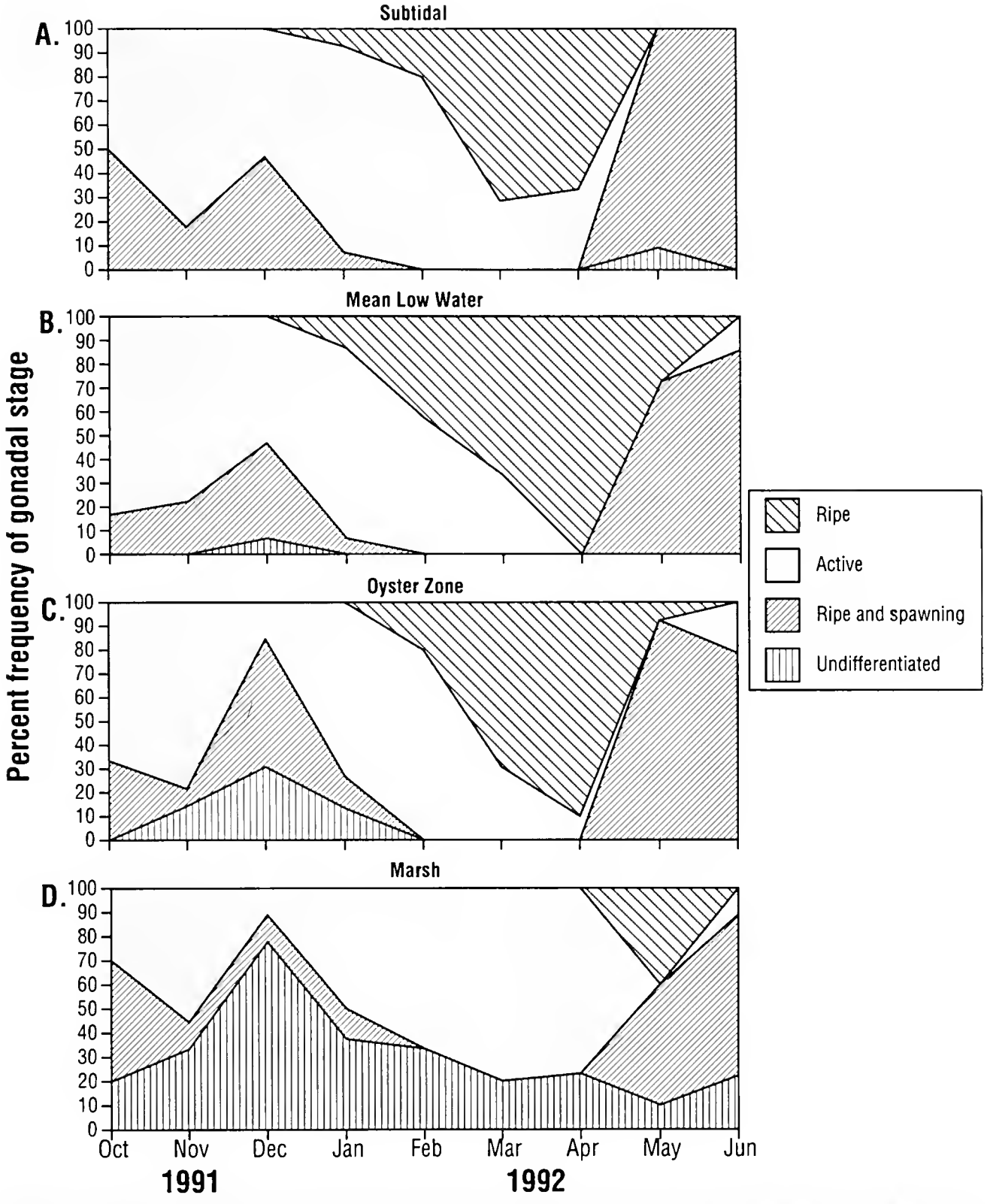

Figure 1. Qualitative data illustrating the sex and gonadal developmental stages of quahogs, *M. mercenaria*, sampled from subtidal (A), mean low water (B), 2 hours above mean low water (C), and the marsh zone (D) from the Skidaway River, Georgia, from October 1992 to June 1993. The height of each the shaded areas represents the percent frequency of clams in each developmental stage.

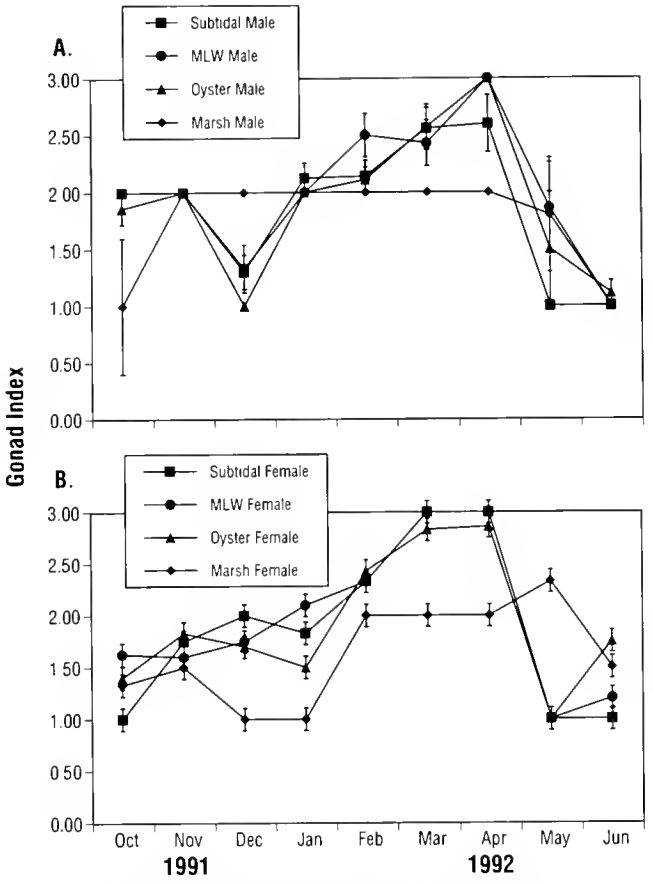

Figure 2. GI ± 1 standard error (SE) for male (A) and female (B) quahogs, *M. mercenaria*, planted at four tidal heights in the coastal waters of Georgia. MLW, mean low water.

heights, whereas a markedly different pattern exists for the clams from the marsh-grass tidal height. A decrease in the GI for males from the three lower tidal sites occurred from November to December, possibly indicating the end of fall spawning in clams (Fig. 2A). An increase in GI values followed from December to April, when values peaked. A significant and rapid drop in GI values occurred between April and June for clams from the oyster and mean low-water sites, whereas male GI values from the subtidal area significantly decreased from April to May with no change from May to June. The pattern exhibited by the males from the high intertidal marsh site increased in GI value from 1.0 in October to 2.0 in November and stayed at this value until April, when the value decreased to 1.0 by June. No significant change in GI ($\alpha = 0.05$, *t*-test) values occurred between April (GI = 2.0) and May (GI = 1.8), but decreased significantly to 1.0 by June. Thus, males from the marsh site never achieved the maximum GI values exhibited by clams from the subtidal (GI = 2.6) or other two sites (GI = 3.0). A low percentage of males (20%) and females (20%) from the marsh site were ripe by May. The decrease in GI values between May and June indicates spawning, and 55% of the males and 11% of the females were in the ripe and spawning stage in June. Approximately 92% of the clams from the three lower sites were in the ripe stage in May, whereas 88% were in the ripe and spawning stage in April (Fig. 1).

The pattern in GI values for females was similar for quahogs from all three lower tidal sites. The pattern for the females from the marsh site follows a trend similar to that of females from the other sites, but at reduced levels and lower peak values; also, clams spawned a month later than clams at the other sites (Fig. 2B). The GI values for clams at the three lower intertidal sites gradually increased from low values in October (range, 1.0 subtidal to 1.6 mean-low water) to peak values in April (3.0 for subtidal and mean-low water to 2.86 for the oyster site), before decreasing significantly to 1.0 by May, indicating spawning. GI for the clams from the marsh site were significantly ($p < 0.0001$) lower than values for the other sites in April (GI = 2.0) and significantly higher in May (GI = 2.33). Thus, females from the marsh site achieved a peak in GI value a month later, and apparently spawned a month later, than quahogs from the lower tidal sites.

Quantitative Results

Spermatogenesis

The results of ANOVA tests for the four tidal heights show that significant differences ($p < 0.0001$) occurred for both the percent gonadal area and percent gonadal area occupied by spermatozoa. Quahogs from the marsh had lower levels ($p < 0.05$, Tukey's MRT) of gonadal area and gonadal area occupied by spermatozoa. Data analysis by month showed an alternation of separation of means of reproductive parameters for the three lower tidal heights. The most consistent pattern over time occurred in the quahogs from the mean low-water level, with the most dramatic change in pattern for the subtidal and oyster-level quahogs occurring in December 1991 (Fig. 3). Subtidal clams showed a significant decrease in percent gonadal area and percent gonadal area occupied

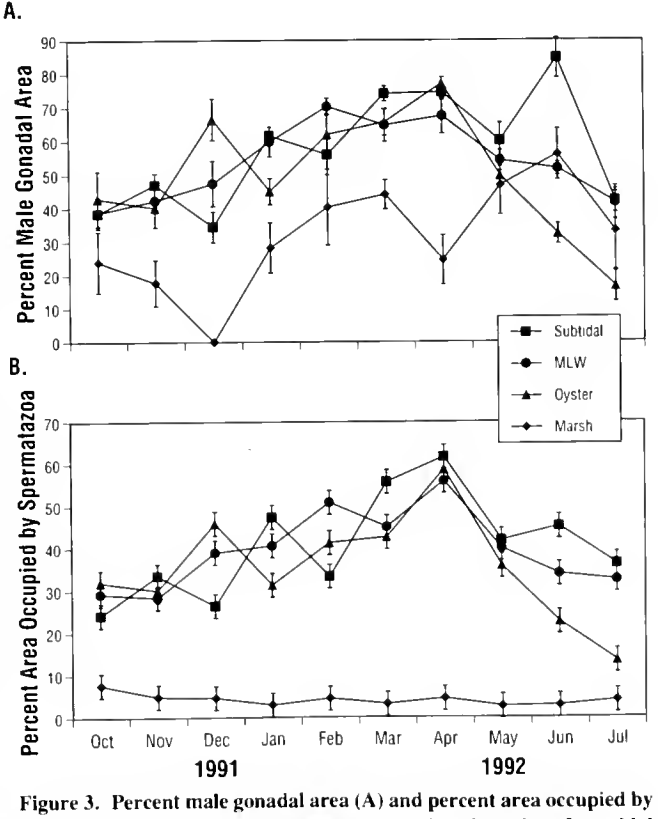

Figure 3. Percent male gonadal area (A) and percent area occupied by spermatozoa (B) for quahogs, *M. mercenaria*, planted at four tidal heights in the coastal waters of Georgia. MLW, mean low water.

by spermatozoa in December, whereas oyster-level clams had a significant increase in both parameters. The decrease in parameters in December for the subtidal males may be explained by adverse effects of cage fouling on gametogenesis (see Discussion below). A similar decrease occurs for female parameters (Fig. 4A and B). The rapid increase in parameters for the oyster-level males and subsequent decrease between December and January is not reflected in the female parameters (Fig. 4A and B).

Oogenesis

The pattern of oogenesis is best seen in the development exhibited by quahogs from the mean low-water and oyster sites. A gradual increase in the percent gonadal area (Fig. 4A), percent gonadal area occupied by oocytes (Fig. 4B), and mean number of eggs per field (Fig. 4C) occurs from October through April. A rapid decline in these parameters from April through July probably indicates spawning. ANOVA of the various parameters reveals significant differences ($p < 0.0001$) among treatments for each reproductive parameter.

Tukey's MRT revealed that, in all cases, data from the marsh site are significantly lower than values from the other three sites. Data from the subtidal site differ from the mean low-water and oyster sites where in October and November, mean percent gonadal area ($p = 0.0012$ and $p = 0.0044$, respectively) and gonadal area occupied by oocytes ($p < 0.0001$ and $p = 0.0010$, respectively) were significantly higher in values. A significant decrease in reproductive values for percent gonadal area and percent gonadal area occupied by oocytes in subtidal clams occurred between November and December, before a rapid increase achieved peak values in April. Furthermore, a more rapid decrease in percent female gonadal area and percent gonadal area occupied by oocytes occurred for clams from the subtidal site (Fig. 4A and B) between April and May compared with the more gradual decrease in parameters for clams from the mean low-water and oyster sites (Fig. 4). In April, mean percent area occupied by oocytes of subtidal clams was significantly higher ($p = 0.0001$) in value than for clams from the intertidal sites. By May, subtidal clams had significantly lower ($p = 0.0001$) area occupied by oocytes than did clams from the intertidal sites. Thus, it appears that the subtidal quahogs had a more intense spawning between April and May than did clams at the other sites. A gradual increase in the mean number of eggs per field of view occurred from December through March-April for quahogs from the mean low-water and oyster-level sites, whereas mean egg number increased from November to December at the subtidal site, but decreased from December to January. A rapid increase in mean number of eggs per field of view occurred in clams from the subtidal site from January ($\bar{x}$ = 2.6 eggs per field) to February ($\bar{x}$ = 14 eggs per field). This increase in egg numbers was correlated with an increase in the mean oocyte diameters found during this time period (Fig. 4D). Finally, mean number of egg patterns (Fig. 4C) showed a temporal shift in peak occurrence. Maximum egg diameter values occurred as early as February in the clams from the subtidal cage compared with peak values in March for mean-low water, March-April for oyster site, and May for marsh cage clams.

Mean ambient water and salinity values taken at 0800 hours at the Marine Extension Service dock on the Skidaway River are given in Figure 5. Ambient river temperatures ranged from a mean high of 28°C in September 1991 to a mean low of 10°C in mid-January before increasing to 29°C by July 1992. River salinity gradually increased from 17 ppt in September 1991 to 30 ppt in January 1992, before decreasing to 23.5 ppt in April, and then rapidly increasing to 30 ppt by July 1992.

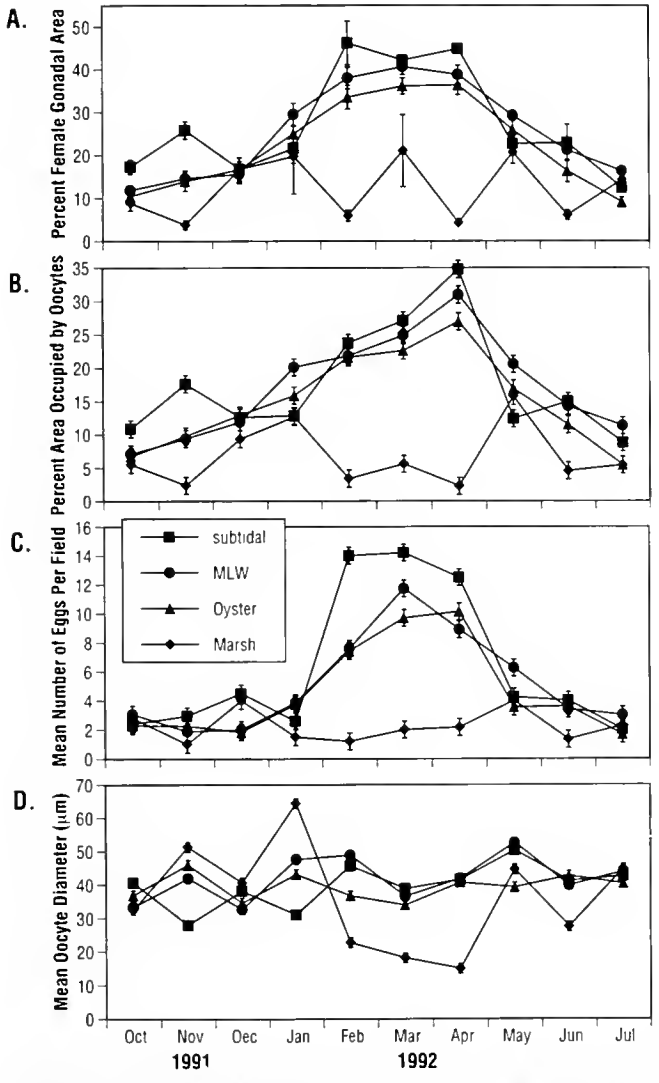

Figure 4. Percent female gonadal area ± 1 SE (A), percent gonadal area occupied by oocytes ± 1 SE (B), mean oocyte number per field of view ± 1 SE (C), and mean nucleolated oocyte diameter ± 1 SE (D) for quahogs, *M. mercenaria*, planted at four intertidal heights in coastal Georgia. MLW, mean low water.

DISCUSSION

The results of this study showed that spatial differences in gametogenesis occurred within a Georgia population of quahogs. Intertidal exposure did affect gametogenesis where animals at the upper limits of its intertidal distribution exhibited lower values of percent male gonadal area, percent gonadal area occupied by spermatozoa, percent female gonadal area, percent gonadal area occupied by oocytes, mean egg numbers per field of view, and mean egg diameters. Furthermore, cage fouling appears to adversely alter the pattern of gametogenesis for clams planted in subtidal areas. Quahogs were found to be reproductively active from October to June, with a major spawning period occurring from April to June for the lower three tidal sites and from May to June for

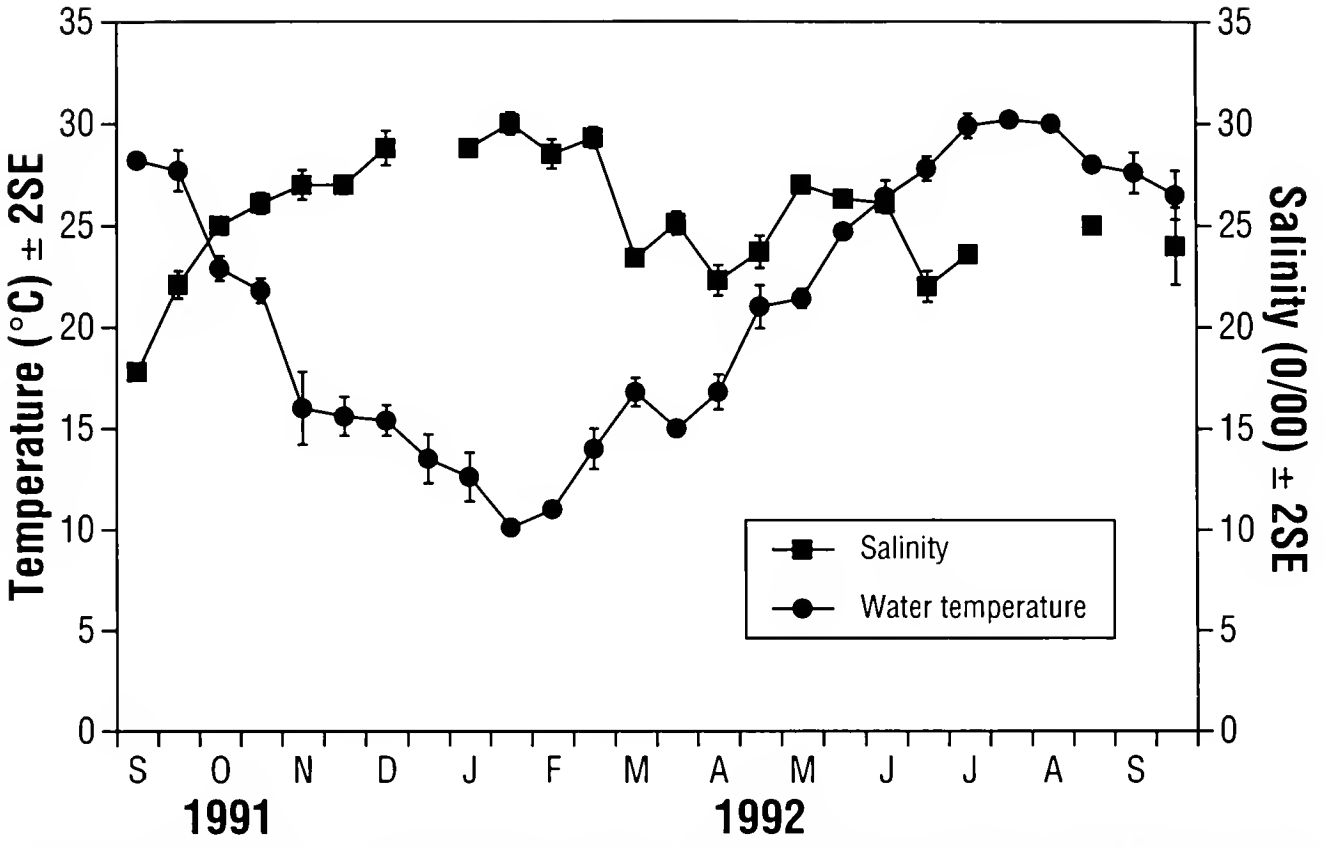

Figure 5. Biweekly means of ambient water temperature (°C) and salinity (ppt) for the site where northern quahogs, *M. mercenaria*, were planted from the subtidal zone to the marsh zone adjacent to the Marine Extension Service's dock located on Skidaway Island, Georgia. Temperature and salinity readings were taken at 0800 hours each morning.

clams from the marsh site. These findings are in general agreement with two earlier reproductive studies showing major spawning season in the spring for quahogs in Georgia (Pline 1984, Heffernan et al. 1989); however, spawning times differed. Various spawning times between these Georgia reproductive studies may result from a number of factors, e.g., sampling in differing years, from dissimilar habitats, and under varying saline or water temperature regimens. In this study, quahogs from the Skidaway River spawned from April through June, whereas quahogs from a Wassaw Island site spawned from March to May in 1982, and chowders spawned sooner and for a longer period than littlenecks (Pline 1984). Quahogs from House Creek site spawned from March to May in 1982, from April to May in 1985, and from April to June in 1986 (Heffernan et al. 1989). These annual variations in spawning patterns are probably tied to environmental parameters (location, habitat, water temperature, and salinity). Quahogs normally spawn when water temperatures increase from 20°C. The Skidaway River temperatures increased from a mean of 21 to 27°C from April to June (Fig. 5).

Temporal and spatial variability in spawning patterns between oyster beds has been documented within the Chesapeake Bay (Kennedy and Krantz 1982) as well as within the James River of the Chesapeake Bay (Cox and Mann 1992). A number of factors can contribute to the variability of spawning patterns, including differences in water temperatures, salinity, disease pressure, size of oyster, and time since last spawning (Cox and Mann 1992), as well as geographical variables (Brown 1984, Newell et al. 1982, Eversole 1989) or depth of occurrence (MacDonald and Thompson 1986). This study shows that the degree of tidal exposure can effect the reproductive pattern of quahogs.

Although, overall, there were no significant differences in the various reproductive parameters for quahogs grown at the oyster-level, mean low-water, or subtidal sites, there is a consistent decrease in the various quantitative values according to tidal exposure. Reproductive variables become statistically lower when clams occurred within the salt marsh—the upper limit of the intertidal distribution of this species in Georgia. Harvey and Vincent (1989) found, for a population of *Macoma balthica*, that clams from the upper limit of its intertidal limit were reproductively senescent compared with individuals from the lower intertidal area. In contrast, only a small percentage (21%) of *Mercenaria* species from the upper intertidal distributional limit in this study were reproductively undifferentiated, with the majority showing some reproductive potential. At peak spawning, all clams showed some differentiation (Fig. 1); however, whether any of the clams from the marsh site produced viable gametes is unknown. Only 40% of the marsh-site clams achieved the ripe stage, all during the month of May, whereas 92% of the clams from the other lower sites were in the ripe stage in April, and 88% were ripe and spawning by May (Fig. 1).

Differences in the maturation of other bivalves according to tidal exposure have been documented. Borrero (1987) found that the onset of gametogenesis and spawning occurred earlier in 10 populations of mussels, *Geukensia demissa*, from low intertidal habitats than in 10 populations from the higher intertidal zone. For a population of *Hiatella* species from the Arctic, subtidal clams

were reproductively active year around, whereas clams from the littoral zone did not produce ova from June through October (Hunter 1949). In a *Modiolus modiolus* population, mussels from the intertidal zone spawned in fall to winter, whereas mussels from the subtidal zone were reproductively active year around (Seed and Brown 1977). For cockles, *Cerastoderma edule*, higher percentages of mature cockles occur in clams collected from the extreme low-water, spring tidal level than in clams collected from mean low-water mark (Boyden 1971).

For *Mercenaria* species, Eversole et al. (1980), using qualitative histological analysis, found no significant differences in reproductive parameters for clams planted subtidally and intertidally in South Carolina. Qualitative GSI analysis revealed that clams from the subtidal zone had higher values than clams from the intertidal zone (Eversole et al. 1984). Landers (1954) suggested that intertidal quahogs spawned earlier than subtidal ones. The quantitative data from this experiment suggest that subtidal quahogs spawned at the same time as clams from the mean low-water and oyster-level sites, but subtidal clams appear to have had a more intense initial spawn from April to May (Figs. 2 through 4), followed by a resting phase from May to June, with another rapid decline in reproductive parameters in June to July. In clams from the oyster and mean low-water sites, a gradual decline in all parameters occurred from April to July.

A decline in reproductive parameters for quahogs according to increases in tidal exposure is expected. In intertidal growth studies (Walker and Heffernan 1990), growth rate of quahogs decreased according to increase in intertidal exposure time. No significant differences in survival occurred in that experiment for quahogs planted from the spring low-water mark up to the 3 hours above the mean low-water mark. In this experiment, quahogs from the marsh site were significantly (as determined by Tukey's MRT) smaller than animals from the lower intertidal sites. This size difference is not associated with any difference in age of clams sampled. Clams were not significantly different in age.

Greater initial mortalities occurred for quahogs at the marsh site, resulting in only 10 clams being sampled per month. The heavy mortality experienced within the marsh site may be due to substrate differences, as well as increased physiological stress, compared with earlier intertidal quahog growth and survival studies (Walker and Heffernan 1990). In this study, quahogs were replanted in a muddy substrate occurring at the base of live *Spartina alternaflora*, whereas in the previous study, clams were planted in a substrate of predominately sand material. Sand substrates are the preferred substrate of quahogs for greater growth and survival (Pratt 1953). Because quahogs roughly allocated equal amounts of energy to gamete production as to flesh production (Ansell and Lander 1967, Hibbert 1977), the observed decreases in growth rate associated with increases in intertidal exposure are interpreted as representing decreased total energy availability, with similar negative effects on gametogenic development expected.

Fouling by sea squirts, *Molgula* species, on the subtidal cage presumably had a negative impact on the gonadal development of the quahogs. Decreases in percent gonadal area and percent area occupied by oocytes, followed a month later by decreases in mean egg diameters and mean numbers of eggs, occurred for clams in the fouled subtidal cage. No major epibenthic fouling (excepting some minor oyster spat settlement) occurred on intertidal cages during the course of this experiment, but in November 1991, a heavy set of *Molgula* species was observed on the subtidal cage. By December 1991, *Molgula* species completely covered the top and sides of the subtidal cage, with consequent negative effects on flow rate and food availability presumed for the caged quahogs. Approximately 90% of the cage was cleaned in December, and on the January sample date, the remaining corner had become cleared of *Molgula* species. The negative effect of this fouling can best be seen in females, where a sharp contrast to the patterns in the two intermediate intertidal height cages occurred. There was a significant decline in both percent gonadal area and percent gonadal area occupied by oocytes for clams from the subtidal cage from November to December as compared with increases in these parameters for quahogs from the intermediate tidal cages. These parameters showed a recovery, presumably associated with cage cleaning and increased food supply, by February, with oocyte area showing a staggered resurgence. Similarly, but delayed by 1 month, there were significant decreases in mean egg numbers observed in the subtidal quahogs in December-January, whereas the mean egg-size pattern was shown to be 1 month out of synchrony with the two intermediate tidal levels. Became gametogenesis is coupled with growth and feeding, we believe that this pattern observed for the subtidal quahogs is a direct result of the cage fouling that restricted growth and feeding during the November-to-December time period.

One can only speculate as to the results if cage fouling on the subtidal cage had not occurred. In October and November, the mean percent female gonadal area and percent gonad occupied by eggs at the subtidal level were significantly higher than values at the mean low-water mark and oyster site. Given the higher gametogenic output at the subtidal level before the impact of cage fouling, it is reasonable to speculate that peak values may have been attained earlier and/or at higher levels in the absence of cage fouling.

Although there is a consistent decrease in the various reproductive parameters according to tidal placement, the results of this study show that no significant differences in reproductive parameters were detected between quahogs planted from the subtidal to the 2 hours above mean low-water mark. This zone is where most quahogs occur naturally in the coastal waters of Georgia. A significant reduction in gamete production does occur for clams grown at the upper limit (i.e., in the salt marsh) of its tidal distribution, and it is unknown if the gametes produced there would produce viable offspring. Thus, reproductive senescence does not occur for all individuals from the upper limits of its distribution, as was observed in *M. balthica* populations (Harvey and Vincent 1989). In light of the results of this study, resource managers can assume that spawning occurs during single events (fall and spring) for most of the Georgia quahog population, with the major reproductive contribution coming from clams within the lower intertidal and subtidal populations.

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THE EFFECT OF AQUACULTURE ON THE GENETICS OF NATURAL POPULATIONS OF THE HARD CLAM, *MERCENARIA MERCENARIA* (L)

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ABSTRACT From 1980 to 1984, *Mercenaria mercenaria* seed clams from Aquaculture Research Corporation (ARC) in Massachusetts were imported by Trident Seafarms Company, located on the Folly River near Charleston, South Carolina. Although broadly similar genetically to naturally occurring *M. mercenaria* populations, ARC stocks show substantially elevated frequencies of two ordinarily rare glucose phosphate isomerase (GPI) alleles and have been marked with *notata*, a shell coloration gene. The purpose of this investigation was to estimate the extent to which the ARC genome has become established in natural populations of *M. mercenaria* remaining 3 years after Trident Seafarms discontinued aquaculture operations in 1989. I examined seven samples of over 300 individual clams each: large and small individuals from Trident Seafarms itself and from two of its former grow-out sites, plus small clams only from a fourth site where no known aquaculture had occurred. I obtained one significant result—apparent linkage disequilibrium between *notata* and a GPI marker at one grow-out site. Over 12 tests, such findings may be attributable to type I statistical error. So, although some follow-up study may be indicated, generally, the effect of aquaculture on the genetics of natural populations of hard clams in South Carolina seems to have been negligible.

KEY WORDS: clams, *Mercenaria*, electrophoresis, isozymes, *notata*, introductions, aquaculture

INTRODUCTION

Important questions have recently arisen regarding the frequency and consequences of genetic interaction between introduced aquaculture stocks and native populations. One possible outcome would be the selectively neutral spread of a foreign genome through the host population until it is diluted beyond detection (Dillon 1988). A second outcome would be that introduced genomes prove deleterious and disappear more rapidly than expected from simple diffusion (Helle 1981, Krueger et al. 1981, Verspoor 1988, Hindar et al. 1991). A third possibility would be that introduced genomes prove selectively advantageous and increase in frequency, to the benefit or detriment of other species, including mankind (Helle 1981, Krueger et al. 1981, Strand and Lavan 1990, Hindar et al. 1991). An analysis of the Trident Seafarms operation, which from 1980 to 1989 raised a New England stock of *Mercenaria mercenaria* near Charleston, South Carolina, affords an opportunity to examine the genetic consequences of one aquaculture operation in some detail.

The establishment and operation of Trident Seafarms has been reviewed by Brown et al. (1983), Manzi et al. (1984), Stevens et al. (1984), and Manzi et al. (1986). In September 1980, *M. mercenaria* seed clams were first imported from Aquaculture Research Corporation (ARC) in Massachusetts to the Trident Seafarms facility on Folly Island, 15 km south of peninsular Charleston, South Carolina. Here, clams were grown to about 8 to 10 mm in nursery systems of various design. Older clams were also held in vinyl-coated wire trays in the adjacent Folly River marshes. From 1980 to 1981, experimental field units were placed at Little Oak Island, also near the Folly River, about 4 km east of the central facility. In 1982, field units were planted in Bass Creek at Kiawah Island, 8 km west. The clams at Bass Creek were removed in 1985. Trident Seafarms maintained larger clams in the marshes of the Folly River near its central facility until going out of business in 1988. The nursery holding ARC seed clams was also discontinued at that time. Hurricane Hugo caused some damage to experimental clam pens remaining in place in the marshes around Trident Seafarms in September 1989, after which all remaining cages were recovered by state personnel (N. H. Hadley, personal communication).

With repeated planting of ARC stocks in local waters over a 9 year period, it seems possible that these clams may have been able to spawn and hybridize with the local wild population of *M. mercenaria*. Dispersal in the veliger larval stage may have spread the ARC genome substantial distances from original nursery and grow-out sites (Dillon and Manzi 1989a, McCay 1990).

Although allozyme studies have not generally detected genetic divergence among natural populations of *M. mercenaria* inhabiting most of the Atlantic coast of North America, ARC stock may be characterized by the loss of some rare alleles and by allele frequencies significantly different from the wild at several enzyme loci (Dillon and Manzi 1987). The difference is most striking at the glucose phosphate isomerase (GPI) locus. Dillon and Manzi (1987 and 1989b) reported that a sample of 213 *M. mercenaria* wild collected in the vicinity of Charleston showed a frequency of 0.038 for an allele they designated "GPI 70" and did not show a "GPI 60" allele. Their estimates for the ARC broodstock (N = 110) were 0.195 for GPI 70 and 0.027 for GPI 60. Adamkewicz et al. (1984) have verified that the inheritance of GPI isozyme phenotype is Mendelian in *Mercenaria* species.

A striking shell color polymorphism, *notata*, may also be used as a marker for ARC contribution to natural stocks. Chanley (1961) reported that this trait is controlled by a single allele inherited in Mendelian fashion. *Notata* homozygotes are dark "red" colored with white longitudinal bands, heterozygotes have thin zig-zag stripes, and clams homozygous for the normal allele are uncolored. *Notata* coloration has been selectively bred into ARC stocks as a marketing tool. In their survey of heterozygosity and growth in hatchery stocks of *Mercenaria* species, Dillon and Manzi (1988) sampled 248 pure ARC clams aged 1 year. An examination of 239 of these individuals showed 16 homozygotes

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and 108 heterozygotes, for a gene frequency of 0.293. The gene is quite rare in the wild, however. Eleven South Carolina populations showed from 0.71 to 2.17% *notata* heterozygotes, with a mean gene frequency of 0.006 (Eldridge et al. 1976, Humphrey and Walker 1982).

The ARC stocks of *M. mercenaria* have been selectively bred for improved growth under aquaculture conditions. Released to the wild, such clams might be able to reach reproductive maturity faster than their neighbors and release propagules earlier, giving ARC offspring an advantage over other larvae in terms of space and food acquisition. Therefore, placing ARC genes into the natural gene pool might be considered advantageous to humans, as it has been in "stock enhancement programs" for other species in the past (Helle 1981, Strand and Lavan 1990, Gaffney and Allen 1992). On the other hand, the movement of the New England *M. mercenaria* genome may model other accidental introductions that have, in the past, proved catastrophic (Groves and Burdon 1986, Mooney and Drake 1986, Strayer 1991).

In this investigation, I examine the evidence that aquaculture operations have affected the genetics of natural *Mercenaria* populations in the Charleston area. Data on GPI and *notata* frequencies at sites where ARC clams were formerly cultured by Trident Seafarms will constitute a baseline against which future studies can assess any long-term genetic consequences of aquaculture.

MATERIALS AND METHODS

The four samples locations are shown in Figure 1. Site 1 is the site of the former Trident Seafarms, where ARC clams (and several other aquaculture stocks) were held from 1980 to 1989. From March 21 to June 2, 1992, I sampled in the drainage ditch where water flowed from the nursery system and holding tanks into the Folly River. Site 2 is located in Bass Creek at Kiawah Island, where clams where held in grow-out units from 1982 to 1985. I sampled clams from this site between December 4, 1992, and March 11, 1993. At Little Oak Island, site 3, clams were planted from 1980 to 1981, and I sampled here from December 8, 1992, to March 23, 1993. Some data from this site were from McMillan (1993). Site 4 was a mud flat on Cole Island, 2.75 km from site 1, 4 km from site 2, and 6 km from site 3. I sampled here from July 23 to November 18, 1992. Although no aquaculture operations are known to have taken place at site 4, it seemed possible that intro-

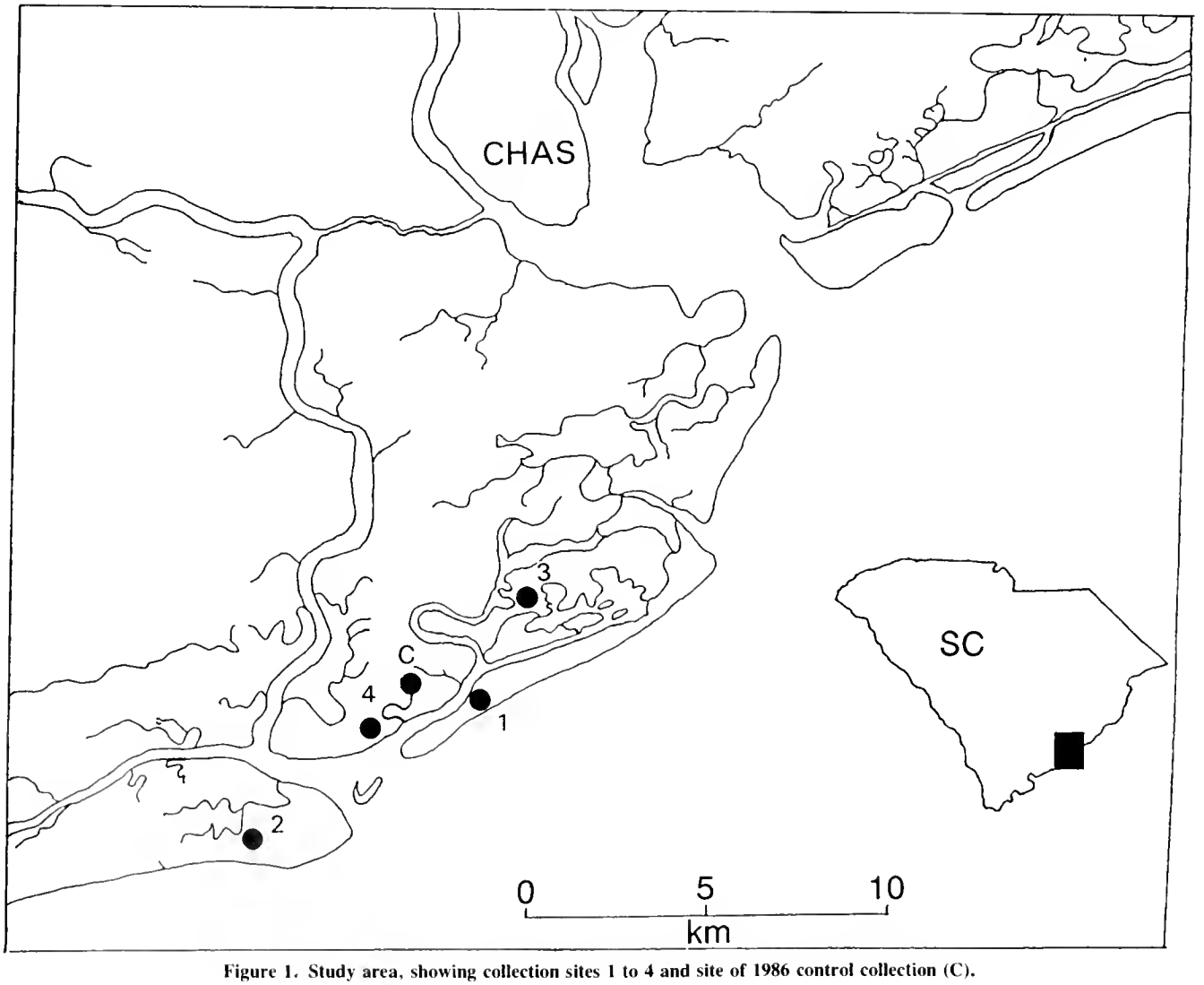

Figure 1. Study area, showing collection sites 1 to 4 and site of 1986 control collection (C).

duced genomes might, in one generation, spread here from site 1 or 2.

At sites 1 to 3, I collected about 600 clams, half "large" and half "small." The large sample included individuals with maximum shell dimension greater than 50 mm, and the small sample included individuals 50 mm or less. At site 4, I sampled over 300 clams, all small. My sample size of 300 was chosen in order to be 95% certain of obtaining a statistically significant contingency chi-square ($\alpha = 0.05$), given the control frequency of GPI 70 estimated by Dillon and Manzi (1989b) and a 10% contribution by the ARC genome. Each small individual collected was scored as to the presence of *notata* markings. Because shells tend to wear and darken with age, I did not score large individuals for coloration.

Methods for horizontal starch gel electrophoresis were generally those of Dillon (1985 and 1992). I ground frozen cuttings of siphon tissue and resolved isozymes in the supernatant by electrophoresis in an N-(3-aminopropyl) morpholine (pH 6) buffer system. Gels were stained for GPI (E.C. 5.3.1.9) by an agar overlay technique.

The 213 *M. mercenaria* serving as GPI controls for this study were collected by Dillon and Manzi (1989b) near the origin of Cole Creek on November 12, 1986 (Fig. 1). This site was located 5 km distant from the nearest known aquaculture site and seems to show no significant divergence from wild Virginia or Massachusetts *Mercenaria* populations at the GPI locus. Because their average standard shell length was 75.4 mm, many of these individuals may have been born before the establishment of Trident Seafarms. Humphrey and Walker's (1982) estimate of 0.006 for the natural frequency of the *notata* gene in South Carolina, based on N = 1,539 individuals pooled from 11 sites, was used as the *notata* control.

My first statistical tests were for temporal heterogeneity in GPI allele frequency. I combined alleles into two categories: the markers GPI 60 + GPI 70 and all others. Then at each of the sites 1 to 3, I compared gene frequencies in the large clams with those in the small clams using Fisher's exact tests (two tailed). Where no significant heterogeneity was detected, samples of large and small individuals were combined. In my second set of tests, I compared GPI allele frequencies obtained at all four of the sites to the control frequencies of Dillon and Manzi (1989b). I used Fisher's exact tests on the one-tailed hypothesis that present frequencies of GPI 60 + GPI 70 are greater. My third set of tests were also one tailed, assuming that the present frequencies of the *notata* allele at each of the four sites are greater than the control frequencies derived by Humphrey and Walker (1982). Here, I used chi-square contingency tests, with Yate's correction. My final tests were for disequilibrium between *notata* and GPI marker alleles. All individuals were categorized by the presence or absence of GPI 70 or GPI 60 (together) and by the presence or absence of *notata* coloration. Then, the significance of the composite linkage disequilibrium (Δ_{AB}) was tested using chi-square (program LD86.FOR of Weir 1990).

RESULTS

Figure 2 shows the size distributions (standard length of shell) of the *Mercenaria* species sampled for this study. Note that these are not random samples of the population at each site. Because smaller clams are more difficult to find, a 300 individual sample of clams smaller than 50 mm taken from the wild will be strongly biased to the 40 to 50 mm size class. The GPI gene frequencies observed at the four sites are shown in Table 1. Combining alleles into marker (GPI 60 + GPI 70) and nonmarker classes, Fisher's exact tests uncovered no significant differences between large and small clams at any site. Two-tailed values of p were 0.76, 0.36, and 0.85 for sites 1, 2, and 3, respectively.

There is no evidence of ARC contribution to the stocks of *Mercenaria* species currently inhabiting the four sites, judging by

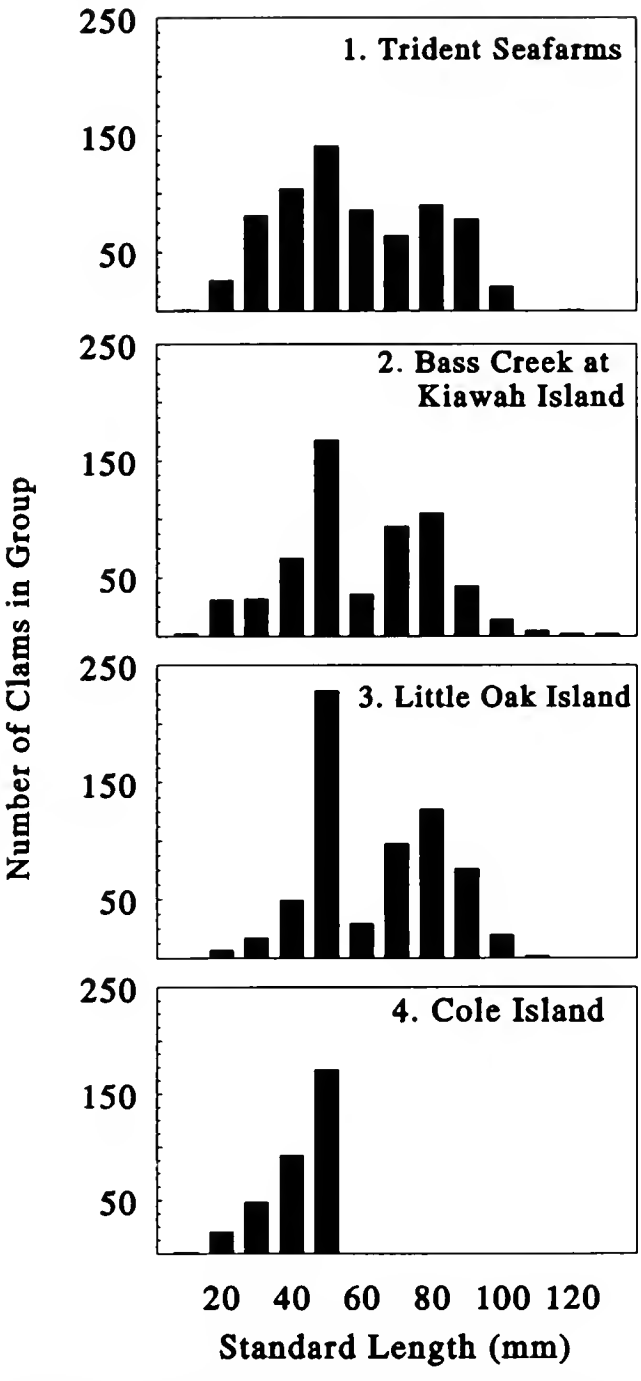

Figure 2. Size distribution (standard shell lengths) of *Mercenaria* species sampled at four sites in the vicinity of Charleston, SC. Individuals are graphed by 10 mm increments; the size given is the maximum for each category.

TABLE 1.

Gene frequencies and sample sizes (N) at the GPI locus in large (L) and small (S) *M. mercenaria* from the four sites shown in Figure 1.

GPI Alleles	Site 1		Site 2		Site 3		Site 4
	L	S	L	S	L	S	S
110	0.020	0.024	0.003	0.005	0.000	0.003	0.022
105	0.006	0.006	0.018	0.022	0.021	0.028	0.002
100	0.910	0.900	0.915	0.893	0.866	0.859	0.920
90	0.021	0.023	0.028	0.030	0.063	0.055	0.027
80	0.009	0.017	0.023	0.032	0.029	0.031	0.019
70	0.029	0.020	0.007	0.017	0.017	0.024	0.006
60	0.005	0.010	0.005	0.002	0.004	0.000	0.003
N	328	351	301	300	350	309	314

TABLE 2.

The frequency of the *notata* phenotype in clams less than 50 mm at the four sites, the gene frequencies implied, and the incidence of marker GPI alleles.

Site/Location	N	*notatas*	Gene Frequency	*notatas* showing GPI 60 or 70
1. Clam Farm	351	8	0.0114	0
2. Kiawah Island	300	5	0.0083	0
3. Little Oak Island	309	6	0.0097	2[a]
4. Cole Island	314	7	0.0111	0

[a] Significant linkage disequilibrium.

allele frequencies at the GPI locus. Counter to expectation, Table 1 shows that the combined frequency of marker alleles was lower at all of the sites than the control 0.037 observed by Dillon and Manzi (1989b). The incidences of *notata* coloration in my four samples of small clams are given in Table 2. None of these is significantly greater than the control frequency (one-tailed p = 0.08, 0.34, 0.21, and 0.11, respectively).

However, two of the small individuals collected at site 3 (Little Oak Island) carried both *notata* coloration and the marker allele GPI 70. The composite disequilibrium coefficient (Δ_{AB}) was 0.0028, highly significant (chi-square = 10.74, one-tailed p = 0.0005). Such disequilibrium between *notata* and GPI markers is consistent with the expectation from an ARC contribution to the *Mercenaria* population inhabiting site 3.

DISCUSSION

At least some escape of foreign gametes, larvae, juveniles, and even adults would seem almost inevitable during the course of normal commercial bivalve aquaculture. The water outfall from the Trident Seafarms facility would be expected to carry veligers from uncontrolled broodstock spawning and early juveniles from the nursery. "Seed" clams were certainly lost in transfer to pens, and older individuals were lost as the result of pen failure, especially during Hurricane Hugo. To the contribution by direct escape would be added gametic gene flow. ARC clams may mature at 20 mm, well before normal harvest, and seem to spasm under conditions similar to native populations, as temperatures reach 25°C. Thus, one might reasonably expect hybridization between caged and native clams to be commonplace.

I was, however, almost entirely unable to detect elevated frequencies of ARC marker alleles in the *Mercenaria* population currently inhabiting the estuaries around Charleston, over all sites and size classes examined. Either the ARC genome has generally proved to be disadvantageous in the wild, or it has by now become diluted beyond my ability to detect it.

I did obtain one significant result. The apparent linkage disequilibrium between *notata* and GPI 70 at site 3 (Little Oak Island) constitutes some indirect evidence of ARC contribution to natural populations. However, it should be remembered that I examined by data 12 times in my effort to detect this phenomenon: four direct tests for GPI 60 + 70, four direct tests for *notata*, and four tests for disequilibrium between the two markers. Because Bonferroni correction would require an adjusted value of p = 0.05/12 = 0.004 for significance, my single significant result (p = 0.0005) may be attributable to type I statistical error.

Some further survey of the *Mercenaria* population around Little Oak Island would seem warranted in the future. However, viewed over the entire Charleston-area population of *M. mercenaria*, the genetic effect of aquaculture from 1980 to 1989 would seem to have been negligible.

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INTERNAL SHELL STRUCTURE AND GROWTH LINES IN THE SHELL OF THE ABALONE, *HALIOTIS MIDAE*

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ABSTRACT The internal shell structure of the South African abalone, *Haliotis midae*, was studied using scanning electron microscopy. Growth lines were counted. The shell consists of the periostracum, prismatic, and nacreous layers. The outer, noncalcified periostracum consists of a single, honeycombed layer. Calcified layers include a simple prismatic and a nacreous layer, the latter being composed of sheet nacre, which is uncharacteristic of gastropods. Growth lines were deposited in the nacreous layer of animals, even under controlled laboratory conditions. In animals of known age, three lines were deposited in the first year and one in each subsequent year. Rate of deposition of lines was not related to diet, temperature, or photoperiod.

KEY WORDS: abalone, shell structure, growth lines

INTRODUCTION

Age and growth rate of commercially exploited molluscan stocks are important factors in the determination of the exploitation status of such stocks (Ricker 1977). Growth rate is also an important consideration in molluscan mariculture because of the relationship between growth and production costs (Day and Fleming 1992). Although aging techniques and growth rates of haliotid populations have been extensively studied (e.g., Sainsbury 1982, Prince et al. 1988, Day and Fleming 1992, Nash 1992), no completely reliable and validated aging techniques have been published (Ward 1986).

Growth checks appearing on the external shell surface have been used to estimate age in various abalone species (Forster 1967, Poore 1972, Kojima et al. 1977, Hayashi 1980, Saito 1981, Kim and Cheung 1985, Prince et al. 1988), but age estimates resulting from such studies have been extremely variable. Although several of the listed studies reported difficulty in interpreting external growth marks because of variations in the timing of the formation of such marks or because of shell damage caused by boring organisms (Forster 1967), the majority concluded that growth checks, at least in larger animals, were deposited annually. Munoz-Lopez (1976), however, stated that external growth checks did not always occur in abalone, whereas Shepherd and Hern (1983) noted that checks were not necessarily annual.

An alternative method of aging abalone shells was developed by Munoz-Lopez (1976), who ground the tip of the spire to expose alternate light and dark layers of concholin and aragonite. He found that, in the Mexican abalone *Haliotis corrugata* and *Haliotis fulgens*, concholin was formed during winter whereas aragonite was formed during the summer. Although he concluded that layers were annual, no independent growth data were presented to validate this assumption. Prince et al. (1988) related similar layers to independent growth data for a Tasmanian population of *Haliotis rubra* and concluded that three minor growth lines were deposited during the first 16 months of life and that, thereafter, lines were deposited approximately annually. This aging method has not, however, found universal acceptance, and Prince et al. (1988), for example, noted that it could not be applied to populations of *H. rubra* from warmer waters of New South Wales. Beamish and McFarlane (1983) stressed the importance of validating all aging techniques with independent growth data for each species and each area studied, whereas McShane and Smith (1992) stated that the use of shell growth checks as a method for age determination of *H. rubra* was not reliable because of variation in frequency of growth checks between populations and the unreliability of growth checks in the shells of individuals that had been infested with boring organisms. Schiel and Breen (1991) also cast doubts on the usefulness of growth lines, but Shepherd et al. (1994) suggested that, despite the variation that occurred in the number of growth lines deposited annually, counts could still be a useful aid to aging shells, particularly if loss of lines from abrasion could be taken into account.

Working on ocean quahogs and using methods developed by Kummel and Raup (1965), Ropes (1984) suggested that some of the ambiguities associated with counting molluscan shell lines could be reduced by cutting complete longitudinal sections of shells and counting lines from acetate peels. Thompson et al. (1980) used the same method to age shells of ocean quahogs and provided independent growth data to validate the technique. Mutvei et al. (1985) also commented on the contradictory data present in the literature on the interpretation of molluscan growth lines and suggested that, in the case of haliotids, this could be partly explained by extremely variable rates of shell mineralization. There data showed that the pallial layer of the abalone mantle was capable of secreting aragonite and calcite simultaneously and that rates of mineralization could change rapidly during growth. Because shell ultrastructure can vary markedly between species, each species requires its own description of shell structure before shell checks can be used to estimate age or growth rates.

Growth rate of the South African abalone *Haliotis midae* was estimated previously by Newman (1968), who measured growth, over a 2 year period, in animals that had been tagged, returned to the sea, and then recaptured. Tarr (1990 and 1993), having listed several criticisms of this work, reassessed growth rates for this species, using a more reliable mark-recapture method. No attempt has, however, been made to validate the use of growth lines to age shells of *H. midae*. The aim of this study, therefore, was to examine the ultrastructure of the shells of *H. midae*, using scanning electron microscopy (SEM) in order to evaluate the use of acetate peels to count growth lines as an aging technique for these shells.

MATERIALS AND METHODS

With the exception of the 37 month old animals, all shells used in this investigation were from animals reared in an experimental

hatchery in Sea Point, Cape Town. Therefore, the exact age of the animals was known. In the case of the 37 month old shells, animals were collected from the wild at a mean length of 10 mm and an estimated age of 6 months (Newman 1968) and were thereafter transferred to the experimental hatchery for the rest of the experiment. Shells were used to investigate the ultrastructure of shell layers, to elucidate the relationship between age and the number of growth lines, and to investigate the possible influence of some environmental variables (e.g., photoperiod, food availability) on the deposition of growth lines. All hatchery animals were kept at a constant temperature of 18 ± 0.5°C. Other experimental conditions for each group, listed in Table 1, were maintained throughout the life of each group from the time of settlement to the time of sampling.

The area of the shell used in this study was the nacreous layer (Fig. 1a), because according to Lutz (1976), preservation of growth lines is better in the nacreous than in the prismatic layer. The area used within the nacre of *H. midae* was the section from the apex of the shell to the outer lip (X–Y, Fig. 1b). This area was chosen because lines near the margin of the shell (Z, Fig. 1b) often condense, making interpretation difficult (Lutz 1976), and because the shell grows radially from the apex so that all growth lines are contained within the area X–Y (Lutz and Rhoads 1980).

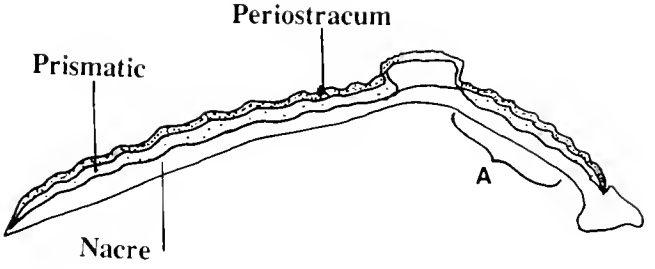

Figure 1a

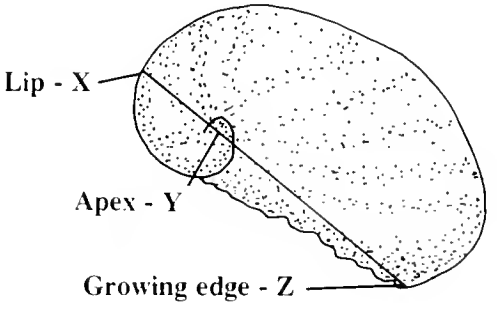

Figure 1b

Figure 1. (a) Layers of the shell of *H. midae* and area in nacre where growth lines were counted. (b) Regions of the shell to show where each section was cut.

Acetate Peels

Sections of the shell were cut to give a flat surface along the axis from X to Z (Fig. 1b). The procedure used to produce acetate peels was that proposed by Ropes (1984) for the ocean quahog *Arctica islandica*, except that polished abalone shells were etched for 25 seconds with 1% HCl. Acetate peels were viewed under a light microscope to examine the internal structure of the whole shell and the growth lines. Growth line counts were accepted only after at least two of three separate investigators counted the same number of lines.

Because peels were made from an entire season of shell, they provided a range means to determine the location and arrangement of the different structural layers (Wise and Hay 1968). Specific areas were selected for detailed SEM study on the basis of the acetate peels.

Scanning Electron Microscopy

SEM was used to investigate the fine ultrastructure of the growth lines, nacre, prismatic, and periostracum. The periostracum was not clearly visible in acetate peels because it is a thin, noncalcified layer. SEM also shows more relief and spatial relations of the shell crystals than the acetate peels (Wise and Hay 1968).

Shells that had been used previously for the preparation of acetate peels were cleaned in an ultrasonic bath and prepared for SEM following the procedure described by Wise and Hay (1968). Shells were mounted onto stubs and coated with 20 μm of gold palladium using a Polaron sputter coater (Polaron Equipment, Ltd., Watford, U.K.) Cambridge S-200 microscope (Cambridge Instruments, Cambridge, U.K.) S2 100 sputter coater. They were then scanned and photographed under a Cambridge S-200 microscope.

TABLE 1.

Experimental conditions of groups.

Group	Age (months)	Light (L):Dark (D)	Food[a]	N[b]	R (SD)[c]
A	8	Ambient	E	4	1 (0)
B	13	Ambient	E	10	3.2 (0.4)
C	23	Ambient	E	4	4.5 (0.6)
D	37	24 h dark	E	7	5.1 (1.2)
E	37	12 h L:12 h D	E	7	5.7 (0.5)
F	37	Ambient	E	5	5.2 (0.8)
G	37	Ambient	L	5	6.0 (1.0)

[a] E, diet of *Ecklonia maxima;* L, diet of *Laminaria pallida*.
[b] Number of abalone per treatment.
[c] Mean number of lines (±standard deviation [SD]).

RESULTS

Shell Structure

Acetate Peels

An acetate peel (Fig. 2) of the region from the lip (X) to the apex (Y) (Fig. 1b) of the shell, shows the three shell layers that are common in molluscs. The aragonitic crystals of the nacre are deposited parallel to each other between X and Y (Fig. 2) and obliquely between Y and Z (Fig. 3a and b). The change in depo-

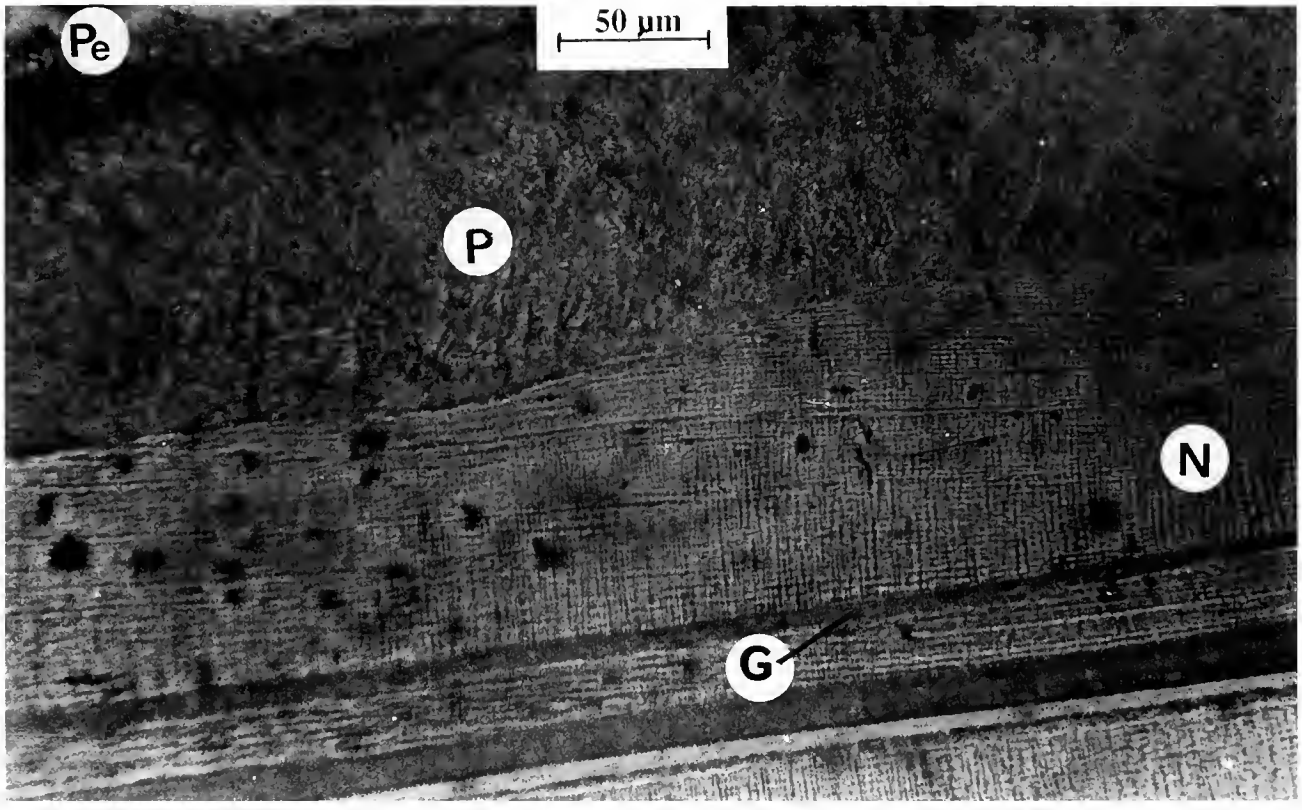

Figure 2. Acetate peel between X and Y (Fig. 1b) of a shell showing three layers. Aragonite crystals, in nacre, are deposited in parallel layers. Pe, periostracum; P, prismatic; N, nacre; G, growth line. Original magnification, ×40.

sition could possibly be to strengthen the shell (R. W. Day, personal communication). Occasionally, a parallel layer of aragonite was seen and it is possible that this was deposited after a predator attempted to bore into the shell. The difference in the deposition of the aragonite indicates that the nacre is growing differently between XY and YZ. The aragonite grows progressively thicker from the lip to the apex as the parallel layers are deposited on top of one another. The nacre grows wider from the apex to the growing edge (YZ) as the oblique layers are deposited.

Scanning Electron Microscopy

Further details of shell structure were apparent from the SEM. The nacre is composed of tabular blocks of aragonite (Fig. 4). This type of nacre is known as sheet nacre (Clark 1974), because the aragonitic tablets resemble a brick wall. Spaces between the blocks of aragonite are filled with organic matrix; this was not examined here. The growth line has a somewhat different structure from the nacre (Fig. 4), the tabular blocks of aragonite appearing to be interspersed with deposits of calcium (Lutz and Rhoads 1980). The growth lines are continuous through the nacre and have a higher topography than the surrounding nacre.

The lip of the shell is also composed of tabular aragonite (Fig. 5), similar to the nacre. Growth lines in the lip have a higher topography than the surrounding aragonite, probably a result of differential etching. Growth lines in the lip are not continuous and could be a reflection of tides or daily rhythms (Lutz and Rhoads 1980).

The prismatic layer in *H. midae* has a typical prism structure (Fig. 6) and is classified as simple prismatic because calcitic prisms are composed of a stack of disc-shaped crystals deposited on top of one another (Watabe 1984). The calcitic crystals develop from spherulites seen on the undersurface of the periostracum (Fig. 7). The periostracum itself is a tanned protein layer which has a honeycomb appearance (Fig. 8).

Validation of the Acetate Peel Aging Technique

Acetate peels were used to count the number of growth lines in each shell. Growth lines on acetate peels are delimited by dark lines that run from the lip (X) of the shell to the apex (Y) (Fig. 1b). A summary of the mean number of lines for shells of known age is given in Table 1. Shells that were 8 months old had one growth line, whereas 13 month old shells had an average of 3.2 lines. By 26 months, shells had deposited an average of 4.5 lines, and by 37 months, an average of 5.5 lines had been laid down.

Data on the number of growth lines counted in the shells of the 37 months old animals were tested for a normal distribution, and a one-way analysis of variance was performed to determine whether there were significant differences between the number of growth lines deposited under the different experimental conditions (groups D, E, F, and G), each group being compared with every other group. The result showed that there were no significant differences between groups (95% confidence limits). This showed that growth lines were deposited under conditions of constant temperature and that differences in diet and photoperiodicity did not affect the number of growth lines formed.

DISCUSSION

Mollusc shells vary in structure and composition, differences being most evident when bivalves are compared with gastropods

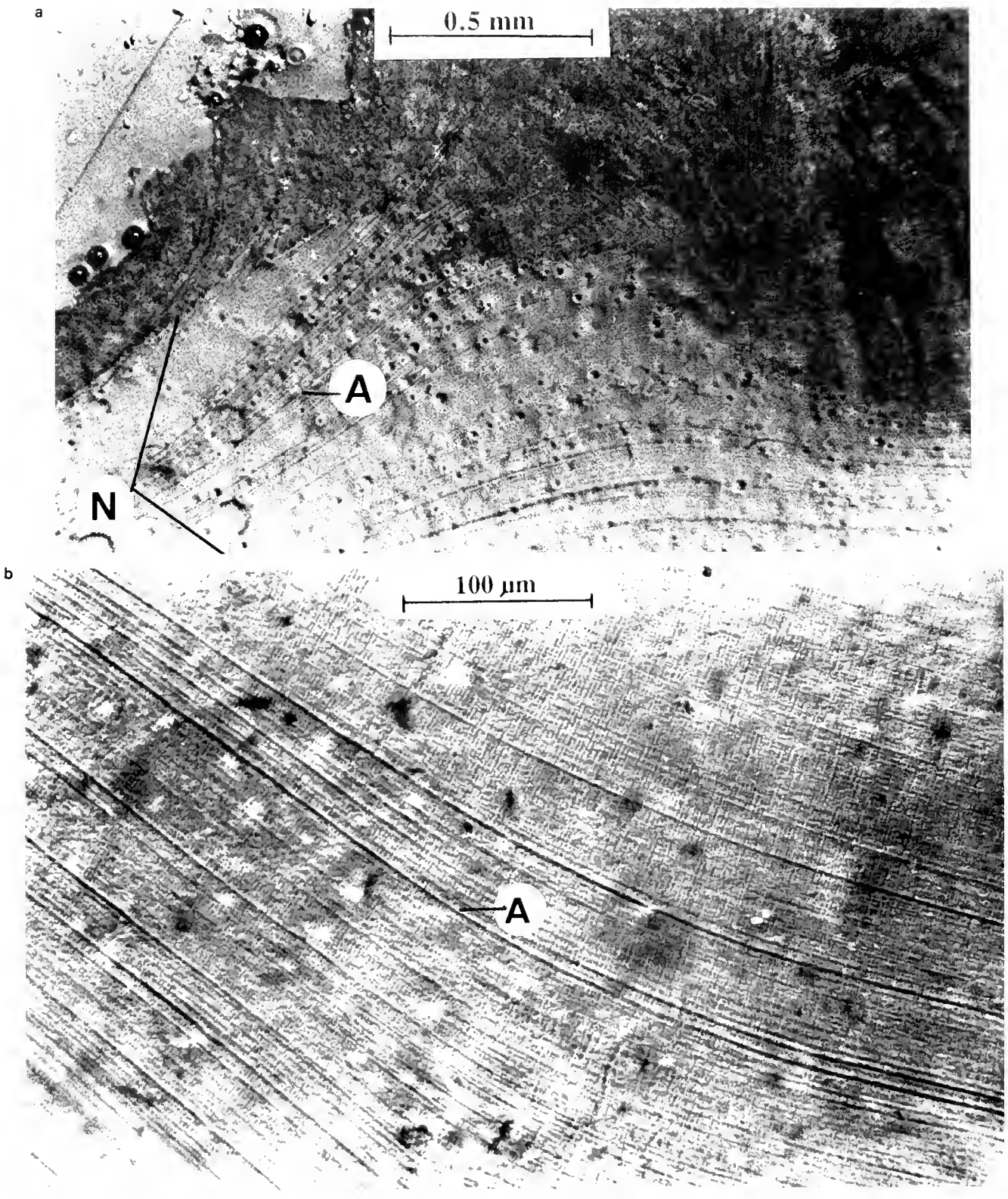

Figure 3. (a) Vertical peel of a shell from Y to Z (Fig. 1b). Aragonite is deposited in oblique layers. N, nacre; A, oblique layer of aragonite. Original magnification, ×20. (b) Higher magnification of layers of aragonite shown in panel a. The oblique deposition can be clearly seen. Original magnification, ×60.

(Watabe 1984). The mollusc shell has two major components, namely, the noncalcified periostracum and the calcified inner layers. This SEM study has shown that the periostracum of *H. midae* has a honeycomb appearance, similar to that described by Saleuddin and Petit (1983) for other species. Of the five molluscan calcified layers described by Clark (1974), only two, the prismatic and the nacreous layers, were observed in *H. midae* (Fig. 4).

Mutvei et al. (1985) examined the shell structure of eight spe-

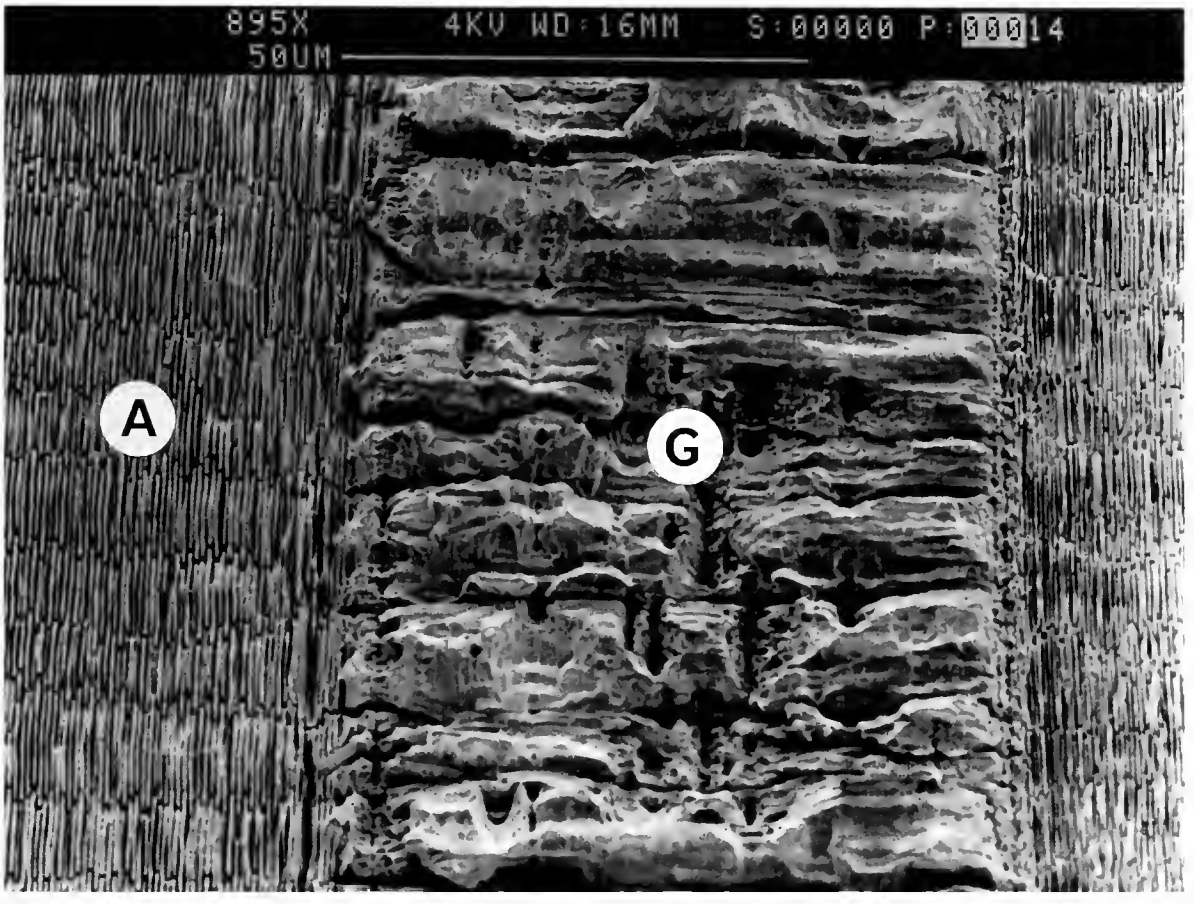

Figure 4. Nacre of shell and growth line. Nacre is composed of tabular blocks of aragonite, and growth line is a deposit of calcium. A, tabular aragonite; G, growth line. SEM, original magnification, ×895.

cies of abalone and found the prismatic layer of each of the eight species to be different, being composed of calcite, aragonite, or a combination of the two. The fact that the pallial epithelium in the mantle, which secretes the prismatic layer, is capable of secreting calcite and aragonite simultaneously and that the rates of mineralization can change rapidly during growth (Mutvei et al. 1985) could partially explain why the prismatic layer could be so different among species of abalone.

In the gastropod family *Littorinidae*, variations in the structure of the prismatic layer have been attributed to differences in water temperature, which could influence the physiological control of shell mineralogy (Taylor and Reid 1990). Those authors suggested that prismatic shell could be calcitic at lower temperatures and aragonitic at higher temperatures. Whether the same reasoning can be applied to explain differences in prismatic composition in abalone is unknown at present. The structure of the prismatic layer of *H. midae* is consistent with the structure of the simple prismatic described by Watabe (1984).

There are three different types of nacre found in mollusc shells, all of which are composed of aragonite (Clark 1974) and consist of numerous horizontal lamellae deposited on top of one another (Watabe 1984). Generally, gastropods have a columnar nacre and bivalves have a sheet nacre, but an exception to this is the gastropod *Cittarium pica*, which has a sheet nacre (Wise and Hay 1968). Columnar nacres were described in an unidentified haliotid species by Mutvei (1978) and in *Haliotis rufescens* by Nakahara et al. (1982). This study shows that the nacre of *H. midae*, being a sheet nacre, is uncharacteristic of the usual gastropod pattern (Fig. 4). The spaces around the tabular blocks of aragonite are filled with organic matrix, and such spaces can also be seen in Figure 4.

The nacre of *Mollusca* may contain growth lines that, according to Koike (1980), are zones of high calcium concentration and low sodium concentration. Although Koike (1980) was working on growth bands of *Meretrix lusoria* that resulted from daily or tidal rhythms, it is possible that the bands she described are similar in structure to the annual rings described here, that is, raised strips of shell matter that were left by HCl etching, the surface material being more easily eroded than the growth lines. The growth lines in the lip of the shell of *H. midae* are higher than the surface relief of the nacre (Fig. 7), suggesting that they are also less easily etched than the surface material. This could be because of a higher concentration of organic matter in the growth lines.

The width of the growth lines in abalone shells is variable (Watabe 1984), being controlled by the rate of deposition of the nacre, which precedes the formation of the line. Watabe (1984) attributed the change in width to seasonal variation in the environment. This suggestion is supported by results of this study, in which the widths of growth lines in shells from animals grown at constant temperature in the experimental hatchery were uniform.

This study has shown that growth lines in the nacre of *H. midae* can be visualized and counted by using the acetate peel method. Studies on counting growth rings, in acetate peels, have been validated previously by comparison with tagging data, but the disadvantage of this method is that, as suggested by Day and Fleming (1992), tags on shells could influence the growth rate of the animals or add disturbance rings. In this study, individual

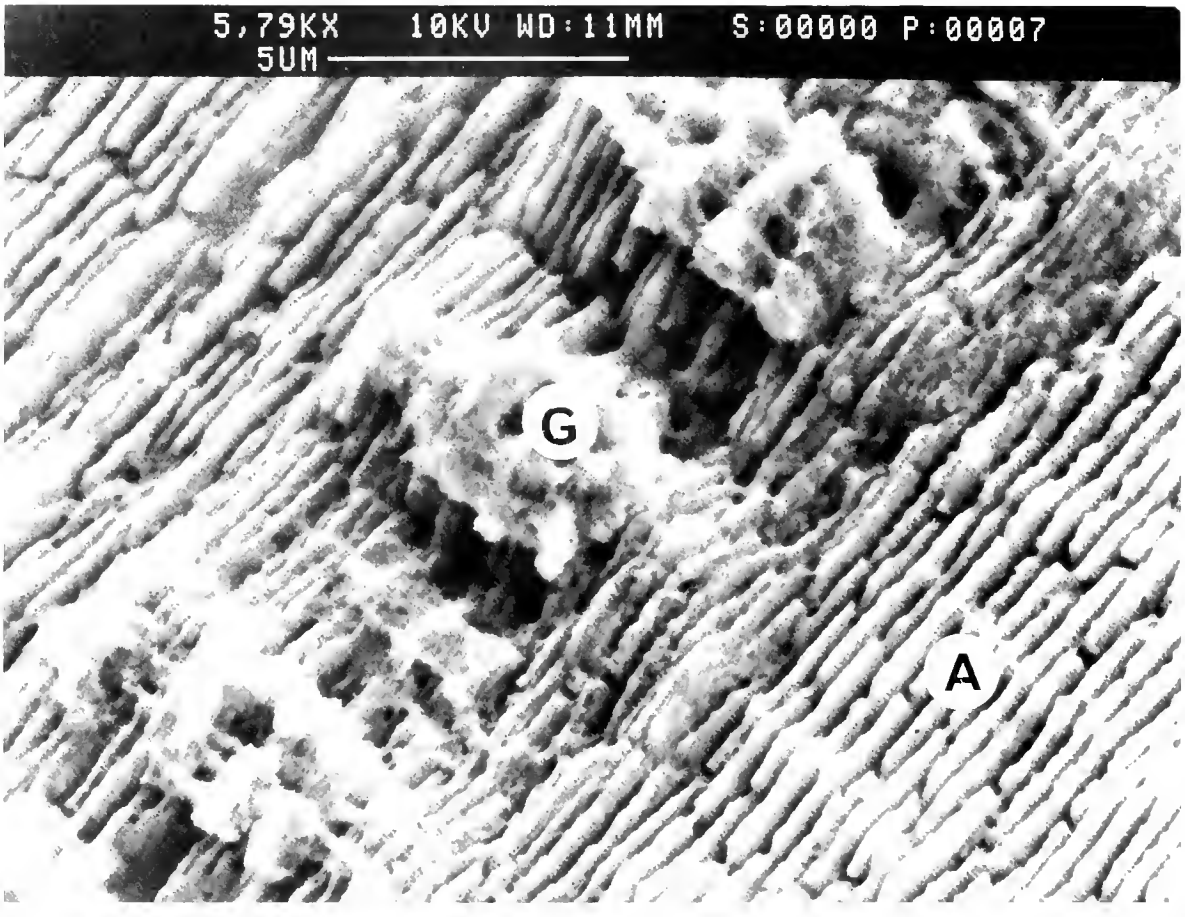

Figure 5. Lip of a shell, composed of aragonite, and growth line of calcium. A, aragonite; G, growth line. SEM, original magnification, ×5,790.

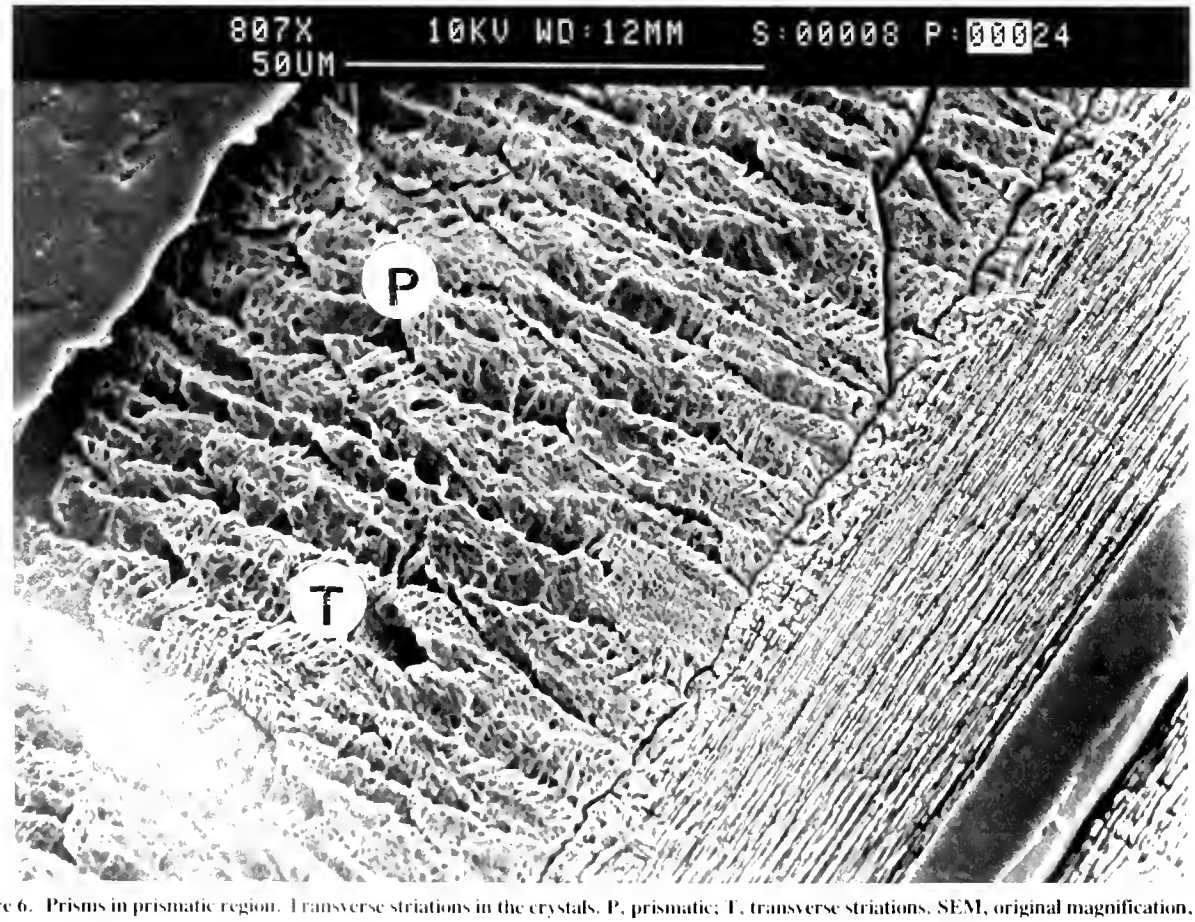

Figure 6. Prisms in prismatic region. Transverse striations in the crystals. P, prismatic; T, transverse striations. SEM, original magnification, ×807.

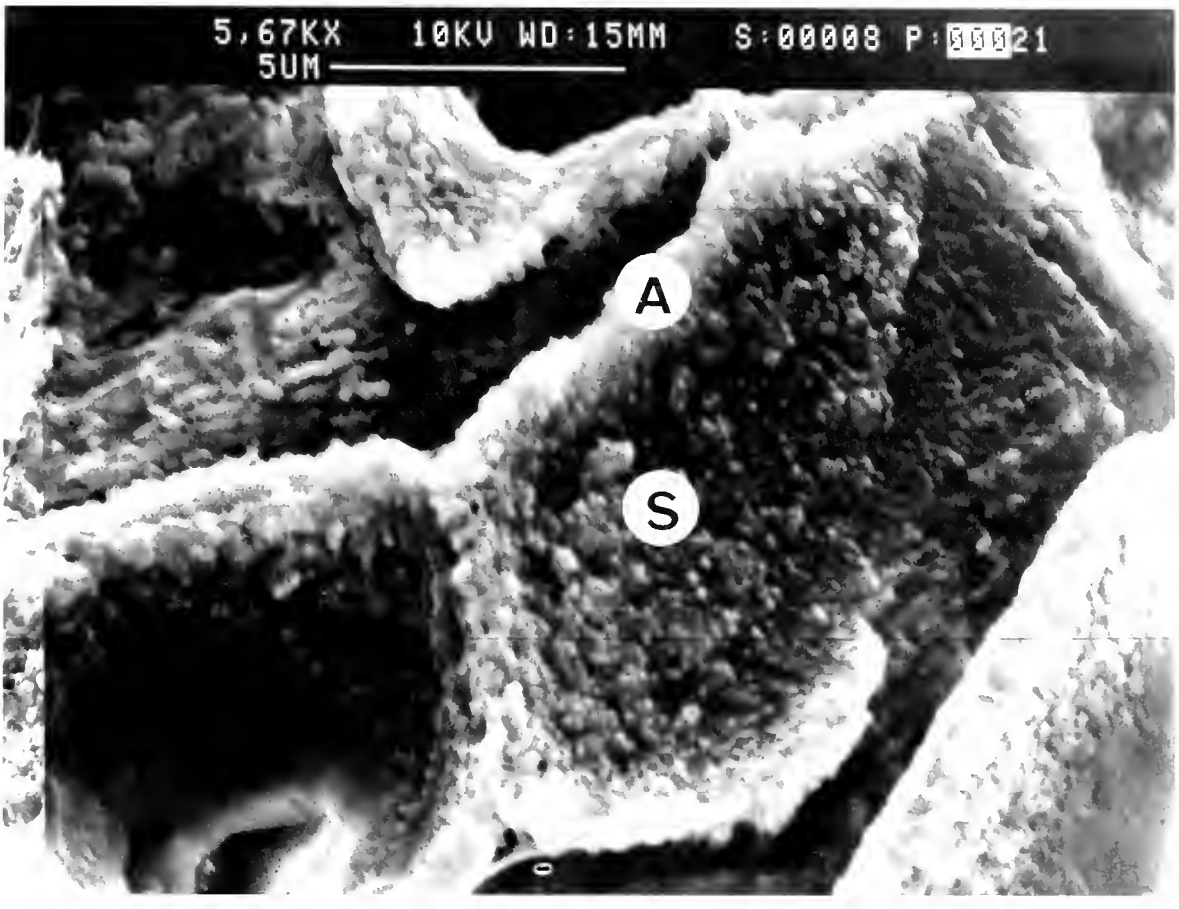

Figure 7. Under surface of periostracum. Antrum (A), on which spherulites (S) form. SEM, original magnification, ×5,670.

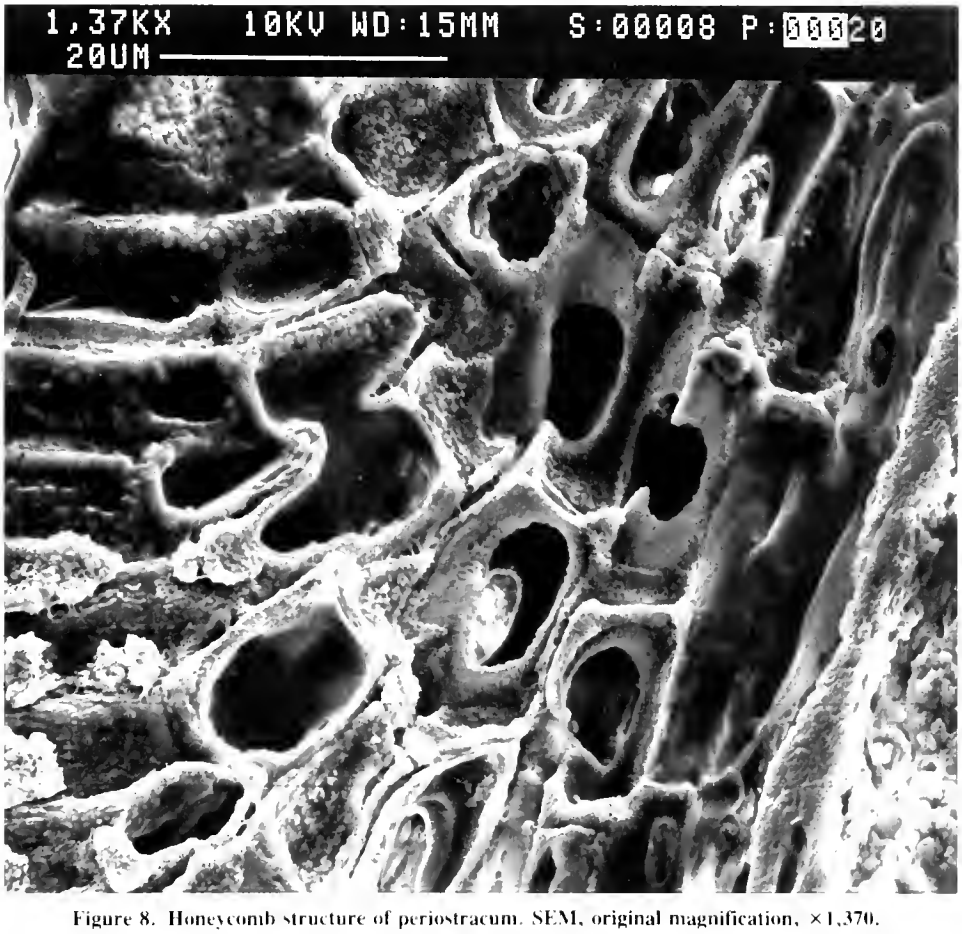

Figure 8. Honeycomb structure of periostracum. SEM, original magnification, ×1,370.

shells were not tagged but were grown under controlled conditions in an experimental hatchery and were, therefore, of known age. The influence of variations in temperature, diet, and light on the rate of deposition of growth lines could, therefore, be directly assessed. Results showed that none of these factors influenced the rate at which growth lines were deposited. The results are similar to those described by Richardson (1988), who although working on microgrowth bands of possibly daily or tidal origin, found that deposition of growth bands in the clam *Tapes philippinarum* was not related to environmental factors. The clams were placed under different constant conditions (temperature, water flow, light, and diet), and none of the factors influenced band formation. Richardson (1988) concluded that the bands were deposited because of an innate rhythm, which was controlled by the growth of the clam.

A possible factor that could influence formation of growth lines in abalone is related to the spawning season. Sakai (1960) and Forster (1967) found that growth lines in *Haliotis discus* and *Haliotis tuberculata* were deposited when the animals spawned. Thompson et al. (1980) suggested that this resulted from energy being channeled into gonad production and not growth. In this study, however, growth ring deposition cannot have been related to spawning because all animals were subadults and none had reached spawning age.

From the results obtained in this study, the most likely pattern of growth line deposition in *H. midae* appears to be very similar to that described by Prince et al. (1988) for *H. rubra*, that is, the deposition of three lines during the first year, followed by annual deposition thereafter. Growth line deposition was not, however, directly related to environmental variables, and it seems likely, therefore, that it could be controlled by an endogenous rhythm related to the growth cycle of the animal. Carter (1980) reviewed the relation between shell microstructure and mechanical properties, and it is possible that the laminated shell structure that results from the deposition of growth lines and from the alternate layers of calcite and aragonite may enhance shell strength and help to localize or deflect shell fractures.

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SURVIVAL, GROWTH, AND GLYCOGEN CONTENT OF PACIFIC OYSTERS, *CRASSOSTREA GIGAS* (THUNBERG, 1793), AT MADEIRA ISLAND (SUBTROPICAL ATLANTIC)

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ABSTRACT Pacific oysters, *Crassostrea gigas*, were introduced to Madeira Island (subtropical Atlantic) and grown at depths of 10 to 25 m. There were no significant differences between oysters grown at 10, 15, and 25 m depth for any of the parameters analyzed. Live weight and shell size increased significantly, whereas dry meat weight and condition declined significantly by 45 and 70%, respectively. Glycogen content decreased by 90% within 5 weeks; this was not linked to gametogenesis. Overall mortality attained 73% in 5 months. The oysters' poor performance is attributed to a combination of stress factors.

KEY WORDS: bivalve, oyster, *Crassostrea*, introduction, aquaculture, condition

INTRODUCTION

The Pacific oyster, *Crassostrea gigas* (Thunberg), is being grown with commercial success in a variety of environments worldwide. In temperate zones, its growth decreases in winter (e.g., Askew 1972, Héral and Deslous-Paoli 1991), but under subtropical conditions, continuous growth can be achieved during the entire year (e.g., Hughes-Games 1977). At Madeira Island, water temperatures are favorable at 17 to 23°C year around. Oysters (*Ostrea* species) have been found occasionally (Abreu, personal communication), but there has never been any local fishery or culture. A small-scale grow-out trial with imported Pacific oysters, however, led to encouraging results (Waschkewitz, personal communication) and motivated this present study.

MATERIALS AND METHODS

Healthy half-grown oysters, *C. gigas*, of British origin, that had been grown in Flensburg Fjord (Western Baltic) from spat size, were introduced to Madeira at a mean live weight of 9.6 g. They were grown at 10, 15, and 25 m depth in six lantern nets with 1 cm mesh that were anchored at a depth of 27 m in a small bay near Funchal Harbour. Each net was stocked with about 320 oysters, at a density of less than 1 g cm^{-2} (as recommended by Spencer 1990).

Every 2 or 3 weeks from July until December 1991, the water temperature at the site was determined and 30 oysters from each depth were sampled randomly by SCUBA diving. Dry weight was determined after drying for 24 h at 60°C, and ash weight was determined after incineration for 24 hours at 550°C. Glycogen was determined after Keppler and Decker (1984); pieces of mantle tissue of about 100 mg were excised from six randomly selected live oysters in each sample, stored in micro-test tubes at −60°C, and later transported on dry ice to IfM Kiel for analysis. To assess the oysters' overall performance, the condition index (CI) of Lawrence and Scott (1982), recommended by Bodoy et al. (1986) and Crosby and Gale (1990), was calculated as: CI = (dry meat weight × 1,000)/(live weight − dry shell weight).

RESULTS

Sea water temperature varied between 24°C in August and September and 19°C in December. There were no significant differences between oysters grown at all three depths (Mann-Whitney *U* test, 0.95 level), and the results for 15 and 25 m have been pooled; the lantern nets at 10 m disappeared 8 weeks after the beginning of the experiment and have been excluded from further analysis.

There was an increase in live weight and shell size and a simultaneous decrease in dry and ash-free dry meat weight of the

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TABLE 1.

Growth (mean ± standard deviation) and mortality of oysters (*C. gigas*) during grow out at Madeira Island. Pooled data for oysters grown at 15 and 25 m depth. N = 60 (week 21, N = 30).

Weeks in Culture	Shell Height (mm)	Live Weight (g)	Wet Meat Weight (g)	Dry Meat Weight (g)	Ash-Free Dry Meat Weight (g)	Dry Shell Weight (g)	Cumulative Mortality (%)
0	41 ± 3	9.6 ± 1.7	2.4 ± 0.6	0.37 ± 0.14	0.31 ± 0.13	5.5 ± 1.1	
7	43 ± 4	10.4 ± 1.8	2.2 ± 0.5	0.23 ± 0.08	0.16 ± 0.07	5.8 ± 1.1	39
14	47 ± 6	12.6 ± 3.3	2.5 ± 0.7	0.23 ± 0.07	0.15 ± 0.05	7.6 ± 2.1	55
21	47 ± 6	13.5 ± 4.0	2.4 ± 0.8	0.20 ± 0.06	0.12 ± 0.04	7.8 ± 2.4	73

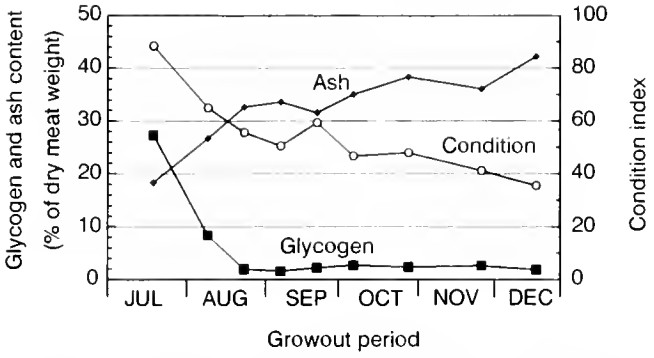

Figure 1. Glycogen and ash levels (left scale) and condition index (right scale) in oysters (*C. gigas*) during grow out at Madeira Island. Pooled data for oysters grown at 15 and 25 m depth. N = 12 for glycogen; N = 60 for ash and condition index (except December, where N = 6 and N = 30, respectively).

oysters during the rearing period; wet meat weight remained constant. The mortality exceeded 70% (data summarized in Table 1). The ash content of the meat increased insignificantly in absolute terms, but it more than doubled as a percentage of the dry meat weight. The condition index and the glycogen level in the meat declined, respectively, to less than 40% and less than 10% of the initial values (Fig. 1).

DISCUSSION

We have no indications that the stock might have been diseased, nor has there been any record of disease or mortality in the original Baltic stock in the years before, during, or after the introduction to Madeira (the source of the oysters was once intended as a quarantine station for imports to Germany). The oysters' initial condition index was in the normal range, and the values found at the end of the experiment are not unusually low (cf. Table 2), except that they did not result from spawning activity.

Oysters are usually cultured in the intertidal zone or in shallow depths, but cultivation at depths of 10 m and more is often advantageous (Marteil 1979); we have not found any reports on oyster cultivation at 15 to 25 m, but the absence of any depth correlation in our data makes negative effects seem unlikely.

The temperature and salinity stress brought about by the transfer from the Baltic to Madeira can by itself hardly account for the oysters' poor performance. It brought an increase in salinity (by 15 to 20 ppt) and in temperature (by about 5°C) for the oysters. Oysters from the identical Baltic stock were, however, subjected to the same stress by Waschkewitz and performed better. Salinities

TABLE 2.

Comparison of condition (determined after Lawrence and Scott 1982) in oysters (*C. gigas* and *Crassostrea virginica*).

Source	High Values	Low Values	Remarks
Lawrence and Scott (1982)	61	41	*C. virginica;* sampled Jan.–June
Bodoy et al. (1986)	60–120	20–30	*C. gigas;* 4-year study of 21 condition indices
Crosby and Gale (1990)	79–88		*C. virginica;* sampled in May–June
This study	90	35	*C. gigas;* sampled July–Dec.

at Madeira are high (36 to 37 ppt year around; INIP 1982), but Hughes-Games (1977) obtained excellent growth of *C. gigas* at 42 ppt and at summer temperatures of 20 to 34°C.

The facilities and equipment available for this investigation were insufficient for the analysis of phytoplankton and nutrients, but the waters of Madeira are oligotrophic (Chl. values of 0.05 to 0.15 mg m^{-2}; INIP 1982), and starvation may have been an important additional stress. In a 6-month starvation experiment with *C. gigas*, Riley (1976) also found an important decline in dry meat weight; carbohydrate levels declined more than lipid and protein, but in the mantle (its main storage site), carbohydrate declined only from 28 to 23% (28 to 2% glycogen in this study). Seaman (1991) kept oysters from the same stock without food for a similar period of time with similar results as Riley (cf. Table 3).

The steep decline in glycogen content that followed the oysters' transfer to Madeira was not associated with gametogenesis. This indicates that the animals were more stressed than in the examples cited above and were thus forced to consume their reserves entirely (cf. Gabbott 1983). We think that the oysters' poor performance resulted from high metabolic demand (due to salinity and temperature stress) in combination with nutritive stress. Better results might be obtained if the oysters were introduced at another time of year, as had been the case with Waschkewitz' introduction.

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TABLE 3.

Performance of oysters (*C. gigas*) after 20 to 25 weeks of starvation, as compared with this study.

Source	Duration of Experiment (Weeks)	Decline in Meat Weight (% of Initial Value)	Decline in Carbohydrate[a] (% of Initial Value)		Cumulative Mortality (%)	Remarks
			Mantle	Whole Body		
Riley (1976)	25	39	20	38	40	Starvation at 13.5°C
Seaman (1991)	20	30		20–40	20–48	Air storage at 7°C
This study	21	46	93		73	Same stock as Seaman (1991)

[a] Total carbohydrate in Riley (1976); glycogen in Seaman (1991) and in this study.

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THE BREEDING AND SECONDARY PRODUCTION OF THE FLAT TREE-OYSTER *ISOGNOMON ALATUS* (GMELIN 1791) IN TROTTS POND BERMUDA

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ABSTRACT *Isognomon alatus* (Gmelin), the flat mangrove oyster, is very abundant in Trotts Pond, Bermuda. The oyster occurs mainly on the submerged prop roots of the red mangrove *Rhizophora mangle* (L.), averaging 250 oysters root^{-1} or 2,700 m^{-2}. The sex ratio found was 68 female:26 male:6 indeterminate. Mature oysters of both sexes were present throughout the year, but maximum breeding activity, shown by maximum gamete volume fraction followed by a fall and by a peak in spatfall, occurred in the spring. No larvae were detected in the plankton during the course of the study. The mean caloric content of the oysters was 5.23 cal mg^{-1}. The mean dry weight biomass was 399.3 g m^{-2}, and the total of somatic gonadal and shell production was 863.9 g m^{-2} per year giving a production:biomass ratio of 2.2:1.

KEY WORDS: oyster, mangrove, breeding, production, ecology

INTRODUCTION

Isognomon alatus (Gmelin) [Isognomonidae], the flat mangrove oyster, is common in tropical and subtropical coastal waters of the western Atlantic Ocean (Abbot 1974, Siung 1980, Rehder 1981, Morton 1983). Its typical habitat is in clumps on the submerged prop roots of the red mangrove *Rhizophora mangle* (L.), although it also occurs on rocks and manmade structures. In Bermuda, it was formerly common on coastal mangroves (Sterrer 1986) but is now confined to the mangrove habitat of the two largest anchialine ponds, where it is very abundant (Thomas et al. 1992, Thomas 1993). The species is harvested only in Jamaica but is of potential commercial importance elsewhere (Siung 1980).

I. alatus has been the subject of only sporadic research. Trueman and Lowe (1970) looked at the relation between temperature and heart rate, and Kolehmainen et al. (1973) investigated the response of the species to thermally elevated discharge water, finding the oyster resistant to elevated temperature. Sutherland (1980) discussed the epibenthic community dominated by *I. alatus* and drew attention to its capacity both to colonize new surfaces and, through its shells, to provide a large area of substrate for associated species. Siung (1980) investigated at the breeding biology and its potential for cultivation in Jamaica. She found that peak spawning occurred at a time of decreasing water salinity at the onset of the rainy season in the autumn, but that some reproduction took place throughout the year. She also found that the presence of eggs and/or sperm in the water acted as a stimulus to spawning.

Trotts Pond is an anchialine pond with an area of 3.85 ha, a low-tide volume of 103,565 m^{-3}, a tidal range of 1.5 cm, a peripheral mangrove swamp with an area of 0.84 ha, and sediments consisting of a matrix of organic mud with large quantities of *I. alatus* shell; it has a small connection to the ocean at about mean pond surface level (Thomas et al. 1991). Temperatures have been reported to range from about 16 to 31°C, and salinities range from 23 to 34‰ (Thomas et al. 1991).

The occurrence of this species in the anchialine ponds of Bermuda is of particular interest. Thomas et al. (1991, 1992) have pointed out that the pond environment is an extreme one and that the diversity of life therein is low. Dissolved oxygen shows an extreme range between anoxia and slight supersaturation. In summer, the combination of high temperature and low dissolved oxygen results in difficult conditions for many aquatic organisms. Nevertheless, *I. alatus* are the dominant attached organism in the two largest ponds, Mangrove Lake and Trotts Pond, colonizing most of the available mangrove roots. This study was designed to investigate their reproductive and secondary production ecology in this extreme environment.

MATERIALS AND METHODS

Studies on *I. alatus* took place from February 1991 to May 1992. Environmental conditions in Trotts Pond were monitored from February 1991 to August 1993.

Temperature was monitored at two sites in the pond. A Branckner XL-100 (R. Branckner Research, Ottawa, Canada) electronic data logger, sampling at hourly intervals, was installed to hang at 50 cm depth in the center of the mangrove swamp on the north side of the pond (Fig. 1). It was operated from February 1991 to May 1992 and downloaded to a computer at 6 month intervals. On the west side of the pond, temperature and dissolved oxygen were monitored continuously from an electrode array, suspended at a depth of 40 cm, in the center of the mangrove swamp (Fig. 1). Two temperature sensors were used, one within a Rosemount (Rosemount Analytical, Irvine, CA) dissolved oxygen analyzer equilibrium electrode and the other a Cole-Parmer (Cole-Parmer Instrument Co., Niles, IL) thermistor sensor attached to the outside of the electrode. The thermistor sensor data were recorded on a Cole-Parmer vertical chart recorder; the electrode data could be displayed on the oxygen meter as a check. They were always within 0.2°C of each other. The dissolved oxygen meter displayed either percent saturation or ppm but recorded in ppm on the same chart as temperature. The oxygen meter-electrode system was recalibrated at 3 month intervals and reconditioned after the first year. Readings showed linear drift with time and were corrected on this basis. Salinity was monitored with a Biomarine Refractometer (Biomarine Inc., Hawthorn, CA) during biological operations.

The reproductive condition of oysters was determined from four samples of randomly chosen oysters, 25 for each sampling period. The gonad of each measured specimen was removed, weighed, and then embedded in Paraplast for sectioning. Sections

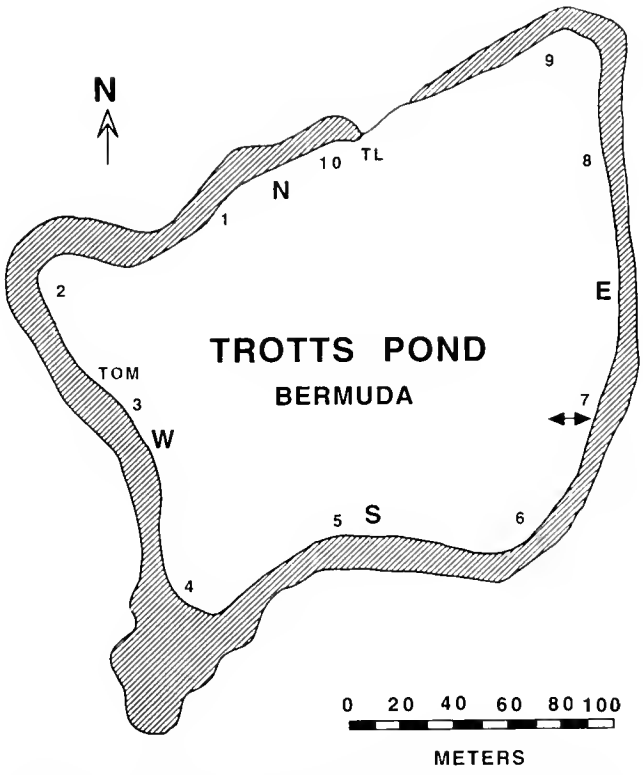

Figure 1. Trotts Pond, Bermuda, showing the locations of the temperature logger (TL), temperature and dissolved oxygen monitor (TOM), the connection to the ocean (↔), biomass and demography sampling satations (N, W, E, S) and spat collector stations (1 to 10). The shaded area is the mangrove swamp.

were cut as follows. The first section was 8 μm thick followed by 10 10 μm thick sections, then a second 8 μm section, and so on. The 8 μm sections were mounted on slides and stained with Mallory's stain. The 10 μm sections were discarded. Sectioning was continued until the entire gonad was sampled. The sex and stage of gonad development were determined from the sections by reference to similar previous studies on other molluscan species (Williams 1964, Fish 1972, Chase 1991). The gamete volume fraction within the gonad was determined by stereology (Steer 1981).

Plankton in the pond were sampled weekly during study periods with a 10 cm mouth Hensen (Kahl Scientific Instrument Corp., San Diego, CA) egg net fitted with 64 μm mesh. The net was used for 5 minute tows in the pond at both 5 cm deep and 20 to 50 cm deep, being towed behind a small Zodiac (Zodiac Corp., Mississauga, Canada) inflatable boat outside the aquatic margin of the mangrove swamp. It was also deployed for 5 to 10 minute periods in the inflowing current of the connection to the ocean (Fig. 1).

Spat settlement was monitored on collectors prepared in February 1991. Two types of collector were used, 21 mm diameter, 600 mm long polyvinyl chloride (PVC) pipes roughened with sand paper and hung vertically from 10 cm below the surface and natural, living red mangrove prop roots carefully lifted from the water, scrubbed free of all living macroorganisms and returned to the water. One of each collector type was situated at each of 10 points around the margin of the pond (Fig. 1), each site being marked with fluorescent flagging tape. Collectors were sampled at each subsequent sampling period, and all spat were counted and measured.

Biomass was sampled in four plots, one each at the centers of the W, N, S, and E sides (Fig. 1) of the pond, having areas of 23.6, 35.8, 26.3, and 67.2 m^{-2}, respectively, and containing 411, 515, 301, and 388 red mangrove prop roots (counted at the surface), respectively. At each sampling period, nine prop roots were selected in a stratified random pattern, three each from the inner, middle, and outer portions of the aquatic part of the mangrove swamp. Each root was stripped of all aquatic organisms, which were sorted to species and weighed fresh. Oysters were counted and then weighed wet and dry with the bodies and shells separated; finally, samples of both bodies and shells were ashed at 435°C to give organic content for production calculations. Biomass was calculated from biomass $root^{-1}$ and root abundance.

Demography was studied from five samples of oysters, one from each sampling period. Each sample consisted of pooled oysters from the centers of each of the W, N, S, and E shores (Fig. 1) derived from randomly selected natural clumps. The samples contained 553, 363, 628, 754, and 875 *I. alatus* respectively. The length of each specimen was measured with Mitutoyo (M.T.I. Corp., Paramus, NJ), digital Vernier calipers, which automatically accumulated the data in a computer. Length frequency histograms were plotted with SYSTAT (Systat, Evanston, IL), and the polymodal distributions were separated into cohorts by the method of Harding (1949), incorporating modifications of Cassie (1954) and Cerrato (1980). Secondary production was estimated by the removal summation method originally described by Boysen-Jensen (1919) but modified by many authors (e.g., Downing and Rigler 1984). Production was considered as the sum of somatic, gonadal, and shell production following MacDonald and Thompson (1985). Calorimetry was carried out on about every 10th oyster collected for demographic analysis (319 in all). Powdered, mixed material from whole-body samples was used to make pills burned in a Phillipson Microbomb calorimeter. Standardization with benzoic acid was performed after every 10th sample.

RESULTS

The maximum water temperature recorded during the study period was 34.7°C in August 1991, and the minimum was 12.9°C in February 1992. The daily mean temperature was 28.4°C in summer and 18.2°C in winter. The diurnal range in summer averaged 1.9°C (max., 3.7°C), whereas in winter, the average was 1.1°C (max., 2.9°C). Surface water salinities ranged from 27 to 34‰, with a mean of 29.5‰. Dissolved oxygen levels in Trotts Pond were exceedingly variable, with a maximum of 9.93 ppm (139% sat.) in December 1991 and minima of 0 ppm on numerous summer nights from July to October 1991. Daytime levels usually rose above zero, but on four separate days in mid-August 1991, continuous anoxia prevailed day and night at the meter site.

The overall sex ratio (female:male) of *I. alatus* in Trotts Pond was 2.6:1, with individual samples ranging from about 4:1 to 2:1. Six percent of the specimens examined could not be sexed. The mean gonad weight was 0.16 ± 0.12 g; there were no significant differences in gonad weight among samples or between sexes. Stages of gonad development were identified as: I, immature; II, developing; III, mature 2; and IV, spent. All four stages could be identified in females but only the last three in males. Figure 2 shows the distribution of the four stages in males, females, and the population at the five sampling times. Mature individuals of both sexes were present throughout the year but peaked early in the year. This suggests that spawning was continuous, with a maximum in the early spring. Gamete volume fraction (GVF) data (Fig.

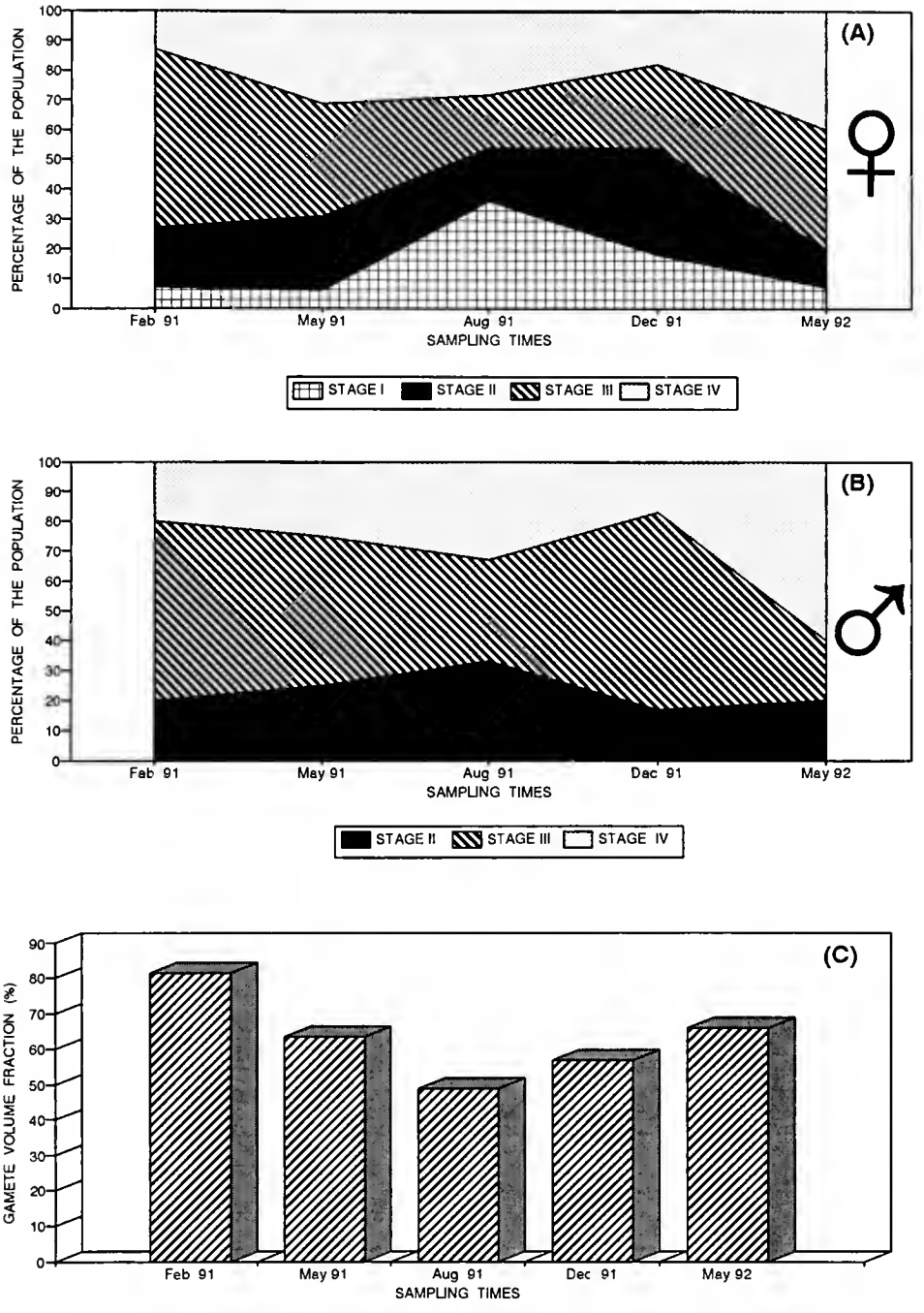

Figure 2. Breeding cycle for (A) female and (B) male *I. alatus* and (C) GVF for both sexes in Trotts Pond, Bermuda.

2) supported this conclusion, showing high values at all times and including an early spring peak.

Plankton samples failed to show the presence of *I. alatus* eggs or larvae in the water at any time. The main constituents of the plankton in order of decreasing frequency of occurrence were: cyanobacteria, dinoflagellates, diatoms, copepods, cladocerans, mysids, and chaetognaths.

The total numbers of spat that settled on the 10 natural prop-root collectors by the end of each observation period were 181, 73, 92, and 89 for May 1991, August 1991, December 1991, and May 1992, respectively. For the PVC pipes, the totals were 6, 1, 2, and 1, respectively. These data support the continuous nature of spawning with a spring peak suggested by the gonad studies.

The population of mangrove oysters on the prop roots of the red mangrove was dense all around Trotts Pond. Mean values were 250 ± 11 root^{-1} or $2{,}696 \pm 896$ m^{-2}, with a mean dry weight biomass of 399.3 ± 89.6 g m^{-2}. Length-frequency data for each sampling period failed goodness of fit tests for normal distributions in all but one case (May 1991), showing the polymodal nature of the population; however, a large overlap between cohorts prevented their separation by length frequency alone. Cumulative frequency plots analyzed by the modified Harding (1949) method suggested the presence of five cohorts at all sampling times. It is assumed that these represent annual groups dominated by the spring spatfall but also containing oysters from earlier and later settlement. It is concluded that the maximum lifespan is 5½ years

at this location. In all but one case (February 1991), the majority of the population fell in cohort 3, suggesting a dominant spatfall in the spring of 1988. Assuming that a spring spatfall dominates each cohort, the mean lengths attained each year are about 17.5, 23, 32.5, 40.5, and 47.5 mm, with a maximum mean length of 52.5 mm. Individual oysters attain up to about 65 mm maximum length. Caloric contents of individual oysters ranged 2.85 to 8.02 cal mg^{-1}. Means for each sampling period were 4.98 ± 0.64, 4.70 ± 0.03, 4.64 ± 0.53, 5.70 ± 0.70, and 5.73 ± 0.77 cal mg^{-1} for February, May, August, and December 1991 and May 1992, respectively. Tukey's multiple range test confirmed that the data fell into two significantly different groups comprising the first three and last two samples, respectively, and showing higher levels in the winter and spring of 1991 to 1992.

Table I gives the values calculated for somatic, shell, gamete, and total production for each cohort during the period from 19 February 1991 to 28 May 1992, the grand total being 2.31 g m^{-2} per day. The majority of the production was contributed by cohort 3. Annual production varied somewhat according to how the year was defined within the total sampling time, but averaged 843 g m^{-2} per year, yielding an annual production:biomass (P:B) ratio of about 2.2:1.

DISCUSSION

The physical data for the pond collected in this study and combined with that of Thomas et al. (1991) show a subtropical anchialine pond with a pronounced annual temperature range of about 13 to 35°C that varies little from year to year and a rather constant salinity ranging from 24 to 34‰. Thomas et al. (1991) attributed the low salinity range to the fact that the connection to the sea at about mean pond surface level allowed fresh water from rainfall and drainage to drain off the surface. Nevertheless, the pond is somewhat hyposaline with respect to the coastal ocean at about 36‰ (Morris et al. 1977). Dissolved oxygen, however, showed extreme variation from slight supersaturation to anoxia. The latter condition occurred regularly in summer, when temperatures were close to a maximum, particularly frequently at night, and lasting up to 3 days continuously at the sensor site. Observations suggested that anoxia was a patchy condition, never involving the entire pond during the period of study. However, residents close to the pond have reported past conditions when a smell of H_2S was associated with the pond and when there were massive mortalities of pond biota. However, total anoxia has never occurred from 1980 to the end of this study and may therefore be considered an infrequent occurrence. However, because the pond has a tidal range of less than 2 cm, the attached biota of the roots are always submerged. The mangrove oysters and their associated, sessile biota are therefore exposed to anoxic conditions for days at a time during the height of summer, and it is assumed that the oysters shift to anaerobic respiration at such times. Such an ability has been demonstrated for other oyster species but is usually a response to winter inactivity rather than summer environmental conditions (e.g., Wilbur and Yonge 1966). Where this species is exposed by the tide, the valves gape slightly in air, allowing a period of normal respiration (Littlewood 1994). The ability of this species to exist without dissolved oxygen for periods in excess of a day at over 30°C in Trotts Pond shows remarkable adaptation to these harsh environmental conditions.

TABLE I.

Total production (gm^2) of the population of *I. alatus* in Trotts Pond, Bermuda during the period from 19 February 1991 to 26 May 1992.

Cohort	Somatic (Pg)	Shell (Ps)	Gamete (Pg)	Total (P)
Cohort 1	7.0632	5.1901	0.8802	13.1335
Cohort 2	79 7561	60 1829	12.8977	152.8367
Cohort 3	298.5551	174.7528	53.5776	526.8854
Cohort 4	177.4929	102.2235	38 2069	317.9233
Cohort 5	47.0579	25.5166	11.8924	84.4669
Total	609.2952	367.8658	117.4548	1095.2457
Daily	1.2687	0.7926	0.2531	2.3144

The preponderance of females over males in the ratio of over 2:1 is similar to that recorded for *Crassostrea rhizophorae* by Littlewood and Gordon (1988) and suggests that sex reversal is taking place in this species. Such a process is quite common among oysters (Andrews 1979). Examination of the gonads from a point of view of both maturity and GVF suggested that spawning was a continuous process in Trotts Pond but that there was a pronounced spring peak as temperatures rose from the winter minimum. This was confirmed by a similar pattern in the settlement of spat. Siung (1980) also reported continuous spawning of this species in Jamaica, but there, a spawning peak coincided with a period of low salinity, falling to a minimum of about 15‰ in September to November as temperatures declined from their peak. In Jamaica, the temperature range was only 26 to 30°C compared with the 13 to 35°C range in Bermuda. In contrast to Jamaica, salinity is fairly constant, without seasonal trends. Evidently, spawning occurs under different environmental conditions in the two locations. The absence of larvae in the plankton is curious. Siung (1980) described pelagic larvae of this species in Jamaica but was unable to determine the time spent in the plankton. In Bermuda, we know that breeding occurred and spat settled, but pelagic larvae were absent both in the open water of the pond and in the tidal inflow water. Two explanations of this are possible. The pelagic larval period may be very short, and settlement may be immediate. However, Siung's observations suggest that it is at least several days. Alternatively or additionally, the larvae may be strongly photonegative and remain under the dense canopy of the mangrove swamp. The lack of any significant tidal or wind-driven currents in the very sheltered environment of Trotts Pond would aid in any swimming behavioral adaptation in the larvae. Photonegativity in sessile benthic invertebrate larvae is a common and widespread phenomenon (e.g., Keough and Downes 1982).

The interpretation of demography from length-frequency data is always difficult and in tropical populations often impossible. However, in the case of the mangrove oyster in Trotts pond, it was felt that the pronounced seasonal variation in temperature should result in annual periodicity of phenomena related to growth and reproduction. This assumption proved to be correct, but the continuous nature of spawning inevitably attenuated the length-frequency range of annual groups. The conclusion from the length-frequency data that there are five annual age groups present is supported by the spawning maximum and maximum spatfall in spring as well as by data from thin sections of shells, which showed a maximum of five annual growth interruptions (Dangeubun 1994). Production calculations were carried out on the basis of the five identified cohorts, but the total would not be significantly affected if the assumption of five cohorts were incorrect because production would just be allocated differently. Both total production and the P:B ratio seem rather low for tropical invertebrates. However, it is very likely that total production is underestimated, particularly for the reproductive component. This

aspect of production was calculated from data on the seasonal reduction in the mean weight of the gonads; it would therefore only include the spring maximum. The steady spawning throughout the rest of the year would not be included by this method. It is not possible to apply a correction for this, but if spat settlement is a good index of production, then reproductive output is at about half the spring value in the other three seasons. On this basis, reproductive production would rise by a factor of about 1.5 to about 276 g m^{-2}, and the overall P:B ratio would rise to about 3:1. This is somewhat lower than the average for univoltine zoobenthos reported by Waters (1977) but higher than the trend shown by Warwick (1980) for benthos with a 5 year lifespan. The crowding of the oysters and the environmental instability would both tend to reduce secondary productivity (Mann 1967). The significantly higher caloric content of oysters from the last two samples is difficult to explain because it is not a seasonal trend, but it is probably related to feeding conditions. Further work on the larval life of this species is certainly needed, but it is evidently a successful and productive species in very severe environmental conditions.

ACKNOWLEDGMENTS

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LABORATORY STUDY OF FOOD CONCENTRATION AND TEMPERATURE EFFECT ON THE REPRODUCTIVE CYCLE OF *ARGOPECTEN VENTRICOSUS*

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ABSTRACT A laboratory study was carried out in Lewes, Delaware, to assess, first, the relationship of gametogenesis in *Argopecten ventricosus* to seasonal changes in temperature and phytoplankton densities, and second, the correlation of the gametogenic cycle with relative changes in the dry weight of digestive gland, adductor muscle, and mantle-gills. The laboratory study showed that *A. ventricosus* increased significantly in total weight by 30 days in high phytoplankton densities. Gonadal dry weight increased significantly after 40 days at high food ration, but gonadal index (dry weight of gonad/dry weight of whole animal × 100) declined. The digestive gland declined sharply in dry weight under high and low phytoplankton densities, possibly suggesting that this organ was providing energy for reproduction. The adductor muscle index (dry weight of muscle/dry weight of whole animal × 100) was higher at a high than at a low food ration. These laboratory studies suggest that gametogenesis in *A. ventricosus* is developed primarily during the wet season at low phytoplankton densities, and somatic growth (especially of the adductor muscle) is enhanced during either dry or wet seasons at high phytoplankton densities.

KEY WORDS: scallop, *Argopecten ventricosus*, reproduction

INTRODUCTION

Environmental Factors Affecting Bivalve Reproduction

Reproduction in the bivalve is coupled to seasonal changes of environmental factors, such as water temperature and food availability. Less dramatic seasonal changes in environmental factors have been observed in coastal tropical zones as compared with those in temperate areas. Despite less dramatic seasonal changes in the tropics, temperature and phytoplankton densities still affect the reproduction of some bivalves as Wilson and Hodgkin (1967) report in *Amigdalum glaberrimu; Brachidontes* cf. *variabilis* and *Septifer bilocularis*, by Carvajal (1969), Lunetta (1969), and Berry (1978); Vélez and Epifanio (1981) in *Perna perna*, and by Vélez (1976, 1985) in *Crassostrea rhizophorae* and *Donax denticulatus*.

Meteorological and Oceanographic Conditions in Panama

The climate on the Isthmus of Panama is characterized by pronounced seasonal changes in rainfall and wind velocity. Seasonality is driven by the passage of the intertropical convergence zone across the equator. This passage is related to alternation of wet (April–December) and dry season (January–March). A distinct upwelling event in the Gulf of Panama on the Pacific side of the isthmus is observed at the dry season. Life cycles of phytoplankton, anchovies, tunas, and shrimps are related to upwelling. Annual gross production rate in the Gulf of Panama ranges from 255 to 280 g of C m^{-2} (Forsberg 1969), and net primary production is 180 g of C m^{-2}, of which 90 is fixed during upwelling (Smayda 1966).

The major development of Panamanian fisheries during late 1985 and 1986 was an increase in scallop catches. Shipments for 1985 totaled 41.0 t and for the first 6 months of 1986 were 1,700 t, valued at $10 million (Marine Fisheries Review 1987). Depletion of the scallop population in the Bay of Panama after 1986 was probably due to a combination of high temperatures, overfishing, and predation.

Reproductive Biology in Bivalves

Reproduction requires energy that is partitioned between several physiological processes. The literature indicates the process by which a bivalve can obtain energy either directly from food or from storage substrates in organs and tissues, such as the digestive gland (Taylor and Venn 1979), the adductor muscle (Ansell 1974, Epp et al. 1988), or the mantle (Barber and Blake 1981, Lowe et al. 1982).

Reproductive cycles in tropical bivalves have been studied either by using histological techniques by such authors as Vélez (1976) in *C. rhizophorae* and Joseph and Madhyastha (1984) in *C. madrasensis* or by using gonadal weights in *Argopecten purpuratus* by Wolff (1988). This points to the need for a comprehensive study over time of the reproductive cycle and the composition of organs and tissues of tropical bivalves relative to major environmental factors.

Objectives

The objectives of this study were, first, to determine in controlled experiments in the laboratory the effect of environmental factors on reproduction in *A. ventricosus*, and second, under controlled experiments in the laboratory, to relate seasonal changes in the gametogenic cycle of *A. ventricosus* to relative changes in the digestive gland, adductor muscle, mantle-gill, and gonad.

MATERIALS AND METHODS

Reproductive Condition

Morphometric data were obtained from dry weights of digestive gland, adductor muscle, mantle-gill, and gonad. Indexes for each organ were defined as dry weight of body organ/dry weight of whole animal × 100.

Culture

Scallops were collected off of Farallon Beach, Panama Bay, Panama, with a 1 m dredge, in 10 m depth (Fig. 1). Animals were

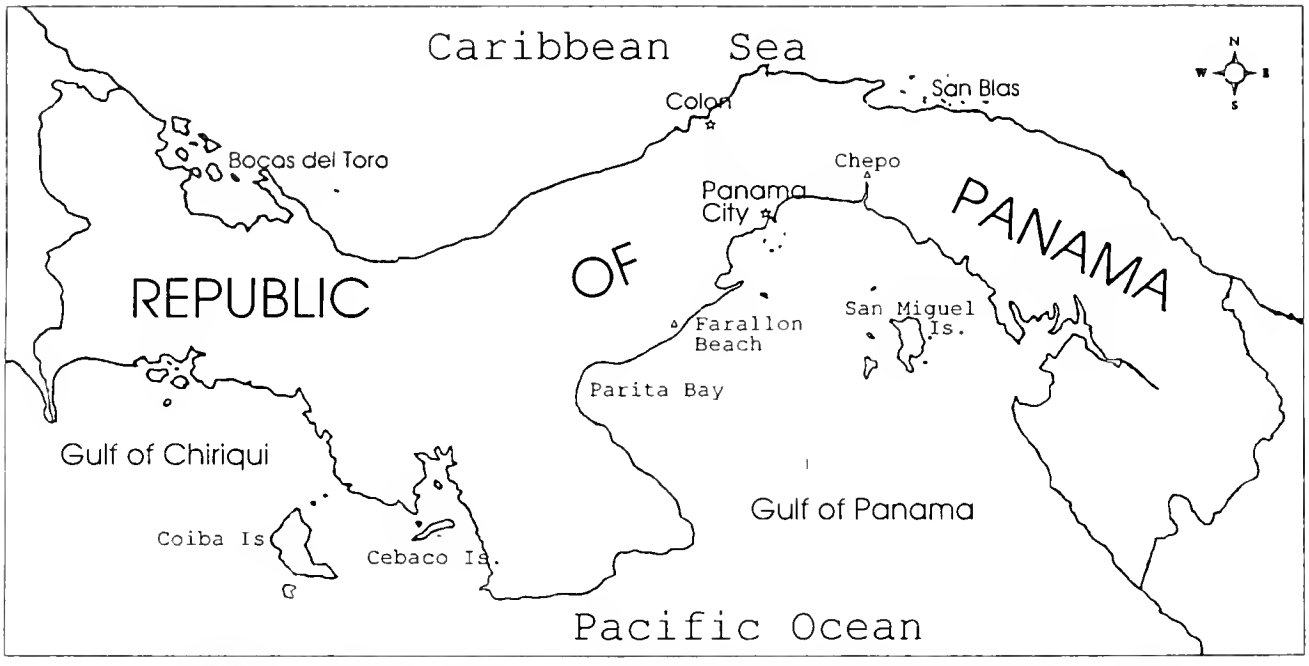

Figure 1. Map of study area in the Isthmus of Panama, including Farallon Beach on the Gulf of Panama. Is, island.

kept in fiberglass tanks in running sea water in the marine laboratory of the University of Panama on Naos Island. Scallops were transported by airplane to the College of Marine Studies in Lewes, Delaware, in September 1988.

In Lewes, 40 scallops were conditioned and held in a 200 l recirculating seawater tank at 19°C and 30‰ salinity. A combined algal diet (50:50) of *Isochrysis* species (C-ISO) and *Chaetoceros calcitrans* was provided daily. In February 1989, 20 scallops were induced to spawn by thermal stimulation (26°C) and frequent water changes. Larvae were reared in a 200 l tank at 24°C, and a combined algal diet was provided daily.

In October 1989, 540 8 month old scallops from the broodstock group spawned in February 1989 were separated into 18 groups (30 scallops each), and their sizes and weights were determined. Initial condition was analyzed for 10 individuals by determining wet and dry weights of the digestive gland, adductor muscle, mantle-gills, and gonad.

Thirty of the 540 scallops were placed in each of 18 aquariums (40 l each) (six treatments × three replicates) (Table 1) containing aerated filtered seawater (seawater was passed through a sand filter and a diatomaceous-earth filter to remove particles ≥5 μm). A combination by cell (50:50) of C-ISO and *Chaetoceros gracilis* was added daily. A high ration (5×10^4 cells ml^{-1} per day) was added to nine aquariums, while a low ration (1.25×10^4 cells ml^{-1} per day) was added to the other nine aquariums; these rations were calculated from data from Panama Bay during the dry and wet seasons, respectively. In addition, as a control, I placed several scallops in the normal densities of phytoplankton observed during the dry and the wet seasons, and the phytoplankton cleared by scallops in each condition was determined. This experiment was performed every other week to determine if sufficient food was being provided to the experimental scallops. Quantities of phytoplankton consumed by experimental scallops were determined daily by direct count with a hemocytometer and weekly with a Coulter counter.

TABLE I.

Experimental design for two rations (high and low phytoplankton densities) and three temperatures (20, 24, and 28°C) in the culture of *A. ventricosus*.

	Phytoplankton density	
Temperature (°C)	High	Low
20	X	X
24	X	X
28	X	X

Six of the 18 aquariums (3 with high and the other 3 with low phytoplankton concentrations) were maintained at 20°C, 6 other aquariums were maintained at 24°C, and the last 6 aquariums were maintained at 28°C (Table 1) (these temperatures were those observed in the field in Panama during the dry, prewet, and wet seasons, respectively). Water temperature was maintained in a circulating bath (Forma Scientific). The pH of the water was recorded daily. Salinity was measured daily with a refractometer.

In each of the 18 aquariums, 10 scallops were sacrificed at 40 and again at 58 days. The condition of each scallop was determined by the dry weight and index of the digestive diverticulum, adductor muscle, mantle-gills, and gonad. Morphometric measurements of the scallops were compared with analysis of variance (ANOVA). To satisfy the assumption of normality, weight data were transformed (ln ×) and index data were transformed (arcsine ×) before ANOVA. Symbols in figures represent means, and vertical bars represent standard errors. Confidence intervals for means are 95%.

RESULTS

pH and salinity are presented in Table 2. In general, pH and salinity were similar to those recorded in the Gulf of Panama.

Morphometric Analysis

Scallops significantly increased in shell height and total weight after 31 days on high phytoplankton densities compared with those

TABLE 2.

Data of environmental factors on aquariums during the experiment.

Parameter	Mean ± SE	Range
pH	7.94 ± 0.26	6.66–8.11
Salinity (‰)	33 ± 1.6	30–37

maintained on low phytoplankton densities (Figs. 2 and 3) ($p < 0.01$).

Dry weights and indices of gonads showed a significant interaction between time, food ration, and temperature (Figs. 4 and 5) (Tables 3 and 4) ($p < 0.01$). The greatest increase in gonadal dry weight was detected in animals exposed to high levels of food at a temperature of 20°C for 58 days. High phytoplankton densities and low temperatures are conditions found in Panama Bay during the upwelling season.

On day 40, at 24°C in low phytoplankton densities, *A. ventricosus* had a higher gonadal dry weight and gonadal index than at 20°C or at 28°C in low phytoplankton densities (Figs. 4 and 5). Gonadal dry weight was higher at high phytoplankton densities than at low phytoplankton densities at the 20 and 28°C treatment. Only on day 40, did scallops have a higher gonadal index at low phytoplankton densities than at high densities, at all temperatures (Fig. 5).

Temperature, as a single factor, used in the experiment seemed to have no effect on dry weights of the digestive diverticulum, adductor muscle, and mantle-gills (Table 3). Rather, temperature seemed to be related with time and food rations. The digestive gland in the scallop declined sharply in dry weight under high and low phytoplankton densities (Fig. 6).

A. ventricosus exposed to high phytoplankton densities increased significantly in shell and total weight (Figs. 2 and 3) (especially dry weights of adductor muscle and mantle-gills) (Figs. 7 and 8). Indices of the digestive diverticulum and adductor muscle were significantly affected by interaction of time, temperature, and food ration (Figs. 9 and 10) (Table 4). Scallops had a higher digestive gland index at low phytoplankton densities than at high densities, at any temperature (Fig. 9). The digestive gland index was higher on day 8 compared with days 40 and 58, at any temperature. The adductor muscle index was higher at a high food ration than at a low food concentration. Scallops at 24°C and high phytoplankton density had the highest adductor muscle index (Fig. 10).

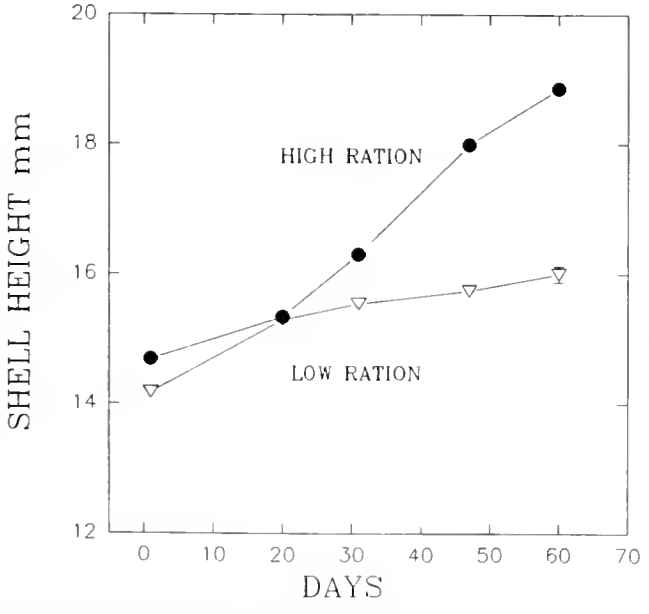

Figure 2. Comparison of shell height of *A. ventricosus* fed two rations during 58 days of experiment.

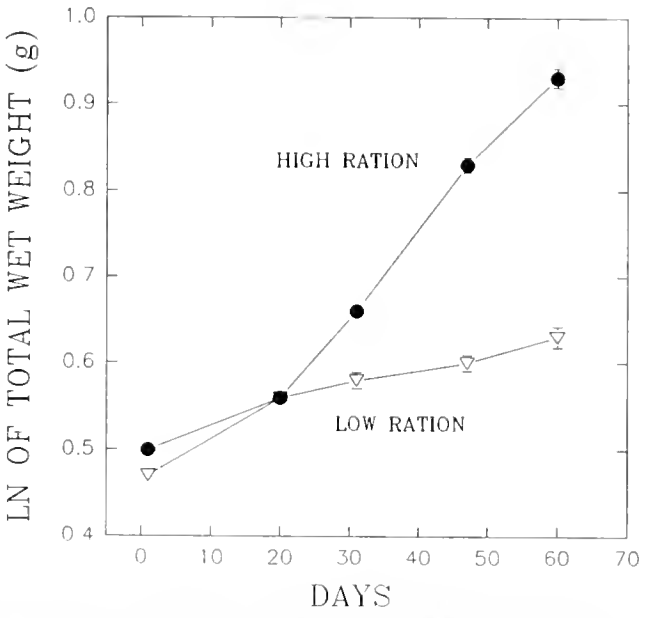

Figure 3. Comparison of natural logarithm (LN) of total wet weight versus two different rations during 58 days of experiment.

DISCUSSION

Temperature

Temperature probably has a greater influence on reproduction in temperate than in tropical bivalves, because thermal fluctuations there are greater. The effect of temperature on metabolism is documented by Sastry (1968) in *Argopecten irradians*, by Keck et al. (1975) in *Mercenaria mercenaria*, by Kennedy and Krantz (1982) in *Crassostrea virginica*, and by MacDonald and Thompson (1986) in *Placopecten magellanicus*.

Less dramatic seasonal changes in temperature have been observed in coastal tropical zones, but they may still affect the reproduction of some bivalves in the tropics. Studies by Carvajal (1969) in *P. perna* and Vélez (1976) in *C. rhizophorae* show a seasonal pattern of gametogenesis related to temperature. This effect of temperature is expected in bivalves in Panama Bay, because pronounced seasonal upwelling occurs in this region, accompanied by changes in water temperature.

In the laboratory study, a relationship between reproductive condition and water temperature per se was not observed in *A. ventricosus*. Temperature, as a single factor, in *A. ventricosus* could be important in the initiation of gametogenesis and as a spawning stimulus. In the laboratory, temperature interacted with food ration and time, promoting changes in scallop gonads. The adaptive response of *A. ventricosus* to a wide range of temperatures could explain the broad distribution of this species, from California to Peru (Keen, 1971).

Food

This experimental study did demonstrate a significant difference between animals at high and those at low phytoplankton

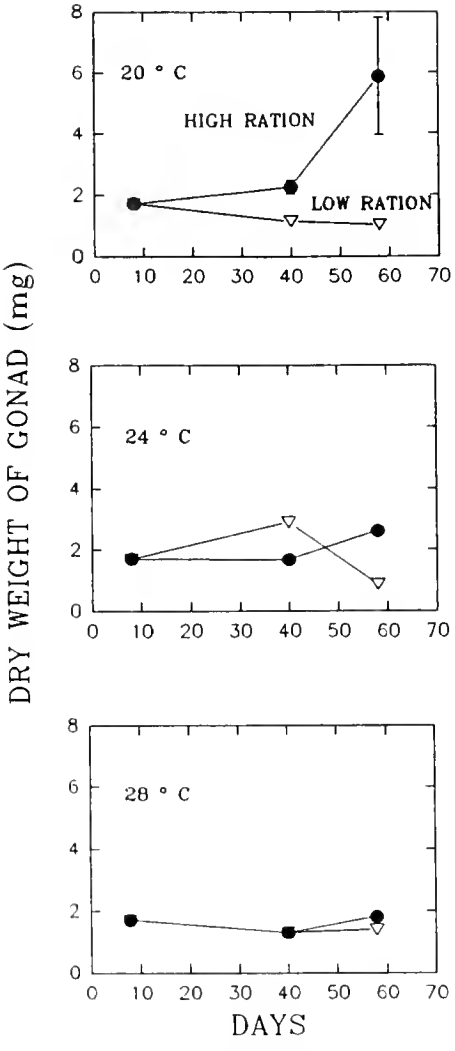

Figure 4. Comparison of natural logarithm of gonadal dry weight versus two rations during 58 days of the experiment at three temperatures.

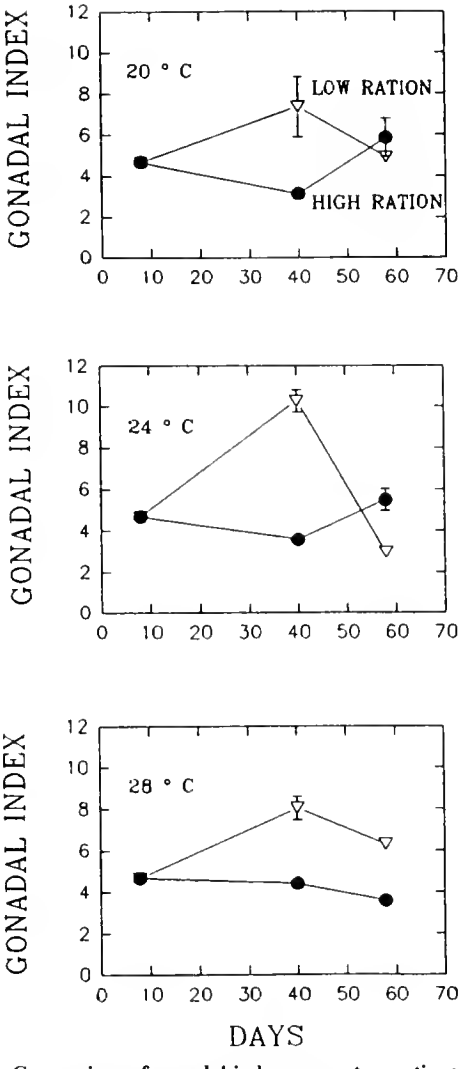

Figure 5. Comparison of gonadal index versus two rations during 58 days of the experiment at three temperatures.

densities. Tropical coastal systems are characterized by some fluctuation in phytoplankton densities, but peaks of abundance are smaller than those observed in temperate and boreal latitudes. However, in the Gulf of Panama, a distinct upwelling brings nutrient-rich waters surfaceward, which leads to increased phytoplankton production. The annual net production in the Gulf of Panama is 180 g of C m^{-2}, of which 90 is fixed during 130 days of upwelling (Smayda 1966). This annual net production is almost double that in Florida (93 g of C m^{-2} per year) (Johansson and Hopkins, 1972) and is close to that in North Carolina (153 g of C m^{-2} per year) (Thayer 1971), and these areas support scallop fisheries. With its annual net productivity, the Gulf of Panama has enough food to support a substantial population of scallops.

In my laboratory study, scallops at different food rations showed significant differences in shell height, total weight, digestive gland weight, adductor muscle weight, mantle-gill weight, and gonadal weight. Scallops increased significantly in total weight after 31 days in high phytoplankton densities. Among these organs, gonadal dry weight increased significantly after 40 days at high food ration, but the gonadal index declined.

The literature reports that, in bivalve reproduction, energy can

TABLE 3.

Study of environmental factors. Results of three-factor ANOVA on days 8, 40, and 58, among three different temperatures and between two food rations for natural logarithm of dry weight of gonad, digestive diverticulum, adductor muscle, and mantle-gills of *A. ventricosus*.

Source of Variation	$p > F$			
	Gonad	Digestive Diverticulum	Adductor Muscle	Mantle-Gills
Time	0.707	<0.001	0.125	0.206
Temperature	0.441	<0.001	0.282	0.276
Food	<0.001	0.011	<0.001	<0.001
Interactions	0.002	<0.001	0.003	0.122
Time × Temperature	0.281	0.093	0.556	0.623
Time × Food	0.001	0.005	<0.001	0.007
Temperature × Food	0.030	0.001	0.183	0.026

TABLE 4.

Study of environmental factors. Results of three-factor ANOVA on days 8, 40, and 58, among three different temperatures and between two food rations for indices of gonad, digestive diverticulum, adductor muscle, and mantle-gills (arcsine transformation) of *A. ventricosus*.

Source of Variation	$p > F$			
	Gonad	Digestive Diverticulum	Adductor Muscle	Mantle-Gills
Time	0.197	<0.001	0.003	<0.001
Temperature	0.670	0.266	0.827	0.026
Food	0.001	<0.001	<0.001	0.178
Interactions	0.003	0.030	0.053	0.090
Time × Temperature	0.319	0.428	0.867	0.306
Time × Food	<0.001	0.002	0.003	0.368
Temperature × Food	0.409	0.693	0.248	0.029

be obtained directly from food in the water or from energy stored in different organs. In scallops, energy for reproduction can come from the digestive gland (Sastry 1970) or adductor muscle (Taylor and Venn 1979, Barber and Blake 1981).

I found that the digestive gland decreased significantly in dry weight, possibly suggesting that this organ was providing energy for reproduction. This agrees with Sastry's findings (1970) in Massachusetts that *A. irradians* had a statistically significant negative correlation between digestive gland index and gonadal index. These findings may also support the idea that *A. ventricosus* is a subtropical species where reproductive strategies are similar to those of scallops living in cooler ecosystems.

Interaction of Food and Temperature

In the experimental laboratory study, *A. ventricosus* exposed to high phytoplankton densities at all temperatures (20, 24, and 28°C) developed only moderate gonadal and digestive gland indexes, while building considerable tissue in the adductor muscle. Also, high phytoplankton densities appeared to inhibit the allocation of energy from the digestive gland to the gonad, but enhanced adductor muscle growth. This would suggest that, during high phytoplankton densities during the dry or the wet season, scallops allocate energy primarily for growth of the adductor muscle.

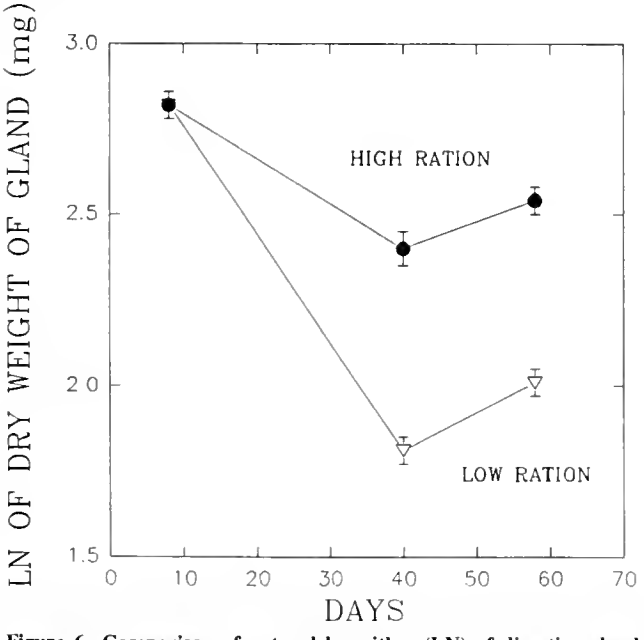

Figure 6. Comparison of natural logarithm (LN) of digestive gland dry weight versus two rations during 58 days of the experiment.

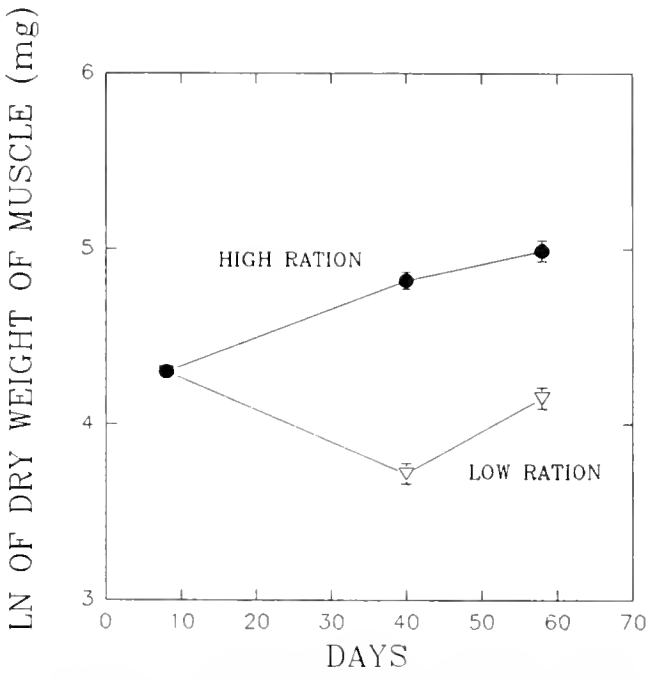

Figure 7. Comparison of natural logarithm (LN) of adductor muscle dry weight versus two rations during 58 days of the experiment. Means of all temperatures.

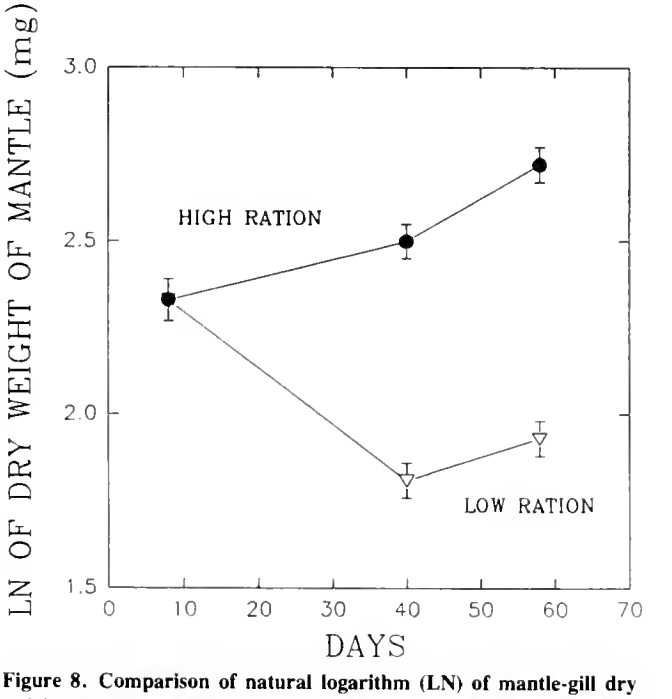

Figure 8. Comparison of natural logarithm (LN) of mantle-gill dry weight versus two rations during 58 days of the experiment. Means of all temperatures.

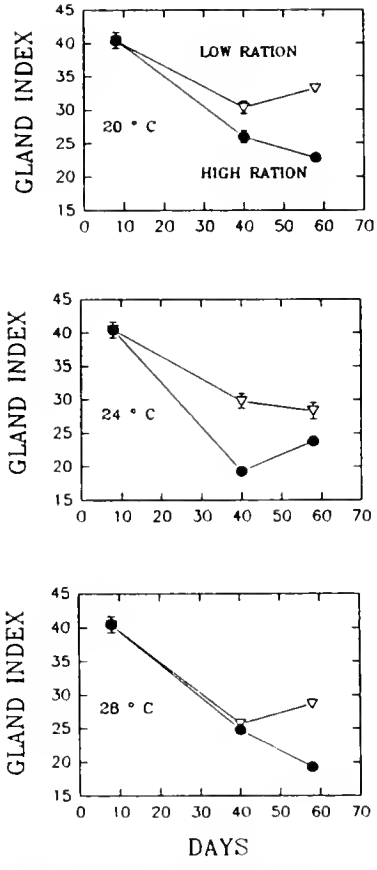

Figure 9. Comparison of digestive gland index and two different rations during 58 days of the experiment at three temperatures. Means of all temperatures.

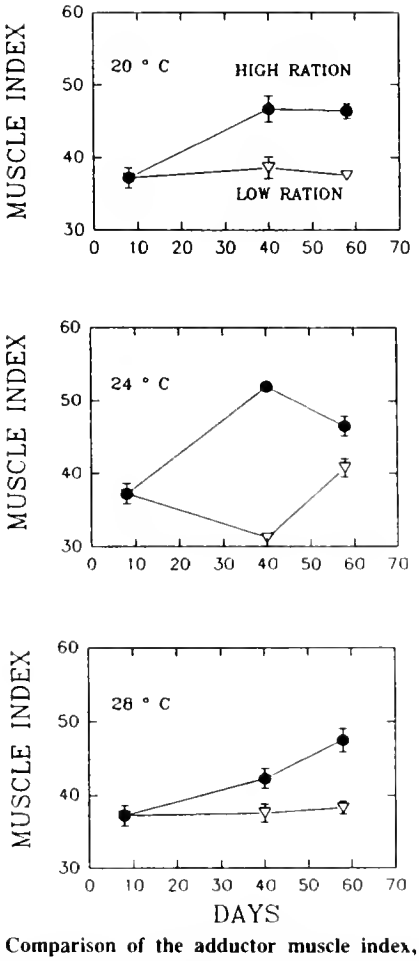

Figure 10. Comparison of the adductor muscle index, two phytoplankton concentrations, and three temperatures during 58 days of the experiment.

The laboratory study showed that scallops exposed to low phytoplankton densities at 24 and 28°C developed a maximal gonadal index. Energy to support gonadal growth was provided mostly by the digestive gland under these conditions. A decline in adductor muscle index was also observed. This suggests that low phytoplankton densities and temperatures higher than 24°C promoted allocation of energy from the digestive gland to the gonad. Thus, maximal gonadal condition could have related to a decrease in adductor muscle growth. In the Gulf of Panama, low phytoplankton densities and temperatures above 24°C occur during the wet season.

Sastry and Blake (1971) observed that the transfer of reserves from the digestive gland to the gonad is regulated by temperature and the stage of gametogenesis. Reserves from the digestive gland are transferred to gonads and adductor muscle, depending on the activity of gonads or the temperature (Sastry and Blake 1971). Gametogenesis in this species is thus developed primarily during the wet season at low phytoplankton densities, and somatic growth (especially adductor muscle) is enhanced during either dry or wet seasons at high phytoplankton densities.

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COMPARISON OF INFECTIVITY AND PATHOGENICITY OF MERONT (TROPHOZOITE) AND PREZOOSPORANGIAE STAGES OF THE OYSTER PATHOGEN *PERKINSUS MARINUS* IN EASTERN OYSTERS, *CRASSOSTREA VIRGINICA* (GMELIN, 1791)

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ABSTRACT Two experiments were conducted to compare the infectivity and pathogenicity of two life stages of the parasite *Perkinsus marinus*, meronts (trophozoites) and prezoosporangia (hypnospores), in eastern oysters, *Crassostrea virginica*. Oysters were inoculated with 5×10^4 meronts or prezoosporangia per oyster by injection into the shell cavity. Prevalence and intensity of *P. marinus* infections, condition index, serum protein concentrations, and lysozyme activities were measured in oysters after 15, 25, 40, and 65 days in experiment 1 and after 20, 40, 50, 65, and 75 days postchallenge by *P. marinus* cells in experiment 2. Controls were injected with filtered York River water. In the first experiment, *P. marinus* infections were initially detected in oysters exposed to prezoosporangia after 15 days postchallenge. In the second experiment, infection was not detected in oysters until 40 days postchallenge with either meronts or prezoosporangia. Intensity and prevalence of *P. marinus* infection were significantly higher ($p < 0.002$) in oysters challenged by meronts compared with prezoosporangia-challenged oysters at the end of both experiments. In experiment 1, a significant decrease ($p < 0.05$) was observed in serum protein in infected oysters challenged by prezoosporangia compared with uninfected oysters. Condition index was higher in uninfected oysters compared with infected oysters challenged by prezoosporangia. The differences in condition index and protein were insignificant between oysters infected by meronts or prezoosporangia. Lysozyme activities were significantly lower ($p < 0.05$) in infected oysters than in uninfected oysters challenged with meronts. No significant differences were observed in condition index, protein concentrations, and lysozyme activities between oysters challenged by meronts and prezoosporangia in experiment 2. Lower condition index and protein concentrations in the groups of oysters infected with prezoosporangia compared with the groups infected by meronts and nonchallenged at the end of experiment 1 suggest a higher energetic demand on these oysters.

KEY WORDS: *Perkinsus marinus*, *Crassostrea virginica*, oyster disease, lysozyme, condition index, protein

INTRODUCTION

The once-thriving oyster industry in the Chesapeake Bay and the East Coast of United States has been threatened by overfishing and diseases caused by two protistan parasites, *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo). The effects of the diseases caused by the two protists have been well documented (Andrews 1988, Barber et al. 1988, Ford 1988, Ford and Figueras 1988, Chu et al. 1993, Chu and La Peyre 1993a and b, Paynter and Burreson 1991). Since 1986, *P. marinus* has reportedly caused greater oyster mortalities in lower Chesapeake Bay than *H. nelsoni* (Andrews 1988).

The life history of *P. marinus* was studied in detail by Perkins (1966). Three life stages were identified, namely, merozoites, prezoosporangia, and the biflagellated zoospores. Immature meronts (merozoites), usually found in the phagosomes of hemocytes are 2 to 4 μm in size and coccoid, with a fibrogranular wall. As the cells mature, they enlarge to about 10 to 20 μm with an eccentrically placed vacuole, which often contains a refringent vacuoplast. The mature meronts, on repeated karyokinesis and cytokinesis, yield sporangia (schizont, 10 to 40 μm in size), an 8 to 32 cell stage enclosed within a mother cell wall (Perkins 1966). Enlargement of meronts to form prezoosporangia is achieved by incubating the meronts in fluid thioglycollate medium (FTM) (Ray 1952). The prezoosporangia are characterized by an extremely large vacuole, which compresses the cytoplasm into a thin layer against the cell wall. On enlargement, the vaculoplast disappears and the nucleus attains a sausage shape, with numerous small lipoid droplets dispersed inside the cell.

Numerous field (Soniat 1985, Craig et al. 1989, Soniat and Gauthier 1989, Crosby and Roberts 1990, Gauthier et al. 1990, Burreson 1989 and 1990) and laboratory studies (Mackin 1951, 1956 and 1962, Andrews and Hewatt 1957, Perkins 1966, Chu and La Peyre 1989, Ragone 1991, Ragone and Burreson 1993) have investigated the effects of temperature and salinity on the disease processes of *P. marinus* in eastern oysters. Other previous laboratory experiments induced *P. marinus* infection through exposure of oysters to meronts, merozoites, and schizonts contained in unpurified or partially purified infected oyster tissue (Chu & La Peyre 1993a, Hewatt & Andrews 1956, Mackin 1962). For convenience, the cellular stages found in oyster tissue will hereafter be termed meronts, with the recognition that merozoites and schizonts are also present.

In nature, the meronts (3 to 15 μm) rarely enlarge to a size of 15 to 100 μm in moribund oysters and when enlarged are called prezoosporangia (Perkins 1966). Prezoosporangia, when placed in seawater, divide by successive bipartitioning and form biflagellated zoospores (Perkins 1966, Chu and Greene 1989). Whereas slightly enlarged cells, believed to be prezoosporangia, can be found in moribund oyster, such cells have never been isolated and induced to form zoospores. The presumption is that they have the capability to zoosporulate. Because the exposure of oysters to minced oyster tissue containing meronts or freshly isolated and partially purified meronts results in a high prevalence of *P. marinus* infection, Perkins (1966) suggested that meronts and merozoites may be the primary infective agents transmitting disease among oysters in the field, with the recognition that zoospores also can induce infections. However, similar infection rates were found by exposing oysters to prezoosporangia and biflagellated zoospores in our laboratory (Chu et al., unpublished results). These

results suggest that all three life stages, namely, meronts, prezoosporangia, and biflagellated zoospores, are capable of inducing infection in oysters, although some of the previous studies have used minced infected oyster tissue (Hewatt and Andrews 1956) or minced infected oyster tissue incubated in thioglycollate medium in 1 day (Mackin 1962). Therefore, the infective cells used in previous studies would mostly be meronts with some prezoosporangia. None of the previous studies have examined purified prezoosporangia as an infective agent, nor were the physiopathological effects investigated. This article reports the results of experiments in which the infectivity and pathogenicity of meronts and prezoosporangia were compared. The physiological responses of oysters challenged by these two infective stages were also determined.

MATERIALS AND METHODS

P. marinus Diagnosis

P. marinus infections were diagnosed using hemolymph and tissue assays (Gauthier and Fisher 1990, Ray 1952 and 1966). The hemolymph assay was as follows: 300 µl of hemolymph containing hemocytes was obtained and incubated in FTM containing antibiotics (penicillin and streptomycin) for 4 days. After incubation, the thioglycollate medium was separated by centrifugation at 800 × *g* and incubated with 1N NaOH for 1 hour to remove tissue debris and hemocytes. The suspension was then washed twice with water, and prezoosporangia were stained with Lugol's iodine and counted. Disease intensity was ranked from 1 to 5 (light to heavy). At the end of each experiment, infections were also diagnosed according to the method of Ray (1952) by incubating pieces of rectal and mantle tissue in FTM. Infection intensities were rated as light to heavy (1 to 5), and weighted indices were calculated based on Ray (1954) and Mackin (1962).

Lysozyme Activity

Lysozyme activity (L) was determined spectrophotometrically according to Shugar (1952) and modified by Chu and La Peyre (1989). Briefly, 0.1 ml of cell-free oyster serum was added to 1.4 ml of bacterial (*Micrococcus lysodiekticus*) suspension. The decrease in absorbance at 450 nm on a Shimadzu UV 600 spectrophotometer was measured after 1 minute. Results are expressed as units per ml of oyster serum. One unit is defined as decrease in absorbance of 0.001 in the bacterial suspension per minute at room temperature (22–23°C).

Serum Protein Concentration

The concentrations of serum protein (P) were measured spectrophotometrically according to Lowry et al. (1951) with bovine albumin as a standard.

Experiments

Two experiments were conducted to compare the pathogenic effects of meronts and prezoosporangia.

Experiment 1

Eastern oysters were collected from the Ross' Rock area of the Rappahannock River, Virginia (ambient salinity, 6 ppt; ambient temperature, 19°C). Oysters from this location have the lowest prevalence of *P. marinus* infection of any oyster bed in Virginia (Ragone Calvo and Burreson 1994). Oysters were gradually acclimated over a period of 6 weeks to the test conditions (temperature, 25.6 ± 1.3°C; salinity, 20.7 ± 1.04) in a 200 l tank. Ninety-six oysters were then randomly placed in aerated individual chambers with flowing 1 µ filtered York River water (YRW). Oysters were fed daily during the acclimation and the experimental period with algal paste (0.1 g per oyster, using a mixture of *Isochrysis galbana, Pavlova lutheri,* and Tahitian *I. galbana*), and water was changed every other day. Meronts were partially purified from infected oyster tissue according to Chu and La Peyre (1993a). Prezoosporangia were cultured on the basis of the method described by Chu and Greene (1989). One hundred microliters of filtered YRW containing 5×10^4 meronts or prezoosporangia cells (meronts cultured in FTM and enlarged to size range of >100 µm) was injected into the shell cavity of each oyster. Controls were injected with 1 µm filtered YRW. There were three treatments: control, meront-challenged, and prezoosporangia-challenged oysters. To monitor infection development, eight oysters were randomly sampled from each treatment at 15, 25, 40, and 65 days postchallenge. Hemolymph samples were withdrawn from the anterior adductor muscle of individual oysters with a syringe with a 27 gauge needle. Serum L and P concentration were measured. Hemolymph was also assayed to evaluated *P. marinus* infection (Gauthier and Fisher 1990). After withdrawal of hemolymph samples, oysters were sacrificed and condition index (CI) (dry meat weight/dry shell weight × 100; Lucas and Beninger 1985) was determined. *P. marinus* infections in oysters were also diagnosed using rectal and mantle tissue according to the tissue assay described by Ray (1952).

Experiment 2

The experimental conditions were similar to those of experiment 1, with the exception that oysters were collected from the Damariscotta River, Maine, a region out of the range of *P. marinus* (ambient salinity and temperature, 32 to 35 ppt and 12 to 14°C, respectively). As in experiment 1, oysters were gradually adjusted to the test conditions (temperature, 21.78 ± 0.84°C; salinity, 20.5 ± 1.19 ppt) in 6 weeks, and then, 135 oysters were randomly placed in individual chambers with 1 µm filtered aerated YRW. Nine oysters from each treatment were sampled at the end of 20, 40, 50, 65, and 75 days after being challenged with infective particles. Measurements of CI and serum L and P were conducted in individual oysters as indicated above.

Statistical Analyses

A one-factor analysis of variance (ANOVA) followed by a Tukey-Kramer test was used to determine the differences in CI, L, and P among treatments. The data were first analyzed for differences among treatments and sampling times. Some of the oysters were not infected after they were challenged with meronts or prezoosporangia. CI, L, and P data from challenged oysters at all sampling times from experiment 1 were split into infected and uninfected oysters. Data from uninfected oysters from each treatment at all sampling times were pooled with the controls. This resulted in three groups, namely, uninfected, meront infected, and prezoosporangia infected. Data were then reanalyzed with one-way ANOVA to determine differences among groups. In experiment 2, CI, L, and P data were analyzed with one-way ANOVA without being split into infected and uninfected groups. Logistic regression (Agresti 1990) was used to determine differences in prevalence of infection between treatments and sampling times in both experiments.

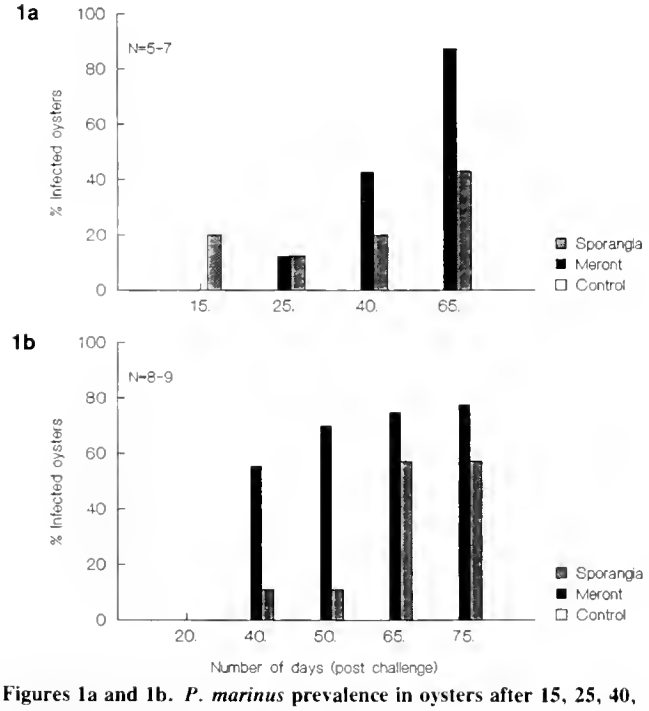
RESULTS

In experiment 1, infection first appeared in oysters 15 days after being challenged with prezoosporangia and 25 days after

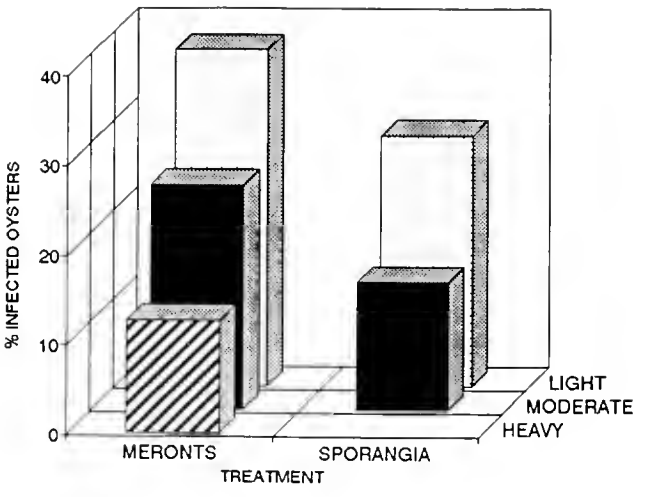
challenge with meronts (Fig. 1a). Prevalence, at 65 days postchallenge, was higher in oysters challenged by meronts (87.5%) compared with oysters challenged by prezoosporangia (43%) (Fig. 1a). Prevalences of both groups significantly increased with time ($p < 0.05$). Prevalence was not significantly different between meront-challenged and prezoosporangia-challenged oysters. Intensities of infections ranged from light to heavy (1 to 5) in meront-challenged oysters, whereas no heavy infections were detected in prezoosporangia-challenged oysters (Fig. 2a). When intensity of infection was expressed as weighted incidence (sum of disease code number/total number of oysters examined), it showed a trend similar to that of prevalence. Weighted incidence (Table 1) at the end of the experiment was higher in oysters challenged with meronts (2.13) compared with oysters challenged with prezoosporangia (0.86).

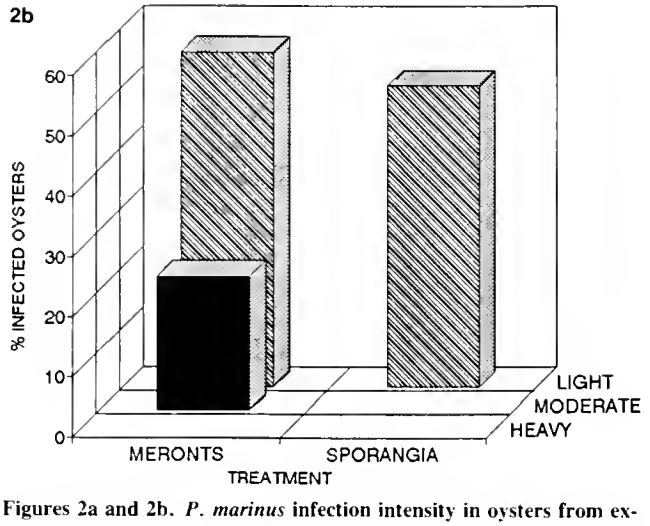
In experiment 2, the first infections appeared after 40 days in both meront- and prezoosporangia-challenged oysters. Prevalence (Fig. 1b) was significantly ($p < 0.002$) higher in meront-challenged oysters (77.5%) compared with prezoosporangia-challenged oysters (57.2%). As in experiment 1, infection in both groups increased with time ($p < 0.0001$). Intensities of infections ranged from light to moderate heavy (1 to 4) in oysters challenged with meronts, whereas only light infections (1) were observed in prezoosporangia-challenged oysters (Fig. 2b). Weighted incidence (Table 1) at the end of the experiment was higher in meront-challenged oysters (0.86) as compared with prezoosporangia-challenged oysters (0.5).

There were no differences in CI, L, and P among treatments at different sampling times in experiment 1 ($p > 0.05$). In experiment 1, within the prezoosporangia-challenged group, CI of infected oysters was lower than that of uninfected oysters (Fig. 3). The CI of infected oysters from the group challenged by meronts was not different from that of infected oysters from the group challenged by prezoosporangia ($p > 0.05$). Serum P concentrations in infected



Figures 1a and 1b. *P. marinus* prevalence in oysters after 15, 25, 40, and 65 days (a) and 20, 40, 50, 65, and 75 days postchallenge (b) by meronts or prezoosporangia.





Figures 2a and 2b. *P. marinus* infection intensity in oysters from experiment 1 (a) after 65 days and experiment 2 (b) after 75 days postchallenge by meronts and prezoosporangia.

oysters challenged with prezoosporangia were significantly lower ($p < 0.05$) than those in the uninfected oysters (Fig. 4). However, no significant difference in P concentrations was observed between infected and uninfected oysters in the group of oysters challenged with meronts. No differences ($p > 0.05$) were observed in serum P concentrations between meront- and prezoosporangia-challenged oysters. Also, no significant difference in P concentrations was observed between infected oysters challenged with meronts or with prezoosporangia. In oysters challenged by meronts, L was significantly higher ($p < 0.05$) in uninfected than in infected oysters (Fig. 5). No such differences were observed between infected and uninfected oysters challenged with prezoosporangia.

In experiment 2, CI and serum P concentrations significantly decreased ($p < 0.05$) in all treatments with time. The CI of oysters at the end of 20 days was significantly higher than the

TABLE 1.

Weighted incidence of *P. marinus* infection and experimental conditions.

Infective Cell	Weighted Incidence		Experimental Conditions[a]	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Meront	2.13	1.33	T = 25.6 ± 1.33°C	T = 21.78 ± 0.84°C
Prezoosporangia	0.86	0.5	S = 20.7 ± 1.04 ppt	S = 20.5 ± 1.19 ppt

[a] T, temperature; S, salinity.

CI of the oysters at the end of 50 and 75 days (Fig. 6). P concentrations in oysters from all treatments decreased with time (Fig. 7). P concentrations at the end of 20, 40, and 50 days were significantly ($p < 0.05$) higher than at the end of 65 and 75 days post-challenge (Fig. 7). No significant differences were observed in L between treatments at any sampling time.

DISCUSSION

Results of this study show that both meronts and prezoosporangia infect oysters, with meronts being more infective than prezoosporangia. This supports the hypothesis (Perkins 1966) that meronts are the primary agents of disease transmission of *P. marinus* in oysters. The higher prevalence of infection in oysters challenged with meronts might have been due to the higher virulence of meronts. The meronts may multiply rapidly in oysters at warm temperatures, such as those (Table 1) used in this study. The cause for the lower infection rate of prezoosporangia is not clear. Although the prezoosporangia injected into the oysters were >95% viable at the time of infection, viability may drop after injection into the oyster tissue, resulting in lower infections. The prezoosporangia used in this study have been cultured in FTM, which may have affected their infectivity. In the field, the infectivity of prezoosporangia could be different. Oysters challenged with cells from pure cultures of *P. marinus* (meronts, merozoites, and schizonts) did not exhibit as heavy infections as those obtained with meronts in homogenized oyster tissue (Volety and Chu, unpublished results, Bushek et al. 1993). Culture of *P. marinus* in artificial media may reduce virulence of the cell stages.

Division of prezoosporangia into meront-like structures by schizogony has been observed in culture (La Peyre 1993; Perkins, personal communication). Although sporangia divide and release biflagellated zoospores in seawater (Perkins 1976, Chu and Greene 1989), the production of zoospores by meronts or prezoosporangia in oyster tissue or in cells isolated from oyster tissue without FTM treatment has not been documented. Indeed, the production of biflagellated zoospores and their subsequent release into seawater may not take place in oyster tissue. Furthermore, the fate of inoculated prezoosporangia in oyster tissue is not known. The lower prevalence in oysters challenged with prezoosporangia may be the result of a long lag time in the division of sporangia into meronts and/or the high mortality rate of cells induced to form prezoosporangia.

Dittman (1993) reported insignificant differences in CI between lightly infected and uninfected oysters. However, in the same study, significantly lower CI values were observed in heavily

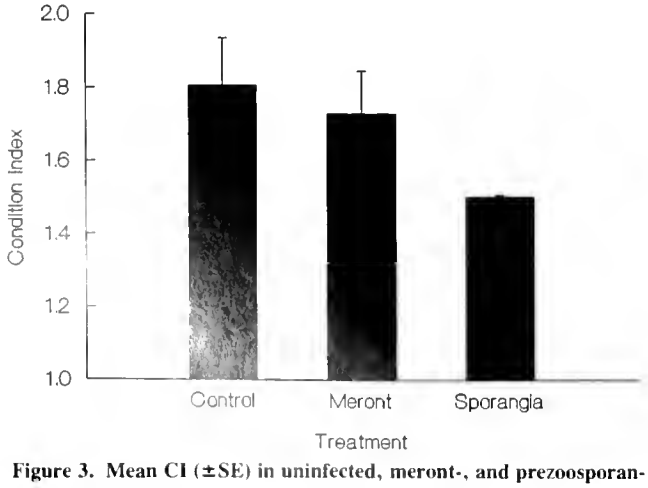
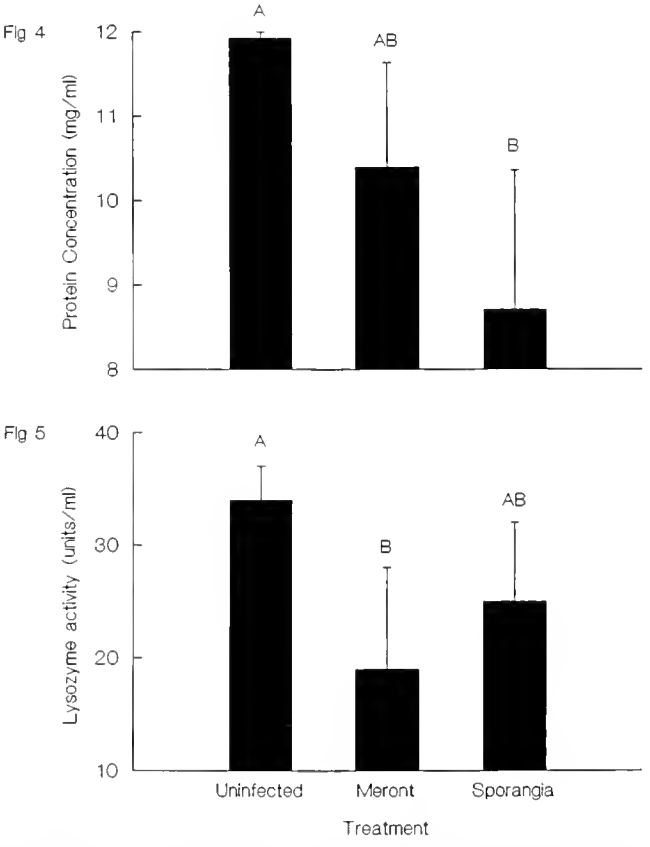
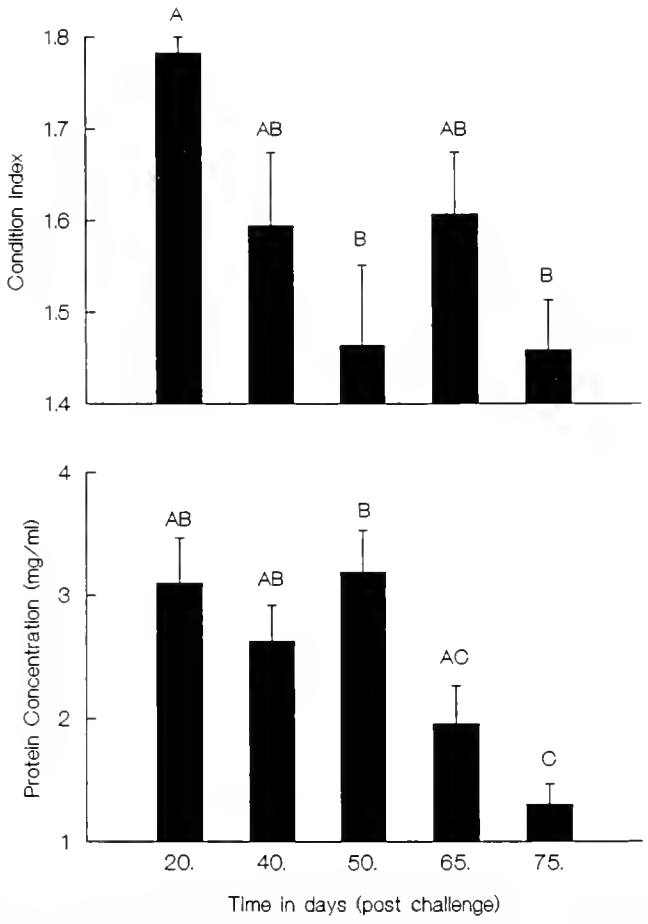


Figure 3. Mean CI (±SE) in uninfected, meront-, and prezoosporangia-challenged oysters.



Figures 4 and 5. Mean serum P concentration (±SE) (Fig. 4) and mean serum L (±SE) (Fig. 5) in uninfected and infected oysters challenged by meront and prezoosporangia. Bars with similar letters are not significantly different ($p > 0.05$).



Figures 6 and 7. Mean CI (±SE) (Fig. 6) and serum P concentration (±SE) (Fig. 7) in oysters at the end of 20, 40, 50, 65, and 75 days postchallenge. Bars with similar letters are not significantly different ($p > 0.05$).

infected oysters compared with uninfected ones. Lower CI in infected oysters challenged by prezoosporangia compared with uninfected oysters in experiment 1, although not statistically significant (Fig. 3), may be because only a few of the oysters were heavily infected. The decrease in CI of oysters with time in experiment 2 may be due to the stress in the confined environment.

The results from experiment 1 indicated that infected oysters challenged by prezoosporangia had significantly lower P concentrations than did uninfected oysters. Lower tissue and hemolymph protein have been observed in oysters heavily infected by *H. nelsoni* (Ford 1986a and b, Barber et al. 1988, Ling 1990). However, no significant differences in P concentrations were observed in oysters lightly infected by *P. marinus* as compared with uninfected oysters (Chu and La Peyre 1993a).

Lysosomal enzymes are believed to play a role in defense in both vertebrates and invertebrates (Ingram 1980, Jolles and Jolles 1984), including molluscs (McDade and Tripp 1967a and b, Cheng 1981 and 1983, Huffman and Tripp 1982, Moore and Gelder 1985, Chu 1988). L in oysters was observed to be negatively correlated with *P. marinus* infection and temperature (Chu and La Peyre 1993a). L of uninfected oysters in experiment 1 had significantly higher activities than infected oysters challenged with meronts (Fig. 5). Lysozyme is hypothesized to be an important enzyme in resistance to *P. marinus* infection (Chu et al. 1993). The absence of *P. marinus* infection in some of the oysters may have been as a result of higher serum L, which may explain the significantly higher L in uninfected oysters. However, no difference in L was observed between meront-challenged and prezoosporangia-challenged oysters.

The higher prevalence, intensity, and weighted indices of *P. marinus* infections in experiment 1 compared with experiment 2 may be due to the higher temperature in the former experiment (Table 1). Temperature is one of the two most important factors (the other being salinity) influencing the geographic distribution of *P. marinus* in oysters. Chu and La Peyre (1993a) reported that prevalence and intensity of *P. marinus* infection increased with increasing temperature. In their study, the prevalence of *P. marinus* in oysters was 23, 46, 91, and 100% at 10, 15, 20, and 25°C respectively. *P. marinus* infection is positively correlated with temperature in the field (Soniat 1985, Craig et al. 1989, Soniat and Gauthier 1989, Crosby and Roberts 1990, Gauthier et al. 1990). The batches of *P. marinus* meronts used for challenging the oysters in the two experiments were isolated from different infected oysters. Their relative infectivity and virulence could differ, contributing to the different infection rates. The difference in the source of oysters may also have been one of the factors for the lower incidence of *P. marinus* infection. Differences in the susceptibility of oysters from different populations to *P. marinus* infection have been reported (Chu and La Peyre 1993b, La Peyre 1993). Their studies have shown differences in the prevalence of *P. marinus* infection in oysters from three locations in the Chesapeake Bay and between Chesapeake Bay and Gulf oysters. Hab-

itat and genetic dissimilarities were suggested as the reasons for the differences in prevalence of infection.

Because only light infections were detected in experiment 2 in both oysters challenged with meronts and prezoosporangia, the insignificant differences noted in CI, L, and P between different treatments were not surprising. These results agree with the findings by Dittman (1993) and Chu and La Peyre (1993a). Neither found differences in CI, L, and P concentrations between lightly infected and uninfected oysters.

In summary, meronts are more infective than prezoosporangia and are possibly the principal agents of disease transmission in the field. The lower CI and P values in the treatment of infected oysters challenged with prezoosporangia, compared with uninfected and meront-challenged oysters, suggest that prezoosporangia may be exerting a higher energetic demand on the host than do meronts. Further studies are needed to examine the causes for the lower P concentrations in prezoosporangia-challenged oysters.

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MORPHOLOGICAL AND GENETIC VARIATION AMONG THREE POPULATIONS OF CALICO SCALLOPS, *ARGOPECTEN GIBBUS*

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ABSTRACT We surveyed morphological and genetic variation among three populations of *Argopecten gibbus* from the Marquesas Keys, Florida; Cape Canaveral, Florida; and Cape Lookout, North Carolina; in order to determine the extent of the genetic isolation of these populations and to examine the hypothesis for larval transport between populations. Burnaby size-adjusted principal component analysis of 14 morphological characters revealed significant differences among sites for the third principle component, which explained 6% of the total variation. Analyses of electrophoretic loci showed significant allele frequency heterogeneity among sites for one of seven polymorphic loci. The magnitude of the morphological and genetic differences between the Marquesas Keys sample and both of the Atlantic coast samples was generally greater than between the geographically more distant Cape Canaveral and Cape Lookout samples, although overall levels of variation among sites were small. Estimates of gene flow suggest that relatively frequent migration, sufficient for panmixia in the absence of historical divergence, may occur between populations. Our results suggest that oceanographic processes play a critical role in the transport of *A. gibbus* larvae between populations. We recommend that management of the *A. gibbus* fishery should include consideration of these processes.

KEY WORDS: *Argopecten*, calico scallop, population structure

INTRODUCTION

The calico scallop, *Argopecten gibbus* (Linnaeus), occurs in open coastal waters throughout the Gulf of Mexico and off the western Atlantic Coast from Florida to Cape Hatteras, North Carolina, in depths of 1 to 370 m (Waller 1969, Blake and Moyer 1991). During the 1940s and 1950s, large "beds" of calico scallops were discovered near Cape San Blas and Cape Canaveral, Florida, and Cape Lookout, North Carolina (Allen and Costello 1972). Those beds have been commercially exploited on a more or less continuous basis since the late 1950s and remain the most productive harvest areas. Since its inception, the commercial fishery has followed a trend of increasing landings, but as is typical of many short-lived scallop species, there has been considerable variability in landings among years. For example, annual landings from the Cape Canaveral calico scallop beds have ranged between 39,000 and 11,000,000 pounds of meats over the last 5 years (Blake and Moyer 1991). The Cape San Blas and Cape Lookout beds are even more transient, typically producing commercial quantities every 2 to 4 years.

Management of the calico scallop fishery has proved difficult because of the extreme year-to-year variability in landings and because the extent to which populations are self-sustaining or are dependent on allochthonous larval recruitment is unknown. Although the species' range is broad, dense concentrations of calico scallops are primarily associated with coastal prominences, including the capes mentioned above and, to a lesser extent, the Florida Keys (Cummins 1971, Allen and Costello 1972). This discontinuous distribution suggests that oceanographic processes may play an important role in structuring calico scallop populations. Capes and headlands, such as those found near large beds of scallops, are capable of generating downstream eddies, which can cause aggregations of zooplankton (Alldredge and Hamner 1980, Wolanski and Hamner 1988). High local abundances of other scallop species, including *Patinopecten yessoensis*, *Amusium bolloti*, *Chlamys tehuelcha*, and *Chlamys patagonica*, have been attributed to larval retention in the presence of such eddies (Yamamoto 1964, Heald and Caputi 1981, Orensanz et al. 1991a). Eddying in the vicinity of Cape Canaveral has also been suggested to enhance the retention of calico scallop larvae until settlement (Bullis and Cummins 1961, Allen 1979). North Carolina stocks of calico scallops, in particular, may be strongly influenced by oceanographic processes that affect larval transport. Kirby-Smith (1970) proposed that a considerable proportion of the North Carolina stock may result from larvae originating from the Cape Canaveral, Florida, beds, carried by the Gulf Stream, and retained by eddying in coastal bays. Similar transport and retention mechanisms may be responsible for the concentrations of calico scallops that occasionally occur off of the Georgia coast near Savannah (Anderson and Lacey 1979). Although the 14 to 16 day planktonic period estimated for *A. gibbus* larvae (Costello et al. 1973) provides the potential for considerable transport by offshore currents, the hypothesis of such larval transport has not been tested.

Extreme annual variation in commercial landings of *A. gibbus* supports the assertion that oceanographic processes are important

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in controlling the distribution and abundance of calico scallop populations. Orensanz et al. (1991b) observed that many scallop species have irregular fluctuations in abundance when recruitment is strongly dependent on hydrographic conditions. If processes such as larval retention by eddies or transport by currents are important for calico scallop recruitment, successful recruitment will depend on the concurrence of such events with spawning. The relatively short lifespan of *A. gibbus* (18 to 24 months, Roe et al. 1971) makes this species particularly vulnerable to fluctuations in recruitment success, which may also contribute to interannual variability in stock abundance (Orensanz et al. 1991b). Failure of a single recruitment event can thus potentially cause the local extinction of scallops. North Carolina calico scallops spawn once a year, from January to April (Singhas 1992), whereas Florida populations of calico scallops have two spawning periods: the main spawn occurs in late spring when the majority of spawning scallops are small, which is followed by a smaller event in the fall when most spawning scallops are large (Blake and Moyer 1991). In 1984, the failure of the fall recruitment in the Cape Canaveral population apparently resulted in the collapse of the fishery in 1985, because the spring 1985 spawning stock was limited to the few large scallops that survived the winter (Moyer and Blake 1986). The extent to which larval recruitment from other populations mitigates such local population crashes and local interannual variability in abundance is unknown.

The relative importance of biological and oceanographic factors in structuring scallop populations, particularly calico scallops, remains largely undetermined. Quantification of the degree of genetic and morphological variation along the scallop's range may provide important clues concerning the extent of larval transport and gene flow between stocks. Waller (1969) characterized the morphology of fossil and extant calico scallops from the entire geographic range of the species. He found no evidence for extensive geographic variation; however, samples from the Florida Keys were slightly distinct morphologically from scallops in other locations in Florida and North Carolina. With the exception of that study, there are no descriptions of morphological, physiological, or genetic variation among *A. gibbus* populations. We present here a preliminary attempt to describe the degree of morphological and genetic variation among three populations of calico scallops sampled from the Gulf of Mexico and Atlantic Coasts of North America. The purposes of this study were to define the extent of isolation among stocks from the eastern and western Florida coasts and from North Carolina and to examine the hypothesis that extensive larval transport occurs between these stocks.

METHODS

Collections

Calico scallops were collected from the vicinity of Marquesas Keys, Florida, in the Gulf of Mexico (24°43′N, 82°10′W) on July 7, 1990; from the vicinity of Cape Canaveral, Florida (28°20′N, 80°10′W), on January 13, 1990, and February 15, 1990; and from the vicinity of Cape Lookout, North Carolina (34°35′N, 76°35′W), on February 2, 1990. In all cases, scallops were collected with modified otter trawls in depths of 20 to 40 m. Samples were either dissected immediately after capture or were returned alive to the laboratory for dissection. Subsequent to dissection, tissue samples were held in a freezer at −70°C. Frozen samples of adductor muscle and digestive gland were lyophilized for storage before electrophoretic analyses.

Electrophoretic Analysis

Samples were prepared for electrophoresis by homogenization in 0.1 M Tris-HCl buffer, pH 8.0, with 20% glycerol w/v and centrifuged for 10 minutes at 5,000 × *g* at 4°C. Electrophoresis of the supernatant was performed on 280 mm horizontal starch gels. Four buffer systems were used to resolve the enzymes. The 11 enzymes studied and the buffer and staining systems used to detect them are shown in Table 1. Two isozymes were present for MDH, AAT, ARK, and IDH, although we were only able to include the most cathodal isozyme in our analyses from AAT, ARK, and IDH because of inconsistent resolution. Alleles were designated according to approximate relative mobility on the gel to that of the most common allele, which was arbitrarily assigned a value of 100.

Statistical analyses of genetic data were performed with programs from the Statistical Analysis System software (SAS Institute, Inc. 1982). For polymorphic loci (frequency of the common allele ≤ 0.95), we compared allele frequencies between samples and genotype frequencies with Hardy-Weinberg equilibrium expectations by pooling rare alleles with the electrophoretically closest common allele to obtain allelic class frequencies with $N \geq 5$. Observed genotypic frequencies were compared with those ex-

TABLE 1.

List of enzymes assayed, their abbreviations and enzyme number, and the buffer and staining protocol used for their detection in the electrophoretic study of *A. gibbus*.

Enzyme	E.C. Number	Abbreviation	Buffer[a]	Staining Reference
Aspartate aminotransferase	2.6.1.1	AAT	1	Johnson and Utter (1973)
Arginine kinase	2.7.3.3	ARK	1	Scopes (1968)
Isocitrate dehydrogenase	1.1.1.42	IDH	1	Schaal and Anderson (1974)
Glycerol 3-phosphate dehydrogenase	1.1.1.8	GPD	2	Shaw and Prasad (1970)
Pyruvate kinase	2.7.1.40	PK	1	Harris and Hopkinson (1976)
Glucose-6-phosphate isomerase	5.3.1.9	GPI	3	Schaal and Anderson (1974)
Phosphoglucomutase	2.7.5.1	PGM	4	Schaal and Anderson (1974)
Octopine dehydrogenase	1.5.1.11	ODH	3	Dando et al. (1981)
6-Phosphogluconate dehydrogenase	1.1.1.44	6PGD	2	Schaal and Anderson (1974)
Malic dehydrogenase	1.1.1.37	MDH	2	Schaal and Anderson (1974)
Mannose phosphate dehydrogenase	5.3.1.8	MPI	1	Schaal and Anderson (1974)

[a] Buffers were as follows: 1, Tris-citrate pH 7.5 (Rodhouse and Gaffney 1984); 2, Tris-citrate pH 7.0 (Rodhouse and Gaffney 1984); 3, discontinuous lithium hydroxide (Koehn et al. 1976); 4, Tris-maleate pH 7.0 (Koehn et al. 1984).

pected under Hardy-Weinberg equilibrium using the G-test and, when necessary, William's correction for small sample sizes (Sokal and Rohlf 1981). Alpha levels were corrected to avoid type I errors from multiple tests of a single hypothesis by use of the sequential Bonferroni technique (Holm 1979, Rice 1989). Heterozygote deficiencies or excesses were calculated using the D statistic = $(H_o - H_e)/H_e$ (Selander 1970). Allele frequencies among sampling sites were compared using the R × C test of independence and the G statistic (Sokal and Rohlf 1981), with significance levels adjusted by use of the sequential Bonferroni technique (Holm 1979, Rice 1989). Effective numbers of alleles were calculated as $n_e = 1/\Sigma\ p_i^2$, where p_i is the frequency of the ith allele in the population (Hartl and Clark 1989). We determined the fixation index, F_{ST}, for all polymorphic loci between pairs of samples and across all samples using the formula of Wright, with correction for limited sample sizes (Wright 1978). Gene flow was estimated from this measure of population differentiation by the relation $Nm = [(1/F_{ST}) - 1]/4$ (Slatkin 1985a, 1987). The degree of population subdivision was also examined by calculating standard genetic distances between populations using the formulae of both Nei (1978) and Rogers (1972) applied to all polymorphic and monomorphic loci scored.

Morphometric Analysis

Morphometric parameters were selected following the criteria of Waller (1969). Only mensural parameters suitable for use in subsequent statistical treatments were included (Table 2). Measures of the pallial scar and assorted muscle scars were not used because of the difficulty of resolution of those features in many of our shell samples. In all cases, we used measurements collected from the right valve of the specimen. A total of 52, 40, and 52 scallops were morphometrically analyzed from the Marquesas Keys, Cape Canaveral, and Cape Lookout, respectively.

Morphological data were quantitatively compared by the use of Burnaby size-adjusted principal component analysis (Burnaby 1966), which removes the influence of variation in the first eigenvector (principal component 1 [PC1]) of the variance-covariance matrix from each population (Rohlf and Bookstein 1987). This approach was taken to minimize the influence of animal size on subsequent statistical analyses. Although the first principal component only gives the direction of maximum variation within the populations, this will be a size-related component if there is a large difference in size among populations (Rohlf and Bookstein 1987); the remaining principal components are then primarily shape related. We next plotted principal component 2 (PC2) against principal component 3 (PC3) to determine the relative distribution of the dominant shape-related principal components among populations. Finally, for each of the second and third principal components, adjusted shape vector scores (ASVS) were subjected to Model I one-way analysis of variance (ANOVA) to compare mean ASVS among populations (Sokal and Rohlf 1981). Significant differences among means were further tested with the Student-Newman-Keuls (SNK) multiple range test (Sokal and Rohlf 1969). Unless otherwise stated, a type I error rate of 0.05 was used for all statistical tests.

RESULTS

Genetics

On the basis of the examination of the 12 electrophoretic loci, the proportion of polymorphic loci, P, in the three samples of *Argopecten gibbus* was 0.64. Allele frequencies for the seven polymorphic loci and sample sizes for electrophoretic analyses are shown in Table 3. *Ark-1*, *Aat-1*, *Pk*, and *6Pgd* were completely monomorphic, whereas *Idh1* exhibited very low levels of polymorphism (frequency of the common allele >0.95) in all of the sampled populations. The average level of heterozygosity (H) over all samples and loci was 0.103, whereas the effective number of alleles per locus ranged from 1.00 to 4.53 (Table 3).

In general, isozyme allele frequencies agreed with Hardy-Weinberg equilibrium expectations (Table 3). With one exception,

TABLE 2.

Description of parameters used in the morphological analysis of *A. gibbus* (from Waller 1969) and the mean and standard deviation (SD) for each parameter for each population.

		Site[a]					
		MK		CC		CL	
Parameter	Description	Mean	SD	Mean	SD	Mean	SD
AM	Height of disk (linear)	20.4	2.1	44.2	1.9	46.5	1.6
AD	Posterior half-diameter of disk	10.3	1.2	24.1	1.1	26.2	1.1
DG	Anterior half-diameter of disk	10.4	1.2	22.7	1.2	23.1	1.3
AK	Posterior dorsal half-diameter of disk	11.0	1.0	23.2	1.6	24.1	1.6
GP	Anterior dorsal half-diameter of disk	10.1	1.1	21.0	1.5	22.4	1.6
LO	Convexity	6.5	0.8	13.6	0.8	13.0	0.8
EI	Height of anterior auricle	3.0	1.1	7.2	0.5	7.5	0.6
BJ	Height of posterior auricle	5.4	1.3	10.7	0.7	10.3	0.8
PW	Plical width	1.3	0.2	2.8	0.2	3.1	0.2
IW	Interplical width	0.6	0.1	1.3	0.2	1.3	0.1
ad	Height of resilial insertion	1.0	0.1	2.1	0.2	2.3	0.1
ce	Length of resilial insertion	1.3	0.2	2.6	0.3	2.9	0.3
DF	Length of anterior outer ligament	5.8	2.3	14.3	1.3	13.8	1.4
CD	Length of posterior outer ligament	5.8	1.8	13.8	0.9	13.9	1.4
DE	Distance between two lines perpendicular to outer ligament, one passing through origin of growth and the other passing through anterior auricular notch	4.6	1.7	12.4	0.9	13.1	1.2

[a] MK, Marquesas Keys, Florida; CC, Cape Canaveral, Florida; and CL, Cape Lookout, North Carolina.

TABLE 3.

***A. gibbus* allele frequencies (f), heterozygosities (H), and effective numbers of alleles (n_e) at seven polymorphic enzyme loci from three sampling sites and G-tests (G) for fit to Hardy-Weinberg equilibrium expectations. For the G-tests, rare alleles were pooled with the electrophoretically closest common allele so that f ≥ 5. N = sample size.**

		Site					
		Marquesas Keys		Cape Canaveral		Cape Lookout	
Locus	Allele	f	G	f	G	f	G
6Pgd	106	0.025	0.601	0.059	7.204[a]	0.011	13.716[b]
	104	0.275		0.118		0.109	
	100	0.625		0.794		0.826	
	96	0.050		0.029		0.054	
	rare	0.025		0.000		0.000	
	H	0.275		0.118		0.073	
	n_e	2.13		1.54		1.43	
Mdh1	110	0.400	0.623	0.044	1.612	0.000	0.028
	104	0.075		0.088		0.042	
	100	0.150		0.647		0.583	
	90	0.050		0.044		0.135	
	80	0.325		0.162		0.229	
	rare	0.000		0.015		0.011	
	H	0.400		0.206		0.240	
	n_e	3.38		2.19		2.43	
Mdh2	104	0.125	—[c]	0.132	2.285	0.135	1.546
	100	0.850		0.794		0.813	
	94	0.000		0.059		0.031	
	rare	0.025		0.015		0.021	
	H	0.050		0.206		0.240	
	n_e	1.35		1.53		1.47	
Pgm	102	0.075	0.032	0.044	0.111	0.052	0.397
	100	0.625		0.382		0.458	
	96	0.175		0.221		0.219	
	95	0.075		0.118		0.125	
	92	0.025		0.088		0.021	
	90	0.000		0.029		0.052	
	88	0.000		0.059		0.031	
	rare	0.025		0.059		0.042	
	H	0.225		0.294		0.365	
	n_e	2.31		4.53		3.56	
Odh	106	0.000	0.055	0.073	0.031	0.021	0.145
	100	0.950		0.853		0.865	
	94	0.025		0.029		0.042	
	rare	0.025		0.045		0.072	
	H	0.050		0.147		0.146	
	n_e	1.11		1.36		1.33	
Mpi	100	0.650	0.292	0.368	0.089	0.531	0.709
	96	0.350		0.618		0.458	
	rare	0.000		0.014		0.011	
	H	0.200		0.235		0.229	
	n_e	1.83		1.93		2.03	
Gpi	100	0.950	0.055	0.971	0.031	0.948	0.145
	rare	0.050		0.029		0.052	
	H	0.050		0.029		0.052	
	n_e	1.11		1.06		1.11	
N		40		68		96	
H		0.104		0.103		0.106	

[a] $p < 0.05$ after adjustment with the sequential Bonferroni technique.
[b] $p < 0.01$ after adjustment with the sequential Bonferroni technique.
[c] Insufficient data for analysis.

there was no tendency for loci to exhibit significant, consistent heterozygote deficiencies or excesses. The *6Pgd* locus, however, showed significant heterozygote deficiency in both the Cape Canaveral and Cape Lookout populations (D = 0.460 and 0.546, respectively).

Allele frequency comparisons revealed relatively little genetic differentiation among the sampled populations of calico scallops (Table 4). Of seven polymorphic loci, only *Mdh1* differed significantly among all three populations. The observed differences were generally greater between Cape Canaveral and Marquesas Keys scallops, which differed significantly in allele frequencies for both *Mpi* and *Mdh1*, than between Cape Canaveral and Cape Lookout populations, which showed statistically homogeneous allele frequencies for all loci. These relationships among sites were confirmed by the genetic distances between the samples calculated using all scored loci (Table 5). In order to further assess the degree of genetic differentiation among populations, F_{ST} values determined for each locus including *Idh1* were used to derive a rough estimate of gene flow, Nm, which may be interpreted as the number of diploid individuals exchanged among populations per generation. Values of Nm varied considerably among loci, ranging from 1.7 to 1935.0 (Table 6), with an estimate across loci and populations of 5.5. The locus that had significant allele frequency differences among populations, *Mdh1*, contributed disproportionately to the overall F_{ST} estimate. Exclusion of *Mdh1* from the calculations of F_{ST} and Nm resulted in a twofold higher overall estimate of gene flow of 12.25. Pairwise F_{ST} calculations suggested that gene flow was, in general, much greater between the Cape Lookout and Cape Canaveral populations than between the Marquesas Keys and either of the Atlantic Coast samples (Table 6).

Morphology

Fifteen mensural characteristics were deemed suitable for inclusion in the morphometric analysis of calico scallop shells (Table 2), including measures of disc shape (AM, AD, DG, AK, GP, and LO), auricular shape (EI and BJ), plical shape (PW and IW),

TABLE 4.

G-test statistic comparisons of *A. gibbus* allele frequencies at seven polymorphic enzyme loci from three sampling sites: Marquesas Keys (MK), Cape Canaveral (CC), and Cape Lookout (CL). For frequency comparisons, rare alleles were pooled with the electrophoretically closest common allele so that f ≥ 5.

	MK × CC × CL		CC × CL		CC × MK		CL × MK	
Locus	G	df[a]	G	df	G	df	G	df
6Pgd	7.480	4	1.499	2	3.757	2	6.475	2
Mdh1	52.321[b]	6	7.958	3	28.931[b]	2	45.227[b]	3
Mdh2	1.337	4	0.315	2	1.313	2	0.595	2
Pgm	15.95	10	5.641	4	8.714	2	7.819	4
Odh	3.395	4	0.481	2	2.744	2	2.410	2
Mpi	7.812	2	3.565	1	7.308[c]	1	1.642	1
Gpi	1.911	2	1.767	1	1.112	2	0.003	1

[a] Degrees of freedom.
[b] $p < 0.01$ after correction of α levels with the sequential Bonferroni technique.
[c] $p < 0.05$ after correction of α levels with the sequential Bonferroni technique.

TABLE 5.

Nei's (1978) genetic distance (upper diagonal) and Rogers (1972) genetic distance (lower diagonal) between the three populations of calico scallops, calculated from electrophoretic data.

	North Carolina	Cape Canaveral	Marquesas Keys
North Carolina		0.009	0.050
Cape Canaveral	0.061		0.070
Marquesas Keys	0.105	0.136	

dimensions of resilial insertion (ad and ce), and ligament shape (DF, CD, and DE). All characters were normally distributed.

The mean disc height (AM) of the three populations was significantly different (type I ANOVA, $p < 0.001$). The Marquesas Keys specimens were generally less than half the size of the Cape Canaveral and Cape Lookout populations, and the mean disc height of the Cape Canaveral population was 2.3 mm less than that of the Cape Lookout population (Table 2). Mean disc height of each population was significantly different from that of the other two populations (SNK, $\alpha = 0.05$). The observed difference in overall size necessitated the application of the size-adjusted Burnaby analysis, which legitimized the interpretation of PC2 and PC3 as the primary shape vectors.

Of the three principal components considered in this analysis, PC1 contributed 39.0%, PC2 contributed 21.3%, and PC3 contributed 6.9% to the total variance of the model. Within PC2, the model was dominated by the influence of the auricular shape variable BJ, which contributed 81.2% of the observed variance. The ligament shape variable DE contributed 7.2% to the model, and each of the other variables contributed less than 2.5% to the remaining variance. Within PC3, the model was influenced by the ligament shape variables DF (40.1% of total variance) and CD (23.3% of total variance). The variables GP (9.5%), AD (6.3%), PW (4.0%), AK (4.0%), LO (2.9%), and IW (2.5%) all contributed at least 2.5% to the total variance.

Results of the Burnaby size-adjusted principal component analysis provided no clear discrimination among the Cape Canaveral, Cape Lookout, and Marquesas Keys populations, particularly along the PC2 axis, although the spread of the Marquesas Keys population along the PC2 axis was considerably greater than the spread for the Cape Canaveral and Cape Lookout populations (Fig. 1). There was a significant difference ($p = 0.0001$) among mean ASVS along the PC3 axis; SNK test results indicate that the mean ASVS of each of the three populations was significantly different from that of the other two populations. The Marquesas Keys population showed the largest spread along the PC3 axis and the least overlap with the other two populations. However, mean ASVS for Cape Canaveral and Cape Lookout were also significantly different from one another, although considerable overlap among ASVS for the Cape Canaveral and Cape Lookout populations was observed.

DISCUSSION

The study reveals relatively low overall levels of morphological and genetic variation among three geographically distant *A. gibbus* populations, although significant heterogeneity was present among samples for a small proportion of the characters examined. In general, the magnitude of differentiation was greater in comparisons of the Marquesas Keys population with both the Cape Canaveral and Cape Lookout populations than in comparisons between these two East Coast samples. These data appear to indicate that the Florida peninsula serves as a partial barrier to gene flow between the Gulf Coast and the Atlantic Coast *A. gibbus* populations, although relatively frequent migration may occur between all populations.

The samples of calico scallops measured showed only slight morphological differentiation, with the exception of the small size of the Marquesas Keys scallops; however, the Marquesas scallops may have been juveniles. This agrees with earlier observations by Waller (1969) that calico scallops generally lack the geographic morphological variation exhibited by other pectinid species such as *Argopecten irradians* and *Placopecten magellanicus*. After corrections for size differences, *A. gibbus* populations were only distinguished by one of two shape-related principal components (PC3), and this difference was greatest between the Marquesas Keys and Atlantic Coast samples. Because this component contributed only 6% to the total morphological variance, these results suggest that *A. gibbus* populations are similar in their overall morphological composition, although there are subtle differences among populations, particularly between the Gulf and the Atlantic Coasts, within a small suite of morphological characters. The specific characters that differed among populations were principally measures of ligament shape, which often exhibit geographical variation in members of the genus *Argopecten* (Waller 1969). Waller (1969) suggested that such variation may represent local environmental influences on morphology, but this has yet to be confirmed.

In agreement with our results based on shell morphology, genetic differences among the three *A. gibbus* populations were small, although the relative magnitude of differentiation was greater between the Marquesas Keys population and Cape Canaveral population than between the Cape Canaveral and Cape Lookout populations, which are more geographically separated. Genetic distances between *A. gibbus* populations fall within the ranges of those calculated for several other scallop species (Kijima et al. 1984, Beaumont and Zouros 1991), which typically reveal moderate levels of genetic differentiation but no evidence for extensive population isolation. Concordantly, although significant allele frequency heterogeneity was observed among all samples for *Mdh1*, and additionally for *Mpi* between the Marquesas Keys and Cape Canaveral samples, our estimates of gene flow indicate that relatively frequent migration occurs, or has historically occurred, among all of the populations. In an island model of equilibrium population structure, frequencies of neutral alleles will not diverge among populations when $Nm \gg 1$, where N is the effective population size, and m is the migration rate among populations (Slatkin 1985b). Our overall and pairwise values of Nm show that migration around the Florida Peninsula occurs at a reduced rate compared with that along the Atlantic Coast, but all Nm values are well above one, suggesting that gene flow is sufficient for panmixia in the absence of historical divergence. However, the significant heterogeneity among populations revealed by a small proportion of the morphological and genetic characters examined indicates that, on an evolutionary time scale, stochastic or selective factors have allowed for some degree of population differentiation. One possible hypothesis to account for these observations is that historical biogeographical processes may have led to some degree of divergence among geographically distant populations, particularly between the Gulf and Atlantic Coasts, that is partially counteracted by migration between these populations. Results from

TABLE 6.

Pairwise and overall F_{ST} values and estimated numbers of migrants (Nm) between populations per generation on the basis of calculated F_{ST} values for *A. gibbus*. See Table 2 for population abbreviations.

	MK × CC × CL		CC × CL		CC × MK		CL × MK	
Locus	F_{ST}	Nm	F_{ST}	Nm	F_{ST}	Nm	F_{ST}	Nm
6Pgd	0.027	9.01	0.001	226.4	0.021	11.52	0.032	7.6
Mdh1	0.118	1.9	0.004	62.3	0.131	1.7	0.116	1.9
Mdh2	0.001	199.1	0.001	288.1	0.002	115.8	0.001	428.1
Pgm	0.014	18.0	0.001	222.4	0.020	12.3	0.009	28.8
Odh	0.009	26.5	0.002	153.3	0.015	16.9	0.007	33.8
Mpi	0.043	5.6	0.020	12.4	0.066	3.6	0.005	52.3
Gpi	0.002	101.4	0.001	180.2	0.001	1935.0	0.001	205.8
Idh1	0.012	20.2	0.008	30.3	—[a]	—[a]	0.008	30.3
Average	0.043	5.5	0.005	50.1	0.053	4.5	.038	6.4

[a] Both populations were monomorphic for the same allele.

studies of a number of coastal species whose ranges span the Gulf and Atlantic Coasts of North America also show genetic discontinuities in the region of the Florida Peninsula, although often to a much greater extent than the oceanic *A. gibbus*. These observations support the hypothesis of historical isolation resulting from climatic and sea level changes (Saunders et al. 1986, Avise et al. 1987, Reeb and Avise 1990, Karl and Avise 1992). Alternatively, various selective scenarios could be advanced in order to account for the disparity between the population heterogeneity indicated by a small proportion of our genetic and morphological characters and the homogeneity indicated by the majority of characters examined. Although the data presented in this initial study are inadequate to rigorously examine such hypotheses, it is clear that overall levels of divergence among *A. gibbus* populations are small, particularly along the Atlantic Coast, and that migration from Florida populations to more northern populations may occur at a relatively frequent rate.

The influence of oceanographic processes may be particularly critical to the dispersal of *A. gibbus* larvae and may maintain the relative genetic and morphological homogeneity among populations for the majority of traits we examined. This species is found primarily in deeper shelf water from depths of 10 to 400 m (Waller 1969, Allen and Costello 1972), which contrasts with the shallow-water habitat occupied by *Argopecten irradians* and *Crassostrea*

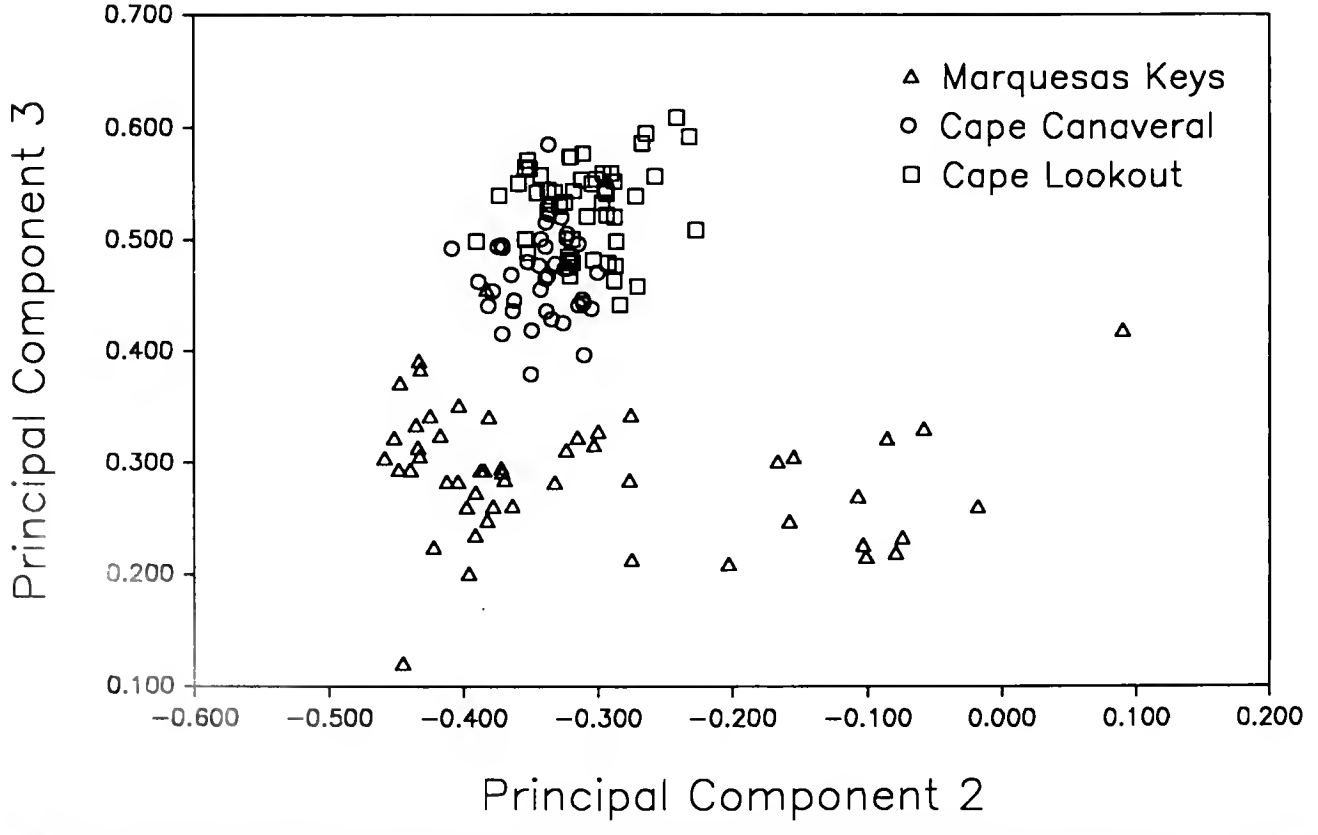

Figure 1. Bivariate plot of adjusted shape vector scores for PC3 against PC2 from results of Burnaby (1966) size-adjusted principal component analysis of *A. gibbus* morphology.

virginica along the East and Gulf Coasts of North America. This oceanic distribution places *A. gibbus* more directly under the influence of the Loop Current, Florida Current, and Gulf Stream than those estuarine species. Several specific features of this current system may enhance larval migration between the studied areas near the Marquesas Keys, Cape Canaveral, and Cape Lookout, supporting our hypothesis for high rates of gene flow among these populations. In particular, the Florida Current exhibits occasional wavelike meanders along its western edge (Boudra et al. 1987), and the Gulf Stream also shows characteristic wavelike meanders as well as large frontal eddies (Lee and Atkinson 1983, Lee et al. 1985 and references therein). These eddies and meanders prevail, are strengthened in regions near capes and shoals (Blanton et al. 1981) that are associated with calico scallop concentrations, and induce upwelling of nutrient-rich waters through intrusions of cold bottom water over shelf regions (Blanton et al. 1981, Hofmann et al. 1981, Lee and Atkinson 1983). In order to spawn, calico scallops require relatively cool waters (<22°C) compared with the typical summer and fall coastal water temperatures (Blake and Moyer 1991). The upwelling caused by meanders and eddies provides the opportunity for shelf waters to reach these lower temperatures (Miller et al. 1981) and allows for larvae to become associated with these water masses. Scallop spawning near Cape Canaveral is known to be associated with such intrusions of cold water (Allen and Costello 1972, Miller et al. 1981).

Water masses associated with the meanders and eddies of the Florida Current and Gulf Stream maintain their integrity over large distances and for extended periods of time (Leming 1979, Hofmann et al. 1981, Lee et al. 1981, Tester et al. 1991). After scallop spawning, these water masses may transport and concentrate large numbers of larvae from the west to the east coasts of Florida and to North Carolina. Direct evidence of the capability of Florida Current and Gulf Stream meanders to transport plankton from the west coast of Florida to North Carolina comes from extensive observations of a recent red tide (*Gymnodinium breve*) event (Tester et al. 1991). A red tide bloom was observed off of the southwest coast of Florida in September 1987. Red tide cells from this bloom were apparently transported by the Florida Current–Gulf Stream system to North Carolina, where a large bloom occurred in October 1987. The occurrence of the bloom in North Carolina coastal waters was associated with a large Gulf Stream meander that persisted and apparently concentrated *G. breve* cells over this region for a period of almost 3 weeks. Subsequent observations have revealed that the transport of red tide cells from western Florida to eastern Florida and North Carolina may occur more often than previously recognized and suggest that the occasional transport of calico scallop larvae by this system may be quite plausible (Murphy et al. 1975, Tester et al. 1991). In fact, on the basis of a range of flow rates from 40 to 100 cm s^{-1} in the Gulf Stream (Brooks and Bane 1983), it is possible for larvae to travel the 500 km from Cape Canaveral to Cape Lookout in approximately 8 to 23 days. Costello et al. (1973) reported 14 to 16 days as the planktonic larval period of calico scallops in the laboratory, so it is not unreasonable to hypothesize that competent larvae spawned off Cape Canaveral could successfully settle off of the North Carolina coast. Similarly, estimated flow in the Florida Current averages approximately 60 cm s^{-1} (Brown et al. 1989). Although the time required for larvae to enter the Loop Current–Florida Current system from the shelf may vary considerably because of midshelf currents and the presence of local gyres (Lee et al. 1992), larvae entrained in this system would be able to traverse the distance between the Marquesas Keys to Cape Canaveral in approximately 5 to 6 days.

One of the assumptions of using allozyme frequencies for estimates of gene flow between populations, as presented in this study, is that they are neutral with respect to selection, reflecting only the stochastic forces of drift and migration (mutation is assumed to be negligible). A number of recent studies of geographic variation in marine organisms that used either restriction fragment length polymorphism analysis of mitochondrial DNA (mtDNA) or single-copy nuclear DNA (scnDNA) call into question the generality of this assumption, and hence, the utility of allozyme data for such estimates (e.g. Karland Avise 1992). Allozyme surveys of geographic variation in American oysters (*C. virginica*) revealed relatively little population subdivision and high rates of gene flow throughout the range of the species along the Gulf of Mexico and Atlantic Coasts (Buroker 1983). In contrast, surveys of both mtDNA and scnDNA demonstrated sharp discontinuities between Gulf and Atlantic oyster populations, with the genetic "break" occurring along the eastern coast of Florida (Reeb and Avise 1990, Karl and Avise 1992). The pattern of differentiation determined from mtDNA and scnDNA was ascribed to vicariant biogeographic processes that initiated the current population structure and is maintained by the Florida Peninsula serving as an effective barrier to gene flow between populations in the Gulf of Mexico and those along the Atlantic Coast (Reeb and Avise 1990, Karl and Avise 1992), whereas Karl and Avise (1992) attributed the relative genetic homogeneity described by allozyme surveys to balancing selection that maintains allele frequencies despite constraints on gene flow. Obviously, the results of this study of *A. gibbus* populations should be interpreted with caution until supported by analyses with other types of genetic markers. On the other hand, the oceanic distribution of this species, as well as the intimate interaction of oceanographic processes with calico scallop life-history attributes, suggests that the Florida Peninsula may serve only as a partial barrier to gene flow for *A. gibbus* and that the relative overall uniformity of allozyme frequencies and morphology may indeed reflect high rates of migration between populations compared with more estuarine species.

With these caveats in mind, the apparent genetic and morphological similarity between populations of North Carolina and Florida *A. gibbus*, particularly those on the east coast of Florida, implies that migrants from Florida populations may be important for sustaining or reestablishing North Carolina stocks. Proper management of the North Carolina fishery may require consideration of this process. Although the data presented here can only support the hypothesis that migration occurs at a sufficient level to maintain relative genetic and morphological homogeneity on an evolutionary time scale, the actual frequency and intensity of present-day migration from Florida to North Carolina remain to be quantified. Additional work combining recruitment studies with more sensitive genetic techniques will be needed to define more clearly the forces that maintain the geographically isolated North Carolina calico scallops and the extent of the contribution (via larval dispersal) of Florida scallops.

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GROWTH AND MORTALITY OF THE TROPICAL SCALLOPS: *ANNACHLAMYS FLABELLATA* (BERNARDI), *COMPTOPALLIUM RADULA* (LINNE) AND *MIMACHLAMYS GLORIOSA* (REEVE) IN SOUTHWEST LAGOON OF NEW CALEDONIA

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ABSTRACT Major growth relationships were computed for *Annachlamys flabellata*, *Comptopallium radula*, and *Mimachlamys gloriosa*. The size-weight relationship for *M. gloriosa* is isometric; for other species studied, the relationship is decreasingly allometric. The edible part (muscle + gonad) represents about 50% of the fresh weight for the three species studied. The estimated parameters of Von Bertalanffy's equation on N capture-recapture measurements were estimated. Growth rate, during the year, is greatest when the temperature is increasing; it is reduced at maximum temperatures and remains stable during the cool season. Pectinids can be separated into two groups according to growth rate. Natural mortality was estimated for two species (*C. radula* and *M. gloriosa*) of pectinidae in Southwest Lagoon.

KEY WORDS: Growth, mortality, scallops, New Caledonia, lagoon

INTRODUCTION

Observation of the growth of bivalves is usually carried out by recording shell measurements: the criterion most widely used is the greater dimension (=height, dorsoventral distance), because it has the advantage of being easy to record without damaging the specimens subjected to examination. Other allometric characteristics can then be derived from such measurements.

A mathematical description of such growth revolves around two major concerns: on the one hand, one wants to find a relationship that best describes the observed growth, and on the other hand, one wants to define as precisely as possible the parameters of a growth function, which then can be included into a population dynamics model. Hoepe (1959) mentioned about 100 functions that had been used to describe growth. Von Bertalanffy's (1938) model is still the most widely used when describing the growth of bivalves in general and that of *Pectinidae* in particular (Theisen 1973, Broom 1976, Ralph and Maxwell 1977, Taylor and Venn 1978, Heald and Caputi 1981, Williams and Dredge 1981, MacDonald and Bourne 1987). This model is convenient in two ways, because it is both heuristic and simple: knowing the K (growth coefficient) and H_∞ (maximum height estimated) parameters is enough to thoroughly reconstruct the geometry of a growth curve.

On the soft bottoms of New Caledonia lagoons, the molluscs form a large and very diverse group and 30 *Pectinid* species are recognized so far (Dijkstra et al. 1989). The more common species are *Mimachlamys gloriosa* (Reeve 1853) (=*M. subgloriosa*, Iredale 1939), *Bractechlamys vexillum* (Reeve 1853), *Comptopallium radula* (L. 1758), and *Juxtamusium coudeini* (Bavay 1803). Only the first three species are large enough for human consumption. Most of these filter feeders live on the substrate, covered by a thin sediment layer. Only *M. gloriosa* is fixed to various substrates by a byssus. In this article, allometric relationships representing the relative growth were defined for three *Pectinidae* in the Southwest Lagoon of New Caledonia (*Annachlamys flabellata* [=*A. leopardus*, Iredale 1939], *C. radula*, and *M. gloriosa*). Total growth and natural mortality were estimated for the two last species.

MATERIALS AND METHODS

Relative Growth

Samples of scallops were collected monthly during 1 year by SCUBA divers in the vicinity of Noumea, New Caledonia (22°15'S, 166°25'E) (Fig. 1). Fifty specimens per species, per sample, were examined. Shells were weighed, measured and dissected as quickly as possible (at the latest three hours following collection). Shells were cleaned, and their epibionts (numerous on *M. gloriosa*) were removed with a wire brush. The height of shells was measured with a slide caliper (±0.1 mm), from the umbo to the ventral margin. Drained shells were weighed on a precision balance (±0.01 g). The viscera, mantle, hepatopancreas, muscle, and gonad were dissected out. Gonad, muscle, and viscera were drained on filter paper for a constant time before being weighed. The dry weight was measured after oven drying at 60°C for 48 hours, and ash-free dry weights (AFDW) were obtained after 3 hours of combustion at 550°C. The amount of organic matter (OM) for any one organ was obtained by dry weight minus ash weight.

Relative growth can then be defined as the relationship between the measurements of two organs. The basic law of growth was expressed in the following way: $y = a \cdot x^b$ where Y represents the dimension of the organ examined and X is that of the reference organ; a and b are constants.

This relationship is usually turned into a linear function by means of a logarithmic relation

$$\mathrm{Ln}(y) = b \cdot \mathrm{Ln}(x) + \mathrm{Ln}(a) \quad \text{or} \quad Y = b \cdot X + A.$$

The slope (b) of such a line corresponds to the rate of relative growth of examined organ as opposed to that of the reference organ. According to the values of b, three types of growth were defined: isometric ($b = 1$ or 3), decreasingly allometric ($b < 1$ or 3), and increasingly allometric ($b > 1$ or 3).

Total Growth

Two experimental sites were built in order to (1) follow the growth of Pectinidae in a natural environment and (2) estimate their natural mortality rate. These sites were set up in biotopes that

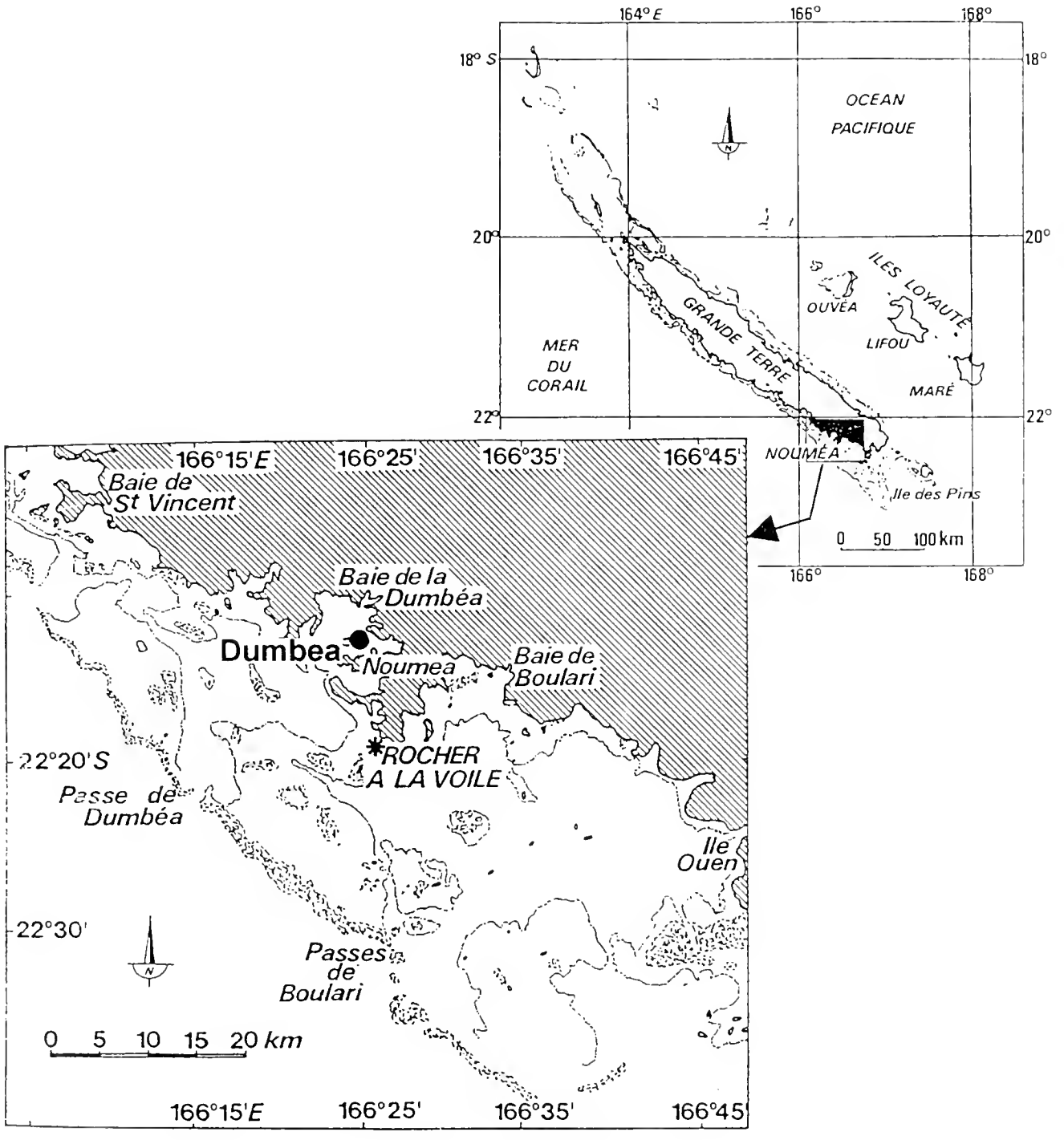

Figure 1. Location of study site.

were characteristic for the various species studied. One site was set up in "Rocher a la Voile" (near Noumea) for *M. gloriosa* and the other was located in Dumbea Bay for *C. radula* (Fig. 1), at depths of 12 and 6 m, respectively. A wire mesh size of 2 cm was selected so that it would not foul too rapidly and yet Pectinidae could not escape. Respective dimensions of the two experimental areas were 5 × 5 m for the "Rocher à la Voile" and 10 × 10 m for the one in Dumbea Bay. The sizes of these experimental areas have been imposed by the deepness and its structure. The sites were monitored weekly; the parks were cleared (of seaweed) every 2 weeks.

The natural population (all benthic species) was removed from the site. A label was glued on the shells of the experimental specimens with cyanoacrylate glue after cleaning and drying the shells. Scallops were labeled, measured, and returned to their respective sites: *C. radula* in Dumbea Bay and *M. gloriosa* in the "Rocher à la Voile." Scallops were measured every 2 months, and dead individuals were removed. Bimonthly measurements were chosen

as a compromise between frequent measurements, which could stress the pectinids and retard their growth, and infrequent measurements, which could reflect the effects of external events, such as storms (cyclones) or diseases.

The mathematical expression used almost everywhere to describe the overall growth of molluscs is Von Bertalanffy's equation (1938), which is written out as followed: $H_t = H_\infty - [H_\infty - H_o) \cdot e^{-kt}]$ or $H_t = H_\infty \cdot (1 - e^{-k(t-t_o)})$, where H_t = height at time t; H_∞ = maximum asymptotic size; H_o = height at t_o; k = constant; t_o = theoretical time where height is equal to 0.

Values of Von Bertalanffy's equation parameters were determined by using the SAS/STAT software (Cary, NC) and the NLIN (Nonlinear regression least squares iterative method) Newton procedure, which allows the model parameters to be estimated in an iterative way using the least squares method.

Data on growth were obtained from "mark and recapture" experiments. The initial equation had to be modified in order to express the measurements as a function of Δt (time interval between two measurements: 2 months) rather than t.

At time t, one finds: $H_t = H_\infty(1 - e^{-k(t-t_o)})$. At recapture time, the equation becomes:

$$\begin{aligned} H_{(t+a)} &= H_\infty \cdot (1 - e^{-k(t-t_o+a)}) \\ &= H_\infty - H_\infty \cdot e^{-k(t-t_o)} \cdot e^{-ka} \\ H_{(t+a)} - H_t &= H_\infty - [H_\infty \cdot e^{-ka} \cdot e^{-k(t-t_o)}] \\ &\quad - H_\infty + [H_\infty \cdot e^{-k(t-t_o)}] \\ &= [H_\infty \cdot e^{-k(t-to)}] \cdot (1 - e^{-ka}) \end{aligned}$$

but we have:

$$H_\infty \cdot e^{-k(t-t_o)} = H_\infty - H_t,$$

and equation becomes:

$$\begin{aligned} H_{(t+a)} - H_t &= (H_\infty - H_t) \cdot (1 - e^{-ka}) \\ &= [H_\infty \cdot (1 - e^{-ka})] - [H_t \cdot (1 - e^{-ka})], \end{aligned}$$

which can be expressed in the following way:

$$H_{(t+a)} = [H_\infty \cdot (1 - e^{-ka})] + H_t \cdot (e^{-ka}) \qquad \text{equation 1}$$

With: $b_o = H_\infty$; b1 = k; $y = H_{(t+a)}$ and $x = \Delta t = a$.

First and second derivatives of the equation 1 were calculated so as to be able to use the Newton method.

$$\begin{aligned} \text{der.}b_o &= 1 - \exp(-b_1 \cdot x); \\ \text{der.}b_1 &= x \cdot (b_o - l_1) \cdot \exp(-b_1 \cdot x); \\ \text{der.}b_o \cdot b_1 &= x \cdot \exp(-b_1 \cdot x); \\ \text{der.}b_1 \cdot b_1 &= -x^2 \cdot (b_o - l_1) \cdot \exp(-b_1 \cdot x); \\ \text{der.}b_1 \cdot b_o &= x \cdot \exp(-b_1 \cdot x); \\ \text{der.}b_o \cdot b_o &= 0. \end{aligned}$$

In order to discover any possible seasonal variation in growth, weekly relative growth (instantaneous growth) between two measurements was calculated according to the following relationship: $(H_{t+1} - H_t)/(H_t \cdot \Delta t)$ (Ricker 1980).

H_t was the height of shells at the moment of recording, H_{t+1} was the height at the moment of the following measurement, and Δt was the time interval in weeks separating these two measurements. To reduce variation, only those specimens that had reached sexual maturity were measured (Lefort 1991).

Mortality

Experimental beds, identical to those studied to estimate growth, were used to estimate the death rate of *M. gloriosa* and *C. radula*. The experimental protocol was the same. On each of our visits, the dead individuals were removed.

Labeled specimens were prevented from leaving the experimental site by a wire mesh. After a first diver had searched, a second would come and collect individuals overlooked by the first; capture efficiency was estimated at 100%.

RESULTS

Relative Growth

Major allometric relationships as computed for *A. flabellata*, *C. radula*, and *M. gloriosa* are shown in Tables 1, 2, and 3. Allometric coefficients were checked against theoretical values ($H_o = 3$ or $H_o = 1$) according to the type of relationship involved: $t_{theo} = 1.96$ ($p < 0.05$). The results of these tests appear in the last column of Tables 1 to 3. The total-weight relationship for *M. gloriosa* is isometric, but for other species studied, this relationship was decreasingly allometric.

In *C. radula*, muscle accounts for 39%, in *A. flabellata*, it accounts for 34%, and in *M. gloriosa*, it accounts for 32% of total dry weight. The edible part (muscle + gonad) represents about 50% of the wet weight for the three species studied. The muscle of *A. flabellata* consists of 90% OM, 89.5% for *C. radula*, and 88.8% for *M. gloriosa*. The gonad is 85.6% OM for *C. radula*, 84% for *A. flabellata*, and 83.3% for *M. gloriosa*, without significant seasonal variation (Lefort 1991). The byssus of *G. gloriosa* is made up of 66.7% OM, and its average length is 21 mm.

Total Growth

The growth of *M. gloriosa* was observed for a full year; that of *C. radula* was studied for 10 months. Different measurements were obtained for the same scallop. Growth may, therefore, be measured over different periods (2 months, 4 months, etc., up to 12 months). The parameters of Von Bertalanffy's equation have not been calculated for *A. flabellata* because the data are not sufficient; the estimated parameters of Von Bertalanffy's equation for the two other species on N mark-recapture experiments were: *M. gloriosa* (N = 1,543): H_∞, 73.89 ± 1.31; k, 1.01 ± 0.07 and *C. radula* (N = 984): H_∞, 92.40 ± 2.14; k, 0.35 ± 0.03.

The relative weekly growth rate was estimated for *M. gloriosa* (Fig. 2). The various rates were also expressed as a percentage of

TABLE 1.

Allometric relationships for *M. gloriosa*.

Relation	N	*r*	a	b	t_{obs}
$TW = a \times H^b$	1,006	0.99	$0.98 \cdot 10^{-6}$	3,068	—
$FW = a \times H^b$	1,006	0.99	$7.62 \cdot 10^{-6}$	3,378	—
$MW = a \times H^b$	1,006	0.91	$5.27 \cdot 10^{-6}$	3,154	—
$FW = a \times (TW)^b$	1,006	0.98	$20.0 \cdot 10^{-2}$	1,096	—
$MW = a \times (TW)^b$	1,006	0.91	$5.00 \cdot 10^{-2}$	1,086	—

Note: TW, total weight (g); H, height (mm); MW, muscle weight; FW, fresh weight (g); —, *t* test significant ($p > 0.05$); NS, not significant; *r*, correlation coefficient.

TABLE 2.

Allometric relationships for *C. radula*.

Relation	N	*r*	a	b	t_{obs}
$TW = a \times H^b$	632	0.96	$21.2 \cdot 10^{-5}$	2,896	NS
$FW = a \times H^b$	632	0.91	$1.92 \cdot 10^{-5}$	3,072	—
$MW = a \times H^b$	45	0.86	$0.14 \cdot 10^{-5}$	3,395	—
$FW = a \times (TW)^b$	632	0.93	$16.2 \cdot 10^{-2}$	1,045	NS
$MW = a \times (TW)^b$	45	0.89	$3.6 \cdot 10^{-2}$	1,128	—

Note: For abbreviations, see footnote to Table 1.

the total annual growth. Growth is important when temperature is increasing, but it reduces when temperature reaches a maximum and then remains stable during the cool season (Figs. 3 and 4).

Mortality

Labeled specimens were prevented from leaving the experimental site by a wire mesh. After a first diver had searched, a second would come and collect individuals overlooked by the first; capture efficiency was estimated at 100%.

Two hundred fourteen *M. gloriosa* were labeled on 30 May 1989; nine of them had died on 2 June 1989. On 6 December 1989 (after 27 weeks of freedom), a count allowed us to recover 156 live specimens that were numbered and 11 live individuals found without labels but whose shells showed evidence of wire brushing.

For *C. radula* two experiments were carried out:

Length of trial (weeks)	16	17
Number of labeled scallops	86	128
Scallops dead in the first week	5	6
Live scallops recovered with label	54	91
Live scallops recovered without label	15	13

If we discount the individuals that died from handling and those that lost their tags, a total of 205 (214 − 9) *M. gloriosa* were present at the start of the experiment, thus giving a 167/208 = 81.46% survival rate over a 192 day period (38 scallops dead), i.e., a weekly death rate of 6.76×10^{-3}. Therefore, the natural mortality rate for *M. gloriosa* was 0.35. Analogous calculations applied to the first case (16 weeks) give a natural mortality rate of 0.48 for *C. radula*, and in the second case (17 weeks) give a coefficient of 0.45; thus, the average for *C. radula* was equal to 0.47.

DISCUSSION

The main interest of Von Bertalanffy's parameter estimations becomes obvious only if their use is other than descriptive, for example, for productivity calculations or biomass evolution study for fishery management. The total growth of *M. gloriosa* and *C. radula* cannot be completely represented by Von Bertalanffy's model, because it can only correctly express the growth of species after sexual maturity has been reached. This is not peculiar to New Caledonian Pectinidae. The growth rate of New Caledonia Pectinidae is very quick: sexual maturity is achieved during the second year for *M. gloriosa* and during the third year for *C. radula* (Lefort and Clavier 1994).

TABLE 3.

Allometric relationships for *A. flabellata*.

Relation	N	*r*	a	b	t_{obs}
$TW = a \times H^b$	213	0.97	$67.3 \cdot 10^{-5}$	2,708	NS
$FW = a \times H^b$	213	0.94	$8.51 \cdot 10^{-5}$	2,916	—
$MW = a \times H^b$	51	0.99	$7.68 \cdot 10^{-5}$	2,686	—
$FW = a \times (TW)^b$	213	0.95	$23.2 \cdot 10^{-2}$	1,064	NS
$MW = a \times (TW)^b$	51	0.99	$5.40 \cdot 10^{-2}$	1,176	—

Note: For abbreviations, see footnote to Table 1.

The growth of Pectinidae, and more generally that of bivalves, cannot be described by any single model. Theisen (1973) also found that Von Bertalanffy's model was well adapted to shellfish, such as *Pecten maximus*, as long as these were mature. Williams and Dredge (1981) reached the same conclusion, i.e., Von Bertalanffy's classic model can correctly describe the growth of *Amusium balloti*, but only when the size is larger than 50 mm. Many models of growth have been improved without success; however, Von Bertalanffy's model was the most adjusted to the studied pectinids (Lefort 1991). Even when an adequate model has been chosen, parameters still need to be evaluated. For Von Bertalanffy's model, numerous methods have been worked out, the best known of which are those of Walford (1946), Beverton (1954), Gulland and Holt (1959), Fabens (1965), and Allen (1966). Calculating growth parameters with these various methods can lead to significantly different results. The choice of a model and the choice of an estimation method for parameters are crucial (and often interconnected).

Within the Pectinidae, there is a great deal of diversity in growth rates (Table 4; Fig. 3) and longevity. Growth parameters vary from one species to the next. They can also vary within the same species, depending on the geographical site (Antoine et al. 1979; Llana 1988) and/or depending on depth (MacDonald and Thompson 1988). However, an examination of published values of k (Table 4) made it possible to compare species on the basis of growth rate. Thus, the Pectinidae listed in Table 4 can be subdivided arbitrarily into two groups ($k > 1$ and $k < 1$). Individuals displaying a high rate of growth ($k = 1$ to 2.8) are: *A. balloti*, *B. vexillum*, and *M. gloriosa*. The second group is composed of the great majority of Pectinidae where $k < 1$.

It is generally agreed that the greater the growth rate (k), the lower the longevity rate. This was particularly true for species such as *A. balloti*, where longevity is only 3 years (Heald and Caputi 1981). On the contrary, *P. maximus* (Antoine 1979) and *Crassadoma gigantea* (MacDonald et al. 1991) live for 15 years or more; as for *Placopecten magellanicus* (which has the lowest growth coefficients with $k = 0.156$), longevity is 20 years (MacDonald and Thompson 1988). New Caledonian Pectinidae have relatively high growth coefficients. *B. vexillum* ($k = 1.41$ to 1.85) has a growth rate clearly superior to that of *M. gloriosa* ($k = 1.015$), but for the former, longevity is 3 to 4 years whereas it is 8 years for the latter *C. radula* has a lower growth coefficient ($k = 0.35$), but this is offset by a high longevity rate compared with that of the two other species. This is in agreement with the generally accepted theory of the relation between longevity and the growth rate.

Weekly relative growth was calculated for *M. gloriosa* (Fig. 2). As was the case with *A. balloti*, no interruption of growth was observed in that species (Heald and Caputi 1981). There are several cases of substantial variations from month to month or from year to year (Borkowski 1974, Kojima 1975, Lewis et al. 1979). Williams and Dredge (1981) observed seasonal variations in growth among *A. balloti*, especially in the adductor muscle, which

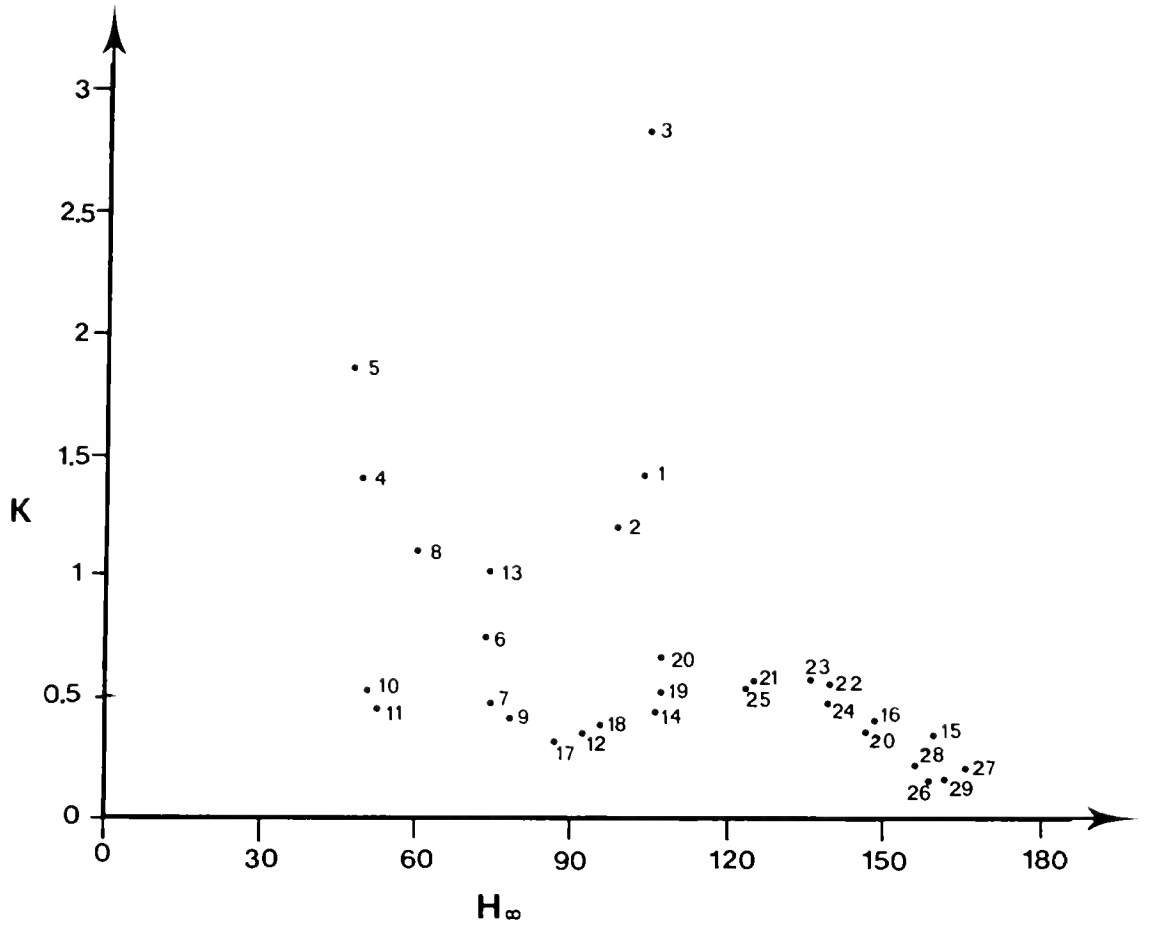

Figure 2. Relative rate of weekly growth for *M. gloriosa*.

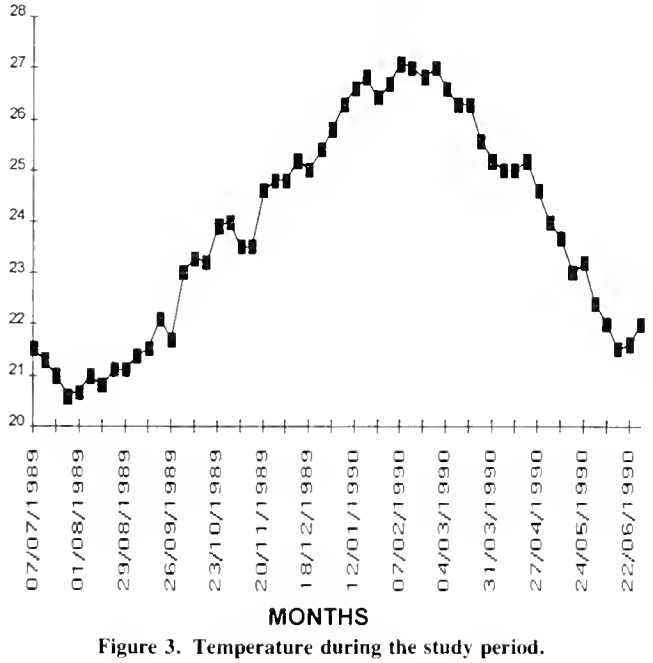

Figure 3. Temperature during the study period.

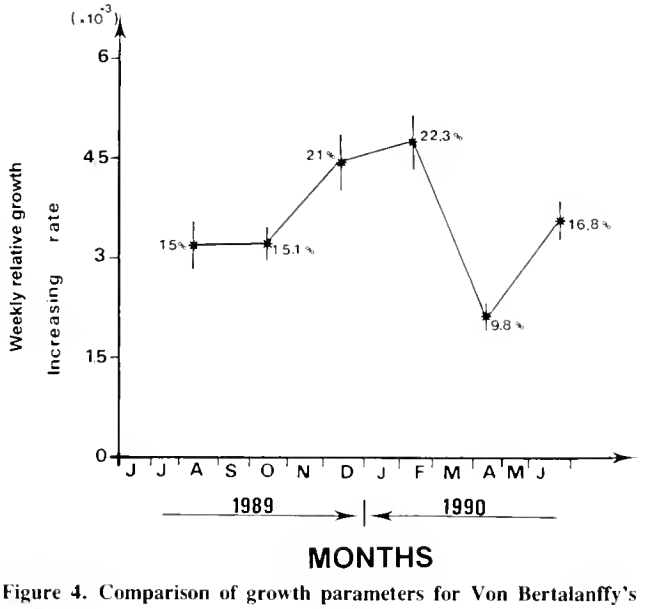

Figure 4. Comparison of growth parameters for Von Bertalanffy's equation.

TABLE 4.

Comparison of growth parameters for von Bertalanffy's equation.

Species	H	k	Locality	References
A. balloti	103.8	1.42	Shark Bay (Australia)	Heald and Caputi 1981
	98.7	1.20	North lagoon (New Caledonia)	Clavier personal communication
	104.9	2.83	Queensland (Australia)	Williams and Dredge 1981
B. vexillum	49.4	1.41	South-west lagoon (New Caledonia)	Luro 1985
	47.4	4.86	South-west lagoon (New Caledonia)	Clavier personal communication
Chlamys operularis	73.5	0.75	Aberdeen (Scotland)	
	74.5	0.48	Kilmore (Irish sea)	Antoine 1979
	60.3	1.10	Jersey (English channel)	
Chlamys varia	78.0	0.41	Brest Harbour (France)	
	52.0	0.46	Brest Harbour (spring cohort)	Antoine 1979
	52.3	0.44	Brest Harbour (autumn cohort)	
C. radula	92.4	0.35	South-west lagoon (New Caledonia)	This study
M. gloriosa	73.9	1.02	South-west lagoon (New Caledonia)	This study
P. caurinus	106.0	0.44	Washington coasts (U.S.A.)	Antoine 1979
	106.4	0.34	Georgia Strait (Canada)	
Patinopecten yessoensis	148.1	0.40	Saroma Lake (Japan)	Antoine 1979
Pecten sulcicostatus	86.7	0.32	Mossel Bay (Bonne esperance)	Antoine 1979
	95.5	0.38	False Bay (Bonne esperance)	
	107.1	0.52	Armen (France)	
	106.7	0.66	Brest Harbour (France)	
P. maximus	124.6	0.56	St. Brieuc Bay (France)	Antoine et al. 1979
	139.8	0.56	Seine Bay (France)	
	135.4	0.58	Seine Bay (France)	
	138.9	0.47	Seine Bay (France)	
	123.5	0.53	Dieppe (France)	
	158.4	0.16	Sunnyside (Newfoundland)	
	166.0	0.21	St. Andrews (New Brunswick)	MacDonald and Thompson 1988
P. magellanicus	155.9	0.22	New Jersey	
	162.0	0.16	Bay of Fundy	Antoine 1979
	146.4	0.35	Georges Bank	

went through its maximum growth during the summer months, whereas the gonad developed little. Seasonal variations in shell growth for *M. gloriosa* are tied to the temperature of seawater, the highest rate being observed during warmer months. However, *B. vexillum* underwent much greater seasonal changes in growth, which were correlated with temperature and with particulate organic carbon (POC) (Clavier et al. in preparation).

Seasonal variations of growth are frequent among Bivalvia (*P. maximus*, Mason 1958; *Mizuhopecten yessoensis*, Golikov and Scarlato 1970, Pickett and Franklin 1975; *Argopecten irradians*, Broom 1976). Such variations were related to abiotic factors such as depth (MacDonald and Thompson 1988), type of substrate (Gruffydd 1974), temperature (Theisen 1973), and currents (Kirby-Smith 1972, Kirby-Smith and Barber 1974). A maximum growth rate is observed in *M. gloriosa* when seawater temperature goes up (October to January); it then drops when temperature reaches its maximum. In contrasts with *A. irradians*, maximum growth (6 to 8 mm per month) occurs when temperature is at an optimal level; growth at 10°C is three times as high as that achieved at 10°C (Castagna and Duggan 1971).

A potential index of growth Ω ($\Omega = \log_{10}(k \cdot W_\infty)$) (Pauly 1982) was calculated for the three New Caledonian Pectinidae. According to Pauly, this index must be more or less constant for species of similar taxonomy; it should therefore be possible to subdivide the Pectinidae family according to this index. The highest value was obtained for *M. gloriosa* ($\Omega = 1.64$), the next was for *B. vexillum* ($\Omega = 1.60$), and the lowest value ($\Omega = 1.57$) was found for *C. radula*. The potential index of growth for *A. balloti* in the northern lagoon of New Caledonia was $\Omega = 1.89$ (Clavier unpublished data).

The estimates for natural mortality rate reported in this article are lower than those usually reported. Our estimations of natural mortality are approximately one and are valid only for the Noumea area. *M. gloriosa* is a subtidal bivalve living on muddy or muddy-sand bottoms, covered by sponges. Sponges could be a good protection against predators (Dijkstra et al. 1989). *C. radula* lives on muddy substrata in shallow water (about 5 to 15 m) and particularly likes the inner part of bays. *C. radula* lives under heads of living coral or in their immediate proximity, lightly covered so that only the ring of sensorial tentacles can be seen by the predators. These species live inside a lagoon, i.e., a semiclosed environment, and this may protect them from predation.

The estimation of the natural mortality of species raised for commercial purposes is usually more difficult than that for non-commercial species. Most methods used to do so require knowledge of the extent of fishing (Beverton and Holt 1956) or the annual yield of fisheries (Csirke and Caddy 1983). In many cases, empirical formulae were used, notably that of Pauly (1982) and of Rikhter and Efanov (1976). Natural mortality estimation of non-commercial species was much easier, especially if regular records of population density can be kept, and it was possible for the two species in the Southwest Lagoon of New Caledonia. However, it seemed interesting to calculate such mortality rate with some more commonly used formulae with our data.

Species	Pauly	Rikhter and Efanov
B. vexillum	M = 2.19	M = 1.0
M. gloriosa	M = 1.31	M = 1.8
C. radula	M = 0.61	M = 3.5

Empirical patterns give variable results and differ from the applied results. Some authors have already mentioned this problem and advised the use of these methods only for an approximate estimate of natural death. The results achieved through these patterns are sometimes too remote from the experimental results; they have to be used only in a limited way with extreme caution. The result obtained with Pauly's formula was the closest to that found for *C. radula* (M = 0.47). In the case of *B. vexillum*, the formula of Rikhter and Efanov corresponded best to the values found experimentally (M = 0.8; Clavier, personal communication). However, for *M. gloriosa*, none of the mathematical results obtained could approach the value we had found. Natural mortality varies with the number of predators and probably with the geographic situation. The main predators we could identify were starfish, gasteropoda (*Muricidae* species) fish (*Lethrinus* species), octopuses, and crustaceans. It is common knowledge that pectinids clap their valves; doing so enables the bivalve to move around. *M. gloriosa* and *C. radula* can make "leaps" when they are disturbed by predators.

CONCLUSION

The growth rate of New Caledonia Pectinidae is very quick; the stage of first sexual maturity is achieved during the second year for *M. gloriosa* and during the third year for *C. radula*. The annual increase is then very low and is more or less constant in 1 year's time. Their growth is mainly achieved during the first 2 years. The growth rate is higher when the seawater is temperature increases, it is lower when the seawater temperature is at its maximum, and it is steady during the cool season.

Our estimates of natural mortality rate reported in this article are lower than those usually reported (valid only for Noumea area). This can be explained by the ecology of the species studied and because they live in a semiclosed environment, which provides them with effective protection against some predators and the external environment.

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THE USE OF RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS IN GENETIC STUDIES OF THE SEA SCALLOP *PLACOPECTEN MAGELLANICUS* (GMELIN, 1791)†

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ABSTRACT This study represents the first published application of the random amplified polymorphic DNA (RAPD) technique to bivalve DNA. A total of 222, 10 base (10 mer) primers were screened against one DNA sample from the sea scallop, *Placopecten magellanicus*, under predetermined optimal reaction conditions. One hundred thirty RAPD primers were found to be positive (59%). When 40 of these positive primers were randomly selected and used to compare RAPD profiles among 24 individuals collected from different scallop beds, at least 15 primers revealed clear polymorphisms. Inheritance of RAPD alleles was examined by the analysis of banding patterns from a pair-mated family, in which almost all alleles segregated in a Mendelian fashion. Although none of the RAPD markers was unique to a single population in our small samples, the frequencies of polymorphic bands at different loci varied between populations. Thus, genetic similarity based on allele frequencies can be estimated and used as an additional tool for understanding the genetic structure of sea scallop populations.

KEY WORDS: RAPD, polymorphism, inheritance, genetic segregation, *Placopecten*

INTRODUCTION

The sea scallop (*Placopecten magellanicus*) supports a valuable commercial fishery on the coasts of Atlantic Canada and the northeastern United States. This species is contiguously distributed within its range, forming spatially discrete aggregates or "beds" (Bourne 1964, Sinclair et al. 1985). In Atlantic Canada, there are a number of scallop beds (Fig. 1) that are commercially exploited (Black et al. 1993), and these are each managed as separate "stocks" (Kenchington and Lundy 1992, Robert et al. 1993). In order to better understand the relationships between these beds, morphological (Kenchington and Full 1994) and enzyme electrophoretic data (Volckaert and Zouros 1989; Beaumont and Zouros 1991; Volckaert et al. 1991) have been collected and analyzed. The morphological characters identified scallops on St. Pierre Bank and in the Bay of Fundy (Fig. 1) as being significantly different from those on the Georges Bank and Sable Island beds in a suite of separate analyses using Fourier shape descriptors of the scallop upper shell (Kenchington and Full 1994). However, morphological differences may be the result of genetic and environmental differences that cannot be studied separately.

Enzyme electrophoresis of scallops from these same beds identified a high degree of genetic similarity (Beaumont and Zouros 1991) in contrast to the shell shape analyses. However, for certain enzyme systems, a pronounced heterozygote deficiency was observed, suggesting that allozyme-specific selection may be acting on this species (Gartner-Kepkay and Zouros 1985). With the availability of new molecular biological techniques, there has been an increasing emphasis on the use of DNA characteristics as markers. DNA variation may provide answers to the questions of genetic relatedness between scallop stocks, which are of direct concern to resource management.

Most methods for the study of DNA variation rely on the use of nonspecific probes that reveal multiallelic profiles unique to each individual (minisatellite DNA fingerprints) or on the use of specific probes that recover locus-specific multiallelic variation (e.g., microsatellites). Minisatellites are not suitable for population studies because they do not allow quantification of genetic differences and measure of heterozygosities and genetic distances. Microsatellites are suitable for those purposes but are difficult and expensive to develop and apply in large-scale population studies.

A relatively new technique, using markers referred to as random amplified polymorphic DNA, or RAPD, has been described (Welsh and McClelland 1990, Williams et al. 1990, Caetano-Anollés et al. 1991a). It is a technique based on the polymerase chain reaction (PCR; Saiki et al. 1988) in which single, short oligonucleotide primers of arbitrary sequence are used to amplify anonymous regions of genomic DNA. The amplification products are then resolved by gel electrophoresis, and amplified polymor-

†NRCC 38025.

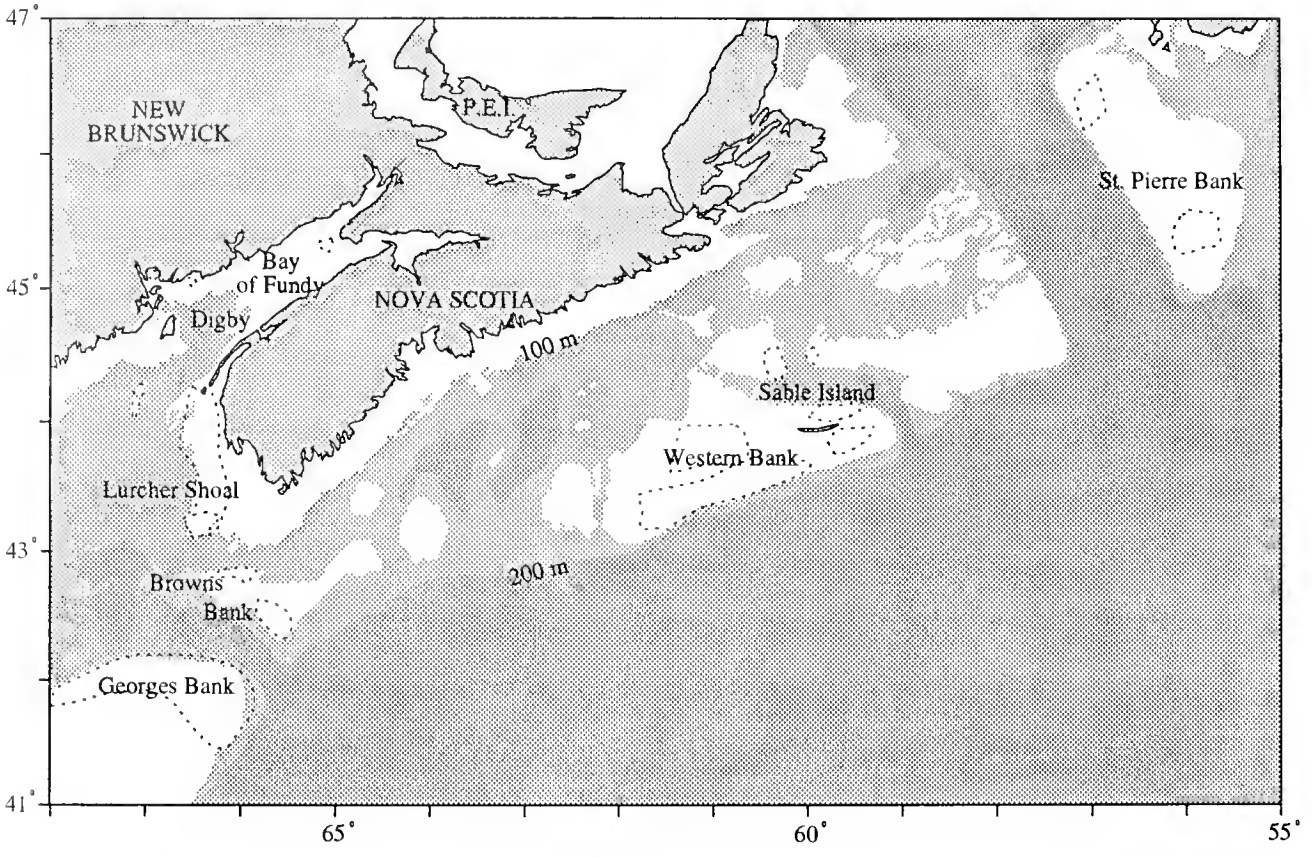


Figure 1. Locations of commercial sea scallop (*P. magellanicus*) beds along the coast of Atlantic Canada.

phic products are used as genetic markers. Compared with other DNA-based techniques, the detection and use of RAPD markers is faster and less expensive. It requires a small quantity of template DNA per reaction; many reactions can be done simultaneously in commercially available thermocyclers, and the reaction products can be resolved and documented easily. The technique has been applied successfully in a variety of genetic, phylogenetic, and population genetic studies (Caetano-Anollés et al. 1991b, Bassam et al. 1992, Hadrys et al. 1992, Tingey and del Tufo 1993). It has been used to study genetic segregation (Carlson et al. 1991, Durand et al. 1993, Kazan et al. 1993, Yu and Pauls 1993), characterize hybrids (Baird et al. 1992, Patwary and van der Meer 1994), identify strains (Goodwin and Annis 1991, Hu and Quiros 1991, Welsh et al. 1991a), assess genetic diversity in culture collections (Akopyanz et al. 1992, Patwary et al. 1993), obtain information on parentage (Welsh et al. 1991b, Hadrys et al. 1993, Scott and Williams 1993), identify markers linked to a disease resistance gene (Martin et al. 1991, Paran et al. 1991), and study speciation (Arnold et al. 1991, Crawford et al. 1993, Crossland et al. 1993) and pollination (Philbrick 1993), and in phylogenetic (Halward et al. 1992, Strongman and MacKay 1993), genetic mapping (Williams et al. 1990, Reiter et al. 1992), and DNA fingerprinting (Welsh and McClelland 1990, Wilde et al. 1992, Eskew et al. 1993) studies.

This article represents the first application of the RAPD technique to bivalve DNA. We identify a number of 10 mer RAPD primers that show polymorphisms in the sea scallop, *P. magellanicus*, and test their suitability for genetic studies of this species.

MATERIALS AND METHODS

The sea scallops [*P. magellanicus* (Gmelin)] used in population comparisons were collected by the Department of Fisheries and Oceans, Canada, from five commercial scallop beds (Fig. 1): Browns Bank, Sable Island Bank, Western Bank (offshore), Lurcher Shoal, and Digby Gut (Bay of Fundy, inshore). Adductor muscles were dissected from the scallop at sea and immediately frozen in liquid nitrogen. Some scallops were brought back alive and maintained in seawater tanks until used for DNA extraction from fresh muscle.

Mendelian inheritance of the RAPD bands was tested by comparing samples from a pair-mated family (Marine Gene Probe Laboratory, Dalhousie University, Halifax). The parents and 12 randomly selected offspring were analyzed.

DNA was extracted from individual adductor muscle tissue. Approximately 0.2 g of tissue was ground to a fine powder in the presence of liquid nitrogen in 10 ml Falcon culture tubes (Elkay Products Inc., Boston, MA) with a steel plunger as a pestle. The frozen powder was immediately mixed with 750 μl of lysis buffer containing 10 mM Tris (pH 8.2), 1 mM NaEDTA, and 400 mM NaCl. To the lysate, solutions of sodium dodecyl sulfate (SDS) and proteinase K were added to final concentrations of 0.8% and 100 $\mu g\ ml^{-1}$, respectively. The samples were mixed thoroughly, incubated at 37°C for an hour, and then digested overnight at 55°C. Protein present in the lysate was precipitated by adding and vortexing 250 μl of saturated NaCl and was pelleted by centrifugation at room temperature at 800 rpm for 20 minutes. The nucleic

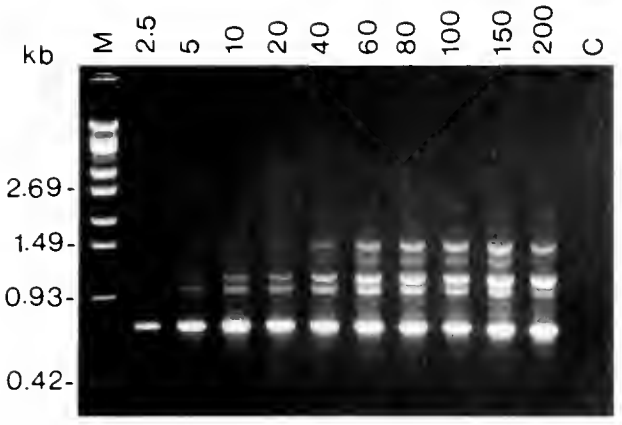
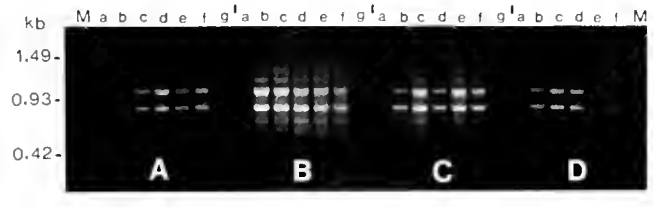


Figure 2. The effect of template DNA concentration on RAPD amplification of sea scallop DNA using a single primer (UBC No. 287). The RAPD products were resolved by electrophoresis through 1.4% agarose gel and stained with ethidium bromide. The numerals above the lanes indicate nanograms of template DNA included in respective 25 µl reactions. The lane marked C is a negative control reaction in which no template DNA was added. The lane labeled M contains DNA size marker bacteriophage λ DNA digested by the restriction enzyme *Sty*I with the size of the λ DNA fragments indicated as the number of kilobase (kb) pairs.

TABLE 1.

RAPD primer composition and the results of screening the primers against the DNA from one individual sea scallop.

% G + C	No. of Primers Screened	No. of Positive Primers	% Positive
50	54	40	80
60	61	25	41
70	58	27	47
80	39	28	71
90	1	1	100



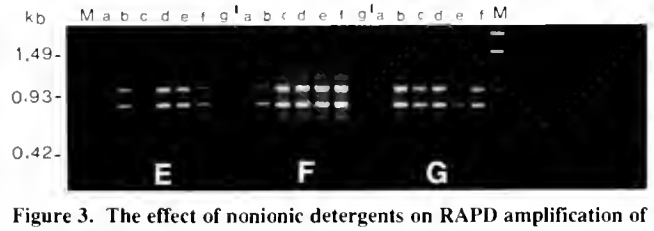


Figure 3. The effect of nonionic detergents on RAPD amplification of sea scallop DNA using a single primer (UBC No. 208). The detergents applied in subpanels are (A) Nonidet P-40, (B) Tween 20, (C) Triton X-100, (D) 1:1 mixture of Nonidet P-40 and Tween 20, (E) 1:1 mixture of Nonidet P-40 and Triton X-100, (F) 1:1 mixture of Tween 20 and Triton X-100, (G) 1:1:1 mixture of all three detergents. The percentage of detergents used in reactions is: lane a, 0.0; b, 0.5; c, 1.0; d, 2.0; e, 4.0; f, 6.0. The lane marked "g" is a negative control reaction. Lane M contains size markers as in Fig. 2. kb, kilobase.

TABLE 2.

A list of primers used in this study.

UBC Primer Number	Nucleutide Sequence	Study
15	CCTGGGTTTG	segregation
25	ACAGGGCTCA	segregation
63	TTCCCCGCCC	population
67	GAGGGCGAGC	population[a]
71	GAGGGCGAGG	population
77	GAGCACCAGG	population, segregation[a]
81	GAGCACGGGG	population[a]
83	GGGCTCGTGG	population
84	GGGCGCGAGT	population
98	ATCCTGCCAG	population
100	ATCGGGTCCG	population
101	GCGGCTGGAG	population
102	GGTGGGGACT	population
104	GGGCAATGAT	population
105	CTCGGGTGGG	population[a]
106	CGTCTGCCCG	population
110	TAGCCCGCTT	population[a]
116	TACGATGACG	population[a]
129	GCGGTATAGT	population
134	AACACACGAG	population
137	GGTCTCTCCC	segregation[a]
145	TGTCGGTTGC	population, segregation[a]
146	ATGTGTTGCG	population
149	AGCAGCGTGG	population[a]
150	GGAGGCTCTG	population, segregation[a]
153	GAGTCACGAG	population[a]
164	CCAAGATGCT	population
166	ACTGCTACAG	population[a]
167	CCAATTCACG	population, segregation[a]
171	TGACCCCTCC	population, segregation[a]
174	AACGGGCAGC	population
181	ATGACGACGG	population
186	GTGCGTCGCT	population
190	AGAATCCGCC	population
198	GCAGGACTGC	population
202	GAGCACTTAC	population
205	CGGTTTGGAA	population, segregation[a]
208	ACGGCCGACC	population
210	GCACCGAGAG	population
211	GAAGCGCGAT	population
212	GCTGCGTGAC	population, segregation[a]
287	CGAACGGCGG	population

[a] Primers revealed clear polymorphisms between individuals.

acids present in the supernatant were precipitated for 30 minutes at −20°C by the addition of an equal volume of isopropanol, collected by centrifugation at 14,000 rpm for 10 minutes, washed in 1 ml of 70% ethanol, dried in a vacuum for 5 minutes, and dissolved in 500 µl of TE (10 mM Tris-HCl, 1 mM EDTA). The RNA present in the nucleic acid preparation was digested by adding 10 µg of RNAse A and incubating at 37°C for 30 minutes. The DNA solution was first extracted with an equal volume of phenol-chloroform [(phenol:chloroform:isoamyl alcohol = 50:49:1), 0.1M Tris (pH 7.5), 0.2% β-mercaptoethanol] and then with an equal volume of chloroform-isoamyl alcohol (24:1). The DNA in the aqueous phase was precipitated by adding one-third volume of 2.5 M NH_4OAc and 2.5 vol of 95% ethanol. The DNA pellet

obtained after centrifugation was washed with 70% ethanol to remove excess salts, dried, dissolved at 37°C in 200 to 300 μL of TE (10 mM Tris-HCl, 0.1 mM EDTA), and quantified with a Beckman DU-64 spectrophotometer that calculates nucleic acids concentrations based on Warburg and Christian coefficients (Warburg and Christian 1942).

A number of PCR using varying quantities of template DNA (2.5 to 200 ng), *Taq* DNA polymerase (0.5 to 2.5 U), $MgCl_2$ (0.5 to 6.0 mM), and nonionic detergents (Nonidet P-40, Tween-20, and Triton X-100 at concentrations of 0.5 to 6.0%) and several temperature profiles were conducted to determine optimal reaction conditions for our preparation of scallop DNA. The final preparation of RAPD reactions was the same as described by Patwary et al. (1993), except that the Mg^{2+} concentration was increased to 2.0 mM and a 1:1 mixture of nonionic detergents (Tween-20 and Triton X-100; Sigma Chemical Co., St. Louis, MO) was added to a final concentration of 2%. The final primer concentration in the reaction mix, in this protocol, is 0.2 μM (Patwary et al. 1993). The detergents were mixed thoroughly in the reaction mix by vortexing for 20 to 30 s before adding DNA and *Taq* polymerase, to obtain optimal results.

A total of 222 primers were screened against one DNA sample (from a single individual) using the optimal reaction conditions described above. These RAPD primers were obtained from Dr. John E. Carlson, Biotechnology Laboratory, University of British Columbia UBC, Canada. The amplification was performed in a Gene Amp PCR System 9600 (Perkin Elmer Cetus, Norwalk, CT) thermocycler programmed for 40 cycles: four initial cycles of 3 minutes each at 94, 36, and 72°C; followed by 35 cycles of 30 seconds at 94°C, 1 minute at 36°C, and 2 minutes at 72°C; and a final amplification of 1 cycle of 30 seconds at 94°C, 1 minute at 36°C, and 10 minutes at 72°C. One-third of each reaction product was separated by electrophoresis at 3 to 4 Vcm^{-1} in 1.4% agarose/TBE (Tris borate EDTA) gels and stained by mixing ethidium bromide with TBE electrophoresis buffer to a final concentration of 0.2 $\mu g/ml^{-1}$. Assessment of the RAPD markers was subjective in that information contained in positive primers, that is, ones producing visible banding patterns, was reduced to those bands that were highly visible and readily scoreable (e.g., bands depicted with arrows on Fig. 5).

RESULTS

Amplification products were obtained in all reactions containing 2.5 to 200 ng of template DNA per 25 μl of reaction, but the number and amount of products were greater in reactions containing over 40 ng of DNA (Fig. 2). Concentrations of $MgCl_2$ between 2.0 and 3.0 mM were found to be most satisfactory; those below 1.0 mM failed to amplify the DNA and concentrations over 3.0 mM produced one or more secondary bands. The addition of either one or a mixture of two or more nonionic detergents (Nonidet P-40, Tween-20, and Triton X-100) to the reaction mix significantly improved the yield of RAPD products (Fig. 3). Amplification occurred in all detergent concentrations (0.5 to 6.0%) examined. Amplification was not inhibited significantly by the addition of detergents even at 6.0%, but the yield was generally better in reactions with 1.0 or 2.0% detergents. Vortexing the detergents after addition to the reaction mix was necessary to obtain satisfactory results. The optimal *Taq* concentrations were found to be 0.75 or 1.0 unit per reaction. The yield of amplified products was insufficient when less *Taq* was used, whereas more than 1.0 U of *Taq* per reaction caused the accumulation of nonspecific background products. The reproducibility of RAPD profiles was improved when four initial cycles with longer denaturation, annealing, and extension periods were used before the regular cycles.

One hundred thirty RAPD primers were found to yield visible amplification products. The G + C content of these primers is given in Table 1. Forty of these positive primers were arbitrarily

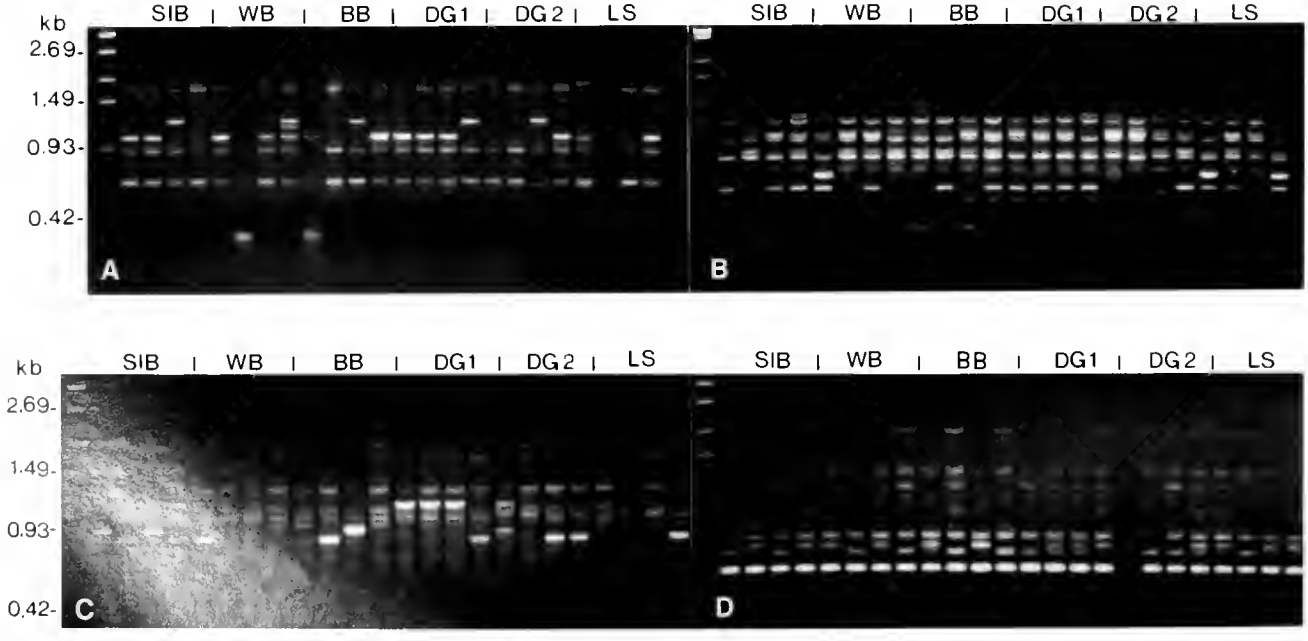


Figure 4. Variable RAPD profiles in the sea scallop. Each lane represents a different animal. The beds from which animals were collected are labelled as SIB (Sable Island Bank), WB (Western Bank), BB (Browns Bank), DG1 (Digby Gut, Bay of Fundy), DG2 (Digby Gut, Bay of Fundy), and LS (Lurcher Shoal, Bay of Fundy). The reactions were performed with primer (A) UBC No. 67, (B) UBC No. 81, (C) UBC No. 171, and (D) UBC No. 205. The left most lane of each set A–D contains size markers as in Fig. 2; kb, kilobase.

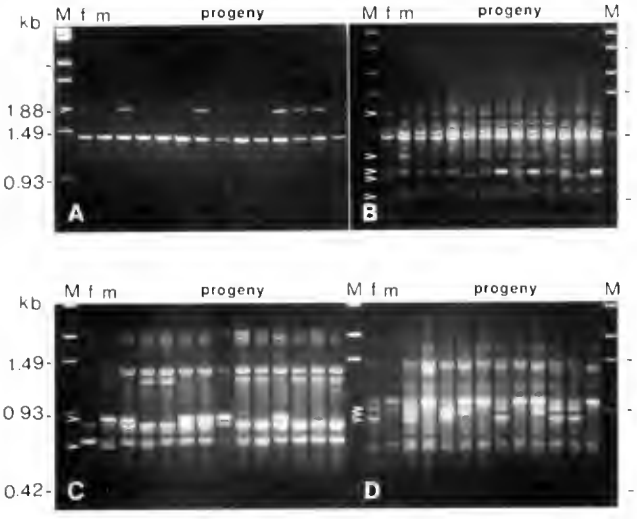


Figure 5. Segregation of RAPD markers (polymorphic bands) generated by single primers (A) UBC No. 25, (B) UBC No. 77, (C) UBC No. 15, and (D) UBC No. 145. The lanes labelled "f" and "m" identify DNA from the female and male parent, respectively. The remaining lanes in each subpanel represent progeny obtained from the mating of "f" and "m." The arrows indicate the positions of segregating loci. Lane M contains size markers as in Fig. 2; kb, kilobase.

selected and used to compare RAPD profiles among 24 individuals from different scallop beds (Table 2). At least 15 primers revealed clear polymorphisms. An example of polymorphism revealed with four of these primers is given in Figure 4. The reactions were repeated, and the polymorphisms were found to be stable and characteristic of each individual animal.

Mendelian inheritance of RAPD alleles was examined by the analysis of banding patterns from a pair-mated family. Thirty of the original positive primers, including the 15 that revealed polymorphisms above, were screened against the parents of this family in order to identify polymorphisms between the parents that could then be examined in the progeny. Twenty of the primers either produced bands that did not differ between the two parents or were too close in size to allow for reliable identification. Ten primers were suitable for progeny analysis. These 10 primers revealed polymorphisms in a total of 21 loci. Almost all alleles in these loci segregated in a Mendelian fashion (Fig. 5; Table 3). Heterozygosity cannot be determined from the banding pattern itself, because RAPD amplification produces one band when amplifying from one or both chromosomes. However, analysis of the progeny identified families in which one parent was homozygous negative and the other was positive/negative heterozygous (e.g., 15–1.2) and families in which both parents were heterozygous (e.g., 77–0.7).

DISCUSSION

One of the problems associated with the RAPD technique has been the reproducibility of bands, particularly, the minor or faint bands (Penner et al. 1993). We have been able to improve the reproducibility of scallop RAPD profiles by optimizing the concentration of reaction ingredients and cycling parameters. The satisfactory application of this technique needs considerable care in preparing reactions to reduce the possibility of contamination from extraneous DNA. The presence of impurities, such as polysaccharides and phenolic compounds, also seriously impairs RAPD performance. Our initial attempts to obtain reproducible RAPD profiles of scallop DNA were mostly unsuccessful. After optimizing reaction conditions and adding nonionic detergents to the reaction mix, the quality of RAPD profiles improved significantly. The addition of detergents appears to enhance the specificity of primer binding with template DNA and at least partially suppresses the effect of impurities. Using these reaction conditions, we have also obtained reproducible RAPD profiles in mussels and lobsters.

In order for the markers identified here to be useful in genetic studies, it must be demonstrated that the bands are heritable and segregate from generation to generation in a Mendelian fashion as in other organisms (Carlson et al. 1991, Echt et al. 1992, Kazan et al. 1993). This has been demonstrated for 10 of the RAPD markers (21 loci) used on sea scallop DNA in this study.

One disadvantage of RAPD polymorphisms compared with allozymes, complementary DNA (cDNA) probes, and microsatellites is that the heterozygote cannot be distinguished from the positive homozygote. The PCR will produce the same product from the DNA of an individual that has twice one site to which the primer binds (a positive homozygote) and from an individual that has the binding site once (a positive/negative heterozygote). The negative homozygote is identifiable by the absence of the band. In pair-mating crosses, this presents no major problem, because the genotypes of the parents can be inferred from the genotype ratios among the offspring. In population surveys, frequencies for the

TABLE 3.

Segregation of RAPD alleles in a pair-mating progeny and χ^2 values for expected Mendelian ratios.

RAPD Allele	Phenotype		Markers in Offspring		Expected Ratio[a]	χ^{2b}
	Female	Male	Present	Absent		
15-1.2	−	+	7	5	1:1	0.33
15-0.8	−	+	5	7	1:1	0.33
15-0.5	−	+	3	9	1:1	3.00
25-1.88	+	−	6	6	1:1	0.00
77-1.2	+	−	6	6	1:1	0.00
77-0.8	+	−	2	10	1:1	5.33[c]
77-0.7	+	+	10	2	3:1[d]	0.44
77-0.6	+	−	4	8	1:1	1.33
137-0.8	+	−	8	3	1:1	2.16
145-0.94	+	−	7	4	1:1	0.83
145-0.92	−	+	4	7	1:1	0.83
145-0.9	+	−	5	6	1:1	0.17
150-0.65	−	+	4	8	1:1	1.33
150-0.55	+	−	12	0	1:0[e]	0.00
167-0.4	+	−	7	5	1:1	0.33
171-1.2	+	−	3	9	1:1	3.00
171-1.1	−	+	9	3	1:1	3.00
171-0.9	+	−	6	6	1:1	0.00
205-0.6	−	+	8	4	1:1	1.33
205-0.5	+	−	12	0	1:0[e]	0.00
212-0.7	−	+	8	3	1:1	2.16
212-0.6	+	−	4	7	1:1	0.83

[a] Expected ratios for progeny on the basis of dominant marker phenotype.
[b] χ^2 at p = 0.05 and df (degrees of freedom) = 1 is 3.84.
[c] Significant deviation from the expected ratio.
[d] Both parents are assumed to be heterozygous at this locus.
[e] Female assumed to be either homozygous dominant or the allele is sex linked.

positive and negative allele at a RAPD "locus" have to be estimated by assuming Hardy-Weinberg equilibrium.

Repeated applications of the RAPD method to a number of individuals from different populations showed that polymorphisms are stable. Although we were not able to detect any RAPD markers that were unique to a population, the results indicate that the frequency of polymorphic bands at different loci can be estimated and used as an additional tool to understand the genetic structure of sea scallop populations. As in most other species, it appears that no single set of markers will suffice for the genetic characterization of stocks of scallops. Allozymes tend to inflate the genetic similarity of populations, because stabilizing selection tends to establish the same equilibrium frequencies in all populations (Karl and Avise 1992). Multibanded minisatellite profiles are not amenable to population genetic analysis because they distinguish variation at the level of the individual. Mitochondrial variation can be very useful for population discrimination (Moritz et al. 1987), but normally, mitochondrial DNA restriction fragment length polymorphism is not abundant and one has to combine PCR amplification and sequencing of the amplified product. Microsatellite assays are expensive to develop and produce polymorphisms that are more useful for the characterization of individuals rather than populations. cDNA probes (Pogson and Zouros 1994) are also expensive to develop but, once developed, have the potential to be very useful tools for population discrimination. RAPDs are much less expensive, but each primer alone produces only a limited amount of information, so that a multiple set of primers must be used (Welsh and McLelland 1990). Experimentation with amplification using smaller primers (5 mers) may produce more variability, but at the cost of a more elaborate detection protocol (Caetano-Anolles et al. 1991a). The final discrimination of scallop populations may have to rely on a combination of such tools. The most useful combination can be determined only through pilot analyses of representative scallop populations.

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SEASONAL GROWTH MODELS FOR GREAT SCALLOPS (*PECTEN MAXIMUS* (L.)) AND QUEEN SCALLOPS (*AEQUIPECTEN OPERCULARIS* (L.))

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ABSTRACT The growth in shell length of two northeast Atlantic scallop species, *Pecten maximus* and *Aequipecten opercularis*, oscillates seasonally. The weights of the marketable parts of these scallops (the gonad and adductor muscle) are also seasonally variable; the weight of the adductor muscle decreases during the winter, and the gonad weight decreases during the protracted summer spawning period. In *P. maximus* > 4 years old and *A. opercularis* > 2 years old, the amplitude of seasonal change in total edible yield from each scallop exceeds mean annual increments. The growth in shell length of both species is modeled with the von Bertalanffy growth function (VBGF), incorporating a sine-wave function to account for the seasonal fluctuations. The growth of the adductor muscle, gonad, and total edible yield is described by combining the seasonalized VBGF for length with seasonalized length-weight relationships.

KEY WORDS: *Pecten maximus*, *Aequipecten opercularis*, seasonal growth, von Bertalanffy, length-weight

INTRODUCTION

The growth of most bivalves from temperate waters, including *Pecten maximus* (L.) and *Aequipecten opercularis* (L.), shows strong seasonality (Comely 1974, Antoine et al. 1979, Taylor and Venn 1979). In the North Irish Sea, the shells of these pectinids show annual growth checks that are associated with the cessation of growth during the winter months (November–March: Mason 1958, Soemodihardjo 1974, Dare and Deith 1991, Allison et al. 1994). Seasonal changes in weight are particularly evident in the gonad, due to spawning and gametogenesis, and in the adductor muscle, which acts as an energy store during the winter months when food supplies are limited (Le Pennec et al. 1991, Thompson and MacDonald 1991). The gonad and adductor muscle are the commercially marketable portions of these pectinids, so that the yield of "meat" from each scallop will vary according to the season. Because these species are of considerable commercial importance in the North Irish Sea and elsewhere in Europe (see reviews by Ansell et al. 1991 and Brand et al. 1991a), explicit consideration of seasonal growth patterns is required to refine yield-based stock assessments and to determine optimal timing of seasonal fishery closures with regard to maximizing the yield from the fisheries.

Seasonal cycles of the somatic and reproductive tissues in pectinids have normally been described with reference to a "standardized animal," with shell length being the standardizing variable (e.g., Ansell 1974, Comely 1974, Taylor and Venn 1979); there have been no previous attempts to incorporate seasonal cycles into growth curves for the edible portions of scallops. Seasonal fluctuations have, however, been previously accounted for in modeling the growth in shell length of French stocks of *P. maximus* (Buestel and Laurec 1976, Antoine et al. 1979).

The von Bertalanffy growth function (VBGF) is the basic model chosen to describe the growth in both length and weight. The VBGF is still the standard model in fishery-orientated growth studies and has previously been shown to give a good fit for shell growth of both *P. maximus* and *A. opercularis* (Orensanz et al. 1991, Ansell et al. 1991, for reviews). The VBGF for weight has also been shown to provide a good fit to the annual growth of the adductor muscle and the total edible yield in these populations (Allison 1993).

The linkage between seasonal temperature fluctuation and growth was pioneered by Ursin (1963), who incorporated temperature in the VBGF and found that the sinusoidal seasonal temperature fluctuations corresponded with the seasonality of the growth pattern. The seasonality of growth has since been simulated by incorporating a sine-wave function in the VBGF (e.g., Pitcher and MacDonald 1973, Cloern and Nichols 1978, Pauly and Gaschultz 1979, Antoine et al. 1979, Hanumara and Hoenig 1987, Somers 1988, Soriano and Jarre 1988, Hoenig and Hanumara 1990).

The recent literature abounds with studies of the fitting of seasonally oscillating VBGFs for length, thanks to the incorporation of this growth curve in recent versions of the widely used ELEFAN routine for extracting growth parameters from length-frequency data (Gayanilo et al. 1987). However, there is little work on seasonal patterns of growth in weight. This is despite the fact that the seasonal patterns of growth in weight are of potentially greater importance. Only Shul'man (1974) has presented growth functions that incorporate seasonal patterns of weight loss and gain. Recent interest in this problem has led Sparre (1991) to present a method of yield per recruit (Y/R) analysis (Thompson and Bell, 1934; Beverton and Holt, 1957) that incorporates seasonality of growth and mortality. Sparre (1991) suggests using empirically determined weights at age, collected at monthly intervals, in a Thompson-Bell type Y/R equation, or combining a seasonalized VBGF for length with season-specific length/weight equations to calculate weights.

In this article, I aim to generate model-based estimates of weight at age for incorporation into subsequent stock assessments. To achieve this aim, I have fitted seasonalized VBGFs to scallop length-at-age data and used a seasonalized model of the length-weight relationship to fit a growth model to the weight at age of the edible portions of the scallop. This growth model accounts for the seasonal changes in yield that occurs as the result of spawning and to the use of the adductor muscle as an energy store during the winter.

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MATERIALS AND METHODS

Sample Collection

Samples of *P. maximus* and *A. opercularis* were collected with "Newhaven" spring-loaded toothed dredges fished from the University of Liverpool's 15 m stern-trawler, the R.V. "Cuma." Additional samples of *A. opercularis* were collected from onboard samples of the catches of commercial vessels fishing with both dredges and otter trawls. The samples of *P. maximus* were taken from an inshore (Bradda Head) and offshore (South-east Douglas) fishing ground (Fig. 1), from January 1987 to October 1988 at approximately monthly intervals. Monthly samples of *A. opercularis* from SE Douglas were also taken during this period. *A. opercularis* were also sampled from commercial vessels fishing the East Douglas ground during the summer of 1988.

The shell length (anterioposterior axis) of all *P. maximus* in each sample was measured to the nearest millimeter with a scallop measuring board. *A. opercularis* catches were generally subsampled if >400 animals were caught. Both species were aged by means of the growth rings on the shell (see Allison et al. 1994).

Weight at age was measured by randomly subsampling approximately 100 to 200 scallops and/or queens from each sample. In addition to measuring shell length and age, adductor muscle and gonad weights (to the nearest 0.1 g) were determined with a Mettler 3000 PC electronic balance, after removing surface moisture by blotting with absorbent paper and leaving the scallops to air dry at room temperature for 1 hour.

Modeling Growth

The growth of the shell of both species was modeled using the von Bertalanffy growth equation (von Bertalanffy 1938 and 1964), modified to incorporate a term representing the seasonal pattern of growth.

$$L_t = L_\infty(1 - e^{-k(t-t_o)+S(t)}) \quad (1)$$

where:

L_t = length at age t
L_∞ = asymptotic length
k = Brody/Bertalanffy growth coefficient
t_o = age at zero length

$S(t)$ is the function describing the seasonal perturbation, derived from the equation for a simple sine wave (e.g., see Batschelet, 1981, p 159):

$$Y = M + A\cos((2\pi/T)\,\phi) \quad (2)$$

where Y = the variable which is oscillating
M = mesor, or mean value of Y
A = amplitude of oscillation of Y
T = period of oscillation
ϕ = acrophase ($t - t_s$, where t_s defines the beginning of the sine wave).

There are several formulae for $S(t)$ in the literature. The version used in this study, developed by Pauly and Gaschultz (1979), modified by both Somers (1988) and Hoenig and Hanumara (1990), gives the best description of the seasonal oscillation with respect to the unbiased estimation of t_o:

$$S(t) = \frac{kC}{2\pi}\sin 2\pi(t - t_s) - \frac{kC}{2\pi}\sin 2\pi(t_o - t_s) \quad (3)$$

where C = amplitude of seasonal perturbation
t_s = time of start of oscillation relative to t_o
k = the Brody/Bertalanffy growth coefficient

In fitting the model to the data, an ordinary VBGF was first fitted to the raw length-at-age (in months) data to obtain initial estimates of the parameters L_∞, k, and t_o. The model was fitted to the data by the use of the nonlinear regression (NLR) procedure in SPSSX, which fits data to user-defined models iteratively using the Levenberg-Marquadt algorithm (SPSSX, 1988). The procedure requires initial estimates of the parameters L_∞, k, and t_o, but, in simple models, is robust with respect to them, converging to the same solution from a wide range of starting values. The fitted ordinary VBGF was then subtracted from the data, and the residuals of the fit of the data to the ordinary VBGF, which should lie on a sine wave of constant mesor and period of 1 year, were examined to obtain starting values for the parameters C and t_s. The seasonal model [equation 1, where $S(t)$ is defined in equation 3] was then fitted to the raw length-at-age data by the use of the SPSSX-NLR procedure. Good initial estimates of the parameters are required to fit this more complex model. The youngest age class in the *P. maximus* samples was not used to fit the equation, because it is likely that only the larger fraction of the length distribution would be retained by the size-selective fishing gear.

The data set for *A. opercularis* from the East Douglas ground was limited. Seasonal oscillations were readily discernible, although the many missing months made it difficult to fit a seasonal curve without the prior assumption of growth cessation during the winter. The seasonal VBGF was fitted by constraining C to 1 to simulate the winter cessation of growth. The curve was fitted using the constrained nonlinear regression procedure (CNLR) in SPSSX. Because the two procedures (NLR and CNLR) use different algorithms and convergence criteria, direct comparison of parameter estimates from the SE Douglas and E Douglas grounds should be undertaken with caution.

The seasonal patterns of growth of the edible portions of the scallop (gonad and adductor muscle) were modeled by converting the seasonally oscillating growth curves for length to weight using

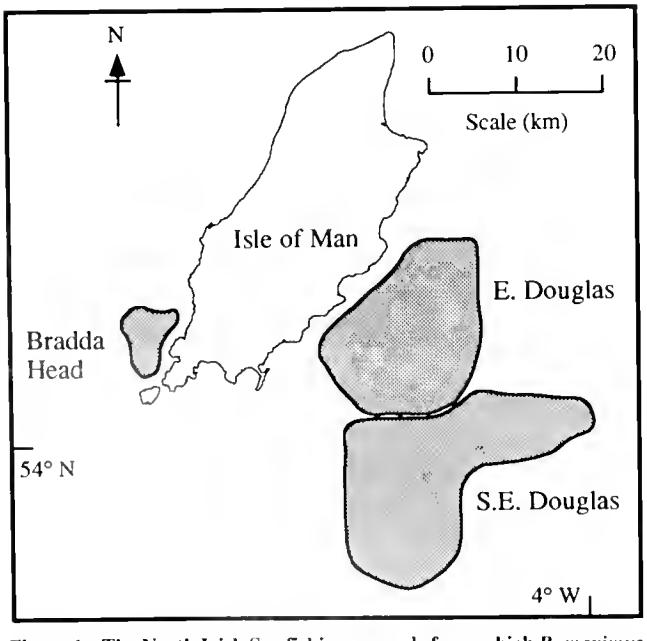

Figure 1. The North Irish Sea fishing grounds from which *P. maximus* and *A. operculoris* were sampled.

season-specific length-weight relationships. Age was expressed in months (entered as decimal fractions of a year). The computational steps were as follows:

1. For each sample date, geometric mean (GM) regression parameters of the relationship between shell length and adductor muscle and between shell length and gonad weight were calculated from the linearized allometric length-weight equation (Ricker 1975a).

$$\ln W = a + b(\ln L) \quad (4)$$

GM regression, rather than ordinary least-squares regression, was chosen for computation of the relationship between these two variables, because they have a functional, rather than a dependent relationship (see Ricker 1973 and 1975a, Saila et al. 1988, for methods of fitting and calculating errors on the model coefficients, and Ricker 1975b, Laws and Archie 1981, for discussion).

2. In order to smooth variability in the data and calculate length/weight relationships for times of year not sampled, a model was then fitted to the calculated values of the exponent (b) and the intercept (a) of the length-weight relationship. The equation for a sine wave (equation 2) was chosen to model the seasonality of the regression parameters and was fitted by least-squares regression using the Quasi-Newtonian search algorithm in the NLR module of SYSTAT (Wilkinson 1990). Starting values for mesor, amplitude, and t_s are easily obtained by visual inspection of the data.

No model was fitted to the length/gonad weight regression parameters for *A. opercularis* from SE Douglas, because the parameters did not show an obvious seasonal cycle. The analysis for these data therefore terminated at this step.

3. Model parameters from step 2 provide smoothed length-weight regression parameters. These were used to convert monthly length-at-age values (predicted from the seasonally oscillating VBGF for length) to predicted weight-at-age values for gonads and adductor muscles from:

$$W_t = a + A_a \cos((2\pi/T)\phi_a) L_t^{(b + A_b \cos(2\pi/T)\phi_b)} \quad (5)$$

where a = the intercept of the functional length-weight regressions equations

A_a = the amplitude of seasonal fluctuation of a (from equation 2)

ϕ_a = acrophase of seasonal pattern of a (from equation 2), and b, A_b, ϕ_b are the equivalent parameters for the slope of the length-weight regression equations.

This model was found to fit the growth pattern of the adductor muscles in all cases and was also applicable to the growth of *A. opercularis* gonads in samples from the SE Douglas ground. The annual increase in weight of gonads was slow in *P. maximus* from Bradda Head beyond the age of 5 years; no annual increase in the weight of gonads was observed in *P. maximus* more than 8 years old from SE Douglas, whereas both shell length and adductor muscle weight continue to increase slowly over the whole age range sampled. This led to predicted gonad weights that fit observed data well in younger scallops, but both mean weight and the amplitude of annual fluctuations were overestimated in older scallops. In order to correct these errors and obtain realistic values of predicted total yield, post hoc modifications to models of gonad weight growth were applied. In order to simulate the changes in weight of the gonads of the older scallops on the SE Douglas ground, a sine-wave equation of constant amplitude (equation 2) and constant mesor (M) was fitted directly to data on weight at age of these gonads. In the case of *P. maximus* from Bradda Head, gonad weight continued to increase, approximately linearly, over the age range from 5 to 11 years, so the mesor (M) in equation 2 was modified to give:

$$GWT = (c + dt) + A \cos(2\pi/T)\phi \quad (6)$$

where c and d are the intercept and slope of the linear regression of mean gonad weights on age, between ages 5 and 11.

4. To obtain predicted total edible yields, model-predicted gonad and adductor muscle weights were summed.

Weight data and length-weight relationships calculated from samples taken during 1987 and 1988 were pooled to give a single series of data covering most months of the year. Mean gonad weight and mean adductor muscle weights at age were plotted with model-predicted growth curves to provide a visual assessment of the fit of the model.

RESULTS

Growth curves fitted to shell length at age of monthly samples of *P. maximus* from the Bradda and SE Douglas grounds show a seasonal pattern, with growth occurring in a series of steps: a plateau corresponding to the winter cessation in shell growth (October to March), exponentially increasing length in spring/early summer, and slowing down through August/September to reach another asymptote the following winter.

The pattern is only clearly visible in scallops aged 2 to 5 years in the Bradda Head samples (Fig. 2a). Between-sample variability masks the seasonal pattern in older age classes, where the annual increment is much less and sample sizes are smaller. The SE Douglas samples show visible seasonal growth fluctuations up to age 8+ (Fig. 2b), reflecting the different growth pattern on this ground, where successive growth increments decrease in size more slowly (lower von Bertalanffy k value). The fit of the seasonal VBGF is good on both grounds, and the pronounced seasonal fluctuation is reflected in the high values of the seasonality parameter C (Table 1). The fact that C exceeds unity produces an apparent slight decrease in length during the winters. This is because of the mathematical form of the curve, which simulates a waveform rather than a series of flat-topped discontinuities.

A. opercularis from SE Douglas monthly samples showed strong seasonality (Fig. 3a). The data set from the East Douglas ground was more limited but less variable, and the seasonal oscillations are also readily discernible (Fig. 3b), although the many missing months make it difficult to fit a seasonal curve without the prior assumption of growth cessation during the winter.

The slopes of the length-weight relationships (b) show considerable variability and large standard errors (Figs. 4 and 5). This is not unexpected; the weight of individual scallops is variable, and wet weight is difficult to measure accurately and consistently. Short-term variability, especially of gonads during summer spawning periods, also accounts for inconsistencies between successive samples. The pooling of 2 years' data may also increase variability, but was considered necessary in order to obtain data for as many months of the year as possible and to increase the likelihood that values were generally applicable, rather than specific to a single year. Despite the variability, an underlying pattern of seasonal fluctuation can be detected, and separate sine-wave models have been fitted to the monthly values of b from each set of samples: the intercept a is simply the inverse correlate of b. The models provide reasonable fit to all but the calculated length/gonad weight parameters for *A. opercularis* from SE Douglas (Fig. 4).

For *P. maximus*, the model predicts the maximum values of the

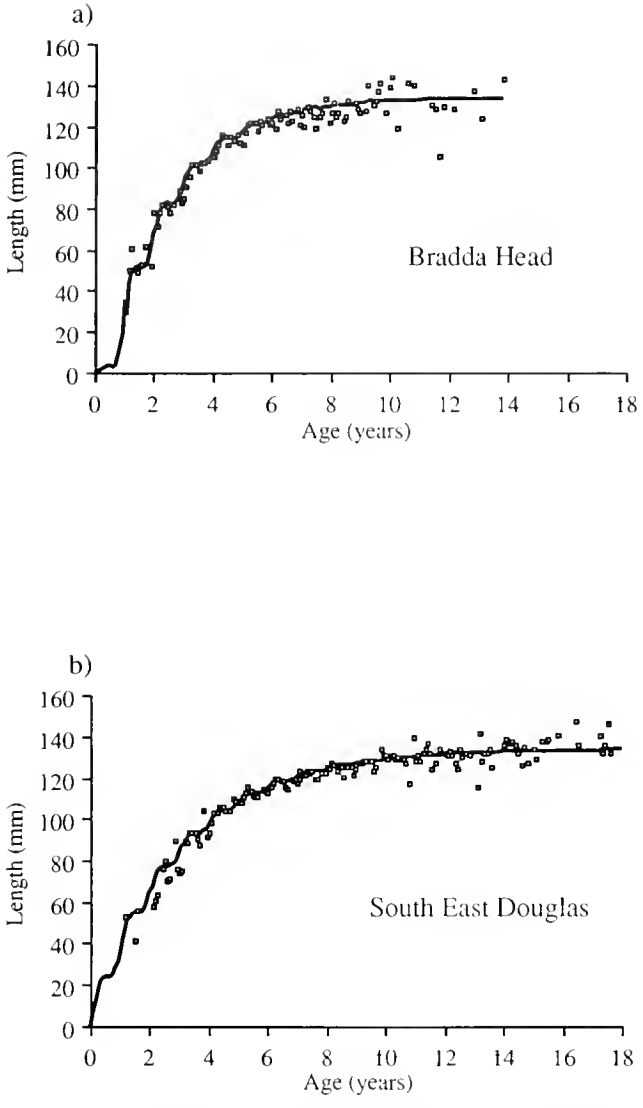

Figure 2. VBGF with seasonal oscillation fitted to *P. maximus* length-at-age data from (a) the Bradda Head fishing ground, and (b) the SE Douglas ground. Ages (to the nearest month) were calculated from the number of growth rings and date of sampling, assuming a July 1st "birthday."

exponent (*b*) of the length/adductor muscle weight and length/gonad weight relationships to occur in midwinter (early December in scallops from SE Douglas, early January in scallops from Bradda Head), with the minima there occurring in May and June, respectively (Fig. 4a). *A. opercularis* from the SE Douglas ground have a maximum exponent of the length/adductor muscle weight relationship in late February (Fig. 4b) and a minimum in August. The parameters of the length/gonad weight equation for *A. opercularis* from SE Douglas do not show clear seasonality (Fig. 4b), so no sine-wave model has been fitted.

Seasonal data for *A. opercularis* from the East Douglas ground are limited, but a sine-wave model can be fitted to provide monthly estimates of the length/weight parameters and to enable a seasonally oscillating model to be fitted to the observed weight-at-age data (Fig. 4b). The parameters of the fitted sine-wave models shown in Figure 4 are given in Table 2.

The growth of *P. maximus* gonads and the growth of adductor muscles in samples taken from Bradda Head (Fig. 5a) and SE Douglas (Fig. 5b) both show strong seasonality. Model-predicted values generally showed good agreement with observed mean weight-at-age data for adductor muscles. There are slight overestimations of adductor muscle weights of scallops aged 2 to 2.5 years from the Bradda Head ground, and underestimation of the weight of adductor muscles of scallops from SE Douglas of ages 4.9 to 5.3 years. Data for the mean weight of muscles and gonads from older year classes show more scatter than for younger year classes, and the fit of the predicted curves is poorer for ages beyond 6 in Bradda Head scallops and 10 in SE Douglas scallops.

Although fitting the models to growth of adductor muscles was relatively straightforward, fitting the model to gonad data was more problematic, because the exponent of the length-weight equation is generally high (5 to 9), reflecting rapid increase in gonad weight with little increase in length as the scallop or queen scallop attains maturity, when further growth of gonads proceeds more slowly. Beyond the age of 5 years, the growth of the gonads in *P. maximus* from Bradda Head is slow (Fig. 5a), and no annual increase in the weight of gonads is observed in *P. maximus* more than 8 years old from SE Douglas (Fig. 5b).

The gonad shows clear seasonality in weight gain and loss, with annual weight maxima being reached in March/April and minima in September, after the autumn spawning. The adductor muscle weights reach annual maxima in October and minima in May.

The seasonal cycles of weight change in adductor muscles and gonads vary out of phase (approximately 160°), and the seasonality of total yield is thus dampened to some extent (Fig. 5a and b). For *P. maximus* from Bradda Head, the annual maxima occur in February to March, while gonad weights are increasing and close to the maxima and adductor muscle weights are decreasing. Lowest yields are found in September, when the gonad is generally spent and the adductor muscle is still increasing in weight. Between the ages of 3.0 and 4.6 years, when the yield is increasing most rapidly and the scallop is becoming vulnerable to exploitation, there is little or no seasonal decrease in yield. However, a period of cessation in growth of yield does occur in scallops of between 3.2 and 3.75 years old. In older scallops, there is marked seasonality in total edible yield per scallop.

P. maximus from SE Douglas show a similar seasonal pattern in growth of the edible yield portions (Fig. 5b). Maxima occur in March; minima occur in September. Seasonality of edible yield is damped up to age 6 years, but still shows cessation of growth over periods of up to 6 months in each year. Above age 6, there are significant annual decreases in yield. These are worthy of consideration on this fishing ground, where, at the time of sampling, over 50% of the exploitable population was aged 6 years or over (Allison 1993).

The limited data set for *A. opercularis* from the East Douglas fishing grounds (Fig. 6a) indicates an increase in both muscle weight and gonad weight during the late spring and early summer. Gonad weights reach maxima in July to August, and adductor muscle weights peak in September to October. Total yield is maximized in September. Predicted gonad weights are at their minima in January, although the data suggest that this minima is in fact reached 3 months earlier. Gonad weights probably do not increase and decrease sinusoidally, which will account for the inability of the model to simulate rapid build-up of gonads and sharp decrease due to spawning. Adductor muscle weights are better predicted by the model for *A. opercularis* from both the East and SE Douglas fishing grounds (Fig. 6).

A. opercularis from the SE Douglas ground (Fig. 6b) show

TABLE 1.

Parameters of seasonally oscillating von Bertalanffy growth models fitted to *P. maximus* and *A. opercularis* length-at-age data by nonlinear least-squares regression.

Species and Area	n	r^2	L_∞ (mm)	k	t_o	t_s	C
P. maximus, Bradda	4120	0.807	133.68	0.466	0.280	0.524	1.192
P. maximus, SE Douglas	2758	0.805	133.92	0.329	0.009	0.558	1.049
A. opercularis, SE Douglas	3153	0.637	75.42	0.696	−0.149	0.428	1.356
A. opercularis, E Douglas[a]	4927	0.856	77.05	0.678	−0.189	0.665	1.000

[a] Seasonality parameter (C) constrained to 1 for model fitting.

strong seasonal cycles in adductor muscle weight, the seasonal change in weight exceeding the annual growth increment in queen scallops older than 24 months. Maxima occur in September to October; minima occur in February to March. The model tends to underestimate muscle weights of *A. opercularis* aged 2 to 2.5. The gonad cycle is more variable than that of *P. maximus*, possibly because of multiple spawning peaks and rapid recovery of gonads in this species. It was not possible to simulate seasonal changes in gonad weight (and therefore in total yield) from these data by the modeling approach applied to *P. maximus* and to *A. opercularis* from the East Douglas ground. Gonad weight is generally higher in spring and early summer, with lower adductor muscle weights during the same period. The gonad makes up a relatively small proportion of the yield, and the yield will therefore follow the pattern of adductor muscle growth closely. Maxima occur in August and September; minima occur in February.

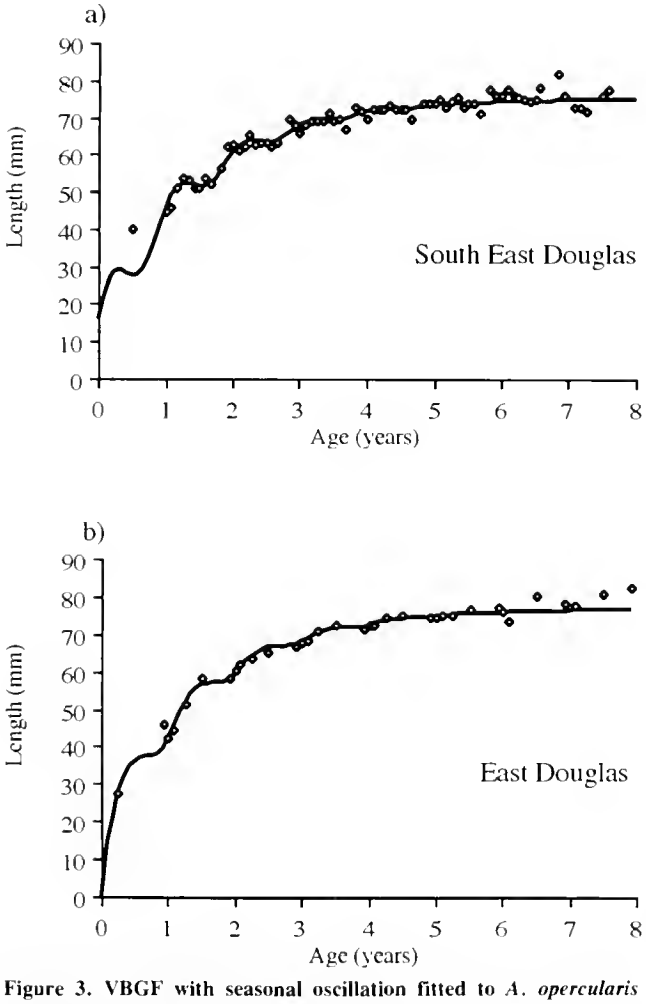

Figure 3. VBGF with seasonal oscillation fitted to *A. opercularis* length-at-age data from (a) the Southeast Douglas fishing ground, and (b) the East Douglas ground. Ages (to the nearest month) were calculated from the number of growth rings and date of sampling, assuming a July 1st "birthday."

DISCUSSION

The seasonality of growth in pectinids, correlated with annual water temperature cycles, is well known (Ansell 1974, Comely 1974, Taylor and Venn 1979). Growth of the shell and increase in body mass cease during the winter months, due either to the effects of lower temperature on metabolic rate or to insufficient food during the winter to maintain metabolic requirements (Broom and Mason 1978). The resumption of growth of the shell and increase in total weight each spring is triggered by rising temperature or increase in food supply after the spring phytoplankton bloom (Broom and Mason 1978, Vahl 1980).

The VBGF with seasonal perturbation provides a good fit to length-at-age data for both species. A slight decrease in length over winter, rather than an extended period of growth cessation, is predicted by the form of the curve, which incorporates a sine wave. Sine-wave models of this type have previously been used to model growth of *P. maximus* in French stocks (Buestel and Laurec 1976, Antoine et al. 1979). Pauly et al. (1992) have developed a seasonal growth model that incorporates a period of zero growth, rather than a simple sine wave; their model may provide a slightly improved fit to scallop length-at-age data than the standard sine-wave model used here.

Models for seasonal growth cycles of the gonad and adductor muscle also fit well. The two constituents of the yield vary 160° out of phase; whereas the gonad weight decreases because of spawning in the summer months, the adductor muscle is increasing in weight. During the winter, the adductor muscle loses weight and the gonad gradually fills, reaching a maximum weight in the spring. The large adductor muscle acts as the main storage site for metabolic reserves in *P. maximus*, and weight loss in winter occurs as the result of mobilization of reserves to contribute to metabolic requirements and development of the gonad (Le Pennec et al. 1991, Faveris and Lubet 1991, Barber and Blake 1991, for reviews). *A. opercularis* adductor muscle weights show the same pattern of seasonal fluctuation, and it is likely that the adductor muscle performs the same function as in *P. maximus*.

Spawning of *P. maximus* takes place in the summer months (June to August). Only one major spawning per year has been observed in this study, and the gonad growth models account for only one, although a more detailed study in the same area (Mason 1958) has indicated two spawning peaks may sometimes occur.

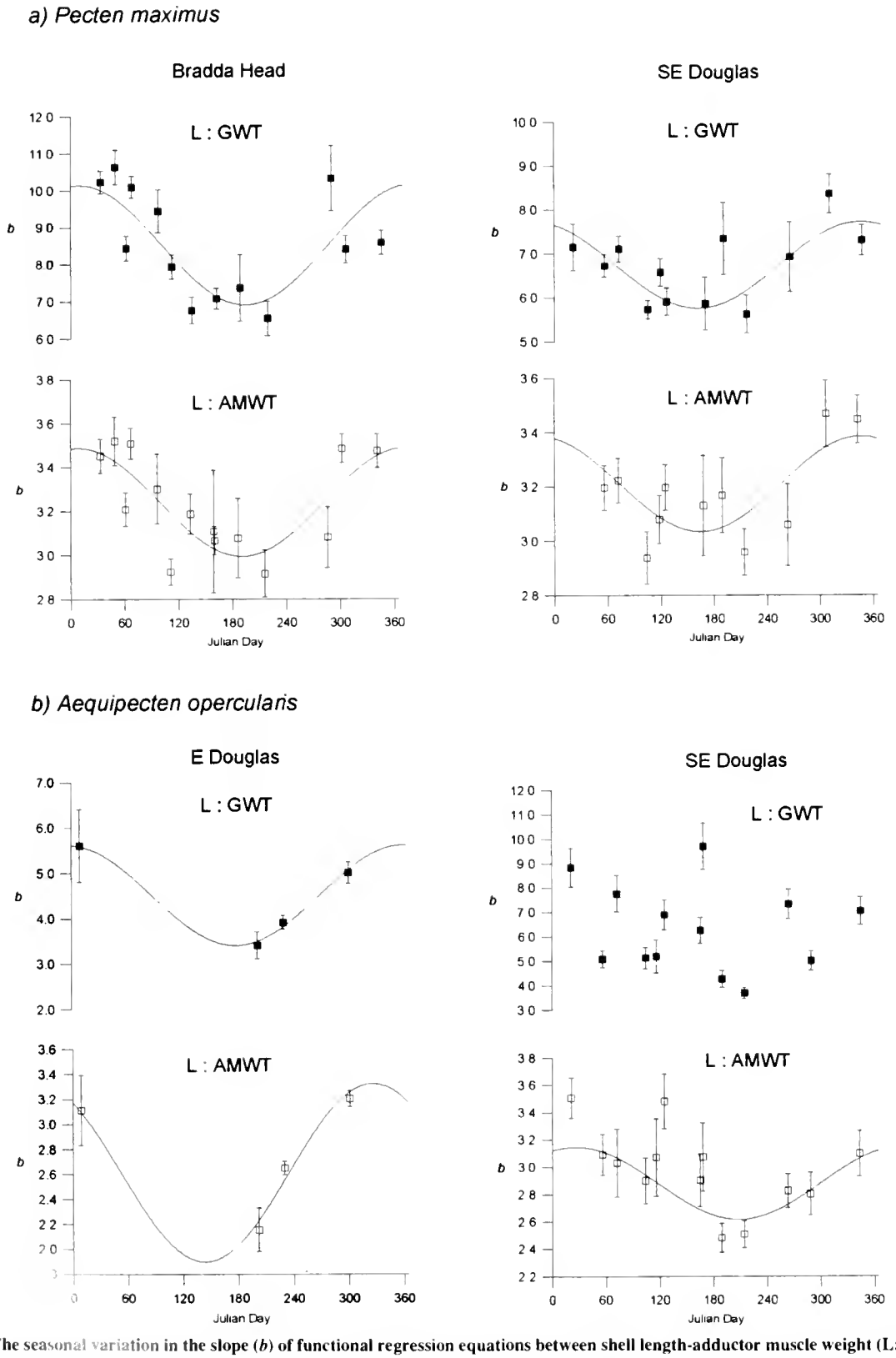

Figure 4. The seasonal variation in the slope (*b*) of functional regression equations between shell length-adductor muscle weight (L:AMWT) and shell-length:gonad weight (L:GWT) of (a) *P. maximus* from the Bradda Head and SE Douglas fishing grounds and (b) *A. opercularis* from the E and SE Douglas fishing grounds. Standard errors of the functional regression parameters are shown, and sine-wave models are fitted to each data set, with the exception of L:GWT of *A. opercularis* from SE Douglas, where data were too variable.

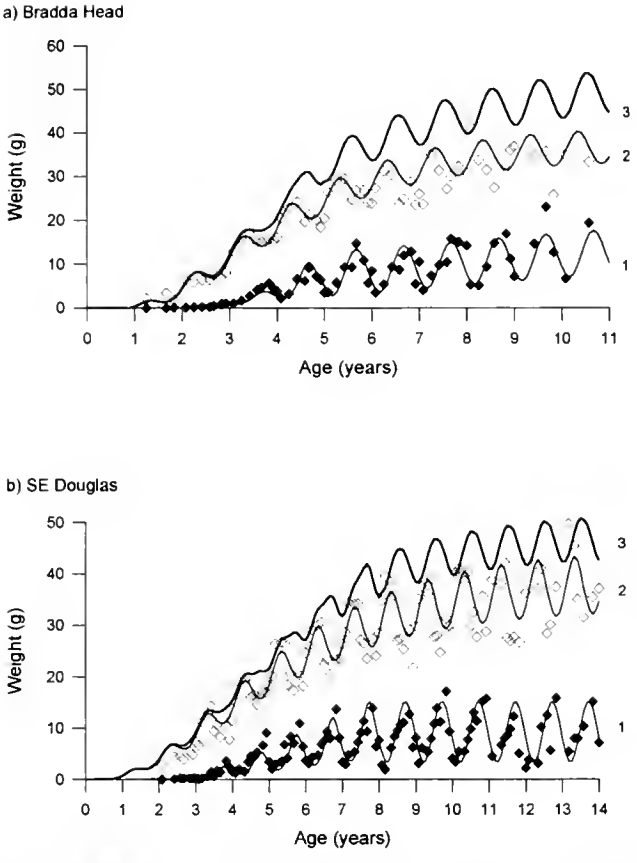

Figure 5. Mean gonad (closed symbols) and adductor muscle (open symbols) weights at age of *P. maximus* from (a) the Bradda Head fishing ground and (b) the SE Douglas ground, with fitted models incorporating seasonal oscillations (1 = gonad weights, 2 = adductor muscle, 3 = total edible yield). Models for adductor muscle weights were VBGFs incorporating seasonal fluctuations (equation 5), as were models for gonad growth up to age 5 on the Bradda Head ground and age 8 on the SE Douglas ground. Gonad weights above age 5 on the Bradda Head ground were modeled by fitting equation 2 empirically, and gonads of scallops of age 8+ on the SE Douglas ground were modeled by fitting equation 6 empirically. Ages were calculated to the nearest month, assuming a July 1st "birthday."

Spawning in *A. opercularis* appears to occur sporadically, with rapid recovery of the gonad. There may be two or three peaks of spawning activity each year in *A. opercularis* from the North Irish Sea, all of which occur over the summer months (Aravindakshan 1955, Soemodihardjo 1974, Paul 1978, Duggan 1987). The simple sine-wave model used here is unable to simulate multiple spawning peaks occurring at irregular time intervals, but is able to describe the basic summer-winter cycle. In populations with more than one distinct spawning period, more complex models would be required to simulate the pattern of weight loss and gain of the gonad.

Beyond the age of 4 years, the seasonal fluctuation in the weight of both the adductor muscle and the gonad of *P. maximus* exceeds the annual growth increment; it is the dominant feature of the growth pattern of older scallops. The amplitude of seasonal weight fluctuations in the edible tissues of *A. opercularis* exceeds the annual growth increment after the second growth season. Because the minimum legal landing size of *P. maximus* in the North Irish Sea (110 mm shell length) and the minimum commercially acceptable size of *A. opercularis* (55 mm) are attained at the ages of 4 and 2 years, respectively (Brand et al. 1991a), these seasonal cycles in yield are of great importance to the fishery.

The models described here serve to highlight the importance of the seasonal cycle in the study of growth in scallops, but should be regarded as preliminary, rather than exemplary, approaches to the problem. Although the incorporation of seasonal cycles into growth curves for length is relatively straightforward by the use of established methods (Hoenig and Hanumara 1990, for review), hypothesis and goodness-of-fit testing suffer from the questionable assumptions made in the calculation of confidence limits after NLR analysis (Donaldson and Schnabel 1987, Cerrato 1990). The techniques used here to model seasonal weight changes require additional development. The models lack an error structure, so no objective goodness-of-fit criteria can be established. Direct fit of a seasonalized VBGF for weight by nonlinear least squares proved difficult; the high value of the exponent of the length/adductor muscle and length/gonad weight relationships in scallops caused successive iterations to diverge so widely that optimal solutions were seldom found. In choosing to fit the models in stages through calculation of length-weight relationships, complexity is increased, and the resultant number of parameters in the models is

TABLE 2.

Parameters of sine-wave models describing seasonal variability in the exponent and intercept of functional length-weight regression relationships for scallops (*P. maximus*) and queens (*A. opercularis*).

Species & Area	Exponent (b)					Intercept (a)				
	M_b	A_b	$t_{s(b)}$	r^2	ν	M_a	A_a	$t_{s(a)}$	r^2	ν
Length: adductor muscle weight										
Scallops, Bradda	3.24	0.247	8	0.640	13	−12.24	1.161	195	0.626	13
Scallops, SE Douglas	3.21	0.176	347	0.507	10	−12.05	0.751	172	0.438	10
Queens, SE Douglas	2.88	0.265	26	0.680	12	−10.34	1.234	215	0.711	12
Queens, E Douglas	2.61	0.713	326	0.974	4	−9.25	2.889	149	0.998	4
Length: gonad weight										
Scallops, Bradda	8.52	1.619	9	0.615	12	−38.63	7.525	187	0.605	12
Scallops, SE Douglas	6.74	0.989	342	0.717	10	−30.40	4.975	152	0.746	10
Queens, SE Douglas										
Queens, E Douglas	4.51	1.102	361	0.993	4	−18.93	5.571	179	0.999	4

Note: M, mesor; A, amplitude; t_w = time at which sine wave begins.

certainly higher than would be required to fit a purely empirical model to the same data. The direct fit of a seasonalized VBGF for weight may be easier with data from whole finfish, where the length-weight exponent (b) can either be fixed at 3, or assumed to approximate it in entering starting values for the parameters. In this study b was found to show considerable seasonal variation, and fixing b and describing seasonality by only allowing a (the length:weight "condition factor") to fluctuate were not possible. Seasonal fluctuations in b imply that the degree of seasonal fluctuation is size dependent, and examination of the data shows that the seasonal fluctuations in the edible yield are greater in larger, sexually mature scallops.

The seasonal growth and reproductive cycles described in this article are of great importance in determining both yield and fishing strategy in these fisheries. *P. maximus* can only be fished from November 1st to May 31st under current legislation. This analysis

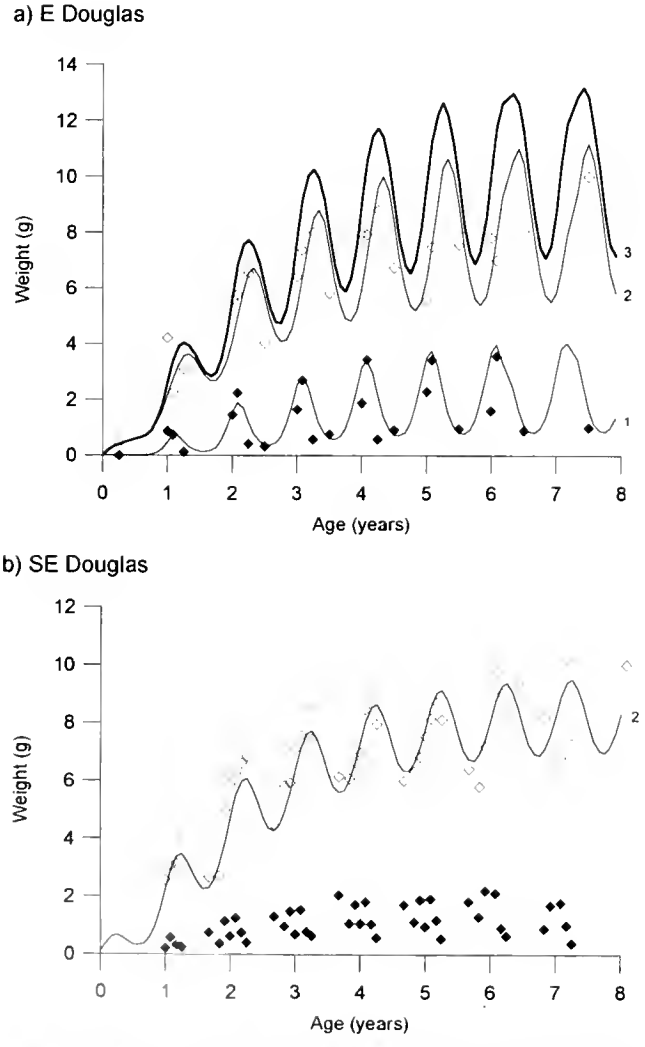

Figure 6. Mean weights at age of gonads (closed symbols) and adductor muscles (open symbols) of *A. operc*ularis from (a) E Douglas and (b) SE Douglas. Model-predicted weight at age of gonads (1), adductor muscles (2), and total yield (3) are shown for *A. operc*ularis from the E Douglas fishing grounds. Predicted weight at age of adductor muscles only (1) are shown for samples from the SE Douglas ground. No model has been fitted to SE Douglas gonads or total yield, because these data were too variable.

of seasonal growth patterns suggests that this period represents the optimal time of year for fishing this species with a 7 month fishing season. The previous year's growth has been completed, and total yield remains relatively constant, despite relative changes in gonad weight and adductor muscle weight. The gonads of >80% of *P. maximus* are full enough to be acceptable to the market throughout the season (Allison 1993), and muscle weight is high until the latter part of the season. Provided that natural mortality does not show strong seasonality, the current fishing season optimizes Y/R for any given age at first capture. The fact that this is the case is largely coincidental; the closed season was originally introduced in the late 1930s to reduce spoilage of scallops transported to markets during the warmer summer months. The closed season has subsequently remained in place with the objective of reducing effort and closing the grounds during the period of spawning and spat settlement in this species (Brand et al. 1991b).

The seasonality of natural mortality is potentially important in determining optimal fishing times. More detailed examinations of the effect of seasonality on yield to the fishery could be performed if monthly or quarterly fishing and natural mortality rates could be calculated. This would help to determine the optimal time of year for harvesting of restocked scallops or closed areas (Brand et al. 1991b), where shorter periods of harvesting may be legislated. Maximum yields are attained just over half way through the current fishing season, in February or March. If a later start to the fishing season is contemplated, or if particular grounds are opened for short periods only, the gains due to increase in weight must be balanced against the losses due to mortality during the period before allowing fishing, bearing in mind that no new recruits enter the fishery over the winter, because shell growth takes place only during the summer.

*A. operc*ularis tend to be fished in the closed season for *P. maximus*, June to October (Allison 1993), and the yield from an individual *A. operc*ularis is maximized in the latter part of this season (late August to September), when the adductor muscle is in peak condition. The shell of *A. operc*ularis grows rapidly during the early summer (June to July), and the 1+ age class does not recruit to the fishery until the latter part of the season on most grounds. On the E Douglas fishing ground, *A. operc*ularis attains the minimum commercially acceptable size of approximately 55 mm shell length during the second growth season (age 1+; Fig. 3b), but not until the third growth season (age 2+) in *A. operc*ularis from SE Douglas (Fig. 3a). Low meat yields for a given shell length and apparently lower catchability of trawled *A. operc*ularis during spring and early summer (due to reduced swimming ability correlated with seasonal energy storage cycles; A. R. Brand unpublished data) mean that fishing for *A. operc*ularis in the early part of the season is a barely viable concern. The option of legislating an extension to the scallop season into June or July to cover this unprofitable period is not tenable, because scallop gonads are spent or partially spent during this period and are therefore unacceptable to the market; meat weights are also low.

In North American *Placopecten magellanicus* (*L.*) fisheries, which are regulated by control of the meat count (maximum number of meats per pound weight), the variability in growth rates and shell length/adductor muscle weight relationships has far-reaching implications for management and has consequently received considerable attention (Worms and Davidson 1986, Shumway and Schick 1987, for reviews), although seasonal growth models have not been constructed. This work highlights the magnitude of seasonal variability in yield from pectinids in temperate waters. In-

corporation of seasonal growth patterns into yield-based stock assessments is recommended for the North Irish Sea scallop fisheries. Investigations of the importance of growth seasonality in other scallop fisheries would be worthwhile.

ACKNOWLEDGMENTS

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DISPERSAL AND MORTALITY OF SEA SCALLOPS, *PLACOPECTEN MAGELLANICUS* (GMELIN 1791), SEEDED ON THE SEA BOTTOM OFF ÎLES-DE-LA-MADELEINE

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ABSTRACT In 1992, a total of 8,980 sea scallops were tagged and placed on a 30 × 30 m seeding site situated at the center of a 150 × 150 m sampling site. The sampling site was divided into nine squares, 50 × 50 m each. Systematic sampling was carried out by video and diving surveys. The density of tagged scallops on the seeding site dropped over 44 days from 10 to 0.29 scallops m^{-2}. On the central square, including seeding site, density change was less dramatic, declining from 3.6 to 1.0 scallops m^{-2} after 25 days. On eight surrounding squares, the density increased from 0 to 0.13 scallop m^{-2} between the beginning and the end of the experiment. Scallop dispersal was extensive and rapid. Movement after 44 days was more than 60 m for 49% of the seeded scallops. Short-term movements seemed to be preferentially toward the south. A diving survey 44 days after the seeding revealed that 12.8% of the observed individuals on the seeding site were dead or tagged shell debris. Predation by crabs appeared to be an important cause of mortality, because about 90% of the dead scallops had broken shells. The most probable causes of dispersal in the scallops were their high initial density and the presence of predators.

KEY WORDS: *Placopecten magellanicus*, scallop, Gulf of St. Lawrence, enhancement, movement, predation

INTRODUCTION

Dispersal and predation of sea scallops, *Placopecten magellanicus* (Gmelin), are two important factors in culturing operations and the restocking of natural beds. Experimental seedings carried out in 1990 and 1991 off Îles-de-la-Madeleine indicated rapid dispersal and heavy mortality of the scallops (Picard and Vigneau, unpublished data). Many scallop species are capable of swimming spontaneously or in reaction to stimuli such as the presence of predators, unsatisfactory substrate, or physical changes in the environment (e.g., salinity, pressure) (Orensanz et al. 1991). Posgay (1981) and Melvin et al. (1985) demonstrated that sea scallop movement for the size examined (≥50 mm) could reach around 10 km $year^{-1}$. Parsons et al. (1992) obtained mean movements of only 3.3 m for juvenile sea scallops over 3 to 4 months.

Most scallop species are not well adapted for making extensive migrations (Brand 1991). Sinclair et al. (1985) maintained that sea scallop aggregations along the Atlantic Coast tend to be in precise geographical locations that are persistent over time. This is supported by the fact that the scallop beds off Îles-de-la-Madeleine have remained spatially stable, even after 20 years of fishing.

As for mortality, Scheibling et al. (1991) observed a high rate of predation on small scallops by the sea star *Asterias vulgaris*. Rock crabs (*Cancer irroratus*), lobsters (*Homarus americanus*), and moon snails (*Lunatia heros*) can also be natural predators of sea scallops (Dickies and Medcof 1963, Caddy 1973, Jamieson et al. 1982, Orensanz et al. 1991). The objectives of this study were to assess the importance of movements of seeded scallops in relation to their size, their survival, and changes in the abundance of predators on a spatial and temporal scale.

MATERIALS AND METHODS

The sampling site was located at the center of a small scallop bed off Îles-de-la-Madeleine in the Gulf of St. Lawrence (Québec, Canada) that has been closed to fishing since 1991 (Fig. 1). The sampling site was between 20 and 22 m deep on a uniform substrate of gravel and sand. A recording current meter (Aanderan), which also recorded temperature and sampled once every 30 minutes, was placed on the sampling site for the duration of the study. The average current speed on the bottom was 3 to 5 cm s^{-1}, and the temperature was between 9 and 13°C during the sampling period. A progressive vector diagram simulating the path of a particle was produced from the current meter data. The vector was traced after low pass filtering the data to remove variations of a factor of 100 due to diurnal and semidiurnal tides, and only the residual current data remained. Because only one current meter was used, the vector did not take into account spatial variations in the current.

The sampling site covered a surface area of 22,500 m^2 and was divided into nine squares of 50 × 50 m. The seeding site was situated at the center of the central square (#5) and was 30 × 30 m. At the corner of each square, there was a cement block with a surface buoy attached. The attaching rope had as little slack as possible to reduce horizontal movement of the buoy. A system of ropes joining the buoys also aided the location of the squares from the surface.

The 8,680 seeded scallops (≤70 mm) were acquired from Newfoundland aquaculture operations at Port-au-Port, and 300 scallops (>70 mm) were collected from Îles-de-la-Madeleine fishing grounds. Approximately 11% of the scallops were measured with an electronic calliper before seeding. In order to distinguish

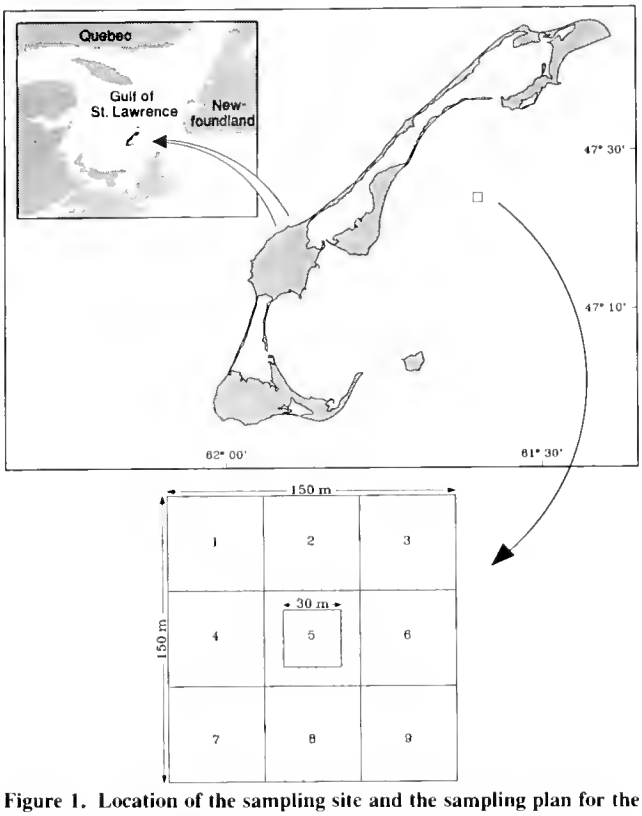

Figure 1. Location of the sampling site and the sampling plan for the video camera during the sea scallop seeding operation carried out in Îles-de-la-Madeleine in 1992.

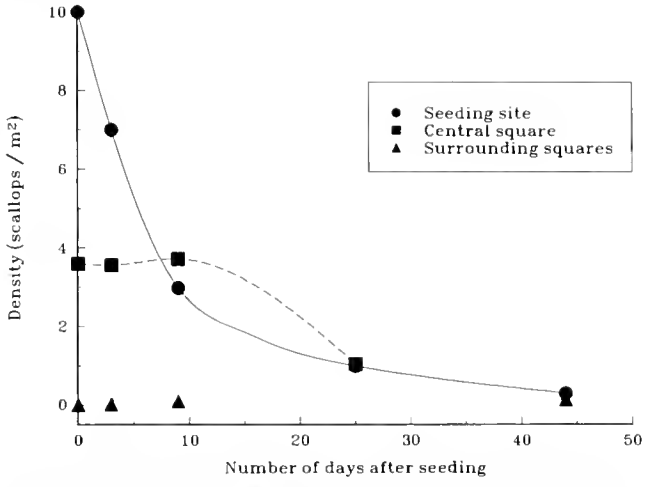

Figure 2. Density of live tagged sea scallops on the seeding site, on the central square (#5), and on the surrounding squares (#1 to 4, #6 to 9). Densities obtained from video sampling and SCUBA diver survey.

the seeded scallops from the indigenous population and to follow movements through time, the seeded scallops were marked with tags. The plastic disc tag was attached near the hinge with cyanoacrylate glue. The scallops were kept in net cages (pearl nets) for about 15 days to verify mortality associated with manipulation and to check the resistance of the glue. Seeding took place on 4 July 1992 by means of a 10 cm diameter flexible tube from the surface. The release of the scallops on the seeding site was controlled by SCUBA divers in order to obtain a uniform distribution ($\sim$10 scallops m^{-2}).

A video camera deployed from the surface was used to monitor the movements of the seeded scallops and to estimate the number of predators present. The camera, enclosed in a waterproof case and fixed to a support, was adjusted so that the square metal base of the support, the sides of which measured 0.5 m, was seen. Thus, each sampling frame was 0.25m^2. Camera adjustments were done from the surface.

The video sampling was conducted on a systematic basis (Fig. 1). Five hundred frames along 10 transects were recorded per sampling period for the central square (#5), and 100 frames on 4 transects were recorded per sampling period for each of the surrounding squares (#1 to 4, #6 to 9). For each sample, 5% of the central square and 1% of the surrounding squares were sampled, totaling 5,000 frames recorded on VHS videocassettes. During analysis, the recordings were played back on a video tape recorder (Panasonic VTR) with stop action. For each video frame, tagged scallops, untagged scallops, and predators were identified and counted; tagged scallops were identified as live or dead. Both cluckers (empty with the two valves attached) and shell fragments with a tag were identified as dead scallops. The shell height of tagged scallops on the central square was measured with the morphometry software Bioquant system IV (R&M Biometrics Inc), which grabs frames from video playbacks for analysis. This method required that scallops be completely visible and correctly oriented toward the camera for precise measurements.

The sampling periods were often spread over several days be-

TABLE 1.

Sea scallop seeding operation carried out in Îles-de-la-Madeleine. Experimental procedure in 1992: type of activity, date, sampling method, and area sampled

Activity	Date	Sampling Method	Area Sampled
Site characterization	1 July–day −3	Video camera	All squares
Seeding	4 July–day 0		Seeding site
Sampling period 1	7 July–day 3	Video camera	Central squares (#5)
Sampling period 1	8 July–day 4	Video camera	Eight surrounding squares (#1 to 4, #6 to 9)
Sampling period 2	13 July–day 9	Video camera	Central square
Sampling period 2	17 July–day 13	Video camera	Eight surrounding squares
Sampling period 3	29 July–day 25	Video camera	Central square
Sampling period 4	17 August–day 44	Video camera	Eight surrounding squares
Sampling period 4	17 August–day 44	SCUBA diver	Seeding site

cause calm seas were required for clear sampling frames. The video data were grouped into distinct blocks from 1 July to 17 August 1992 (Table 1). In addition, on 17 August, the seeding site was sampled by SCUBA divers and included 30 quadrats of 3 m^2 each distributed systematically on three transects of 30 m. This manual sample was done to check the video results and to evaluate scallop mortality, in particular, tagged scallops with broken shells.

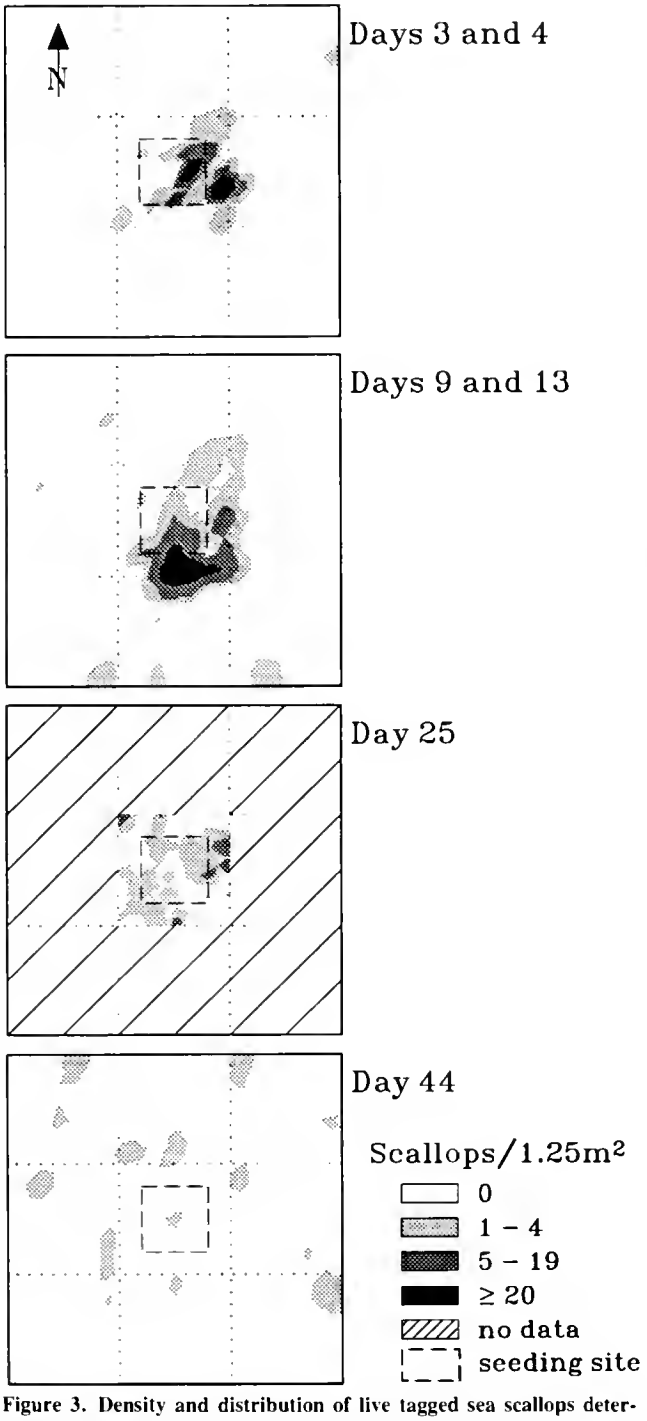

Figure 3. Density and distribution of live tagged sea scallops determined by contour analysis with Axum Data Software (Trimetix Inc.) on sampling site (nine squares) at each sampling period. Data obtained by video sampling and SCUBA diver survey.

RESULTS AND DISCUSSION

Dispersal

The density of indigenous scallops (untagged) was estimated to be 0.24 scallop m^{-2} during the study site characterization a few days before the seeding (day −3) and 0.42, 0.54, 0.87, and 0.69 scallop m^{-2}, respectively for the subsequent sampling periods. The changes in density of indigenous scallops were insignificant and possibly due in part to lack of tag visibility (scallop turned over or partially hidden). The density of live tagged scallops changed over time on the seeding site, on all of the central square (#5), and on the rest of the surrounding squares sampled (Fig. 2). During the first 10 days, seeded scallop movements were important but limited to the central square. At the end of the study, 44 days after seeding, the density on the seeding site decreased from 10 to 0.29 scallops m^{-2}. In the central square, the change was less dramatic, dropping from 3.6 to 1.0 scallops m^{-2} after 25 days. On the surrounding squares (#1 to 4, #6 to 9), the density of tagged scallops increased noticeably from 0 to 0.13 scallop m^{-2} between the beginning and the end of the study. Dispersal of the tagged scallops was outward from the seeding site toward the periphery, and it occurred gradually with a tendency toward uniform density over the sampling site after 44 days. At the end of the study, the total density of scallops (tagged and untagged) over the sampling site was approximately 1 scallop m^{-2}, with between 0.13 and 0.29 tagged scallop m^{-2} and 0.69 untagged scallop m^{-2}. Kalashnikov (1991) noted that, with scallops in culture (*Mizuhopecten yessoensis*), high density stimulated the movement of the scallops, which brought about reduced density. According to Orensanz et al. (1991), scallops adjust their density toward an equilibrium level that reduces competition for a resource in limited supply (most likely, food) without reducing chances of cross-fertilization.

In the last sampling period, the number of tagged scallops remaining alive on the whole sampling site was estimated to be 3,404, which was 38% of the initial number. This estimate is

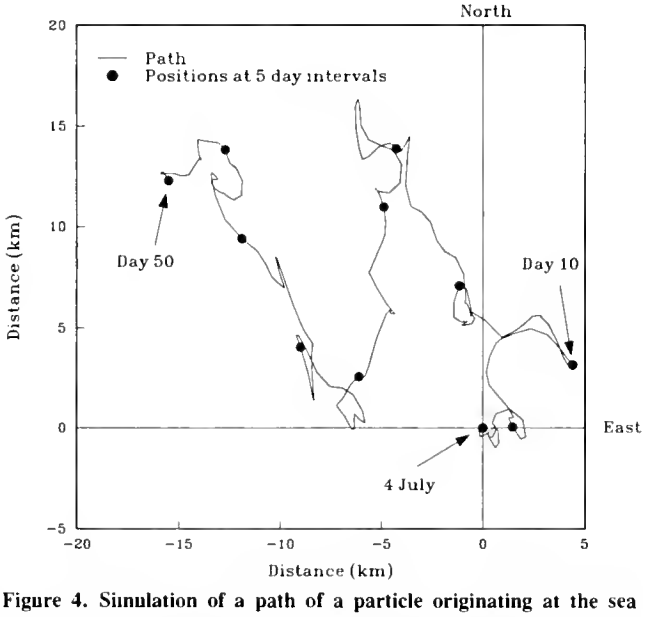

Figure 4. Simulation of a path of a particle originating at the sea scallop seeding site (0,0) on 4 July 1992, according to a temporal series of residual current speed. North indicates magnetic north.

based on SCUBA diver observations on the seeding site and by video observations on the eight surrounding squares. After 44 days, only 2.9% of the seeded scallops remained alive on the seeding site. Thus, 44 days after the seeding, 49% of the tagged scallops were no longer within the sampling site. The strong decrease in the number of tagged scallops on the sampling site cannot be attributed to tag loss. Another experiment demonstrated that the retention rate of tags was 99.5% over 36 days. It appears that a significant number of the seeded scallops moved more than 60 m in 44 days. These are greater movements than those found in Passamaquoddy Bay, which averaged 3.3 m over 4 months for scallops ≤25 mm (Parsons et al. 1992), but our scallops were larger.

The distribution of tagged scallops on the central square changed noticeably with each sampling period (Fig. 3). On day 3, 70% of the scallops were still concentrated on the seeding site. On day 9, only 32% of the individuals remained and were mainly concentrated in the southern part of the central square, suggesting a movement in that direction. On day 25, the majority of the scallops had left the central square, and the distribution of the remaining scallops was fairly uniform. In the last sampling period, tagged scallops were present over the whole sampling site. The southern direction of scallop movements in the first few days after seeding corresponded with the southeast geostrophic currents for the month of July in Îles-de-la-Madeleine, as described by El-Sabh (1976). However, the 1992 current meter data provided a better indication of the currents on a smaller scale. A progressive vector

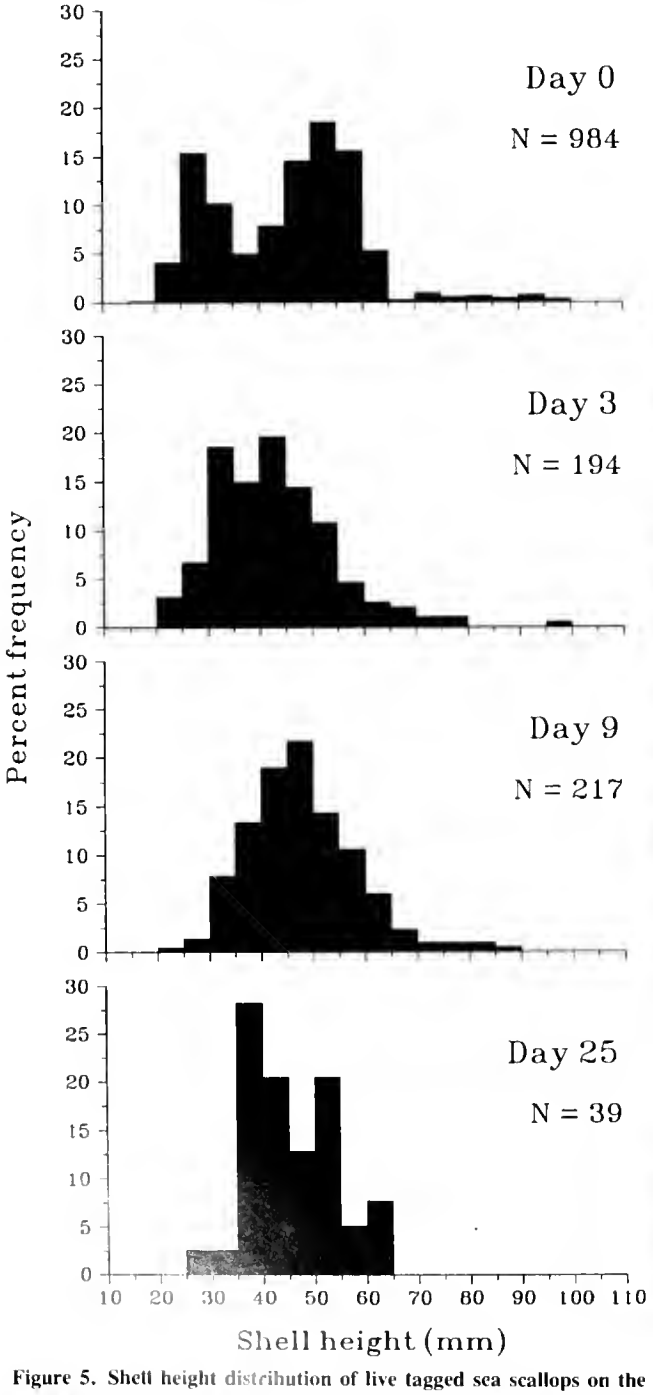

Figure 5. Shell height distribution of live tagged sea scallops on the central square (#5), grouped by size class of 5 mm. Scallops were measured with electronic callipers on day 0 (before seeding) and by morphometric image analysis for the three other sampling periods.

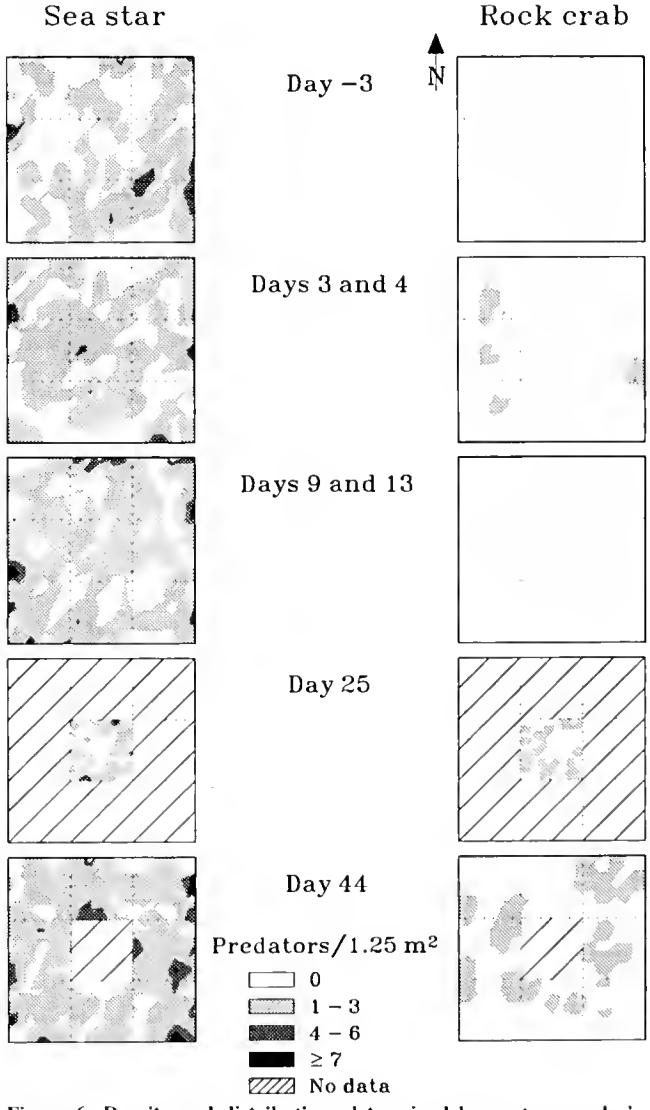

Figure 6. Density and distribution, determined by contour analysis (Axum, TriMetrix Inc.), of predators on the sampling site (nine squares) for each sampling period. Densities obtained by video sampling.

diagram simulating the path of a particle, originating at the seeding site in the bottom layer, indicated a northeast movement of water after 10 days and a general northwest direction after 50 days (Fig. 4). Thus, the general direction of tagged scallop movements did not seem to correspond with the movement of the water mass.

The shell height distributions for scallops measured during the different sampling periods (day 3 to 25) were similar (Fig. 5). However, they differed from the size structure obtained before seeding; small scallops <35 mm and scallops between 50 and 70 mm appeared to be underrepresented during the sampling periods. This is difficult to explain because the densities estimated during the first two sampling periods were similar to the initial density. Limits imposed by experimental conditions and techniques may partially explain the observed difference. Among other things, about 50% of the scallops, mainly small, were unmeasurable in the video frames because they were difficult to distinguish properly. In addition, live and dead scallops were not measured during the SCUBA diver survey 44 days after seeding. It was thus impossible to evaluate the effect of size on scallop dispersal because of the lack of information on the size of live and dead scallops during the sampling periods.

Mortality and Predators

On the basis of video observations, the number of dead tagged scallops on the central square 25 days after seeding was estimated to be 126, which is 1.4% of the original number seeded. On the surrounding squares, the estimated number of dead tagged scallops varied from 0 to 206 between the sampling periods, representing between 0 and 2.3% of the original number seeded. Very few broken shells were observed in the video samples. The diving survey 44 days after seeding estimated 1,150 dead scallops or parts of tagged shells on the seeding site, which is 12.8% of the total number released. Short-term mortality of seeded scallops was therefore quite significant, especially on the seeding site. Mortality estimates based on diving observations were much higher than those based on video observations, suggesting that the video was inadequate in estimating numbers of dead tagged scallops. It was not always evident whether closed shells were empty in video playbacks, and the shells of dead scallops were often disarticulated and fragmented, making it difficult to detect tags.

The fluctuations in the abundance of the principal predators (sea stars and rock crabs) on the sampling site before seeding and during each successive sampling period were marked (Fig. 6). Between days −3 and 44, the predator density on the whole study site increased from 946 to 1,454 sea stars 1,000 m^{-2} and from 0 to 123 rock crabs 1,000 m^{-2}. The density of rock crabs was most likely underestimated because sampling took place during the day, when they are less active and often bury themselves in the substrate (Stehlik et al. 1991, Gendron and Cyr 1994).

Our results showed that the distribution of the sea stars was relatively uniform, whereas the rock crabs seemed to have a patchy distribution on the sampling site. There was no apparent relationship between the distribution of these two predators and that of the scallops. Although the sea stars were the most abundant predators on the study site, they were probably not the principal cause of scallop mortality. Diving observations revealed that 90% of the dead tagged scallops on the seeding site had broken shells, which is consistent with crab predation (Elner and Jamieson 1979, Jamieson et al. 1982). Thus, it appears that crab and/or lobster predation is significant. The main predator is probably the rock crab because very few lobsters and spider crabs (*Hyas* species) were observed on the sampling site.

As summarized by Orensanz et al. (1991), several factors may induce scallop movement, including the presence of predators, unsatisfactory substrate, or physical changes in the environment (e.g., salinity, pressure). The presence of indigenous scallops on the sampling site seems to indicate that the substrate and environmental conditions were satisfactory for sea scallops. Therefore, it appears that displacements of the seeded scallops were principally caused by: (1) an initial density that was too high and beyond the optimal levels for growth and reproduction; or (2) the increasing density of predators, which triggered escape responses in scallops (Thomas and Gruffydd 1971, Winter and Hamilton 1985). In general, our results emphasize the major impact of predation on seeded scallops. The tendency of scallops to disperse and reduce their density greatly affects harvesting yields and the economic profitability of experimental seedings. It is therefore important to chose seeding sites and periods carefully in order to minimize dispersal and mortality of seeded scallops.

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TECHNICAL EFFICIENCY, BIOLOGICAL CONSIDERATIONS, AND MANAGEMENT AND REGULATION OF THE SEA SCALLOP, *PLACOPECTEN MAGELLANICUS* (GMELIN, 1791) FISHERY

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ABSTRACT Achieving social and economic efficiency in a fishery requires that production be technically efficient. Yet, technical efficiency (TE) is rarely examined for a fishery. By the use of detailed trip-level data and information about resource conditions obtained from routine sampling, a stochastic frontier production model relating landings to days at sea, crew size, and resource conditions is specified and estimated for 10 Mid-Atlantic sea scallop (*Placopecten magellanicus*) dredge vessels. TE is shown to depend partly on the mix of controllable inputs such as days at sea and crew size but possibly more on uncontrollable factors such as resource conditions and biological characteristics. Last, we illustrate that two regulations recently implemented by the management authorities should increase TE in the U.S. sea scallop fishery.

KEY WORDS: Technical efficiency, stochastic frontier, biological conditions

INTRODUCTION

Management and regulation of commercial fisheries primarily focus on efficient resource utilization and resource conservation. Efficient resource utilization, however, requires that production be technically efficient or maximized, given input levels and the technology (Fare et al. 1985). That is, technical efficiency (TE) is a necessary, but not sufficient, condition for economic and social efficiency. Unfortunately, achieving TE in fisheries may be difficult because of varying resource and environmental conditions. These are important determinants of landings, which unlike days at sea and crew size, captains cannot easily change. In addition, achieving TE may be complicated by inadequate information about the parameters that define and influence TE.

Studies on TE in fisheries have been limited (Comitini and Huang 1967, Hannesson 1983, Hilborn 1985). TE has most often been analyzed in terms of landings per unit effort (LPUE) or with respect to a TE parameter, the constant term in a multiplicative regression model relating landings to nominal fishing effort, as in Strand et al. (1981). TE has also been analyzed in terms of the proportion of fish that could be harvested by a particular gear type (e.g., a catch of 25 out of 100 possible fish yields an efficiency estimate of 25%). Although these types of analyses provide useful information, they, nevertheless, do not provide adequate information about TE or the ability of a producer to produce the maximum output (frontier) possible from a given set of inputs and production technology.

A serious limitation of previous studies on TE is the use of a deterministic measure of TE. A deterministic measure does not accommodate noise, measurement error, or random shocks beyond the control of the production unit. A deterministic measure, in fact, attributes all noise, random shock, and measurement error to inefficiency in production (Fare et al. 1985). A deterministic measure may, therefore, be a seriously biased measure of TE for a fishery and lead to erroneous policies by fishery managers.

In this article, we illustrate a stochastic approach for estimating and examining TE in a fishery. By the use of a panel data set reflecting production activities for a sample of sea scallop dredge vessels operating in the Mid-Atlantic, a stochastic production function or frontier is specified and estimated. The estimated frontier is used to calculate TE for each trip and vessel. Estimates of TE are subsequently summarized and examined relative to input levels—days at sea and crew size—and biological conditions—stock abundance, meat yield, and reproductive activities. Last, TE is examined relative to two regulations—an annual days at sea restriction per vessel and crew size limits—recently implemented by the New England Fishery Management Council (NEFMC), the U.S. agency responsible for managing the fishery.

MATERIALS AND METHODS

Methods

TE is a measure of the ability of a producing unit to produce the maximum output, given the level of inputs and the technology. A producer that produces in the interior of a maximum output boundary or frontier or requires more inputs than necessary to produce a maximum output is technically inefficient. Consider the hypothetical frontier depicted in Figure 1. Output or landings is measured along the vertical axis, and fishing effort or days at sea is measured along the horizontal axis. The darkened line represents the maximum output possible or the production frontier, given the producer's technology and level of inputs. A producer is said to be TE if production is on the frontier and technically inefficient if production is below the frontier output. A measure of TE, however, is a relative measure in that the production performance of one producing unit is compared with the production performance of a best-practice input–output relationship (Squires and Tabor 1991). TE is, therefore, measured in terms of the deviations of individual vessels from this best-practice frontier.

It is important to recognize, however, that TE is not the same as economic and social efficiency. Economic efficiency is a measure of the ability of a firm or industry to use inputs to produce output, given input and output prices; that is, economic efficiency indicates whether or not production is consistent with the input and output levels required to minimize cost or maximize profit (Fare et al. 1985). The mathematical product of economic or allocative efficiency and TE provides a measure of overall efficiency (Corbo and de Melo 1986). Social efficiency is a measure of how well

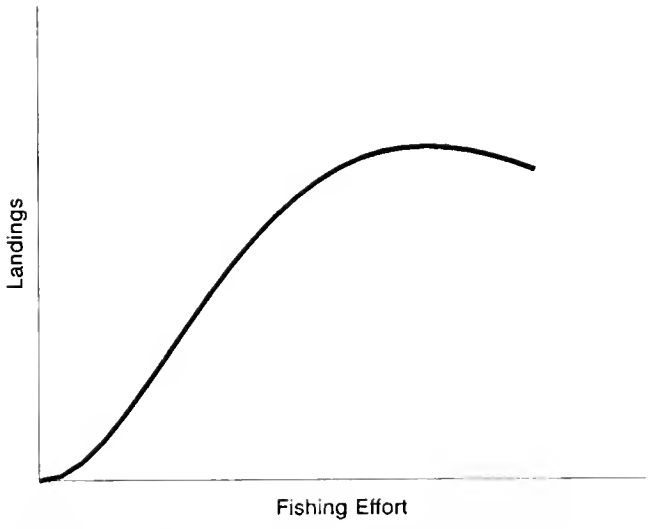

Figure 1. Hypothetical production frontier or maximum output boundary

firms use inputs to produce outputs, such that net benefits to society are maximized; this is also known as the long-run competitive equilibrium output (Fare et al. 1985 and 1994). This study only considers TE.

We assume the best-practice frontier is stochastic, rather than deterministic, and consists of two error terms. One error term is assumed to be normally distributed with a mean of zero and a constant variance; this is the familiar normally distributed error term assumed in conventional regression. This error term captures measurement error and random exogenous shocks beyond the control of the producing unit. Aigner et al. (1976 and 1977) Meeusen and van den Broeck (1977a and b) and Green (1980) have shown, however, that another error term that is one sided may be introduced to represent technical inefficiency.

Following Aigner et al. (1976 and 1977) and Meeusen and van den Broeck (1977a and b), the stochastic frontier production function may be written as follows:

$$Y_i = h(X_1,X_2, \ldots ,X_N;A)u_i,\ i = 1,2, \ldots , M \qquad \text{equation 1}$$

where Y_i is the output of the ith vessel, X_j ($j = 1, \ldots , N$) is the jth of N inputs, A is a vector of parameters, and u_i equals the exponential value, 2.71828, raised to the power of the sum of two independent error terms—ϵ_{1i} and ϵ_{2i}.

The sum of the two independent error terms equals the disturbance term ϵ_i. We let ϵ_1 be a symmetric, normally distributed error term; ϵ_1 is less than, equal to, or greater than zero. This error term allows for random variation of the production function across vessels and reflects statistical noise, measurement error, and exogenous shocks beyond the control of the producer. The error term ϵ_2 is one sided and nonpositive and represents technical inefficiency relative to the stochastic frontier (Jondrow et al. 1982). The technical inefficiency error term may follow a half-normal, exponential, or truncated normal distribution. In the examination of the TE of the sea scallop fishery, we assume the half-normal distribution.

The value of the error term ϵ_2 must be less than or equal to zero (Fare et al. 1985; Jondrow et al. 1982). If $\epsilon_2 = 0$, production lies on the stochastic frontier and is TE ($TE = 1$); if $\epsilon_2 < 0$, production lies below the frontier and is technically inefficient ($0 \leq TE \leq 1$). In applied work, TE for each observation may be measured by calculating the distribution of ϵ_2 conditional on $\epsilon = \epsilon_1 + \epsilon_2$ (Jondrow et al. 1982):

$$TE = 1 + E[\epsilon_2 \mid \epsilon_1 + \epsilon_2] \qquad \text{equation 2}$$

where E is the expectations operator.

The symmetric error ϵ_1 is independently and identically distributed as $N(0,\sigma_{\epsilon 1}^2)$. For estimation purposes, the nonpositive error ϵ_2 is assumed to be distributed as the absolute value of the normal distribution, $|N(0,\sigma_{\epsilon 2}^2)|$ (i.e., half-normal). Use of the absolute value of ϵ_2 requires that the + signs in equation 2 be changed to − signs in order to calculate TE. The variance of ϵ equals the sum of the variances of ϵ_1 and ϵ_2. The variances of the two error terms play an important role in assessing TE. We let $\delta = \sigma_{\epsilon 2}^2/\sigma_{\epsilon 1}^2$. The more δ exceeds one in value, the more production is dominated by technical inefficiency, and the closer δ is to zero in value, the greater is the likelihood that differences between the observed and frontier output are primarily associated with random factors beyond the control of the captain.

Estimating TE for the individual firm (TE_i) requires estimation of the error term (ϵ_2) and decomposing the error ϵ_i for the ith observation into the individual components—ϵ_1 and ϵ_2. Jondrow et al. (1982) suggests a decomposition method from the conditional distribution of ϵ_{2i} given ϵ_i. Given the normal distribution of ϵ_1 and the half-normal distribution of ϵ_2, the conditional mean of ϵ_{2i}, given ϵ_i for each observation i, is as follows:

$$E[\epsilon_{2i}|\epsilon_i] = (\sigma_{\epsilon 2}\sigma_{\epsilon 1}/\sigma)\,[(f(\epsilon_i\delta/\sigma)/(1 - F(\epsilon_i\delta/\sigma)) - (\epsilon_i\delta/\sigma)] \qquad \text{equation 3}$$

where f() and F() are the values of the standard normal density function and the standard normal distribution function estimated at $\epsilon_i\delta/\sigma$ and $\delta = \sigma_{\epsilon 2}^2/\sigma_{\epsilon 1}^2$. The measure of individual TE may thus be calculated for each observation (i) as $e(-E[\epsilon_{2i}|\epsilon_i])$, where ϵ_i is replaced by its estimate and $0 \leq TE_i \leq 1$. The expected value indicates the TE of firm i relative to the practices of the best fishing vessels; the closer TE_i lies to 1 (0), the closer (further) the TE of firm i lies to the best-practice frontier.

A Second-Order Flexible Functional Form Specification of the Frontier

It is advantageous to have estimable production relationships that place relatively few restrictions on the technology or the nature of the relationship between output and inputs (Chambers 1988). Alternatively, it is desirable to have generalized functions or flexible-functional forms so that various hypotheses about production can be examined. A flexible form providing a second-order numerical or differential approximation to the transform of an arbitrary function is particularly well suited for specifying the production technology.

The production frontier may be specified by any well-behaved second-order function. A well-behaved second-order function requires that its parameters may be chosen such that the value of the function, its gradient, and Hessian equal the corresponding magnitudes for any arbitrary second-order function evaluated at any point. We specify a translog production frontier, which is a natural log transform of the generalized second-order quadratic, $G(X) = \alpha_0 + \Sigma_i \beta_i g_i(X_i) + (1/2) \Sigma_i \Sigma_j \beta_{ij} g_i(X_i) g_j(X_j)$, at the level of the individual fishing trip for 10 mid-Atlantic sea scallop dredge vessels operating between 1987 and 1990:

$$\ln Y_{it} = \alpha_0 + \alpha_1 \ln DA_{it} + \alpha_2 \ln L_{it} + \alpha_3 \ln S_{it} + \sum_{j=1,3} \beta_j D_j + \beta_4 DR + \tau_1 (\ln DA_{it})^2 + \tau_2 (\ln L_{it})^2 + \tau_3 (\ln S_{it})^2 + \tau_{12} \ln DA_{it} \ln L_{it} + \tau_{13} \ln DA_{it} \ln S_{it} + \tau_{14} \ln L_{it} \ln S_{it} + \sum_{k=2,12} \Gamma_k D_k \ln S_{it} + \sum_{k=2,12} \Theta_k D_k (\ln S_{it})^2 + \epsilon_{it} \quad \text{equation 4}$$

where i and t index individual scallop vessels and trips, respectively. The variables are landed scallop meat weight (Y_{it}) by the ith vessel on the tth fishing trip, days at sea per trip (DA_{it}), crew size (L_{it}), resource stock size (S_{it}), annual dummy variables for 1988 through 1990 (D_j), dummy variable for dredge size (DR = 1 for 3.96 m dredge and zero otherwise), and dummy variables for the months of February through December (D_k). The parameters to be estimated are α_i (i = 0, . . . , 3), β_j (j = 1,2,3), τ_k (k = 1,2,3), τ_{11} (l = 1,2,3), Γ_m (m = 2, . . . , 12), and Θ_m. The disturbance term ϵ_{it} is assumed to be composed of a normally distributed error (ϵ_1) and a half-normal distributed error (ϵ_2).

The translog function is flexible in that it imposes few restrictions on the underlying relationship between landings and the factors of production (e.g., days at sea, crew size, and resource conditions). Direct estimation of the translog, however, is often difficult because of multicollinearity problems, particularly when there are more than two distinct right-hand side variables. Thus, empirical results obtained from estimates of equation 4 should be evaluated relative to the potential problems of multicollinearity. In addition, not all right-hand side variables used in equation 4 are appropriate measures of factor or input usage. For example, days at sea embodies electronics, fuel, gear, and other inputs and, thus, is assumed to be a composite or aggregate input. Crew size is a stock and not the flow of labor services; labor services, however, are assumed to be proportional to crew size. Despite these limitations, equation 4 offers a convenient framework for examining TE in the mid-Atlantic sea scallop fishery. Moreover, the specification is consistent with biological and bioeconomic specifications typically used to examine the relationship between catch and effort.

Data

Information on production activities and vessel performance was obtained from vessel owners of 10 scallop dredge vessels operating in the mid-Atlantic between 1987 and 1990. Settlement sheets or trip-level financial summaries provided detailed data on landings, days at sea, and crew size. The 10 vessels were relatively homogeneous in vessel characteristics (Table 1). All vessels were constructed between 1979 and 1987, and all were steel hulled. Vessel size ranged from 24.4 to 27.4 m (length overall). Three of the 10 vessels, however, had lower horsepower engines and, therefore, pulled smaller dredges (3.96 vs. 4.57 m). All vessels had two radars, Loran C, and plotters. The 10 vessels made 581 trips between January 1987 and December 1990.

Information on stock size was obtained from data regularly collected as part of a Virginia Institute of Marine Science monitoring program established to determine the gametogenic cycle and resource conditions of sea scallops in the mid-Atlantic resource area; the data collection program is discussed in detail in Schmitzer et al. (1991) and Kirkley and DuPaul (1991). For this study, stock abundance per vessel per trip was calculated in terms of the geometric mean of the number of baskets of scallops caught per hour by approximately 36 vessels fishing the same area during the same period of time and using the same dredge size; baskets of scallops caught per hour were only for the last tow that a group of vessels regularly make for research purposes.

TABLE 1.

Characteristics of 10 mid-Atlantic sea scallop dredge vessels.

Vessel	Year Built	Length Overall (m)	Horsepower	Dredge Width (m)
1	1985	27.4	620	4.57
2	1980	24.4	500	3.96
3	1979	25.9	500	3.96
4	1987	27.4	620	4.57
5	1980	25.9	500	3.96
6	1981	25.9	520	4.57
7	1984	26.5	520	4.57
8	1980	25.9	520	4.57
9	1981	26.5	520	4.57
10	1981	25.9	520	4.57

There is considerable debate about the validity of using catch per unit effort (CPUE) or LPUE to indicate stock abundance for any species (Ricker 1940, Dickie 1955, Paloheimo and Dickie 1964, Westrheim and Foucher 1985, Pennington 1986, Richards and Schnute 1986, Hilborn and Walters 1992). An anonymous referee suggested that CPUE and LPUE may provide a good estimate of density within a scallop bed but not likely a good measure of resource abundance. The referee suggested that CPUE or LPUE may not be an adequate indicator of stock abundance because scallops are generally sedentary and patchily distributed. There are also several other reasons why LPUE and CPUE may not provide valid measures of stock abundance (e.g., the functional form for the short-run catch-effort model may be different than the traditional multiplicative model or the effort may not be properly measured). Recognizing these limitations, we, nevertheless, used the geometric mean of CPUE from the last tow of several vessels fishing the same area during the same periods of time as, at least, a crude indicator of stock abundance. Moreover, we note that Dickie (1955, pp. 805–807) suggested that average catch per vessel per trip may be a valid indicator of the relative abundance of sea scallops.

In this study, all biological parameters other than stock abundance were defined relative to 90 to 94 mm shell height sea scallops. This size was commonly observed in our scallop monitoring program and is a size that is fully recruited. The reproductive or spawning cycle was defined in terms of wet gonadal weight. Meat yields were calculated as the average weight of meats obtained from 90 to 94 mm shell height scallops.

RESULTS

Equation 4 was estimated by maximum likelihood procedures available in LIMDEP 6.0 (Green 1992). Most of the parameters were statistically different than zero at the 5% level of significance (Table 2). For the purpose of assessing the estimated stochastic frontier model, the adjusted R^2 was calculated for the ordinary least-squares regression (R^2 = 0.845). The model was further estimated subject to several structural restrictions; specifically, the structure of the traditional multiplicative model and the lack of technical interactions between crew and fishing effort, crew and

TABLE 2.

Parameter estimates of stochastic production frontier.

Variable[a]	Final Model
Intercept	−2.97
	(2.79)[b]
Days at sea	3.18[c]
	(0.50)
Labor	5.00[c]
	(2.32)
Stock abundance	0.10
	(0.68)
1988 constant	−0.17[c]
	(0.05)
1989 constant	−0.26[c]
	(0.04)
1990 constant	−0.20[c]
	(0.05)
Dredge size	−0.11[c]
	(0.03)
Days at sea squared	−0.18[c]
	(0.06)
Labor squared	−0.82
	(0.51)
Stock abundance squared	0.21[c]
	(0.05)
Days at sea * labor	−0.38
	(0.23)
Days at sea * stock abundance	−0.12
	(0.07)
Labor * stock abundance	0.13
	(0.29)
February * stock	0.22
	(0.19)
March * stock	0.92[c]
	(0.24)
April * stock	0.73
	(0.49)
October * stock	−0.24
	(0.27)
November * stock	−0.37[c]
	(0.13)
December * stock	0.04
	(0.15)
February * stock squared	−0.03
	(0.18)
March * stock squared	−0.65[c]
	(0.22)
April * stock squared	−0.46
	(0.35)
October * stock squared	0.04
	(0.28)
November * stock squared	0.02
	(0.13)
December * stock squared	−0.61[c]
	(0.17)
$\sigma(u)/\sigma(v)$	1.28[c]
	(0.14)
$\sigma^2 = \sigma^2(u) + \sigma^2(v)$	0.41[c]
	(0.02)

[a] All variables except intercept and dummy variables are in natural logarithms.

[b] Numbers in parentheses are standard errors.

[c] Statistically significant at the 5% level of significance.

* indicates product of variables

stock size, and effort and stock size were examined. Calculated chi-square values were 71.12 for the multiplicative model and 12.234 for no interactions; corresponding critical chi-square values for 12 and 3 restrictions were 21.0 and 7.81, respectively. Additional likelihood-ratio tests suggested that all monthly dummy variables except those for February through March and October through December could be omitted from the model (chi-square for 10 restrictions was 17.2; the critical chi-square value was 18.3 at the 5% level of significance).

The ratio $\delta = \sigma_{\epsilon 2}/\sigma_{\epsilon 1}$ was 1.28 and statistically significant at the 1% level of significance (t-statistic was 8.98). Therefore, the discrepancy between observed and frontier output was dominated by technical inefficiency rather than by random factors beyond the control of the captains. This result suggests that there are opportunities for expanding production and increasing TE. Alternatively, sources of technical inefficiency can, at least, be identified.

Following the procedures of Jondrow et al. (1982), TE per vessel per trip was calculated and subsequently summarized over vessels, trips, months, and years. Estimates of TE were compared with input levels, meat yields, and stock abundance. Unfortunately, estimates of TE could not be easily compared with the same variables used to estimate equation 4. Conventional regression in which estimates of TE would be regressed against days at sea, crew size, and stock abundance would be biased because of simultaneous equation bias, and the associated parameters would be inefficient. This is because regressions in which the value of the dependent variable is determined by the same variables against which it is being regressed yield biased, inefficient, and inconsistent parameter estimates.

The usual analysis of variance (ANOVA) also could not be used to assess possible differences in mean TE between vessels, months, and years. TE was nonnormally distributed and censored at 0 and 1; therefore, the normality assumptions required for ANOVA were violated. A tobit or Tobin's (1958) tobit, limited dependent variable regression model could have been used to assess differences in mean TE or to approximate ANOVA procedures but was rejected as being unnecessary because nonparametric procedures were available. Pairwise Kruskal-Wallis or Mann-Whitney tests were, thus, used to further examine TE.

Kruskal-Wallis tests suggested that TE per trip was equal between most vessels. These results are omitted from this article because 45 tests were required to compare the equality of TE among vessels. Differences in mean efficiency between some vessels, however, could not be rejected. The overall Kruskal-Wallis test results indicated that differences primarily occurred in 1988 (chi-square for 1987 to 1990 with 9 degrees of freedom equaled 14.53). No differences in TE were detected between the most efficient vessel (#1 in Table 1; TE = 0.78), which dragged 4.57 m dredges, and the less efficient vessels (2, 3, and 5; $TE_{\#2} = TE_{\#3} = TE_{\#5} = 0.74$), which dragged 3.96 m dredges.

Equality of mean TE between months was tested and rejected by Kruskal-Wallis tests (1987 to 1990, chi-square with 11 degrees of freedom equaled 36.87; 1987, chi-square with 11 degrees of freedom equaled 41.51; 1988, chi-square = 33.32; 1989, chi-square = 27.26; 1990, chi-square = 34.65). Pairwise Kruskal-Wallis or Mann-Whitney tests of the equality of efficiency between months did not indicate a clear consistent pattern in TE. These results are not presented because 320 tests were conducted (64 tests per year and over all years). The most significant differences were detected between May and September (chi-square = 25.3), May and December (chi-square = 10.14), May and No-

vember (chi-square = 20.58), June and October (chi-square = 10.14), June and November (chi-square = 8.17), and June and December (chi-square = 8.19). Significant differences were also found between January, March, April, May, July, August, October, and September (i.e., January vs. September and October vs. September).

Nonparametric Kruskal-Wallis tests were also used to test the equality of mean TE relative to number of days at sea and crew size. Differences in mean TE per trip relative to number of days at sea could not be rejected by the Kruskal-Wallis test (chi-square with 24 degrees of freedom equaled 54.57). Additional Kruskal-Wallis tests, however, failed to reject equality of mean efficiency for trips between 2 and 9 days (chi-square with 7 degrees of freedom equaled 13.03). A Kruskal-Wallis test of mean TE for crew sizes between 6 and 15 suggested no differences in TE (chi-square for 8 degrees of freedom equaled 6.85). The null hypothesis that mean efficiencies for crew sizes of 6 to 15 were equal for trips between 15 and 20 days, however, was rejected (chi-square with 8 degrees of freedom equaled 17.58).

A clear, concise relationship between efficiency and biological conditions was not depicted by the data. Alternatively, high values of TE occurred for low and high values of resource abundance and meat yield and during all stages of reproduction. A simple regression of monthly mean efficiency against monthly mean gonadal weights, meat yields, and stock abundance was not significant at the 5% level of significance ($F_{3,44} = 2.68$ vs. critical value of $F_{3,44} = 3.21$). Further examination of efficiency and biological conditions on a year-by-year basis, however, suggested a possible relationship. Regressions of TE against stock abundance, gonad weight, meat yield, and these variables squared were found to be statistically significant for 1989 and 1990 (1989, $F_{6,11} = 7.23$; 1990, $F_{6,11} = 6.56$).

In general, TE was found to increase as stock abundance and meat yield increased; gonadal weight did not appear to be a significant explanatory variable except during 1990 (Table 3). This latter result may be caused by the possibility that gonadal weight and meat weight may be redundant variables (meat weight changes as gonad weight changes). Kirkley and DuPaul (1989) and Schmitzer et al. (1991) have shown, in fact, that meat weight changes in response to changes in spawning events. Alternatively, changes in meat yields are consistent with gonadal weights only during parts of a year (e.g., Kirkley and DuPaul [1991] demonstrated that meat yields change during spring and fall spawns but not during other periods of the year).

DISCUSSION

In evaluating TE, it is important to recognize that TE must be evaluated relative to the technology or the technical constraints imposed on the underlying technology. That is, differences in vintage of capital, vessel size, and resource conditions should be considered in evaluating TE. TE, for example, may be equal for low- and high-resource conditions if vessel captains use the proper levels of inputs. Alternatively, the TE of a 3.96 m dredge may equal the TE of a 4.57 m dredge, even though the larger dredge realizes higher catches per trip. The critical issue is what changes in the input mix and production strategies can be made to improve TE in the scallop fishery?

Unfortunately, the analysis does not provide sufficient information for making precise prescriptions to improve TE. There are simply too many variables to consider (e.g., days at sea, crew size, dredge size, stock abundance, meat yield, reproductive activities, and temporal weather patterns). We can, however, offer qualitative prescriptions on the basis of examining TE of the more highly efficient trips relative to the least efficient trips. Alternatively, we can offer a "monkey-see, monkey-do" prescription based on examining the general pattern of TE.

Over all trips and fishing vessels, TE, in general, positively varied with input and output levels and resource conditions (Table 4). Higher TEs were associated with higher landings, input levels, stock abundance, and meat yields. There were, however, some substantial differences from these patterns. For example, one of the relatively lowest average TEs (0.38) was associated with the highest average stock abundance (3.19); this group of trips also had a low average number of days at sea and a high average crew size per trip. The maximum or highest average TE (0.91) per trip between 1987 and 1990 was associated with moderately few days (10.6 days) at sea per trip, the maximum average crew size (11.4

TABLE 3.

Statistical results of regressing TE on gonad weight, meat yield, and stock abundance, 1987 to 1990

	Parameter Estimates[a]						
Constant	Stock (baskets per hour)	Stock^2	Meat Yield (g)	Meat Yield^2 (g)	Gonad Weight (g)	Gonad Weight^2 (g)	R^2
1987							
8.37446	0.18789	−0.0196	−1.0957	0.0371	0.00056	0.0015	0.74
[1.96]	[2.81]	[1.31]	[1.78]	[2.77]	[0.008]	[0.23]	
1988							
4.4022	−0.38831	0.0751	−0.4896	0.0195	−0.05625	0.0074	0.47
[1.70]	[1.60]	[1.65]	[1.21]	[1.23]	[0.82]	[0.85]	
1989							
−4.4842	0.06073	−0.0087	0.8876	−0.0371	−0.09342	0.0143	0.89
[2.04]	[0.31]	[0.24]	[3.14]	[4.17]	[0.72]	[0.98]	
1990							
−6.4098	−0.27606	0.0497	1.0967	−0.3873	−0.10038	0.0109	0.89
[2.84]	[2.50]	[2.19]	[3.31]	[3.12]	[2.81]	[2.89]	

[a] Numbers in brackets are *t*-statistics.

TABLE 4.

TE and average catch per trip, days at sea per trip, crew size, stock abundance, and weight of individual scallops, 1987 to 1990.

Efficiency	Catch per Trip (mt)	Days at Sea per Trip	Crew Size (No. of People)	Stock Abundance (Baskets per Hour)	Yield of 90 to 94 mm Scallops (g)	Gonad Weight 90 to 94 mm (g)
0.90–0.94 [5][a]	4.80	10.60	11.40	3.08	14.51	3.57
0.85–0.89 [54]	4.77	14.30	9.50	2.15	13.61	4.31
0.80–0.84 [151]	4.83	15.69	9.46	2.96	13.63	3.93
0.75–0.79 [165]	4.43	15.78	9.66	3.03	13.49	3.91
0.70–0.74 [90]	4.23	16.28	9.87	3.07	13.44	3.53
0.65–0.69 [40]	3.36	16.85	9.50	2.66	13.35	3.46
0.60–0.64 [23]	2.63	14.43	10.48	2.69	12.78	2.94
0.55–0.59 [19]	2.34	15.47	9.42	2.04	13.13	4.00
0.50–0.54 [7]	1.92	14.57	9.29	1.89	12.96	4.13
0.40–0.49 [13]	1.31	12.46	8.77	2.11	12.78	4.02
0.30–0.39 [3]	2.05	13.33	10.33	3.19	13.20	4.50
0.10–0.29 [5]	0.58	9.80	8.88	1.95	12.93	3.75
0.00–0.09 [6]	0.33	9.17	9.83	2.52	12.68	3.30

[a] Numbers in brackets indicate number of observations.

individuals), relatively high stock abundance (3.08), and the highest average meat yield (14.51 g).

Further examination of TE by days at sea and crew size groupings offered some possible prescriptions for increasing TE in the sea scallop fishery (Table 5). Higher TEs given relatively low-resource ($\leq$2.00 baskets per hour) conditions were associated with crew sizes of fewer than 8 individuals; larger crew sizes for low-resource conditions were generally associated with inefficient trips. For higher stock abundances (e.g., $\geq$3.00 baskets per hour), high TEs per trip were generally associated with crew sizes of 11 or more individuals. Over all observations, maximum average TEs generally occurred for trips between 14 and 22 days. Inefficient trips between 14 and 22 days per trip were generally associated with low abundance ($\leq$2.00 baskets per hour) and large crew sizes ($\geq$9 individuals).

A consistent linear association between efficiency, input levels, and resource conditions, however, was not evident from the analysis. Neither Pearson nor Spearman rank correlation coefficients indicated a linear relationship. The lack of a consistent linear pattern suggests that captain's skill and unknown biological and environmental factors are likely to be important determinants of TE in the sea scallop fishery. Alternatively, the relationship between TE, input levels, and resource conditions may be highly nonlinear because of technical, environmental, and biological constraints. For example, a large number of days at sea may be inefficient because of inadequate resource conditions or too few workers, or efficient production for trips with few days requires a large number of workers.

The most discernable pattern between efficiency, catch, days at sea, number of crew, and resource conditions was exhibited on a monthly basis (Table 6). Average TE closely followed vessel performance and resource conditions (i.e., high stock abundance and high TE and low abundance and low efficiency). Maximum efficiency occurred in June, when scallops have just completed their spring spawn (Schmitzer et al. 1991). TE was also high between March and May and in August when meat yields are high. TE declined in July when meat yields also are high; the decline may reflect a product wafering problem, which typically occurs in July because of high air temperatures (DuPaul et al. 1990).

Captain's skill in determining labor needs and other levels of inputs may be responsible for obtaining maximum efficiency in June. Knowledgeable captains recognize that stock abundance is typically high and relatively constant in June, and individual meat yields are predictably low relative to many other periods of the year. Captains can, therefore, more easily predict their labor needs and make changes in their input mix (e.g., days at sea and crew size). In late summer and early fall, resource abundance and spawning appears to be erratic; captains cannot, therefore, easily predict these conditions and change crew size.

Minimum TE primarily occurred between September and February. Low TE in September may have been associated with weather. This is a period of intense storms in the mid-Atlantic

TABLE 5.

TE and biological characteristics, and input utilization per trip, 1987 to 1990.

Days at sea	TE			Meat Weight (g)			Stock Abundance Baskets per Hour		
	≤8	9–10	≥11	≤8	9–10	≥11	≤8	9–10	≥11
5	0.80	0.71	0.77	13.8	13.7	15.0	1.66	2.61	2.81
6	0.89	0.72	0.83	9.6	13.6	14.2	1.12	2.36	4.08
7	0.72	0.74	0.74	14.4	12.9	14.2	1.44	1.92	3.16
8	0.00	0.75	0.75	14.5	13.9	14.7	2.54	1.89	3.04
9	0.75	0.71	0.63	12.6	12.9	12.9	1.56	2.62	3.13
10	0.84	0.72	0.79	13.6	13.5	15.0	2.89	2.76	3.66
11	0.46	0.70	0.68	13.2	12.9	14.1	1.44	2.41	4.03
12	0.68	0.78	0.76	12.6	13.1	14.7	1.38	2.63	3.50
13	0.60	0.76	0.54	12.9	13.0	13.7	1.65	2.54	3.29
14	0.89	0.74	0.71	10.5	12.3	13.1	1.80	2.28	2.70
15	0.79	0.78	0.78	12.7	13.3	14.6	1.81	2.24	3.93
16	0.78	0.77	0.66	11.3	13.0	12.7	1.64	2.55	3.11
17	0.77	0.79	0.76	12.6	13.4	14.8	2.12	2.64	4.00
18	0.79	0.77	0.80	12.9	13.5	15.0	2.09	2.83	4.06
19	0.76	0.76	0.76	12.5	13.2	14.3	2.15	2.93	4.00
20	0.76	0.75	0.74	13.0	13.8	13.7	2.62	3.16	3.19
21	0.77	0.74	0.84	12.6	13.8	13.0	2.39	2.73	3.44
22	0.80	0.78	NA[a]	10.5	14.1	NA	2.11	3.13	NA
23	0.00	0.56	0.50	NA	11.7	9.6	NA	2.01	1.17
24	0.74	0.71	0.75	11.7	15.0	13.4	2.76	2.35	3.98
<14	0.66	0.72	0.74	12.9	13.2	14.2	1.66	2.50	3.27
14–22	0.77	0.77	0.76	12.6	13.4	14.2	2.20	2.84	3.77
>22	0.70	0.67	0.62	13.2	13.6	11.5	2.98	2.28	2.58

Note: results are summarized relative to three groups of crew size (≤8, 9–10, and ≥11 individuals).

[a] NA indicates no observations for days at sea and crew size group.

resource area, and vessels must often ride out the storms at sea or cut trips short and return to port. Low efficiency between October and November likely was associated with reproductive activities and reduced meat yields. The fall spawn, although erratic in the mid-Atlantic resource area, typically occurs between October and November (DuPaul et al. (1989) Kirkley and DuPaul 1991). The minimum average efficiency of December was likely associated with low abundance and meat yields.

Low efficiencies in September, January, and February, however, also may have been caused by poor judgment about crew size by captains. Stock abundance during these 3 months was relatively low but highly variable, as indicated by the coefficient of variation, whereas changes in crew size were infrequent. If captains fail to change crew size in response to changes in resource conditions, TE might be low because of too much or too little labor. In fact, higher mean TEs per trip in each month were associated with lower average crew sizes. For example, mean crew size for TE $< 0.8 = 9.55$ and mean crew size for TE $\geq 0.8 = 8.91$ in February; differences were similar for September and January. It is, thus, quite probable that technical inefficiency during September, February, and January is at least partly caused by excess labor.

In comparison, resource abundance and crew size were both highly variable in December, but the monthly mean TE per trip was the lowest relative to all other months. The coefficients of variation for crew size and stock abundance indicated that captains changed crew size in response to changes in resource conditions. Low efficiencies during December, however, were also associated with high crew sizes (e.g., mean crew size for TE $< 0.8 = 9.56$, whereas mean crew size for TE $\geq 0.8 = 7.96$). It is, thus, likely that captains misjudge their labor needs and use too much labor in December. Alternatively, it may be possible that vessel operating requirements cause excess labor relative to resource conditions in December (e.g., safe operation of a scallop vessel requires seven to nine individuals, and eight or more crew may be excessive, given resource abundance).

Short of curtailing fishing activities between September and February, what prescriptions does the analysis offer for improving TE in the mid-Atlantic sea-scallop, *P. magellanicus*, fishery? In general, vessel captains can consistently achieve high TE by taking trips between 14 and 22 days. High TE may also be achieved with shorter trips, but economic conditions seriously limit net returns for short trips; maximum profit occurs for trips between 15 and 22 days (Kirkley and DuPaul 1992). When resource abundance is relatively low, crew size should be restricted to eight or fewer individuals; when abundance is relatively high, crew size should be sufficient to provide the labor services of nine or more individuals. In essence, the best qualitative prescription for improving TE is for captains to become more aware of changes in resource and environmental conditions and accordingly change input levels.

Thus far, analyses indicate that TE is affected by controllable factors of production and relatively uncontrollable, but not necessarily unpredictable, biological and environmental conditions. Relatively high levels of TE can be maintained over a wide range of resource conditions and input and output levels. Managers designing policies to promote efficient resource conservation, therefore, need to consider the potential interaction between TE, resource conditions, known temporal patterns in variables that might affect efficiency, and input levels.

The NEFMC has required that crew size be restricted to nine individuals to limit total harvest and prevent excess harvesting of small scallops. NEFMC has also proposed restrictions on days at sea per year per vessel to limit total production and size of scallops. Assessing the likelihood of these regulations to accomplish their goals will require considerable detailed knowledge of TE and the importance of biological and economic conditions. For example, what are the interactions between resource conditions and the proposed regulations? Will TE be increased because of the regulations?

By the use of information obtained from eight at-sea experiments in which crew sizes or shucking capacities required for given harvest levels were estimated, it was determined that production levels for only 63 of 581 trips between 1987 and 1990 would have been affected by restrictions on crew size. Crew, however, would have to work more hours per day (i.e., increase the number of hours per individual from 12 up to 16 hours). Relative to production during 1990, a restriction on crew size would have reduced landings for only 4 of 132 trips. A nine-man crew limit would, therefore, only marginally restrict total catch and reduce fishing mortality relative to observed levels; it would, however, improve TE in the mid-Atlantic sea scallop fishery.

On the basis of an assumed proportional relationship between fishing mortality and fishing effort (days at sea), the NEFMC restricted the annual number of days at sea a vessel may fish. Analyses in Kirkley and DuPaul (1992) have shown that an annual restriction on days at sea, depending on the total number of days allowed, will likely cause vessels to stop fishing between October and January, when economic returns are low. This also corre-

TABLE 6.

Average monthly technical efficiency, 1987 to 1990.

Month	Efficiency	Effort	Crew Size	Stock Abundance	Weight of 90 to 94 mm Sea Scallops (g)	Gonad Weight 90 to 95 mm (g)
January	0.74	14.57	9.03	1.73	13.22	3.98
	[21.4][b]	[31.8]	[9.4]	[34.7]	[6.5]	[21.0]
February	0.74	12.89	9.41	2.24	13.77	5.86
	[18.3]	[48.9]	[10.8]	[37.6]	[5.1]	[12.1]
March	0.76	14.53	10.04	2.52	14.46	6.79
	[15.2]	[36.8]	[10.7]	[35.7]	[6.2]	[6.8]
April	0.77	17.02	10.71	3.54	14.13	6.07
	[7.5]	[27.2]	[15.4]	[24.8]	[4.9]	[24.6]
May	0.77	15.57	10.24	3.77	13.85	3.76
	[20.5]	[33.8]	[13.1]	[33.0]	[9.7]	[17.2]
June	0.79	16.71	9.92	3.78	13.09	2.43
	[10.3]	[23.6]	[14.5]	[28.3]	[7.0]	[29.1]
July	0.75	15.25	10.06	3.53	13.97	2.47
	[17.4]	[35.5]	[16.6]	[33.8]	[6.1]	[17.1]
August	0.77	16.23	9.80	3.29	14.09	2.31
	[9.6]	[29.9]	[13.3]	[34.3]	[8.5]	[11.1]
September	0.72	16.02	9.84	2.94	13.88	3.08
	[12.0]	[35.6]	[10.7]	[36.7]	[10.0]	[13.1]
October	0.74	15.70	9.05	2.41	12.89	3.96
	[19.4]	[29.8]	[15.4]	[35.4]	[14.0]	[27.6]
November	0.71	15.91	8.89	2.07	12.19	2.59
	[20.0]	[31.3]	[13.8]	[37.8]	[9.3]	[13.2]
December	0.69	14.60	8.56	1.67	11.76	2.41
	[25.7]	[35.0]	[16.4]	[35.7]	[11.7]	[20.3]

Note: effort is the number of days at sea per trip, crew size is the number of individuals aboard the vessel per trip, and stock abundance is number of baskets per hour.

[a] Numbers in brackets are coefficients of variation.

sponds to the time period when scallops spawn, resource abundance and meats yields are low, and TE is minimum. Given that vessels curtail fishing activities during this period, average TE per trip can be expected to increase in response to a restriction on days at sea per vessel per year. Average TE over all 10 vessels and months between 1987 and 1990 was 0.74. Mean TE per trip increased to 0.77 or by approximately 4.1% when fishing activity was restricted to February through September. A restriction on the number of days at sea per vessel should, therefore, increase TE while overall harvest levels decline and spawning occurs.

This study demonstrated that TE in the mid-Atlantic sea scallop dredge fishery from 1987 through 1990 was maintained over a wide range of input levels and resource conditions. Production was most efficient for trips of 14 to 22 days and made between March and August. Minimum efficiency occurred for trips in excess of 22 days, or in general, less than 14 days, and taken between September and February. Inadequate input levels, given resource conditions or incorrect decisions by captains about input levels, were determined to contribute to technical inefficiency. It was also determined, however, that efficiency more closely followed the temporal patterns of resource abundance, meat yields, and the reproductive cycle. Gains in TE are, thus, highly likely if captains can correctly match input levels to resource conditions.

Efficacious regulatory policy will have to consider the effects on input and output levels and TE, as well as the relationship between TE, input levels, and resource and environmental conditions. Failure to recognize these linkages could result in inadequate regulatory policies, particularly those policies designed to promote social and economic efficiency. Relative to the restrictions on days at sea and crew size, TE should increase while harvest levels decline.

A comprehensive evaluation of TE in the sea scallop fishery must consider stock conditions, input levels, vessel characteristics, and managerial ability. This study did not address managerial ability, and thus, it is possible that levels of TE may have been inappropriately attributed to biological and economic conditions. Alternatively, estimates of TE may be biased because of the omission of captain's skill from the analysis. Managers concerned with TE in the sea scallop fishery must be cognizant of changes in TE, economic performance, and biological and environmental conditions when determining regulations. Although TE is necessary for social efficiency, it is not a sufficient condition. Evaluation of TE in the sea scallop fishery and other fisheries will require considerably more information than is usually available. Additional information on vessel captain, gear, vessel characteristics, environmental conditions, food abundance, and the gametogenic cycle must be obtained to better understand TE in the sea scallop fishery.

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AGE, GROWTH RATE, SEXUAL DIMORPHISM AND FECUNDITY OF KNOBBED WHELK *BUSYCON CARICA* (GMELIN, 1791) IN A WESTERN MID-ATLANTIC LAGOON SYSTEM, VIRGINIA

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ABSTRACT Growth, onset of sexual maturity, and sexual reversal in laboratory-reared *Busycon carica* have been examined. Animals first matured at 9 years of age. The first sign of maturity in all animals we reared was the presence of the penis. At 12.4 years of age, one of the animals laid an egg case that did not contain embryos. This animal, and all others, still retained a penis. At 13.5 years, three egg cases were laid and over half the animals had undergone sex reversal (loss of the penis). Field studies have shown that egg strings are laid in the fall on tidal and intertidal flats and over winter to hatch in the spring. Organisms that require a relatively long time to mature, that lay few eggs per spawning season, and that are vulnerable for a long time are difficult to manage for a sustained yield fishery.

KEY WORDS: *Busycon carica*, whelk, age, growth, sex reversal, sexual dimorphism, fecundity

INTRODUCTION

Busycon carica is a large, predacious gastropod that is commercially harvested along the east coast of the United States and marketed as conchs or, more properly, as whelks. Over 200,000 lbs were landed in Virginia in 1986. The frozen meats are used in salads and chowders or sold in ethnic markets as squingelli (DiCosimo 1986, Kaplan and Boyer 1992).

There are few published data on growth rates, age of onset of sexual dimorphism, or sex ratios of busyconine whelks (Frank 1969, Powell and Cummings 1985, Kraeuter et al. 1989), and no information on the age at which females become sexually mature. Although there are relatively large numbers of studies on epifaunal snails, other than growth rates, little information is available on size at age or the age of sexual maturity of long-lived predaceous infaunal gastropods. Growth information is available for *Polinices duplicatus* (Say 1822) (Edwards and Huebner 1977). Gendron (1992) reported growth rate and the size of sexually mature *Buccinum undatum* (Linne 1758), whereas Santarelli and Gros (1985) examined age structure in *B. undatum* on the basis of opercular striae. Heller (1990) compared longevity throughout the entire mollusca phylum to reveal common patterns of reproduction. This was based on data gleaned from the existing literature on the life durations of 547 species from marine, freshwater, or terrestrial habitats. Gastropods are the second most long-lived of mollusks, after bivalves. Short-lived mode of life is often correlated with: lack of external shell or an external shell that is semitransparent, dwelling in a harsh microenvironment that is exposed to high solar radiation and high temperatures, dwelling in an environment in which reproduction occurs at least once a year, and very minute size. Powell and Cummings (1985) compiled data on longevity of bivalves and gastropods and found that a higher-than-average number of long life spans coincide with periods of long-term cycles in marine communities. These cycles could affect longevity in one of two ways: cyclic phenomena that produce environmental changes beyond the species tolerance limit or cycles that might affect recruitment success and thereby exert selective pressure for longevities longer than the cycle affecting recruitment. Interest in culture of queen conch, *Strombus gigas* (Linne 1758), has provided substantial information on the growth, size-specific mortality, and ecology of that species (Wefer and Killingley 1980, Appeldorn 1988).

Magalhaes (1948) studied *B. carica* growth rate in the field near Beaufort, North Carolina, and Sisson (1972) measured growth of *Busycotypus* (*Busycon*) *canaliculatus* (Linne 1758) in Narragansett Bay, Rhode Island. The general ecology of whelks is best known from Magalhaes (1948), Peterson (1982) in North Carolina, and from Menzel and Nichy (1958), Paine (1962 and 1963), and subsequent studies by Kent (1983) in Florida. Davis and Sisson (1988) have increased the available information on whelks in Rhode Island, Massachusetts, and Georgia (Walker 1988). The work of Magalhaes (1948) remains the most complete ecological study of *B. carica* to date. She reports egg laying in Beaufort, North Carolina, from May to June and again from September to November. Numbers of egg capsules per string ranged from 9 to 156 (mean, 80), and the total numbers of egg per string ranged from 4,000 to 6,000 (Magalhaes, 1948). Ram (1977) found that an extract from the nervous system would cause mature animals to lay egg capsules.

We report on the continuation of a 14 year study of growth rates in which *B. carica* were reared in the laboratory from hatching to sexual maturity to egg laying. Kraeuter et al. (1989) examined the growth rate of a *B. carica* population in Virginia, using three methods: (1) measurement of individuals marked and recaptured in the field; (2) examination of growth lines in the operculum; and (3) measurement of laboratory-reared individuals. Most whelks that were marked and recaptured were larger than 170 mm. The smallest individual tagged and recaptured grew from 138 to 151 mm (0.098 mm/day^{-1}). Growth rates for males were not calculated because too few were obtained to make accurate estimates. Most of the males were in the 170 to 209 mm size classes. Two series of marked individuals were released into the field.

Males were 7.8% (N = 23) of the 190 individuals in the first group and 9.0% (N = 167) of the 1,859 individuals of the second group. No indications of sex reversal were noted in the field studies. Information on numbers of eggs per capsule, numbers of capsules per string, seasonal changes in gonad and nidamental gland precursor (precapsulin; see Goldsmith et al. 1978), and time of hatching for a Virginia population of *B. carica* is presented in this article.

METHODS

Sexual Maturity and Growth at Age

B. carica were hatched from egg cases collected from an intertidal sand flat behind Cedar Island, Virginia, in the winter of 1976 to 1977. Nine egg strings were returned to the Virginia Institute of Marine Science Laboratory in Wachapreague, Virginia, and maintained in a running seawater system. Seawater is drawn from a nearby channel where salinity ranges from 25 to 32 ppt, with short excursions below 20 ppt after extreme rain storms. Hatching from the strings was complete by May 1977. Attempts to keep newly hatched whelks in glass dishes, plastic trays, or shallow trays filled with sand from the intertidal flat failed because the animals continually climbed out of the water and desiccated. Some individuals were hatched in a 2.4 × 0.6 m fiberglass flowing seawater tray in which a miniature sand beach had been made. Some newly hatched individuals from several egg strings were reared inside a polypropylene filter bag that received flowing ambient seawater. When the animals reached approximately 20 mm shell length, they were transferred to the running seawater trays with beach sand substrate and supplied with small, live juvenile clams (*Mulinia lateralis, Mercenaria mercenaria,* or *Mya arenaria*). As the *B. carica* grew, larger size clams were furnished. *M. mercenaria* was the most commonly fed species and was used exclusively after the third year. Dietary supplements may have entered with the flowing seawater.

Length measurements were made on individual whelks removed from the substrate five times during the first year, then two or three times in subsequent years. Measurements were made on a minimum of 25 individuals per sampling period, until 1985, when the number was reduced to 20.

Initially, over 2,000 individuals were maintained but these were reduced to 261 through attrition and sacrifice for other studies by the fall of 1977. Mortality had reduced the total numbers being maintained to 93 after 5 years (February 1982). In the fall of 1989, the remaining whelks were divided into groups of six and placed in four sand-filled 0.6 × 0.6 m boxes with a standpipe supplied with ambient flowing seawater. The organisms were maintained in these boxes until April 1990, when a number of individuals had developed a boring sponge (*Cliona*) infestation in their shells. All individuals were given a 10 second dip in a saturated salt solution and air dried for 1 hour before being returned to flowing seawater. These same individuals were used for the sexual maturation studies.

The cultured whelks were sexed by observing the presence or absence of a penis. The observations were made in three ways. Animals were placed in a shallow plastic tray containing 1 to 3 cm of flowing ambient seawater until they were firmly affixed to the bottom (about 1 hour). The penis, a C-shaped organ, could be observed by gently tilting the shell clockwise. In another method, the whelks' shells were attached dorsal side down and held in place with a small ball of clay or with malleable lead wire. The tray was left in a partially darkened room for 0.5 to 2 hours, allowing time for the animals to attempt to right themselves by extending their foot. The extension of the foot exposed the penal area. A third method was sometimes used for older intractable specimens. With a 1 ml syringe and a 23 gauge needle, 0.4 ml of 2 mM serotonin (5-hydroxytryptamine, creatinine sulfate complex; Sigma Chemical Co., St. Louis, MO) was injected into the foot just posterior to the operculum. The animals reacted within 2 hours by extending the foot and rolling the operculum away from the aperture, exposing the phallus.

Gonadal Analysis

About 10 specimens were randomly collected in 1976 to 1977 at approximately 11 monthly intervals (Σ N = 105) from the study site behind Cedar Island, returned to the laboratory, and frozen. Shell length and width were measured before the removal of the flesh. The animal was extracted from the shell, sexed (presence or absence of eggs or a penis), and weighed to the nearest 0.1 g. The tissue was dissected into four fractions: meat, viscera, nidamental gland (females only), and gonad, and each was weighed to the nearest 0.1 g.

Egg Strings and Egg Capsules

Several studies examined the timing of egg laying, numbers of capsules per egg string, and time and number of egg hatching. Egg laying and paired individuals (copulation) were recorded during mark recapture field studies (approximately monthly during 1976 and 1977). Numbers of egg capsules per egg string were recorded in the field, the location was noted, and the string was marked. These were followed on subsequent visits until hatching was recorded.

To determine hatching success, the shoreline was examined for old egg strings in the fall and winter of 1976 and these were removed. Any egg string that was found on the beach in the spring (late March to May 1977) was returned to the laboratory, and numbers of juveniles per capsule were determined. These data formed the basis for an estimate of the numbers of hatchlings per string and the percent mortality. For these estimates, the total number of eggs per string was estimated on the basis of the largest numbers of juveniles found in capsules from the midportion of the egg string.

A number of strings were returned to the laboratory for more detailed study of the number of embryos per capsule and the number of capsules per string. The number of capsules before the first egg-bearing capsule (anchoring part of string) was determined from these strings. Capsules were randomly removed from the length of the string and measured (height, diameter, and volume). The number of embryos per capsule was determined by removing the top of the capsule and counting the eggs or developing embryos.

Hatching studies were performed on capsules removed from the same strings as those used to determine the distribution of the number of embryos per capsule. Ten capsules from each of four egg strings were placed in baths containing water of three temperature regimens: cold (2 to 5°C) ambient (10 to 15°C), and warm (20 to 25°C). Capsules were examined daily, and the day of hatching for various capsules was recorded.

RESULTS

Sexual Maturity and Growth at Age

Growth rate for individuals maintained in the laboratory was highest in the first year, when the animals grew from 4 to 36.5 mm

shell length. The average size after 10 years of growth was 144 mm, and by 14 years, the average size was 168.7 mm shell length (Fig. 1). All 20 cultured individuals in 1986 at 9 years of age were considered to be males, because 9 had a relatively large, well-developed penis and 11 had a smaller penis. By October 1987, all 20 had either a large (10) or a moderately developed (10) penis, and by April 1989, all had a well-developed penis.

On September 25, 1989, 12 years 5 months after hatching (ca. 13 years after eggs were laid), one whelk laid an egg case, but it contained no embryos. The animal producing the egg case was 172 mm long and 95 mm wide and had a 25 mm long penis in April 1989. By April 6, 1990, 5 animals were female with the penis reduced to a rounded protuberance, 10 were males, and 5 had died, including the original female.

By May 20, 1991, 9 of the remaining 15 individuals were females. Rounded protuberances remained, but the individuals that appeared to have most recently become female had a small, vestigial, flap-like penis. This flap was approximately 5 to 7 mm across the widest point. *Busycon* under 166 mm length had a larger, C-shaped penis and were considered to be males.

In September 1991, 3 of the remaining 14 *Busycon* laid egg cases containing viable embryos. Five whelks were now presumptive males (under 166 mm), 7 were definite females with round, button-like protuberances, and 2 were apparently transitional with neither a flap, a well-shaped button, or a C-shaped phallus.

The three egg cases were maintained in running seawater from September to April, while temperatures ranged from 9.2 to 14.5°C and salinity averaged 30 ppt. During the first 3 weeks in April most of the *B. carica* hatched from the egg cases. Some of the early hatchlings climbed out of the tank and died. The others were removed from the tray and grown in the laboratory for nearly 60 days, during which they approximately doubled in size before being released in early June on the sand flats where the egg strings were collected in 1976.

Gonadal Analysis

Of the 105 individuals used for gonadal analysis, only 8 were males (sex ratio = 0.082), so males were not included in the analysis. Females were collected on June 30, July 29, August 26, October 10, and November 11, 1976, and on January 11, 1977, and a second series was collected on April 15, May 11, June 12, July 7, and August 8, 1979. The range in gonadal weight was 0.3 to 27.4 g. Average gonadal wet weight ranged from 4.1 to 12.2 g and average nidamental gland weight ranged from 21.9 to 52.1 g. The percentage of gonad or nidamental gland to total weight and meat weight are given in Table 1. The data indicate a decrease in these percentages in October, the time of maximum observed egg laying in the field. Although the data suggest a spring spawning, no such spawning was seen in over 5 years of field observations.

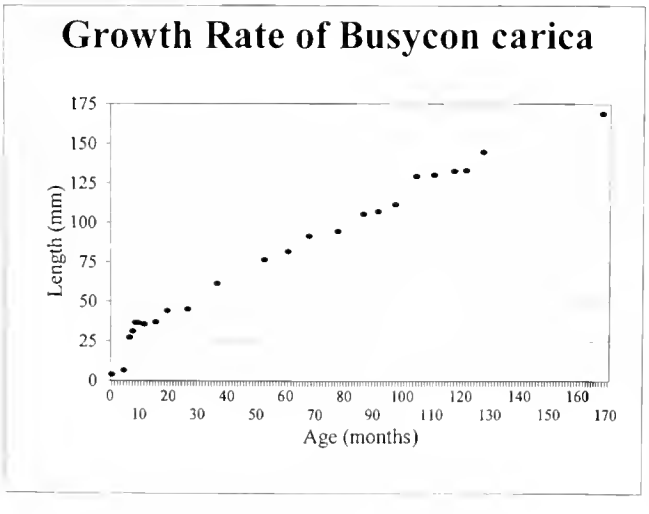

Figure 1. Growth rate of *B. carica*.

Egg Strings and Egg Capsules

The only months in which copulating individuals were observed on the intertidal flats were June and July. Eggs were laid in the field from mid-August to November, with most egg laying from mid-September to mid-October. The largest number of egg strings found on the intertidal flat was 66 in 1976. Capsules remained closed until spring, and hatching (on the basis of open capsules) was observed from mid-March through early May. A few of the earliest laid strings (late August) began hatching by the end of October. In general, strings were present on the intertidal flat throughout the winter, but by April or May, most had disappeared. Some of these egg strings washed ashore in those 2 months, and juveniles that had not hatched were dead.

Capsules per string ranged from 42 to 121. The average number of capsules above the anchor point per string was 99.7 (standard error [SE], 4.68; N = 16) in the winter of 1977 to 1978, 89.4 (SE, 2.44; N = 66) in the winter of 1978 to 1979, and 92.4 ± 4.93 in the spring of 1979. The number of capsules in the anchor portion of the string ranged from 8 to 22 (mean, 13.1; SE, 1.21; N = 16).

On the basis of the random selection of capsules along the length of four strings, the average number of eggs per capsule is less in the first and last 10% of egg-bearing capsules (Table 2). Seventeen egg strings were found washed up on the beach in April to May 1976. These cases were covered with fouling organisms (algae, *Illyanassa* eggs, *Corophium* tubes) and had apparently broken off from their anchor. One case had not hatched (escape plugs were intact); development had not occurred. The percent hatch (hatching success) ranged from 18 to 86% (Table 3). Thirteen of the 16 strings analyzed held hatching rates of more than 60%. Fouling by corophid amphipods or mud snail (*Illyanassa obtusata*) egg capsules may block the escape of whelks in the field.

Laboratory studies on hatching yielded a positive correlation of hatching with temperature. With increasing temperatures, hatching times were reduced: at 2 to 5°C, no hatching occurred; at 10 to 15°C, hatching began at 65 to 78 days, and at 20 to 25°C, it began at 22 to 30 days. One string was excluded because of a drop in water temperatures.

DISCUSSION

Sexual Maturity and Growth at Age

The growth rates and timing of the sex changes in these laboratory studies may not mimic those from the natural habitat; however, the whelks always had a surplus of clams of varied sizes, so food should not have been a limiting factor. The temperatures and salinities were typical of those in the field because of the continuously supplied ambient seawater. The containers in which the whelks were grown, although relatively uncrowded, would certainly not compare with the low-density habitats found in nature.

TABLE 1.

Seasonal gonadal indices (%) for female *B. carica* in Virginia. Indices are all wet weight

	Month and day										
Item measured[a]	J 30	J 29	A 26	O 10	N 11	J 11	A 15	M 11	J 12	J 7	A 8
G/TW	3.1	2.4	2.7	1.4	3.0	2.0	3.1	1.1	1.8	2.5	2.2
N/TW	12.9	10.3	12.3	8.1	12.7	11.3	11.1	7.3	8.6	12.5	11.2
G + N/TW	15.9	12.4	13.3	9.5	15.7	13.3	14.2	8.4	10.4	15.0	13.4
G/N	24.3	23.8	21.7	17.4	23.6	18.0	28.3	15.1	20.9	20.3	19.4
G + N/MW	22.1	16.8	20.5	12.8	21.7	18.2	20.6	11.3	15.1	22.0	19.1
G/MW	4.4	3.2	3.7	1.9	4.1	2.8	4.5	1.8	2.6	3.7	2.8
N/MW	17.7	13.6	16.8	10.9	17.6	15.4	16.0	9.5	12.5	18.3	16.4

[a] G, gonad; N, nidamental gland; TW, total weight; MW, meat weight.

Gonadal Analysis

The sex ratio (0.082) from this study is within the range 7.8 to 9.0% reported by Kraeuter et al. (1989) for whelks from this location and is almost identical to that reported for intertidal populations of *B. carica* in Georgia (Walker 1988). Magalhaes (1948) suggests that eggs are laid in the spring both at Beaufort, North Carolina, and in Connecticut, but her data from farther north are based on "fresh" capsules and not observed egg laying. On the basis of gonadal and nidamental gland data and the small amount of information available from the males, there appear to be two spawning seasons in Virginia. In over 5 years of field study, however, we did not find eggs being laid on an intertidal flat during the spring, although spawning might occur in deeper water.

Ram (1977) used categories of gonadal weights ranging from 0.0 to 0.9 g to >3.6 g. The gonads of his animals were significantly smaller than those on our animals; his largest class is smaller than the average weight in any month in our study and far less than our maximum of 27.4 g. It does not seem plausible that the large percent body weight reduction from egg laying could be recovered within a month, as our data suggest. The rapid recovery of the ratios after May and October could be the result of cohorts of snails moving on and off the intertidal flat. This rapid movement is consistent with mark-recapture studies done on animals from this same location (unpublished data). Similar rapid emigrations of marked snails were reported by Weil and Laughlin (1984) for tagged queen conchs on subtidal tropical grass flats. This explanation is further supported by the evidence that there was never a sample in which all females had spent gonads. It is possible that, in addition to fairly rapid immigration and emigration, not all of the females lay eggs every year.

TABLE 2.

Numbers of *B. carica* eggs per capsule along the length of strings collected from Cedar Island, Virginia, December 1976 to January 1977

Percent distance along string	No. of capsules sampled	Average no. of eggs	SE
0–10	6	20.0	8.53
11–30	5	37.4	5.98
31–60	8	51.5	4.20
61–90	9	37.5	3.64
91–100	5	10.2	3.08

Egg Strings and Egg Capsules

Magalhaes (1948) reported finding copulating individuals of *Busycon* in March, June, August, and September, but her article does not distinguish between *B. carica* and *B. canaliculata*. Kent (1983) found copulating *Busycon spiratum* and *Busycon contrarium* in October and January on tidal flats in northwest Florida. Walker (1988) found whelks mating in the spring and fall on Georgia intertidal flats. Our data fit the general pattern that copulation may be seasonal, but not necessarily at the same time as egg laying.

The range in numbers of egg capsules above the anchor from Beaufort, North Carolina (9 to 156) (Magalhaes 1948) spans the number found in Virginia. The mean number of capsules in North Carolina (80) is slightly less than the average number produced in 2 years on Cedar Island, VA. The numbers of individuals potentially hatching from a single string appear to range up to 5,000 to 6,000 in both North Carolina and Virginia, although 2,000 to 3,000 would be more typical.

The timing of egg laying (September) in the laboratory-reared animals matches that which we have observed in field studies on intertidal flats. Hatching in the laboratory in spring also mirrors what we have observed in both the field and from controlled-temperature laboratory studies. Egg strings washing ashore during winter storms or, more typically, during April and May, were destroyed along with unhatched juveniles. The mechanism that causes the egg strings to weaken and wash ashore around hatching time is not known.

Several factors of importance to management are apparent from this study. Clearly, the length of time required for maturity has far-reaching implications. The presence of a penis does not unambiguously define whether a particular individual is male or female, and thus, attempting to regulate harvests on the basis of sex or sex ratios would be difficult. The length of time before females ap-

TABLE 3.

Egg cases of *B. carica* washed up on the bay beach of Cedar Island, Virginia, in April and May 1977

Category	Range	No. of srings	Average	SE
Whelks/string	1,945–5,508	16	3770	206.50
Whelks/capsule	30–51	16	41.79	1.54
% Hatched	18–86	16		

peared in the cohort was very long (12 years), and their length (172 mm) was large. Harvest selection by size would probably be specific for females. Egg cases remain vulnerable to storms or other forms of bottom disturbance for 7 months before they hatch. This long development further restricts the amount of time an area could be exploited by dredges or other gear that disrupt the bottom. Most egg cases were concentrated in a relatively small portion of the flat. Hatching was controlled by temperature, and some factor also positively associated with temperature allows the egg strings to weaken so that they are more apt to wash away. Although we did not make estimates of posthatch mortality, the rapid loss of individuals in the laboratory suggests that field mortality of small individuals is significant. We were never able to discover small individuals in the field. Laboratory observations suggest that they remain buried most of the time (as do the adults).

This species, which requires a long time to mature, lays relatively few eggs per spawning season, has a low survival rate of its young, and appears to be extremely vulnerable to harvest pressure. Management for this resource for an optimal sustainable yield will be difficult.

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THE OCCURRENCE OF DOMOIC ACID IN RAZOR CLAMS (*SILIQUA PATULA*), DUNGENESS CRAB (*CANCER MAGISTER*), AND ANCHOVIES (*ENGRAULIS MORDAX*).

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ABSTRACT In September 1991, water fowl died in Monterey Bay, CA, after eating anchovies (*Engraulis mordax*) contaminated with domoic acid. Analysis revealed that the anchovies contained up to 485 ppm domoic acid in their viscera. This was the first reported incidence of domoic acid-related mortality of any organism in the United States. After this reported outbreak we obtained frozen samples of anchovies that were harvested near Newport, CA, in April 1991 and found they contained 270 ppm domoic acid in their viscera. By May, average domoic acid levels in frozen anchovy samples from this same area were less than 1 ppm. In October 1991, domoic acid was detected in razor clams (*Siliqua patula*) from Oregon and Washington and appeared to peak (an average of 106 ppm for all Washington State beaches) in the first week of December 1991. The averages then declined to less than 20 ppm without 6 months. However, domoic acid was still present at low levels (averages <5 ppm) in razor clams from Washington state beaches in December 1993. Dungeness crab (*Cancer magister*) in Washington and Oregon were also found to contain domoic acid, but only in their viscera. Domoic acid concentrations in the raw viscera of individual crabs from Washington state in December 1991 averaged 13 ppm and ranged from 0.8 to 90 ppm. The highest average levels of domoic acid in Washington state crabs were in the Grays Harbor and Willapa Bay samples, 32 and 31 ppm, respectively. By 1992 domoic acid level averages were <5 ppm in preseason samples of Dungeness crab taken along the Oregon and Washington coasts, ranging from 0 to 71 ppm. The highest levels of domoic acid in 1992 (36–71 ppm) were recorded in samples taken early in that year (January through April).

KEY WORDS: domoic acid, Dungeness crab, *Cancer magister*, anchovies, *Engraulis mordax*, razor clams, *Siliqua patula*

INTRODUCTION

The first outbreak of domoic acid poisoning in the United States was reported from Monterey Bay, CA, in September 1991 (Work et al. 1991, 1993). In that outbreak, a large number of sick and dead pelicans (*Pelecanus occidentalis*) and cormorants (*Phalacrocorax penicillatus*) were observed. Analyses of the stomach content of the affected birds revealed they had consumed anchovies (*Engraulis mordax*). Chemical and biological assays of the stomachs of pelican and anchovies taken from the bay revealed high levels of domoic acid (T.M. Work pers. comm.; anonymous 1991; Work et al. 1991; Fritz et al. 1992). Examination of water column samples and gut contents of the anchovies from Monterey Bay revealed large numbers of a diatom, later identified as *Pseudonitzschia australis* (Fryxell 1992; Buck et al. 1992). When samples of recovered diatoms were grown in the laboratory, they were shown to produce domoic acid (Garrison 1992; Garrison et al. 1992; Buck et al. 1992; Villac et al. 1993).

Because of the tragic consequences of the 1987 domoic acid outbreak in Canada (Todd 1990, 1993), a rapid response to the threat of domoic acid poisoning was mounted in the United States. The U.S. Food and Drug Administration (FDA) declared an action limit of 20 ppm domoic acid in seafoods entering interstate commerce, and health regulatory agencies in Washington, Oregon, and California began monitoring for the presence of domoic acid in a variety of marine species. As a result of the early warning and rapid response by state and federal agencies, no confirmed human illnesses were reported from Washington, Oregon, or California.

In order to manage rationally the risks associated with domoic acid poisoning, it was necessary to determine both the biological and geographical distribution of the outbreak. Because anchovies were implicated in Monterey Bay, a survey of commercial frozen stocks of anchovies intended for human consumption was performed. Shellfish that consume *P. australis* and their predators were also examined. Finally, it was necessary to determine the longevity or persistence of domoic acid in certain target species. In November 1991, our laboratory began analyses of several marine species that included razor clams (*Siliqua patula*), mussels (*Mytilus edulis*), anchovies (*E. mordax*), and Dungeness crab (*Cancer magister*).

Wekell et al. (1994) reported that domoic acid levels in Washington state razor clams seemed to peak in December 1991 and then declined below the regulatory limit of 20 ppm by June 1992. In addition, we continued to monitor and observe low levels (<5 ppm) of domoic acid in these clams along the Washington coast well into December 1993 more than 2 years after the initial outbreak was reported in 1991. However, in the late fall (November through December) of 1992 and 1993, we observed brief increases of domoic acid levels in some samples of Washington state razor clams.

As potential predators of razor clams and other contaminated sources, it was clear that Dungeness crab might also become contaminated with domoic acid. We report here some information about the geographic distribution of the domoic acid in Dungeness crabs and razor clams along the Washington and Oregon coasts from December 1991 to December 1993. In addition, we present data on mussels and anchovies from Monterey Bay, CA. The anchovies were taken at the time of the initial observations of domoic acid poisoning in September 1991, while the mussels were taken in November 1991. We also include domoic acid data on commercially frozen anchovies from southern California landed in April and May of 1991. This work was part of a combined effort that included health and regulatory agencies in the states of California, Oregon, and Washington, the U.S. Food and Drug Administration, and the National Marine Fisheries Service.

MATERIALS AND METHODS

Reagents

Methanol (MeOH) and acetonitrile (MeCN) were HPLC grade (Baxter Healthcare Corp., Burdick and Jackson Division, Muskegon, MI 49442). Sodium chloride was ACS reagent grade. Water to be used for extractions and HPLC analyses was prepared by passing distilled water through a Milli-Q water system (Millipore Products Division, Bedford, MA 01730). The Milli-Q water used as an eluent in HPLC analyses was filtered through a 0.45-μm HA filter (Millipore Products Division, Bedford, MA 01730) and mixed with 100 mL MeCN in a 1-liter volumetric flask. To this mixture was added 1.0 mL trifluoroacetic acid (TFA) (Sigma Chemical Co., St. Louis, MO 63178) and then diluted to mark. Immediately before chromatography, solvents were degassed with helium for 5 min.

Standards

A working domoic acid standard solution was prepared from the certified standard DACS-1 (Canadian National Research Council, Institute of Marine Biosciences, 1411 Oxford Street, Halifax, N.S., Canada B3B 3Z1; Hardstaff et al., 1990) by diluting it with 10% aqueous MeCN to a final concentration of 4.45 ppm. A 1000 ppm stock solution of tryptophan was prepared by dissolving 100 mg L-tryptophan (Sigma Chemical Co., St. Louis, MO 63178) in 10% MeCN and diluting to 100 mL. A working standard solution of tryptophan was prepared by diluting the stock solution to 100 ppm using 10% MeCN. The tryptophan standard was used only to set initial chromatographic parameters, i.e., resolution of tryptophan and domoic acid, when chromatographic conditions, columns, or guard columns were changed.

Anchovy

Anchovies from Monterey Bay were obtained from Dr. Thierry Work, California State Fish and Game, Wildlife Investigations Laboratory, Rancho Cordova, CA. The samples were frozen and held in storage for about 3 months before we received them. Commercially frozen anchovies from southern California were received from Mr. Glenn Kiel, National Marine Fisheries Service, Seafood Inspection Branch, Bell, CA. To facilitate analysis, the fish were allowed to thaw only partially so that the frozen viscera could be removed as an intact unit for domoic acid analysis. After evisceration, the head and fins were removed from the remaining body. The body and viscera were homogenized and analyzed separately.

Mussels

Mussels from Monterey Bay were obtained from Mr. Kenneth Hansgen, California Health Services, Environmental Health Services Section, Sacramento, CA. Mussels were removed from their shells and the whole animal was taken for analysis. For sampling, a sufficient number of mussels were selected so that about 50 g of shucked meats were obtained, usually 20 to 30 individual animals.

Razor Clams

Razor clams were furnished by the Washington State Department of Fisheries (WDF). Clams were collected from an area that starts at the mouth of the Columbia River and extends north along the Washington coast to the Moclips River. This area is divided into four major management beaches: Long Beach, Twin Harbors, Copalis, and Mocrocks (see Fig. 3). In most cases, six clams were taken from each management site at each sampling period, usually once a month at low tide, kept cool with "gel-ice" packets, and transported to our laboratory for shucking the next day. If clams could not be processed the next day, the specimens were frozen whole in the shell. Razor clams were first rinsed quickly in running water to remove sand and other debris and then shucked. If needed, the clam meat was rinsed briefly in running water to remove any further sand or debris, and then divided into edible meat and viscera. This procedure was similar to the steps that recreational and commercial processors employ for preparing razor clams for consumption or retail sale (Washington State Department of Fisheries pamphlet, no publication date). Clams were analyzed individually and an arithmetic mean determined for each sampling.

Dungeness Crab

Dungeness crab samples from Washington and Oregon were provided by the Washington State Department of Agriculture and Oregon State Department of Agriculture, respectively. In Washington state, crabs were collected in December 1991 by commercial fishermen from areas designated by the WDF representing the crab fishery off the Washington state coast. In 1992 and 1993 crabs were collected in both states as part of a monitoring program to determine the suitability of opening the commercial crabbing season. For a given site, a minimum of six crabs were used for analysis. Crabs were analyzed individually for domoic acid and an unweighted mean determined for each sampling site. Visceral tissues removed for analyses included the hepatopancreas, stomach, and intestinal tract. Heart and the epidermal membrane were also removed and included with the visceral tissues for analysis. Gills, which are not part of the visceral sac, were removed separately and not included in the visceral sample.

Sample Preparation

Tissues were homogenized using a common household type blender or food processor depending on volume of the sample. If sample sizes were too small for the blender, the tissue was mixed with distilled water, in a 1:1 ratio (w/w), to physically increase the volume and ensure thorough homogenization. Allowances were made for this added water in the final calculations of domoic acid content.

Domoic Acid Analysis

All tissue homogenates were extracted according to the method of Quilliam et al. (1991) with modification to the solid phase extraction (SPE) cleanup procedure (Hatfield et al. 1994). It was necessary to subject samples to the SPE procedure to remove compounds that interfered with the domoic acid HPLC analyses. All analyses were performed on a Hewlett-Packard 1090 HPLC, equipped with a Vydac 201TP column (Reversed phase C_{18}, 2.1 mm × 25 cm, Separations Group, Hesperia, CA 92345) and a diode array detector set at 242 nm with a 10-nm bandwidth and reference signal at 450 nm with 10-nm bandwidth. The domoic acid was chromatographed isocratically at 40°C with water MeCN TFA (90:10:0.1) (v/v/v) at a flow rate of 0.300 mL/min. Chromatographic conditions (solvent polarity and flow rate) were adjusted so that domoic acid was separated from tryptophan and had retention times between 6 and 9 min. Sample injections, using a 25-μL sample loop, ranged from 5 to 20 μL. A certified domoic

acid standard (DACS-1) was included before, after, and sometimes within each set of samples for calibration and quantitation.

RESULTS AND DISCUSSION

Anchovies

Chronologically, domoic acid poisoning was first reported in the United States in mid-September of 1991 from Monterey Bay, CA (Work et al. 1991). Our analyses of anchovies taken at this time in Monterey Bay found an average of 275 ppm domoic acid in the viscera and 77 ppm in the body meat (Fig. 1). Levels in the viscera of six fish ranged from 177 to 485 ppm, whereas the body content ranged from 51 to 105 ppm. Whether domoic acid is naturally distributed in the body tissues of these fish is questionable because samples provided to our laboratory for analysis had marked decomposition of the visceral tissues and indication of enzymatic deterioration of the surrounding body tissue. It is possible that the detection of domoic acid in the body tissues may be due to contamination by the postmortem migration of the toxin from the catabolized visceral tissues into the immediate surrounding body tissues.

In November 1991 we analyzed frozen anchovies from southern California, caught in the area between Point Vincente and Newport and east of Santa Catalina Island. In anchovy samples taken on April 21, 1991, 4 months before the domoic acid poisoning was reported in Monterey Bay, we found 192 ppm domoic acid in the viscera and 28 ppm in the body meant (Fig. 1). The body concentration of domoic acid was less in these Southern California anchovies than was found in the Monterey Bay samples (i.e., 77 ppm); however, less decomposition of the body tissue surrounding the viscera was noted in these samples, perhaps reducing migration of domoic acid from the visceral tissue into the surrounding body cavity tissue. Further samplings of anchovies from southern California in May 1991, about 1 month later, showed levels of domoic acid of less than 1 ppm.

Mussels

Mussels taken from sites within Monterey Bay (Fig. 1) on November 25, 1991 showed domoic acid levels of 0.6 ppm at Monterey (Point Pinos), 9.1 ppm at Moss Landing (Elkhorn Creek), and 1.0 ppm at Santa Cruz (Natural Bridges). We note that the highest levels were reported in the inner most part in the Bay at Moss Landing, whereas the lower levels were seen at the more seaward sites. Because mussels are known to depurate domoic acid rapidly (Novaczek et al. 1992), this would suggest that the domoic acid source was still resident in the Bay 2 months after the initial observation in September 1991.

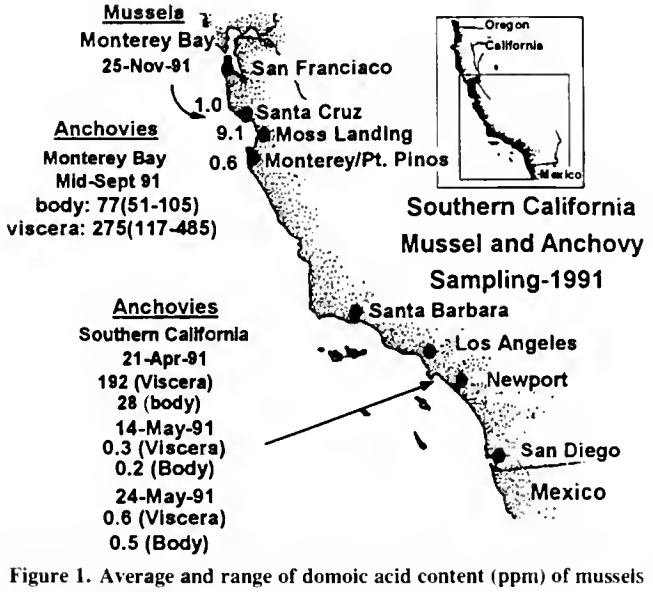

Figure 1. Average and range of domoic acid content (ppm) of mussels and anchovies taken from southern California sampling sites.

Razor Clams

By October 1991, domoic acid was found in razor clams from Oregon and Washington states. Wekell et al. (1994) reported that razor clams in Washington state apparently acquired their highest levels of domoic acid in the first week of December 1991. In that study, razor clams were separated into edible and nonedible (visceral tissue) portions as described earlier in this paper. The highest average concentration of domoic acid observed in edible razor clam tissue was 147 ppm, in samples taken from the Twin Harbors area on December 3, 1991, whereas the lowest average (73 ppm) on this date occurred in samples from the Long Beach area. In the study, the twin Harbors and Long Beach areas represented the two extremes in the collected data. We found that domoic acid levels in samples from Twin Harbors always exceeded the average for all Washington state beaches and the levels in the Long Beach area samples always fell below that overall average. The nature of this phenomenon is not understood at this time. Copalis and Mocrocks areas had values for domoic acid that fell between the two extremes of the Twin Harbors and Long Beach areas. We began our observations of domoic acid concentrations in razor clams in November 1991 and have continued through January 1994. From our observations, it seems that domoic acid achieves its highest levels in razor clams during the late fall (October through December). However, levels in 1992 and 1993 were considerably lower than those observed in December 1991 (Fig. 2). The lowest levels of domoic acid in razor clam samples from all Washington state beaches were observed in the late summer of 1993, with averages below 5 ppm. The late fall domoic acid season in 1993 seemed to produce higher levels of the toxin than was observed in 1992.

It also seems that domoic acid was not a new phenomenon in 1991 but has been present on the west coast of the United States since at least 1985. In 1992, we examined both frozen and canned razor clams dug from 1985 to 1990 in Washington state and found traces of domoic acid (Wekell et al. 1994).

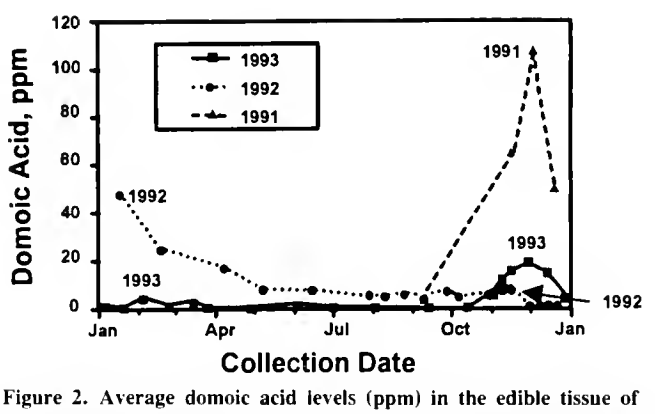

Figure 2. Average domoic acid levels (ppm) in the edible tissue of razor clams from alt Washington state management areas, September 1991 through December 1993.

Dungeness Crab

The commercial Dungeness crab season along the Washington and Oregon coastal areas normally begins on December 1 and can extend through the summer months of the following year. The season is usually closed for a brief period as the crab enter their moulting stage, sometime between June and September. However, the highest fishing activity and the major portion of the total landings occur in the first 6 to 8 weeks of the season, i.e., December and the following January, weather permitting. When domoic acid concentrations in razor clams reached a maximum in the first week of December 1991, a limited survey of Dungeness crabs off the Washington coast was undertaken because, as potential predators of razor clams, they might accumulate harmful levels of domoic acid.

Dungeness crab is marketed in several forms, e.g., live, whole cooked (fresh or frozen), picked meat, and sections. Although most people consume only the cooked leg and body meat, a significant portion of the population also consumes crab viscera either directly, usually the hepatopancreas (crab "butter" or "mustard"), or indirectly as a soup, stock, flavoring ingredient, or condiment. Whole cooked Dungeness crab is one of the more common forms and is prepared by placing the crabs in large baskets and submerging them in boiling salted water for varying lengths of time, usually 20–30 min depending on the processor. After the crabs are cooked, they are allowed to cool, either in cooling water baths or in air. During this cooling process, the crabs may drain or retain any entrapped cooking or cooling water that has entered the visceral compartment beneath the carapace. Because domoic acid is readily soluble in boiling water (Quilliam et al. 1991), it was not clear how much domoic acid may be lost by draining or diffusion into other parts of the cooked crab. The uptake, retention, loss, or diffusion of fluids from the crab will depend on their orientation in the basket during processing and the integrity of the crab's body compartment. Therefore, cooking crab may potentially lower its domoic acid content.

In unpublished studies on raw Dungeness crab, we could not detect domoic in the gills, hemolymph (blood), or in any of the edible body and leg meats of the crab. Domoic acid was found to be confined exclusively to the viscera of these crabs, with the largest portion in the hepatopancreas, a part of the digestive system. Because of the possible influences of the cooking process and other handling procedures and our findings on live crab, all results presented in this report represent visceral concentrations of domoic acid in uncooked Dungeness crab.

When it was noted that domoic acid in razor clams reached very high levels in the first week of December, concern was expressed about similar levels in Dungeness crab, a possible predator of razor clams. We began our survey of Dungeness crabs with samples taken on December 11, 1991. During the following year, our laboratory analyzed more than 400 raw crab viscera samples (Tables 1 and 2).

Although the Dungeness crab season usually extends into the summer months, the major fishing effort of the 1991–1992 season occurred between December and April; therefore we confined our analyses for the season to the period December 11, 1991 to April 3, 1992 (Table 1). The high values of domoic acid, first recognized in razor clams in 1991, were also reflected in the 1991–1992 season sample weighted average for Dungeness crab, i.e., 17 ppm, with an average range of 4.7 to 35 ppm. However, by the latter part of 1992 (Table 2), the preseason weighted average dropped to 2.5 ppm with an average range of 0.4 to 11 ppm. The highest values of domoic acid during the 1991–1992 season were found in samples from Grays Harbor, WA with 90 ppm (December 17, 1991) and a sample from Willapa Bay, WA with values of 78 (December 12, 1991). Both of these samples were collected approximately a week after our estimate of the peak domoic acid content was reached in razor clams. During this period the 1991 commercial crab fishing season in Washington was closed. Later in the spring, a high level of 71 ppm (March 1, 1992) was observed in Willapa Bay.

For the 1992–1993 preseason sampling study, we surveyed

TABLE 1.

Summary of domoic acid levels in Dungeness crab in NMAFS survey: December 1991 through April 1992.

Collection Site Location	Collection Date	Average[a] ppm	Standard Deviation	Minimum ppm	Maximum ppm	N
Willapa Bay, WA	11-Dec-91	0.8	NC[c]	0.8	0.9	2
Willapa Bay, WA	12-Dec-91	30	20	11	78	12
Ocean Shores, WA	17-Dec-91	6.2	7.7	1.4	22	6
Grays Harbor, WA	17-Dec-91	28	36	1.5	90	7
Off Grays Harbor, WA	17-Dec-91	6.1	3.0	2.7	9.5	4
Pacific Beach, WA	17-Dec-91	10	4.0	5.7	16	6
Destruction Island, WA	17-Dec-91	17	5.9	8.1	26	6
North Head Light, WA	17-Dec-91	26	NC[c]	24	29	2
Long Beach, WA	17-Dec-91	6.2	6.5	1.9	19	6
Sea View, WA	17-Dec-91	2.7	NC[c]	1.4	4.0	2
Klipsan Beach, WA	17-Dec-91	9.7	NC[c]	4.4	15	2
Willapa Bay, WA	19-Dec-91	14	10	2.8	29	8
Willapa Bay, WA	20-Jan-92	17	13	2.0	36	6
Willapa Bay, WA	1-Mar-92	20	26	4.5	71	6
Pt. Grenville, WA	27-Mar-92	16	13	3.3	37	6
Willapa Bay, WA	3-Apr-92	31	26	0	70	6
	1991–92 Average	17[b]	14[a]	4.7[a]	35[a]	87

[a] arithmetic mean

[b] sample weighted mean

[c] Not Calculated, since N = 2

TABLE 2.

Summary of domoic acid levels in Dungeness crab in NMFs pre-season survey: July 1992 through November 1992.

Collection Site Location	Collection Date	Average[a] ppm	Standard Deviation	Minimum ppm	Maximum ppm	N
North Head Light, WA	20-Jul-92	0.3	0.3	0	0.7	6
Grays Harbor, WA	24-Jul-92	1.3	2.2	0	8.2	17
Grays Harbor, WA	18-Aug-92	0.3	0.7	0	2.5	12
Columbia River, WA	20-Aug-92	0.9	1.1	0	3.4	12
Ocean Shores, WA	31-Aug-92	3.7	4.9	0	22	18
Alsea Bay, OR	28-Sep-92	0.8	1.5	0	3.8	6
Yaquina Bay, OR	30-Sep-92	0	0	0	0	6
Coos Bay, OR	30-Sep-92	2.8	4.9	0	16	12
Columbia River, OR	1-Oct-92	0	0	0	0	12
Brookings, OR	2-Oct-92	2.9	6.3	0	23	12
Tillamook Bay, OR	3-Oct-92	0.2	0.6	0	2.1	12
Bellingham Bay, WA	13-Oct-92	0.3	0.4	0	0.8	6
Long Beach, WA	26-Oct-92	1.4	1.0	4.0	4.0	12
Brookings, OR	26-Oct-92	6.4	10	1.2	38	12
Grays Harbor, WA	28-Oct-92	1.8	1.5	0	3.4	12
Off Willapa Bay, WA	28-Oct-92	3.1	2.2	1.1	6.6	8
Astoria, OR	28-Oct-92	0.7	0.9	0	2.4	12
N. of Jetty, Coos Bay, OR	28-Oct-92	11	20	1.2	74	12
Tillamook Bay, OR	31-Oct-92	0.2	0.4	0	1.0	6
2mi.S. of Columbia River, OR	9-Nov-92	1.0	1.5	0	4.8	12
3mi.S. of Cape Blanco, OR	10-Nov-92	2.3	2.4	0	7.2	12
Brookings, OR	11-Nov-92	4.6	6.0	0.9	20	12
Newport, OR	12-Nov-92	3.9	5.5	0	15	9
Garibaldi, OR	12-Nov-92	1.9	1.9	0	6.2	12
Coos Bay, OR	12-Nov-92	3.7	5.3	0	19	12
Grayland, WA	15-Nov-92	2.9	1.7	1.0	5.9	6
Grays Harbor, WA	15-Nov-92	2.3	3.0	0	8.0	6
Copalis Rock, WA	15-Nov-92	3.6	2.9	1.6	7.9	4
Off Willapa Bay, WA	15-Nov-92	2.0	1.4	1.2	4.8	6
Ocean Shores, WA	15-Nov-92	1.1	0.7	0	2.1	6
Newport, OR	15-Nov-92	3.9	5.2	0	16	12
	1992 PreSeason Average	2.5[b]	3.1[a]	0.4[a]	11[a]	314
	Combined 1991–1992 Average	5.7[b]	6.2[a,c]	1.9[a]	19[a]	401

[a] arithmetic mean

[b] sample weighted mean[c] excludes standard deviation data where N = 2

Dungeness crabs taken between July 20, 1992 and November 15, 1992. Although domoic acid levels declined in late 1992, occasional high levels samples were observed. For example, we observed one sample containing 74 ppm from the Coos Bay area in Oregon on October 28, 1992. Nevertheless, for our whole sampling effort from December 11, 1991 to November 15, 1992, the overall combined sample-weighted average* was 5.7 ppm (Table 2), with an average† *high* value of 19 ppm and an average *low* value of 1.9 ppm.

Washington

Samples of raw Dungeness crabs taken from all sites along the Washington coast in December 1991 were found to contain some domoic acid (Fig. 3) with a weighted average level below 20 ppm. Three of these samples (Willapa Bay, Grays Harbor, and North Head Light) exceeded 20 ppm. The highest average levels of domoic acid were found in crab taken from inside both Grays Harbor and Willapa Bay, 28 and 30 ppm, respectively (Table 1). However, considerable variation was found within the samples from each area; domoic acid concentrations in individual crab ranged from 1.5 to 90 ppm for samples from Grays Harbor and 0.8 to 78 ppm for crabs from Willapa Bay (Table 1). Lower levels of domoic acid were observed in samples collected just outside of Grays Harbor: 6.2 ppm (range: 1.4–22 ppm) for the northern side and 5.9 ppm (range: 2.7–9.5 ppm) for the southern side (Fig. 3). Destruction Island, about 40 km north of the entrance to Grays Harbor, produced Dungeness crab samples that averaged 17 ppm domoic acid and ranged from 8 to 26 ppm (Fig. 3). Domoic acid levels in viscera from Dungeness crab harvested along the Long Beach Peninsula averaged 9.6 ppm and ranged from 1.4 to 29 ppm. Crab samples from Long Beach were broken down into six sample subsites, with only two to three crabs from each of these sites. Figure 3 shows the results of this subsampling. In the Long Beach area, the highest level observed were in crabs taken near the mouth of the Columbia River (average of 26 ppm); however, because of the small sample size (N = 2 or 3) and the wide range of con-

*Sample weighted mean = $(N * [DA]_{mean})/(\Sigma N)$, where N is the number of samples in the group, $[DA]_{mean}$ is the average domoic acid concentration of the group, and ΣN is the total number of individual samples.

†Arithmetic mean = $\Sigma [DA]_{high\ or\ low}/\Sigma N_{sites}$, where $[DA]_{high\ or\ low}$ is the highest or lowest value of domoic acid concentration for each sample group and ΣN_{sites} is the total number of sites sampled.

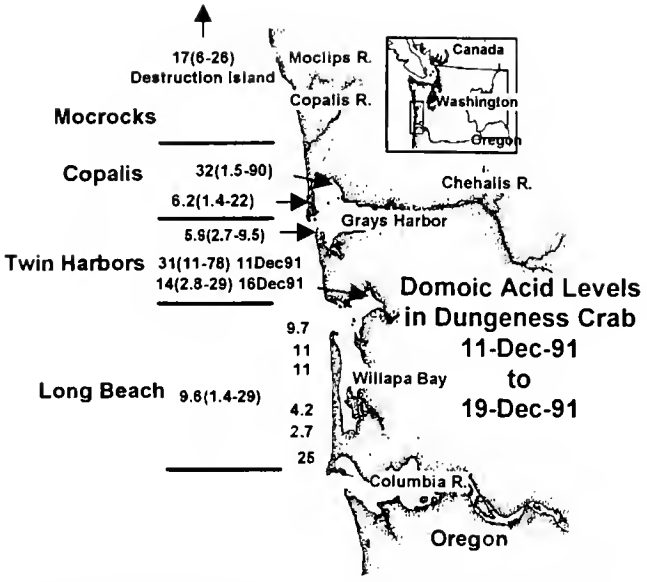

Figure 3. Average and range of domoic acid levels (ppm) in Dungeness crab taken from Washington state preseason sampling sites during December 11, 1991 and December 19, 1991. For Long Beach, values along the shoreline are subsets of the sampling yielding an average of 9.6 and a range of 1.4 to 29.

centrations, the values for these specific locations may not be that informative.

Oregon

Examination of the data on Dungeness crab harvested along the Oregon coast during the last quarter of 1992 (between September 28 and November 15, 1992, approximately one year after the high levels of domoic acid were observed in razor clams and crab from Washington state) still indicated the presence of domoic acid (Fig. 4). During this period, the highest concentrations were in samples taken from the mid to southern Oregon coastal areas, i.e., from Coos Bay south to Brookings, near the northern California border. Although average levels of domoic acid were moderate, i.e., 0–11 ppm, the range of values was wide, with one crab containing a level of 74 ppm. Crab taken from northern sites near the Columbia River (Astoria to Tillamook) contained the lowest levels of domoic acid, averaging less than 2 ppm. The highest concentration seen in this sampling was 6.2 ppm. Samples from the Newport-Depoe Bay area ranged from 0 to 15 ppm, and averaged less than 4 ppm.

Concern for the seriousness of domoic acid poisoning is amply justified. However, public health authorities have incorporated substantial safety margins into the current domoic acid level regulations. During the 1987 outbreak in Canada (Todd 1990, 1993), human toxicity occurred with estimated doses of 5–10 mg/kg (Hynie et al. 1990; Iverson 1992). The FDA set a limit of 20 ppm domoic acid in high-consumption seafoods, based on a review of the Canadian data and published reports (U.S. Food and Drug Administration Health Hazard Evaluation Board Recommendation No. 2734, December 9, 1991). In 1992, the FDA raised the permissible level of domoic acid in cooked Dungeness crab viscera to 30 ppm in 1993 after examining consumer utilization and consumption patterns of crab viscera (U.S. Food and Drug Administration Health Hazard Evaluation Board Recommendation No. 2988, December 21, 1992).

When the Pacific coast commercial Dungeness crab season

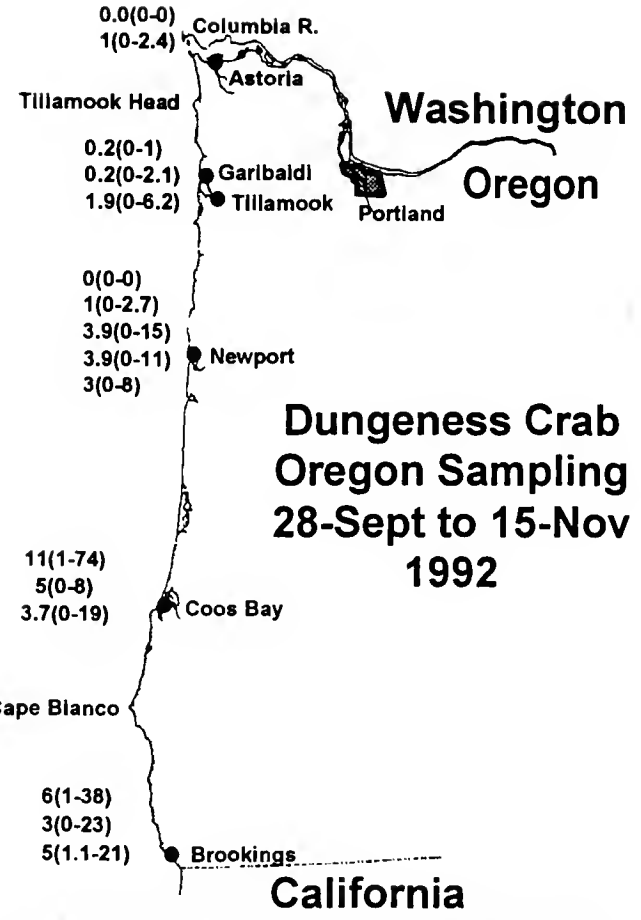

Figure 4. Average and range of domoic acid concentrations (ppm) in uncooked Dungeness crab from Oregon preseason sampling sites from September 28, 1992 to November 15, 1992.

opened in December 1992, cooked crab never became a problem since domoic acid levels in crab, and razor clams as well, continued to decline. However, as a precaution, health and regulatory agencies initiated various consumer education programs to encourage users to exercise caution in the consumption of crab viscera.

CONCLUSIONS

The movement of domoic acid through the marine food web is not well understood. When water samples and gut contents from the anchovies and pelicans from Monterey Bay were examined, large numbers of a diatom later identified as *P. australis* (Fryxell 1992; Buck et al. 1992) were observed. Samples of these recovered diatoms grown in laboratory culture were shown to produce domoic acid (Garrison 1992; Garrison et al. 1992; Buck et al. 1992). Although domoic acid was first found in anchovies from Monterey Bay in September 1991, analysis of anchovy samples landed in April 1991 indicated high levels of domoic acid (approximately 200 ppm) in their viscera. Anchovies are primarily carnivorous but they will consume phytoplankton if other food sources are not available. Although *P. australis* may be a source of domoic acid in Monterey Bay and may be responsible for domoic acid in other West Coast sites, its role as a source of domoic acid in razor clams is less clear. The razor clams available to recreational fishers and the subject of this paper occupy the "surf zone," a unique niche in the marine coastal environment (Lewin 1978; Lewin et al. 1989). Their diet has been reported to consist

entirely of the "surf diatoms" *Gonioceros* (formally *Chaetoceros*) *armatum*, *Asterionellopsis* (formally *Asterionella*) *socialis*, and *A. glacialis* (Lewin and Norris 1970). None of these surf diatoms have been reported to produce domoic acid and *P. australis* is not considered a surf diatom. Nevertheless, in 1994 we have observed significant numbers of *Pseudonitzschia* spp. and *Ceratium* spp. in surf water samples from the Twin Harbors (Fig. 3) area of Washington State (Wekell unpublished data; R. Horner personal communication).

The immediate source of domoic acid for Dungeness crabs is unclear at this time. These crabs are considered opportunistic predator-scavengers in the marine benthos. Our data (Wekell et al. 1994) and the work of others (Drum et al. 1993; Horner et al. 1993) indicate that razor clams retain domoic acid for long periods of time, e.g., 6 months. Although some razor clams live in the "surf zone," others subsist in the subtidal regions. Therefore, it is possible that Dungeness crab can prey on toxic subtidal razor clams, providing at least one source of domoic acid for the crabs. The finding of domoic acid in crabs taken from the Oregon coast, nearly one year after the high levels observed in December 1991, is consistent with razor clams being a possible source of domoic acid for Dungeness crabs. On the other hand, high levels of domoic acid were observed in crab taken from Grays Harbor and Willapa Bay (more than 30 ppm) where few, if any, razor clams are to be found. Therefore, other benthic sources of domoic acid must also be considered, such as scallops or other smaller crustacea, snails, and bivalves.

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ERRATA

Lesser, M. P. & S. E. Shumway. Effects of toxic dinoflagellates on clearance rates and survival in juvenile bivalve molluscs. *J. Shellfish Res.* 12(2): 377–381.

Page 379: Sentence should read: Multiple comparison testing on *Ostrea edulis*, *Argopecten irradians*, and *Placopecten magellanicus* all showed a similar pattern with clearance rates on *Alexandrium tamarense* being significantly higher than *Gyrodinium aureolum* or *Isochrysis* sp., which were grouped together.

The authors regret the error.

The description of the cover photograph for Volume 12(2) should read:

Juvenile pearl oysters *Pinctada margaritifera galtsoffi* produced at Black Pearls, Inc. hatchery in Kona, Hawaii. Approximate shell diameter 5–8 cm. Photo by Dale J. Sarver.

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Vol. 13, No. 2 DECEMBER 1994

CONTENTS

CONTENTS CONTINUED ON INSIDE BACK COVER