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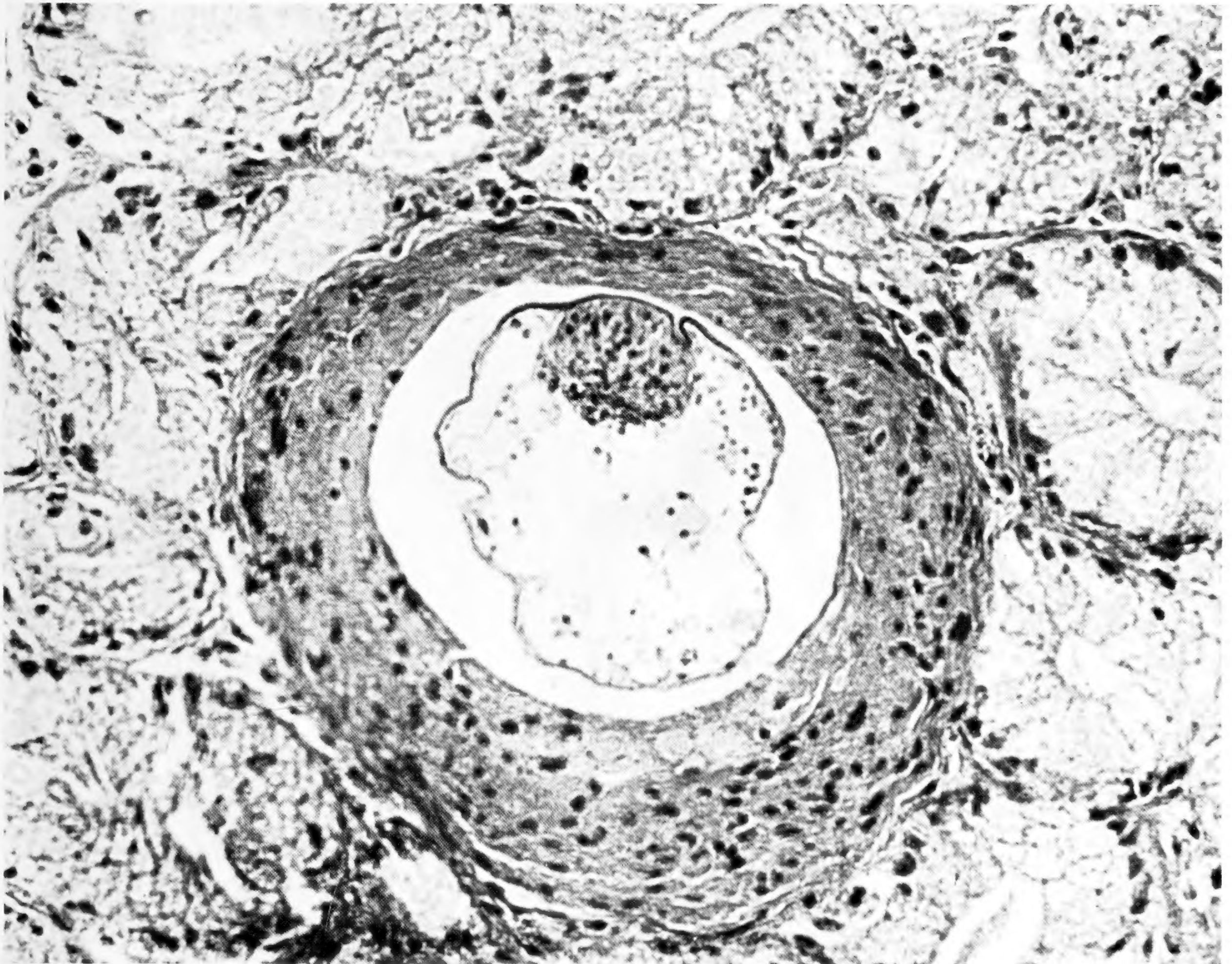

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GROWTH AND REPRODUCTION OF SUBTIDAL AND INTERTIDAL POPULATIONS OF THE GAPER CLAM *TRESUS CAPAX* (GOULD) FROM YAQUINA BAY, OREGON[1]

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ABSTRACT

A study of over 2000 gaper clams (Tresus capax) *from Yaquina Bay, Oregon, showed that clams from subtidal regions grew more rapidly than those from intertidal areas. Allometric relationships among whole weight, shell weight, dry body weight, wet body weight, volume, length, width, and height were examined for intertidal vs. subtidal clam populations and ripe vs. inactive clam populations. Analyses indicated that clams of intertidal and subtidal populations had differently shaped shells, but similar volumes per unit length. Total wet weight relative to length was higher in intertidal than in subtidal clams. Moisture content of the body tissues was also found to be higher in intertidal and inactive clams than in subtidal or active clams respectively. Shell weight was consistently higher in subtidal clams, but increased at a faster rate (per unit length) in intertidal clams. Histological examinations indicated that* Tresus capax *from Yaquina Bay are late winter spawners with spawning being coincident with yearly low temperatures. Spawning was found to be synchronous among subtidal and intertidal populations. Comparisons with other studies on intertidal populations on the Pacific coast indicate that latitude- related differences occur in the time of spawning of* Tresus capax.

INTRODUCTION

A decline in availability of at least two important east coast bivalves (genera *Mya* and *Spisula)* has resulted in a growing demand for clam products from the west coast (Stoker, 1977). The gaper, *Tresus capax* (Gould), is a large clam that occurs commonly both intertidally and subtidally in bays on the west coast from California to Alaska (Morris, 1966). It is currently the target for a pilot subtidal commercial fishery program initiated by the Oregon Department of Fish and Wildlife. Because *T. capax* supports an important intertidal sport fishery we have tried to evaluate the probable effect of a subtidal commercial fishery on the intertidal stocks.

Tresus capax has been the subject of relatively little research, most of it on intertidal populations; studies on reproduction or growth are few. Reid (1969) correlated peak levels of gonadal lipid in *T. capax* with gonad ripening in December, and the decrease of levels with egg release in winter. More recently, thorough examinations of the reproductive cycle of intertidal clams were made at Humboldt Bay, California (Machell and DeMartini, 1971), and near Vancouver Island, British Colum-

[1] This work is the result of research supported by the Oregon State University Sea Grant College Program supported by NOAA Office of Sea Grant, Department of Commerce, under Grant No. 04-7-158-44085.

bia (Bourne and Smith, 1972). These studies indicated that *T. capax* spawned during the late winter or early spring. Other mactrids studied are summer spawners (Ropes, 1968; Calabrese, 1970). Bourne and Smith (1972) found the growth rate of intertidal *T. capax* to be more rapid, over 100 mm/5 yr, than that of other commercial clams. Wendell, DeMartini, Dinnel and Siecke (1976) studied the spatial and age-class distributions, mortality, recruitment, predation, and burrowing of *T. capax* in Humboldt Bay. Their samples included predominantly intertidal clams, although their distribution studies did include subtidal clams.

Using the approach of Wilbur and Owen (1964) to study growth, significant differences have been found between allometric growth rates of inter- and subtidal bivalves. Dame (1972) observed a higher moisture content in intertidal oysters. Brown, Seed and O'Connor, (1976) found a greater emphasis on tissue growth in subtidal bivalves. Rao (1953) found that mussels of a lower tide height had greater shell weight for a given soft body weight than did higher level mussels.

During this study, a concerted effort was made to provide information about growth rates and reproductive cycles of subtidal and intertidal populations of *T. capax* in an attempt to discover any parental relationship between those populations, and, hence, the effect of a subtidal fishery on the total population.

MATERIALS AND METHODS

Gaper clams (*T. capax*) were collected from April 1975, through February 1977, from four areas in Yaquina Bay, Oregon (Figure 1). Stations 1, 2, and 4 were subtidal. Substrate was removed with a suction dredge manipulated by SCUBA divers (Goodwin, 1973), allowing the clams to be collected by hand. Collections at these stations were generally made at daytime high slack tide at depths of approximately 9.1, 4.6 and 11.0 m respectively. Station 3 was located on a tidal mudflat; samples were taken at low tide by digging with clam shovels. Occasionally unfavorable tidal, weather, and sea conditions made uniform sampling difficult; nonetheless, most samples contained 10 clams from each station and the period between samples was approximately two weeks

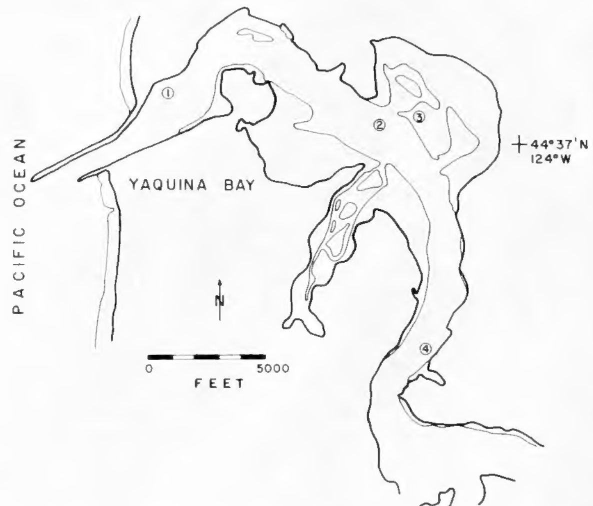

FIGURE 1. *Map of Yaquina Bay, Oregon, showing location of subtidal sampling sites 1, 2, and 4, and intertidal sampling site 3.*

from November through February and one month during the remainder of the year.

Measurements of temperature and salinity were taken with each subtidal collection. Samples of the substrate were taken from all stations with each collection and qualitatively described as: bedrock, rock, gravel, sand, mud, shell, or debris.

Length, height, and width (Figure 2) were measured to the nearest 0.1 mm and rounded to the nearest mm. Age was determined by counting annual growth check rings of both valves and/or by counting annuli in the chondrophore of either valve.

A sample of gonadal tissue, taken from the middle of the foot of each clam, was fixed in Bouin's solution, embedded in paraplast, sectioned at 7 μm and stained with Harris' hematoxylin and eosin. Based on examinations of the slide preparations, the sex of each clam was identified. The stage of the reproductive cycle was assessed as inactive, active, ripe, partially spawned, or spent according to the criteria set by Ropes and Stickney (1965). Five ovarian alveoli were examined in each ripe and partially spawned female clam from Stations 2 and 3. In each alveolus, the diameters of the whole cell and of the nucleus (with apparent nucleolus) were measured with an ocular micrometer for 15 oocytes, and counts were made of oocytes attached to the alveolar walls and free in the lumina.

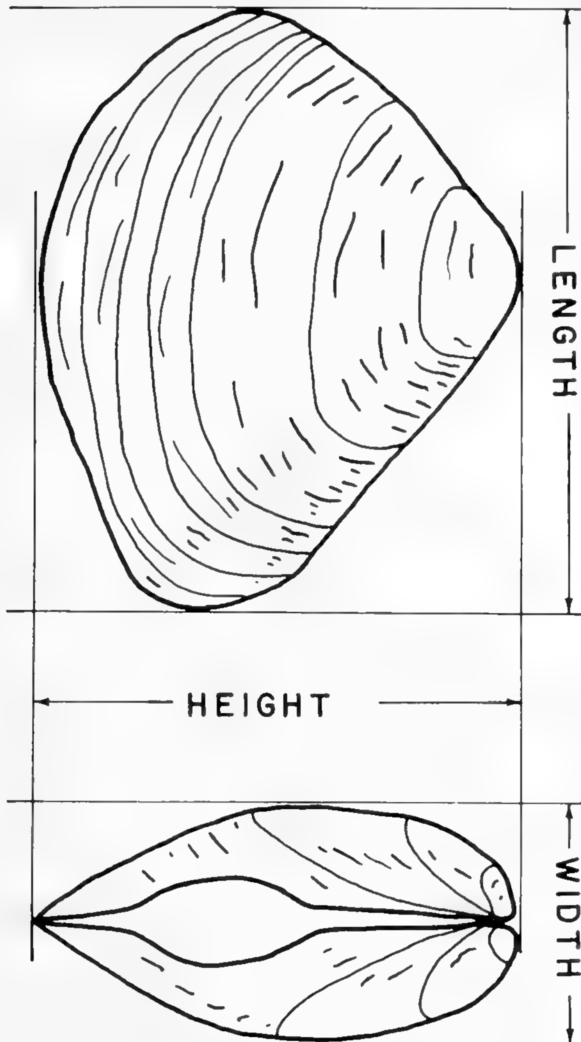

FIGURE 2. *Diagram of* Tresus capax *shell, showing length, width, and height measurements taken.*

When possible, additional clams were collected at the same time and with the same methods. These were used to examine the relation of weight to linear dimension. In addition to the length and width data listed above, measurements of total wet weight and shell weight were taken immediately upon return to the laboratory. Dry body weight was measured after drying in a constant temperature oven (110°C) for 48-72 hr. Volume within the shell was measured by securing the two clean valves of each clam tightly together, pouring sand through the gap between the valves until full, and measuring the volume of sand.

Statistical procedures used during this study follow the methods outlined by Steel and Torrie (1960) and Snedecor and Cochran (1967).

Absolute growth was determined by finding the mean length of the clams and the 95% confidence interval for each age class at each station (a total of 57 means). Student's t-test was used to compare mean lengths for age classes between stations. Generally, two compared means were significantly different when the confidence interval of one did not overlap the other.

Allometric relationships were calculated on a computer. These were compared between subtidal and intertidal populations, and inactive and active populations, using the linear equation:

$$\log y = \log a + (b) \log x,$$

which was transformed from the exponential growth equation,

$$y = a(x^b).$$

The value b is the ratio of specific growth rates of y and x, i.e., the factor of differential growth and the slope of the log regression line. The value a is equivalent to y, when $x = 1$.

The Chi-square (X^2) test criterion was used to test the hypothesis that the frequency distributions of clams in each phase at each site were similar and constant for each two-week interval through the observed duration of each phase in Yaquina Bay.

RESULTS

Growth

Nearly 2000 clams were examined during the growth and reproductive studies. The techniques for determining age of the clams, either counting annual growth checks (rings) on the valves or counting annuli on the chondrophore, gave similar results, indicating that the methods could be interchanged. The oldest clams collected from subtidal sites were 10-12 yr of age; those collected from the intertidal site reached 9 yr of age.

Mean lengths of each age class from each station are shown in Table 1. Subtidal clams (from Stations 1, 2, and 4) over 4 yr of age were significantly larger than similarly aged intertidal clams. The size of intertidal clams 4 yr and younger were not significantly different from those of subtidal clams of a similar age.

TABLE 1. *Mean lengths (mm) of* Tresus capax *of different ages from four sampling sites in Yaquina Bay, Oregon.*

	AGE (yr.)												
	0	1	2	3	4	5	6	7	8	9	10	11	12
STATION NO. I													
$\overline{X}$	8	35	50	61	76	96	111	119	123	123	119		
N	200	41	15	123	122	13	41	98	108	36	6		
s	4.72	5.33	5.89	16.19	12.42	9.68	9.85	6.25	7.45	7.56	10.17		
CI (95%)	0.65	1.68	3.26	2.89	2.23	5.85	3.11	1.25	1.42	2.56	10.67		
STATION NO. II													
$\overline{X}$	5	24	49	86	97	112	119	125	123	123	125	123	122
N	56	59	22	8	23	67	107	85	30	10	2	2	1
s	1.82	7.75	11.37	10.47	9.45	8.02	7.04	6.01	10.75	10.45	4.95	4.95	0
CI (95%)	0.49	2.02	5.04	8.75	4.09	1.96	1.35	1.30	4.01	7.47	44.61	44.61	—
STATION NO. III													
$\overline{X}$	—	35	58	80	92	101	103	108	111	109			
N	—	3	1	45	73	88	75	21	4	3			
s	—	4.66	0	6.82	6.70	6.72	8.21	6.75	114.06	2.77			
CI (95%)	—	11.58	—	2.05	1.56	1.42	1.89	3.07	18.15	6.88			
STATION NO. IV													
$\overline{X}$	7	29	51	73	89	106	114	119	120	120	118		
N	53	22	9	5	11	41	75	95	48	34	5		
s	4.81	7.14	12.34	14.58	10.58	6.93	8.65	7.04	7.74	11.55	9.27		
CI (95%)	1.33	3.17	9.30	18.10	7.11	2.19	1.99	1.43	2.25	4.03	11.51		
STATION NOS. I, II, IV													
$\overline{X}$	7	29	50	63	80	114	116	121	122	122	120	123	122
N	309	122	46	136	156	121	223	278	186	80	13	2	1
s	3.82	6.34	9.89	13.58	11.47	8.01	8.73	6.81	8.37	9.94	8.55	4.95	—
CI (95%)	0.43	3.61	2.94	2.29	1.80	1.45	1.14	0.41	1.20	2.21	5.16	44.61	—

Linear growth rate (Figure 3A) was approximately 22 mm/yr during the first 3 yr and decreased until little growth occurred after 7-8 yr of age. Initial and final growth rates of the intertidal and subtidal groups were similar (Figure 3B); however, the rate of growth of intertidal clams decreased more rapidly between the ages of 4-7 yr (inclusive) than it did for subtidal clams.

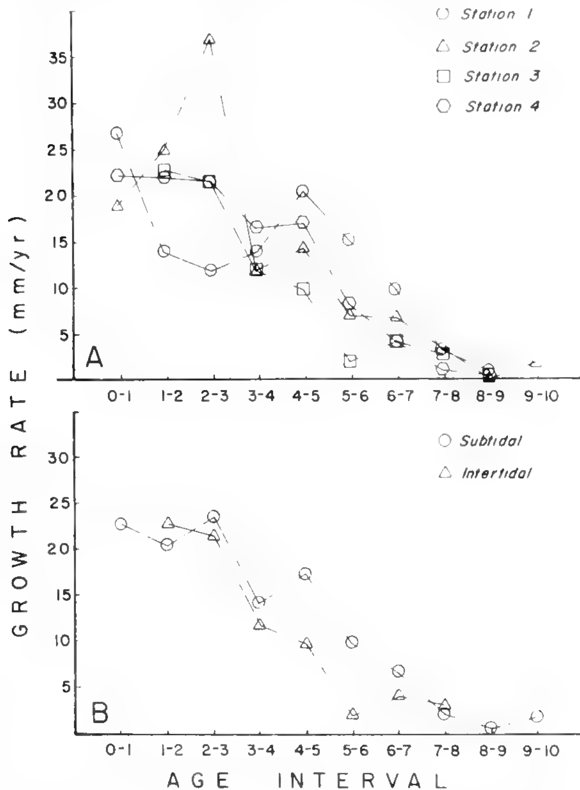

FIGURE 3. *Growth rate of* Tresus capax *from Yaquina Bay, Oregon. A. From four sampling sites; B. From subtidal (sites 1, 2, and 4 combined) and intertidal (site 3) stations.*

Mean volumes of each age class from each station are shown in Table 2. Volumes of subtidal clams were consistently larger than those for intertidal clams of similar age. Volume data were not available for clams under 3 yr of age.

The allometric coefficients for the various morphological relationships given in Table 3 are those which best fit the data by least squares and apply only within the range of the data. Significant differences of a pair were indicated when the 95% confidence intervals of those coefficients were non-overlapping.

Analysis of the width:length and height:length relationships for subtidal and intertidal clams showed significantly greater b values for the subtidal clams in both instances. The coefficients of determination (R^2) were, in all but one case, higher than 95%. No significant difference was found in the volume:length relationship between the a or b values for the subtidal and intertidal clams (Figure 4A). These allometric ratios indicate that for a given rate of linear growth, subtidal clams increased more rapidly in height and width than did the intertidal clams, while the increase in volume for the two populations remained the same.

Growth of total wet weight relative to length was higher in intertidal clams than in subtidal clams (Figure 4B), but was not significantly different between ripe and inactive clams. Rate of increase (the value b) was also greater in intertidal than in subtidal clams, but did not differ between clams of different reproductive phases.

Ratios of wet body weight:length, dry body weight:length and wet body weight:total wet weight were similar among all groups of clams, although low R^2 values rendered these correlations questionable in some instances.

The ratio, wet body weight:dry body weight was significantly higher with a greater rate of increase in the intertidal clams than in the subtidal clams (Figure 4C). The significance of the intersection of the regression lines for ripe and inactive clams is discussed below.

The percent moisture in the body tissues averaged:

82.3% in subtidal clams (N = 163);
84.0% in intertidal clams (N = 48);
82.1% in ripe clams (N = 59);
83.1% in inactive clams (N = 33).

Shell weight:length was slightly higher in subtidal clams than in intertidal clams, although the rate of increase of this ratio was faster in intertidal clams (Figure 4D).

Due to the low R^2 values, the shell weight:dry body weight correlation was questionable and could not be compared between clam populations.

Reproductive Cycle

Histological characteristics of the reproductive phases of *Tresus capax* from Yaquina Bay were

TABLE 2. *Mean volumes (ml) of* Tresus capax *of different ages from four sampling sites in Yaquina Bay, Oregon.*

	AGE (yr.)						
	3	4	5	6	7	8	9
STATION NO. I							
$\bar{v}$	67	136	121	166	170	211	241
N	1	2	13	11	23	15	2
s	0	3.11	5.94	5.67	5.77	6.30	3.06
CI (95%)		27.94	3.59	3.81	2.50	3.49	27.49
STATION NO. II							
$\bar{v}$	70	87	139	188	205	221	
N	2	3	14	14	13	7	
s	4.35	2.91	6.28	6.44	6.48	5.80	
CI (95%)	39.11	7.23	3.63	3.72	3.92	5.36	
STATION NO. III							
$\bar{v}$	54	75	110	130			
N	11	15	17	3			
s	3.97	3.91	4.90	4.16			
CI (95%)	2.67	2.17	2.52	10.34			
STATION NO. IV							
$\bar{v}$		86	140	171	188		
N		3	6	14	4		
s		4.74	6.38	6.54	4.69		
CI (95%)		11.78	6.70	3.78	6.51		
STATION NOS. I, II, IV							
$\bar{v}$	69	99	132	176	183	215	241
N	3	8	33	39	40	22	2
s	2.05	5.14	6.17	6.30	6.20	6.11	3.05
CI (95%)	5.17	4.30	2.19	2.04	1.98	2.71	27.49

essentially the same as those from Humboldt Bay, California (Machell and DeMartini, 1971). Therefore, these phases are described only briefly below.

The sex ratio was 1:1 for the phases in which sex was discernable.

Graphs of the five reproductive phases for the clams from the four collection sites are shown in Figure 5A-D. In all cases, there was some overlap from one phase to the next. The onset of the inactive or undifferentiated phase was rapid, beginning in May, with the phase lasting through November. All gaper clams from Station 1 had inactive gonads in August; from Stations 2 and 3, in July; and from Station 4 in June and July.

The active phase, a period of spermatogenesis in the male and oocyte enlargement in the female, was first recorded in July and lasted, at one site, into March of the following year. Most or all of the clams collected were in this phase in September through November.

Ripe gonads, characterized by more detached than attached oocytes in the ovaries or a majority of spermatozoa radially arranged in the testes, were first observed in October, peaked in occurrence in December-January, and continued into April. Of the five phases, this one continued for the longest period of time. Oocytes of this phase had a mean diameter of 49 μm, and a mean nucleus diameter of 27 μm (Table 4).

Gonads partially emptied of ripe gametes and with disorganized follicular tissue, indicating spawning (termed "partially spawned"), were found in most samples from February through May or June. Peak occurrence was observed in April for Stations 1 and 2, March for Station 3,

TABLE 3. *Allometric growth coefficients for various morphological relationships of populations of* Tresus capax.

Relationship y/x	Clam Population	Log a(±95% C.I.)	b(±95% C.I.)	N	R^2
Height	Subtidal	-0.409(0.010)	1.149(0.006)	653	0.9957
Length	Intertidal	-0.300(0.058)	1.092(0.030)	212	0.9608
	Total	-0.406(0.008)	1.147(0.005)	865	0.9960
Width	Subtidal	-0.716(0.094)	1.212(0.007)	603	0.9941
Length	Intertidal	-0.563(0.012)	1.137(0.048)	188	0.8968
	Total	-0.722(0.011)	1.216(0.006)	791	0.9939
Volume	Subtidal	-3.488(0.329)	2.766(0.159)	146	0.8763
Length	Intertidal	-3.406(0.774)	2.711(0.396)	47	0.8085
	Total	-3.714(0.240)	2.874(0.117)	193	0.9165
Total Wet Weight	Subtidal	-2.972(0.545)	2.586(0.264)	162	0.6958
Length	Intertidal	-3.922(0.654)	3.014(0.106)	47	0.8773
	Ripe	-3.689(0.668)	2.944(0.327)	57	0.8629
	Inactive	-4.319(1.300)	3.208(0.570)	33	0.8050
Wet Body Weight	Subtidal	-2.648(0.618)	2.307(0.300)	162	0.5881
Length	Intertidal	-3.432(0.891)	2.662(0.456)	48	0.7503
	Ripe	-3.176(1.060)	2.581(0.390)	58	0.7590
	Inactive	-3.615(0.818)	2.753(0.399)	33	0.8644
Dry Body Weight	Subtidal	-4.077(0.890)	2.627(0.432)	162	0.4691
Length	Intertidal	-3.683(1.021)	2.386(0.520)	48	0.6492
	Ripe	-4.670(1.302)	2.938(0.478)	59	0.7301
	Inactive	-4.903(1.106)	3.001(0.540)	39	0.8010
	Total	-4.691(0.550)	2.919(0.270)	211	0.6830
Wet Body Weight	Subtidal	-0.192(0.097)	0.974(0.041)	162	0.9258
Total Wet Weight	Intertidal	-0.076(0.107)	0.937(0.054)	48	0.9639
	Ripe	-0.021(0.111)	0.911(0.048)	59	0.9623
	Inactive	0.012(0.138)	0.888(0.060)	33	0.9604
	Subtidal & Ripe	0.027(0.183)	0.891(0.078)	53	0.9114
	Subtidal & Inactive	-0.074(0.227)	0.924(0.097)	25	0.9061
Wet Body Weight	Subtidal	1.130(0.061)	0.731(0.045)	162	0.8601
Dry Body Weight	Intertidal	0.798(0.089)	0.994(0.088)	48	0.9178
	Ripe	1.002(0.090)	0.821(0.067)	59	0.9141
	Inactive	0.921(0.067)	0.885(0.052)	33	0.9738
Shell Weight	Subtidal	-4.038(0.491)	2.930(0.238)	162	0.7839
Length	Intertidal	-5.496(0.427)	3.594(0.218)	48	0.9598
	Total	-5.609(0.350)	3.683(0.172)	210	0.8947
Shell Weight	Subtidal	1.120(0.223)	0.660(0.086)	163	0.5857
Dry Body Weight	Intertidal	0.554(0.116)	1.001(0.216)	48	0.6532
	Ripe	0.641(0.232)	0.967(0.126)	59	0.8061
	Inactive	0.628(0.170)	1.020(0.203)	34	0.7670

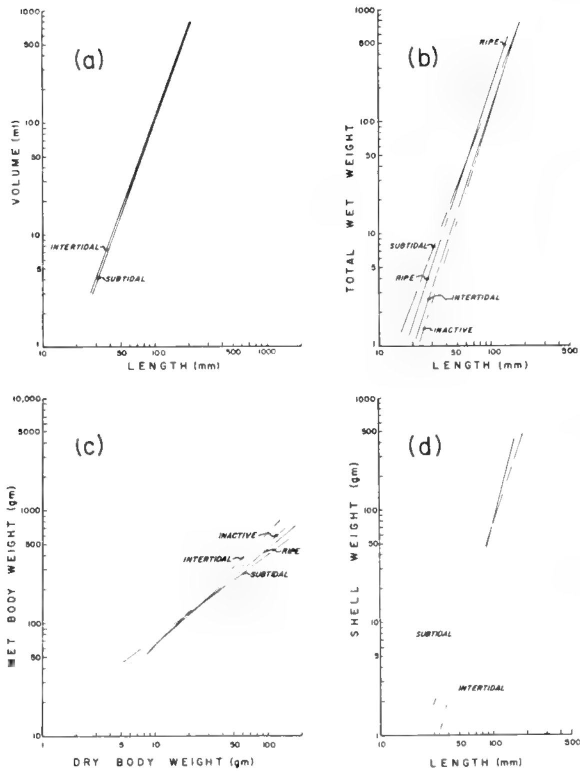

FIGURE 4. *Graphs showing allometric growth relationships in* Tresus capax:

A. Volume: length relationship for subtidal and intertidal clams;

B. Total wet weight: length relationship for subtidal, intertidal, ripe, and inactive clams;

C. Wet body weight: dry body weight relationship for subtidal, intertidal, ripe, and inactive clams;

D. Shell weight: length relationship for subtidal and intertidal clams.

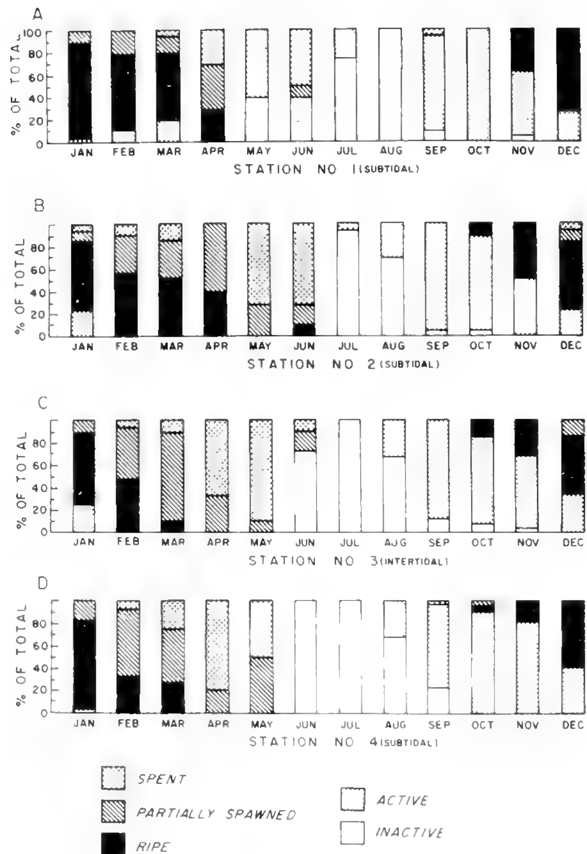

FIGURE 5. *Monthly percent frequency of* Tresus capax *in each reproductive phase. A. Station 1; B. Station 2; C. Station 3; D. Station 4.*

and February for Station 4. Oocytes from clams in this phase had a mean cell diameter of 49 μm, and mean nucleus diameter of 28 μm (Table 4).

Spent clams, with gonads having thick-walled, shrunken alveoli containing debris or a few remaining gametes undergoing cytolysis, were first observed in February at Station 2, later at the others, and were present through May or June. Most clams were in this phase of the reproductive cycle in April (Station 4), in May (Stations 1 and 3), and May-June (Station 2), after which a rapid drop in frequency of this phase occurred.

TABLE 4. *Mean diameters (μm) of oocytes and oocyte nuclei from subtidal and intertidal gaper clams of different reproductive phases.*

	Station #2 (subtidal)	Station #3 (intertidal)
Ripe Clams		
Oocyte Diameter	48.18	48.84
N	885	705
s	5.28	5.07
Nucleus Diameter	27.49	27.05
N	885	705
s	3.07	3.18
Partially Spawned Clams		
Oocyte Diameter	48.90	49.68
N	255	240
s	4.41	4.37
Nucleus Diameter	27.92	27.86
N	255	240
s	3.73	2.78

Results of the X^2 tests showed that each reproductive phase was distinct from other phases at the same station or at other stations. Within each phase, few differences were observed between stations for the inactive, active, or spent phases. These differences appeared to be random, following no pattern from phase to phase or station to station. There were no significant differences between the stations for the ripe or partially spawned phases, indicating that the clams in the bay became mature and spawned at the same time.

Salinity and Temperature

Salinity and temperature data recorded throughout this study at the three subtidal sampling stations are shown in Figure 6A and B.

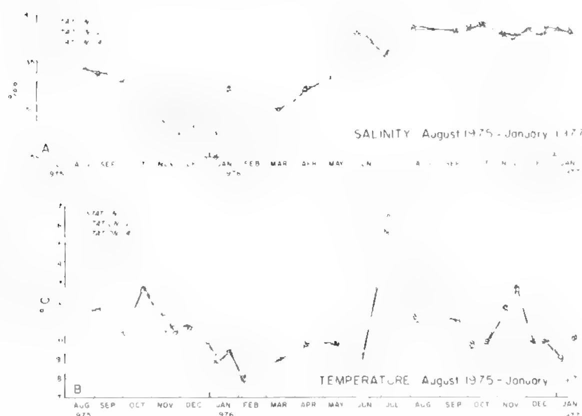

FIGURE 6. *Salinity and temperature at the three subtidal sampling sites, Yaquina Bay, Oregon, July 1975-January 1977.*

Substrate

The substrate of the subtidal stations (1, 2, and 4) was largely composed of sand; that of the intertidal station (3) was of finer grain size (Table 5).

DISCUSSION

Growth

Differences in growth between gaper clams from subtidal and intertidal regions may be attributed to environmental factors associated with the different habitats. Physiological adaptations of the two populations have possibly effected variations in their respective relative growth rates; gonad development and the phase of the reproductive cycle also influence relative growth.

Tresus capax from subtidal areas grow more rapidly than intertidal gapers. Furthermore, growth rates of subtidal clams reported here were slightly lower than those reported earlier for intertidal *T. capax* from Yaquina Bay (Marriage, 1954), yet were similar to those reported for intertidal gaper clams from British Columbia (Bourne and Smith, 1972). These observations indicate that littoral height may not be a factor for growth. However, comparison with the former report is difficult, as no data were published with that study.

Observed differences in bivalve growth rates are, no doubt, due to several environmental factors, including substrate. Differences observed in substrate type at the four stations are corroborated by those reported by Kulm (1965) (Table 5). Our data indicate gaper clams in sandy substrate grew faster than those in muddier areas. However, because differences in growth rates coincide with both substrate composition and tide height, further comparative studies should be made. Substrate may be correlated with food supply, as coarse-grained habitats experience higher current velocities than fine-grained habitats. Pearce (1965), however, observed that intertidal gaper clams from a muddy area of coastal Washington grew as much as 40 mm larger than did clams from

TABLE 5. *Sediment types in Yaquina Bay, Oregon.*

Sample Site (Figure 2)	Substrate Type This study, 1975[a]	 Kulm, 1965[b]
1 (subtidal)	sand-shell	fine & medium sand
2 (subtidal)	sand	fine & medium sand
3 (intertidal)	mud	fine, medium sand & silty sand
4 (subtidal)	sand-shell	fine & medium sand

[a] from categories of: bedrock, rock, gravel, sand, mud, shell, debris.
[b] from categories of: fine sand, medium sand, silty sand, clayey sand, sandy silt, sand-silt-clay.

a sandy area, and concluded that substrate composition influenced burrowing of the clams and their ability to avoid freezing temperatures. Substrate also affected shell growth of *Mya arenaria*, clams from sand-dominated areas growing faster than clams in a mud-gravel-shell substrate (Swan, 1952).

Seed (1967) found that population density had a marked effect on the shape of mussel shells, with animals from dense populations being long and narrow, compared with rounder individuals of more sparse populations. Two conditions exist which suggest that the same may be true of *T. capax* in Yaquina Bay:

a) subtidal clams were consistently longer than intertidal clams of the same age class;
b) subtidal clam populations were denser than intertidal populations (Hancock, et al., 1979).

Because shell shape may be influenced by density, we suggest that intershell volume is a more reliable indicator of comparative size than length. Volume of *T. capax* was strongly correlated to shell length, despite differences in shell shape. Subtidal clams were not only longer, but also had greater volume than similarly aged intertidal clams.

Growth rates have been directly correlated with food availability and/or length of feeding periods by Smith (1928), Coe (1947), Coe and Fitch (1950), Fitch (1950), Stickney (1964), and others. Intertidal gaper clams would experience limited periods of exposure to sea water and therefore of feeding, period length being defined by the clams' height in the intertidal zone. In Yaquina Bay, intertidal clams are subjected to heavy fresh-water run-off, freezing temperatures, and insolation, stresses that are not confronted or are not as extreme in the subtidal regions. Bourne and Smith (1972), Nosho and Chew (1972), and Paul, Paul, and Feder (1976) also suggest temperature as a growth-controlling factor in bivalves.

Tresus capax experiences spurts of growth in the summer and growth checks in the winter (Bourne and Smith, 1972; Wendell, et al., 1976). These periods of growth alternate with periods of gonad activity and spawning, a phenomenon not unusual in pelecypods (Coe, 1947; Fitch, 1950; Ansell, 1961; Galtsoff, 1964; and Lammens, 1967). Reid (1969) noticed an alternation of depletion and accumulation of glycogen in gonads of *T. capax*, coinciding respectively with the period of food scarcity and abundance and with the period of gonad activity and quiescence. It is also probable that in *T. capax*, stored and acquired nutrients are being alternately devoted to growth and reproduction, each or both triggered by seasonal changes of the environment.

It is not only likely that energy is budgeted between growth and reproduction, but that growth, whether linear or by weight, is the balance of the relative growth rates among each individual's component parts. Relative growth of the body parts appears to be dependent upon the amount of exposure to sea water, i.e., the height in the littoral zone, and on the degree of gonad development. The regression analyses suggest that intertidal clams are heavier (total weight) per unit length than are the subtidal clams. Clam weight is distributed among its components: total wet weight is comprised of shell weight and wet body weight, in turn comprised of dry body weight and water. Intertidal clams had a consistently higher moisture content than subtidal clams. Dame (1972) suggested that the higher retention of water in intertidal oysters resulted from a physiological adaptation to the intertidal environment.

Although not as heavy as subtidal clam shells, intertidal clam shells showed a significantly greater increase in weight relative to length. Therefore, if these clams lived longer, it is possible that their shell growth would overtake that of the subtidal clams. Perhaps due to limited exposure to sea water, intertidal clams have become more efficient in absorbing and metabolising calcium from the water. Rao (1953) concluded that shell secretion in intertidal and subtidal mussels occurred at a rate directly proportional to submersion time, sea water being the source of calcium. Subtidal mussels not only had heavier shells, but had more rapid shell secretion rates. However, the findings of Baird and Drinnan (1957) conflict with Rao as to whether higher intertidal *Mytilus* should have heavier or lighter shells than *Mytilus* of lower tide levels. Subtidal oysters had heavier shells, but there was no significant difference between rates of shell secretion of subtidal and intertidal populations (Dame, 1972).

We attribute the higher total wet weight:length ratio of the intertidal clams to: higher moisture

content, higher ratios of wet body weight:length and shell weight:length.

Ripe clams have a higher dry body weight:wet body weight ratio than do inactive clams, correlating with gonad development. Parallel regression lines for this relationship for these two populations would indicate no gonad development. The intersection of these lines theoretically indicates the approximate size at which the clams become sexually mature. In this instance, the intersection corresponds to ~ 90 gm wet body weight, and ~ 80 mm length. Bourne and Smith (1972) reported that gaper clams of ~ 70 mm from British Columbia had developed gonads. It is possible that latitude-related environmental conditions such as temperature, photo-period, and tidal regimen influence the size at which sexual maturity occurs (see also Reproductive Cycle below). Histological studies of local juvenile and young adult gaper clams are necessary before such a generalization can be made.

The reason for the higher dry body weight in subtidal clams is not entirely clear; it is, of course, a function of water retention and could be related to feeding time or substrate stability. Brown, Seed, and O'Connor (1976) studied three species of bivalves: *Cerastoderma edule*, *Mytilus edulis*, and *Modiolus modiolus*, the latter being the only subtidal species. In this study it was found that the two intertidal species had heavier shells and faster rates of shell growth than did the subtidal species. The authors suggested that when "moving from an intertidal to a subtidal position there appeared to be a progressive emphasis on tissue rather than on shell growth," and that the intertidal species tend to be more unstable in their habitat due to the instability of nutrients. However, gonad development was not considered as a factor of growth in the Brown et al. study. Their intertidal and subtidal species were collected and processed at two different seasons of the year; differences in body relationships including dry body weight could therefore be attributed to the stage of gonad development instead of to tidal height. Furthermore, studies comparing aspects of the biology of subtidal and intertidal organisms should include either greater numbers of subtidal and intertidal species, or larger sample sizes from subtidal and intertidal populations of one species.

Reproductive Cycle

Gonad examinations confirm that *Tresus capax* from Yaquina Bay are late winter spawners with spawning being coincident with yearly low temperatures (Figure 6). Gametogenesis was initiated in the late summer and continued through autumn. Gametes developed until ripe gonads predominated. Spawning began in the winter, peaking in March and April. A discrete inactive period followed during the summer. The observation that some clams contained gonads filled with deteriorating ripe gametes suggests that some may either fail to spawn or experience incomplete spawning.

The west coast range of *T. capax* extends from California to Alaska, yet few studies of the reproductive activity of *T. capax* at different latitudes can be found in the literature (Machell and DeMartini, 1971; Bourne and Smith, 1972). It appears that gaper clam populations of more southern latitudes have slightly earlier spawning periods than do more northern clams (Table.6). Differences in spawning time at different latitudes are apparent for other clam species: *Mya arenaria* (Ropes and Stickney, 1965; Porter, 1974), *Mercenaria mercenaria* (Loosanoff, 1937a, 1937b; Porter, 1964), and *Tivela stultorum* (Coe and Fitch, 1950). Temperature may be a latitude-dependent, determining factor (Caddy, 1967; Lammens, 1967).

Bimodal spawning for the gaper clam has not been suggested by this study, by Machell and DeMartini (1971), or by Bourne and Smith (1972); although Wendell, et al. (1976) reported bimodal recruitment peaks. It is the only mactrid clam reported to spawn in the late winter-early spring. Summer high temperatures coincide with spawning of the mactrids *Spisula solidissima* (Ropes, 1968) and *Mulinia lateralis* (Calabrese, 1970); both of these species experience bimodal spawning.

Synchronous spawning of *T. capax* was exhibited at the four sampling sites, with no differences found between the spawning period of subtidal or intertidal populations and allowing cross-fertilization of gametes of the two populations. Multiple spawnings of the clams were not indicated. Nonetheless, results of plankton studies of gaper clam larvae (Hancock, et al., 1978) sug-

TABLE 6. *Spawning seasons of* Tresus capax *at different latitudes on the east Pacific coast.*

LOCATION	Jan	Feb	Mar	Apr	May	June
Seal Island, B.C. (49°12′)[a]			XXXXXXXX			
Yaquina Bay, Or. (44°37′)		XXXXXXXXXXXX				
Humboldt Bay, Cal. (40°52′)[b]	XXXXXXXXXXXXX					

[a] Bourne and Smith, 1972.
[b] Machell and DeMartini, 1971.

gest a lunar periodicity of spawning in the populations. However, as gaper clam larvae are extremely difficult to identify, results of this study are inconclusive and may be an artifact of the sampling technique.

Our studies confirm that *T. capax* from Yaquina Bay are late winter spawners, and suggest that latitude may affect the onset of spawning. Other factors such as temperature may also affect the reproductive cycle.

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LITERATURE CITED

Ansell, A.D. 1961. Reproduction, growth, and mortality of *Venus striatula* (DaCosta) in Kames Bay, Millport. J. Mar. Biol. Assoc. U.K. **41**:191-215.

Baird, R.H. and R.E. Drinnan. 1957. The ratio of shell to meat in *Mytilus* as a function of tidal exposure to air. J. du Cons. **22**:329-336.

Bourne, N.D. and D.W. Smith. 1972. Breeding and growth of the horse clam, *Tresus capax* (Gould). Proc. Natl. Shellfish. Assoc. **62**:35-37.

Brown, R.A., R. Seed and R.J. O'Connor. 1976. A comparison of relative growth in *Cerastroderma (=Cardium) edule, Modiolus modiolus,* and *Mytilus edulis* (Mollusca: Bivalvia). J. Zool. Lond. **179**:297-315.

Caddy, J.F. 1967. Maturation of gametes and spawning in *Macoma balthica* (L.). Can. J. Zool. **45**(6):955-965.

Calabrese, A. 1970. Reproductive cycle in the coot clam, *Mulinia lateralis* (Say), in Long Island Sound. Veliger **12**(3):265-269.

Coe, W.R. 1947. Nutrition, growth, and sexuality of the Pismo clam *(Tivela stultorum).* J. Exp. Zool. **104**:1-24.

Coe, W.R. and J.E. Fitch. 1950. Population studies, local growth rates, and reproduction of the Pismo clam *(Tivela stultorum).* J. Mar. Res. **9**:188-210.

Dame, R.F. 1972. Comparison of various allometric relationships in intertidal and subtidal American oysters. Fish. Bull. **70**:1121-1126.

Fitch, J.E. 1950. The Pismo clam. Calif. Fish and Game **36**(3):285-312.

Galtsoff, P.S. 1964. The American oyster, *Crassostrea virginica* Gmelin. Fish. Bull., U.S. Fish and Wildlife Service **64**:480 p.

Goodwin, C.L. 1973. Distribution and abundance of subtidal hard-shell clams in Puget Sound, Washington. Wash. Dept. Fish. Tech. Rept. No. 14. 81 p.

Hancock. D.R., T.F. Gaumer, G.B. Willeke, G. Robart and J. Flynn. 1979. Subtidal clam populations: distribution, abundance, and ecology. Oregon State University Sea Grant College Program, Final Report, Publ. No. ORESU-T-79-002. 243 p.

Kulm, L.D. 1965. Sediments of Yaquina Bay, Oregon. Ph.D. Diss., Oregon State University, Corvallis, Oregon. 156 p.

Lammens, J.J. 1967. Growth and reproduction in a tidal flat population of *Macoma balthica* (L.). Neth. J. Sea Res. 3:315-382.

Loosanoff, V.L. 1937a. Seasonal gonadal changes of adult clams, *Venus mercenaria* (L.). Biol. Bull. 72:406-416.

Loosanoff, V.L. 1937b. Spawning of *Venus mercenaria* (L.). Ecology **18**(4):506-515.

Machell, T.R. and J.D. DeMartini. 1971. An annual reproductive cycle of the gaper clam, *Tresus capax* (Gould), in south Humboldt Bay, California. Calif. Fish and Game **57**(4):274-282.

Marriage, L.D. 1954. The bay clams of Oregon. Their economic importance, relative abundance and general distribution. Fish. Comm. Oregon, Contrib. 20, 47 p.

Morris, P.A. 1966. A Field Guide to Shells of the Pacific Coast and Hawaii. Riverside Press, Cambridge. 297 p.

Nosho, T.Y. and K.K. Chew. 1972. The setting and growth of the Manila clam, *Venerupis japonica* (Deshayes) in Hood Canal, Washington. Proc. Natl. Shellfish. Assoc. **62**:50-58.

Paul, A.J., J.M. Paul and H.M. Feder. 1976. Growth of the littleneck clam, *Protothaca staminea,* on Porpoise Island, Southeast Alaska. Veliger **19**(2):163-166.

Pearce, J.B. 1965. On the distribution of *Tresus capax* and *Tresus nuttalli* in the waters of Puget Sound and the San Juan Archipelago (Pelecypoda: Mactridae). Veliger **7**(3):166-170.

Porter, R.G. 1974. Reproductive cycle of the soft-shell clam, *Mya arenaria,* at Skagit Bay, Washington. U.S. Fish. Bull. **72**:648-656.

Rao, K.P. 1953. Shell weight as a function of intertidal height in a littoral population of pelecypods. Experientia **9**:465-466.

Reid, R.G.B. 1969. Seasonal observations on diet, and stored glycogen and lipids in the horse clam, *Tresus capax* (Gould, 1850). Veliger **11**(4):378-381.

Ropes, J.W. 1968. Reproductive cycle of the surf clam, *Spisula solidissima,* in offshore New Jersey. Biol. Bull. **135**(2):349-365.

Ropes, J.W. and A.P. Stickney. 1965. Reproductive cycle of *Mya arenaria* in New England. Biol. Bull. **128**(2):315-327.

Seed, R. 1967. The influence of habitat, density and age on the shell shape of *Mytilus edulis.* Rep. Challenger Soc. **3**(19):58-59. (Abstr.)

Smith, G.M. 1928. Food material as a factor in growth rate of some Pacific clams. Trans. Roy. Soc. Canada **25**:287-291.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical Methods. 6th ed. Iowa State University Press, Ames, Iowa. 593 p.

Steel, R.G.D. and J.H. Torrie. 1960. Principles and Procedures of Statistics with Special Reference to the Biological Sciences. McGraw Hill, New York. 481 p.

Stickney, A.P. 1964. Feeding and growth of juvenile soft shell clams, *Mya arenaria.* Fishery Bull. Fish. Wild. Serv. U.S. **63**:635-642.

Stoker, S.W. 1977. Report on a subtidal commercial clam fishery proposed for the Bering Sea. Final Report to U.S. Marine Mammal Commission. Report No. MMC-77/01. 33 p.

Swan, E.F. 1952. The growth of the clam *Mya arenaria* as affected by the substratum. Ecology **33**(4):530-534.

Swan, E.F. and J.H. Finucane. 1952. Observations on the genus *Schizothaerus.* Nautilus **66**(1):19-26.

Wendell, F., J.D. DeMartini, P. Dinnel, and J. Siecke. 1976. The ecology of the gaper or horse clam, *Tresus capax* (Gould 1850) (Bivalvia: Mactridae), in Humboldt Bay, California. Calif. Fish and Game **62**(1):41-64.

Wilbur, K.M. and G. Owen. 1964. Growth, pp. 211 to 242. In: Physiology of the Mollusca Vol. I, K.M. Wilbur and C.M. Yonge, eds. Academic Press, New York.

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COMPARATIVE STUDIES ON GROWTH OF THE PURPLE-HINGE ROCK SCALLOP *HINNITES MULTIRUGOSUS* (GALE) IN THE MARINE WATERS OF SOUTHERN CALIFORNIA[1]

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ABSTRACT

Growth of juvenile rock scallops was observed in cages at depths of 1 m below the surface, 4 m, and 6 m (Mean Lower Low Water) in Quivira Basin in Mission Bay, San Diego, California, and at depths of 3 m, 9 m, 15 m, and 18 m (MLLW) in the Pacific Ocean off the coast of San Diego. Greatest overall growth was observed for rock scallops suspended more than 1 m above the bottom at both the Quivira Basin and open ocean locations. Cages holding scallops 1 m below the surface in Quivira Basin became overgrown with fouling organisms which resulted in reduced growth. Scallops 1 m above the bottom at both the bay and open ocean stations (6 m depth in Quivira Basin and 18 m depth in the open ocean) experienced higher concentrations of suspended matter which resulted in less growth, than the other groups at shallower depths. Rock scallops grew heavier adductor muscles in the ocean than did those in Quivira Basin. This greater muscle growth in the ocean was correlated with the formation of deeper shells.

*Results of a stepwise multiple regression analysis suggest that the size of rock scallops at the beginning of a measuring period and the depth at which the animals were located were inversely correlated with growth of shell diameter. Temperature, chlorophyll-*a*, and particulate organic carbon, as measured in this study, may have had no significant effect on the growth of shell diameter of* H. multirugosus.

H. multirugosus *was stocked at densities of 5, 10, 20, and 40 animals/0.1 m^2 to examine the effects of crowding on growth. The optimum stocking density for this animal appears to be between 5 and 10 animals/0.1 m^2. Rock scallops at higher densities exhibited reduced growth due to competition for growing space and food.*

INTRODUCTION

The purple-hinge rock scallop, *Hinnites multirugosus,* occurs along the Pacific coast of North America from British Columbia to Punto Abreojos, Baja California. It is usually found attached to hard substrates at depths from low intertidal to about 30 m in bays, inlets, and the ocean (Keen, 1937).

The rock scallop appears to have high potential for mariculture. Leighton and Phleger (1977) monitored growth of the rock scallop, *H. multirugosus,* in Mission Bay, San Diego, California, and found that growth of juveniles averaged 6

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cm per year. They predicted that a marketable scallop could be produced in two to three years in the bay, reaching a size of approximately 12 cm in diameter and yielding 30-40 g of adductor muscle meat.

The purpose of this study was to determine and evaluate growth of scallops held at different depths and stocking densities. Temperature, chlorophyll-*a*, and particulate organic carbon were measured to evaluate their possible effects on growth.

DESCRIPTION OF STUDY AREAS

The two study areas were Quivira Basin, located in Mission Bay, San Diego, California, and the Naval Oceans Systems Center (NOSC) oceanographic tower (Figure 1). Quivira Basin is a well-circulated, shallow water marina cove, which is 7 m deep (Mean Lower Low Water) and lies approximately 1.2 km from the Pacific Ocean. This basin receives good tidal circulation with the Pacific Ocean through the Mission Bay entrance.

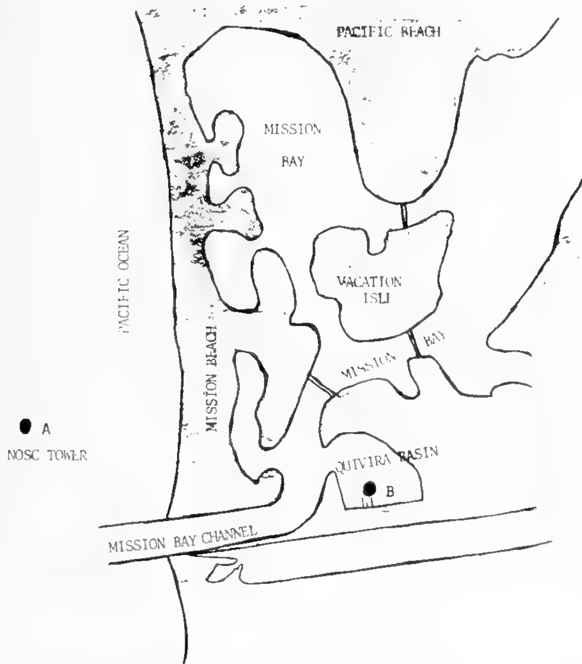

FIGURE 1. *Map of coastline showing the locations of Naval Ocean Systems Center (NOSC) tower station (A) off the coast of San Diego and Quivira Basin stations (B) in Mission Bay, San Diego, California.*

It has extensive natural populations of scallops which are located primarily on pier pilings. The NOSC tower is an oceanographic platform that is operated by the Naval Ocean Systems Center in San Diego. It is located near the entrance of Mission Bay and situated on a sandy bottom approximately 1 km off the coast of San Diego in 19 m of water (MLLW). Total tidal amplitude for these two stations ranged from approximately −0.7 m to +2.5 m.

METHODS AND MATERIALS

Approximately 500 juvenile rock scallops, ranging from 10 mm to 25 mm, were collected from jetty rock substrates around the edges of Bonita Basin and the adjoining channel in Mission Bay during August, 1976. They were held in Quivira Basin on a floating research laboratory until cages were ready for use. The captured juveniles were subdivided into experimental groups with equal initial sizes. One group containing 20 scallops was sacrificed to obtain representative shell diameter, shell depth and total body, shell, soft body, and adductor muscle wet weight. Growth observations were begun in December, 1976. Shell diameter (average of length and width of shell) and shell depth (width of valves in a closed position) were measured every two months.

Scallops were caused to cement to asbestos board substrates by temporary confinement in small plastic cagelets (Leighton, personal communication). These cagelets were removed after the animals had cemented. These substrates were then inserted into cylindrical cages made of polyethylene 2.5 cm mesh (Conwed Corp.). Growth of *H. multirugosus* at different depths in a bay was examined by suspending duplicate cages, 30 cm in diameter and 60 cm in length, from a floating platform at 1 m below the surface and permanently securing cages to a piling at depths of 4 m and 6 m (MLLW) in Quivira Basin. Groups located at 6 m were situated 1 m above the bottom. Each cage contained 20 scallops which were allowed to cement to both sides of the substrates so as to provide enough room for unrestricted growth. Eight replicate cages, 20 cm × 60 cm and partially imbedded in cement, were set at depths of 3 m, 9 m, 15 m, and 18 m (MLLW) on the NOSC tower to compare growth at different depths in the ocean. Scallops located at a depth of 18 m were situated 1

m above the bottom. Each cage contained 5 rock scallops so that there was ample room for unrestricted growth.

The effects of stocking density on the growth of rock scallops were examined by suspending duplicate sets of cages at a depth of 3 m in Quivira Basin. Each set contained animals stocked at densities of 5, 10, 20 and 40/0.1 m^2.

In December, 1977, rock scallops were harvested to obtain final shell diameters and shell depths, plus the wet weights of the total body, shell, soft body, and adductor muscle.

Temperature, chlorophyll-*a*, and particulate organic carbon were measured every two weeks at depths of 1 m below the surface, 4 m, and 6 m in Quivira Basin and at depths of 3 m, 9 m , and 18 m at the NOSC tower. Chlorophyll-*a* was analyzed by spectrophotometry after water samples were filtered through glass fiber filters (Whatman GFC) and extracted by grinding in 90% acetone and $MgCO_3$ (Strickland and Parsons, 1965). Particulate organic carbon was analyzed by combustion of filtered material retained on a sterilized glass fiber filter (Whatman GFC) in a Hewlett-Packard Carbon-Hydrogen-Nitrogen analyzer (Mullin, 1974).

RESULTS

Growth of Rock Scallops at Different Depths

The mean linear and weight measurements from twenty juvenile rock scallops sacrificed at the beginning of this study are summarized in Tables 1 and 4. Table 1 also contains the summary of final linear and weight measurements of *H. multirugosus* grown at 4 m and 6 m depths in Quivira Basin and at depths of 9 m, 15 m, and 18 m at the NOSC tower.

In Quivira Basin, greatest overall growth was exhibited by rock scallops located at mid-depth. The results of a Tukey HSD multiple range test (Tukey, 1953; Nie, et al., 1970) showed that rock scallops held at a depth of 4 m had significantly greater final linear and weight sizes than did those grown at 6 m (Table 1; $p < 0.05$). Scallops grown at 1 m below the surface were not included in the final statistical analysis because of reduced growth due to excessive fouling (Monical, 1980).

At the NOSC tower, best overall growth was shown by rock scallops grown at greater than 1 m above the bottom. The results of a Tukey HSD multiple range test (Tukey, 1953; Nie, et al., 1970) showed that rock scallops held at a depth of 9 m exhibited significantly greater final shell depths and shell weights than did the other two groups at 15 m and 18 m (Table 1; $p < 0.05$). There were no significant differences in final shell diameter, total body weight, soft body weight, and adductor muscle weight between scallops held at depths of 9 m and 15 m ($p > 0.05$). The rock scallops held at depths of 9 m and 15 m had higher final values for all of the linear and weight characteristics, except shell depth, than those grown at 18 m ($p < 0.05$). Scallops grown at 15 m and 18 m depths exhibited no significant differences in final shell depths ($p > 0.05$). Those animals grown at a depth of 3 m were not included in the final statistical analysis. The original group was destroyed by ocean surge and replaced halfway through the study. Growth of the replacement group was monitored for nine months only. This group exhibited increases in shell diameter similar to those held at depths of 9 m and 15 m over the nine-month period (Monical, 1980). It appears that the rock scallops grown at a depth of 3 m would have attained the same final sizes as the scallops held at 9 m and 15 m depths if allowed to grow for one year.

Although growth appeared to be similar between the bay and open ocean sites, rock scallops grown in Quivira Basin produced larger but flatter shells than did those on the NOSC tower (Table 1). Scallops in the ocean had deeper shells and correspondingly heavier adductor muscles than those grown in Quivira Basin. It is doubtful that the different stocking densities between the Quivira Basin and the NOSC tower stations had any effect on growth because there was adequate space for more unrestricted shell expansion.

Environmental Factors

Temperatures recorded at 1 m below the surface, 4 m, and 6 m depths in Quivira Basin are summarized in Table 2. Table 3 contains the summary of temperatures at depths of 3 m, 9 m, and 18 m at the NOSC tower. Temperatures decreased with depth at both locations and were lower in the ocean than in Quivira Basin.

Concentrations of chlorophyll-*a* at 1 m below the surface, 4 m and 6 m depths in Quivira Basin

TABLE 1. *Mean and 95% confidence interval for the mean (95% CI) of shell diameter, shell depth and the wet weights of total body, shell, soft body, and adductor muscle for rock scallops,* Hinnites multirugosus, *sacrificed at the beginning of the study, (SRC; n=20) and those grown at depths of 4 m* (n=32), 6 m* (n=32), 9 m (n=31), 15 m (n=37), and 18 m (n=30). The data from the scallops that were sacrificed initially are the approximate beginning measurements for the groups examined for one year. Groups in a given box are not significantly different from each other ($p>0.05$) but are significantly different from those in other connected boxes ($p<0.05$) based on the results of the Tukey HSD test.*

SHELL DIAMETER (mm)

Group	Mean	± 95% CI
SRC	23.03	± 1.55
18 m	76.69	± 1.93
6 m*	79.12	± 2.84[1]
9 m	81.55	± 2.49[2]
15 m	82.49	± 2.09
4 m*	85.34	± 2.20

SHELL DEPTH (mm)

Group	Mean	± 95% CI
SRC	7.06	± 0.48
6 m*	22.15	± 0.79
18 m	24.45	± 0.70
4 m*	24.91	± 0.71
15 m	25.66	± 0.60
9 m	27.14	± 0.74

TOTAL BODY WEIGHT (g)

Group	Mean	± 95% CI
SRC	1.76	± 0.31
18 m	87.42	± 4.90
6 m*	89.20	± 8.10
15 m	111.67	± 5.18
4 m*	115.77	± 7.64
9 m	122.15	± 8.05

SHELL WEIGHT (g)

Group	Mean	± 95% CI
SRC	0.90	± 0.17
18 m	53.55	± 3.08
6 m*	57.91	± 5.20
15 m	70.36	± 3.12
4 m*	76.31	± 4.56[3]
9 m	79.59	± 5.02

SOFT BODY WEIGHT (g)

Group	Mean	± 95% CI
SRC	0.52	± 0.10
6 m*	18.01	± 2.06
18 m	21.75	± 1.39
4 m*	26.44	± 2.41
9 m	28.34	± 2.28
15 m	28.88	± 1.55

ADDUCTOR MUSCLE WEIGHT (g)

Group	Mean	± 95% CI
SRC	0.12	± 0.02
6 m*	7.55	± 0.79
18 m	9.83	± 0.65
4 m*	10.17	± 0.99
15 m	12.16	± 0.71
9	12.71	± 1.02

* Group located in Quivira Basin.

[1] Group not significantly different from 9 m, 15 m and 18 m groups.

[2] Groups not significantly different from 4 m* and 6 m* groups.

[3] Group not significantly different from 9 m and 15 m groups.

TABLE 2. *Summary of data for temperature, chlorophyll-a, and particulate organic carbon at three different depths in Quivira Basin. Boxes contain the means ($\bar{x}$), standard deviations (SD), and ranges (range) of measurements in the field.*

Location Quivira Basin		Temperature °C	Chlorophyll-*a*	Particulate Organic Carbon
1 m below	$\bar{x} \pm$ SD	18.0 ± 2.2	2.4 ± 1.4	652 ± 318
surface	range	13.9 − 21.7	0.0 − 5.5	279 − 1467
4 m	$\bar{x} \pm$ SD	17.4± 2.0	2.6 ± 1.5	659 ± 245
	range	13.1 − 19.6	0.0 − 5.6	302 − 1170
6 m	$\bar{x} \pm$ SD	16.6± 2.0	2.5 ± 1.8	789 ± 418
	range	12.8−19.0	0.0− 8.1	297 − 1936

TABLE 3. *Summary of data for temperature, chlorophyll-a, and particulate organic carbon at three different depths near the NOSC tower. Boxes contain the means ($\bar{x}$), standard deviations (SD), and ranges (range) of measurements in the field.*

Location NOSC Tower		Temperature °C	Chlorophyll-*a*	Particulate Organic Carbon
3	$\bar{x} \pm$ SD	16.3 ± 1.5	1.2 ± 0.9	537 ± 2401
	range	11.1 − 22.8	0.0 − 4.1	183 − 974
9 m	$\bar{x} \pm$ SD	15.4 ± 1.7	3.3 ± 3.8	609 ± 261
	range	11.1 − 21.3	0.0 − 14.4	170 − 1270
18 m	$\bar{x} \pm$ SD	14.1 ± 1.5	3.1 ± 3.8	741 ± 355
	range	9.4 − 20.0	0.0 − 15.3	385 − 1925

are summarized in Table 2. Similar concentrations of chlorophyll-*a* were observed at all depths in Quivira Basin. Table 3 contains the summary of data for the concentration of chlorophyll-*a* at depths of 3 m, 9 m, and 18 m at the NOSC tower. Greatest concentrations were recorded at 9 m and 18 m depths at the NOSC tower.

Particulate organic carbon represents the standing stock of phytoplankton, zooplankton, and detritus. Table 2 contains the summary of data for the concentrations of particulate organic carbon at 1 m below the surface, 4 m and 6 m in Quivira Basin. There was a greater concentration of particulate organic carbon at a depth of 6 m than at the other depths in Quivira Basin. Concentrations of particulate organic carbon at depths of 3 m, 9 m, and 18 m on the NOSC tower are summarized in Table 3. Highest overall concentrations were observed at a depth of 18 m while the lowest were found at 3 m.

Relationship Between Growth of Scallops and Environmental Conditions

A stepwise multiple regression analysis was employed to determine if the physical factors monitored had any significant effects on the growth of *H. multirugosus* (Sokal and Rolhf, 1969; Nie, et al., 1970). The dependent variable was the mean increase in shell diameter every two months for animals held at different depths at the bay and open ocean stations (CHANGE SD). The independent variables were the mean initial shell diameter of rock scallops at the beginning of each two month growth period (SIZE I_o) and the mean temperature, concentration of chlorophyll-*a*, and concentration of particulate organic carbon for each two month growth period, plus the depths at which the rock scallops were located (DEPTH). The following equation helps to summarize factors affecting growth of shell diameter:

$N = 28$

$$(\text{CHANGE SD}) = 466.84 - 0.22(\text{SIZE } I_o) - 0.13(\text{DEPTH})$$

$\text{ADJUSTED } R^2 = 0.85$

The results of this analysis suggest that the size of scallops and the depth at which the animals were located had a significant but inverse effect on growth as measured in terms of shell diameter ($p < 0.05$). Temperature and concentrations of chlorophyll-*a* and particulate organic carbon appear to have had no significant influence on differences in growth of *H. multirugosus* ($p > 0.05$) within the ranges encountered in the field in this study.

Growth of Rock Scallops at Different Stocking Densities

Table 4 contains the summary of data for the final linear and weight measurements of rock scallops grown at densities of 5, 10, and 20/0.1 m^2. Animals grown at densities of 40/0.1m^2 were not included in the final analysis since they exhibited reduced growth and failed to cement to the substrates after four months of observations. A Newman-Keuls multiple range test (Sokal and Rohlf, 1969; Nie, et al., 1970) was used to determine which groups were significantly different from each other. Rock scallops grown at densities of 5 and 10/0.1 m^2 exhibited similar final measurements for all linear and weight characteristics (Table 4; $p > 0.05$). Animals stocked at densities of 5 and 10/0.1 m^2 exhibited significantly greater final linear and weight sizes, except shell depth, than did those grown at densities of 20/0.1 m^2 ($p < 0.05$). There was no significant difference in final mean shell depths between the groups ($p > 0.05$). These results suggest that the best stocking density for rock scallops under these conditions for up to one year is 10/0.1 m^2.

DISCUSSION

Optimal growth of *H. multirugosus* appears to be at a position greater than 1 m above the bottom at both the Quivira Basin and the NOSC tower locations. The fouling and surge effects were most noticeable near the surface in Quivira Basin and at the NOSC tower, respectively. These two factors exerted less influence on growth as depth increased and other factors began to take effect. Results of the stepwise multiple regression analysis show that depth, or some other factor associated with depth, had a significant, but inverse, correlation with growth. Scallops held at the greatest depths at both the bay and open ocean locations, which were within 1 m of the unconsolidated bottom sediment (6 m depth in Quivira Basin and 18 m depth at the NOSC tower), exhibited least growth (Table 1). Duggan (1973) reported that survival of the bay scallop, *Argopecten irradians*, was greatest in the middle of the water column and least near the bottom. He postulated that survival of this species at 1 m above the bottom was reduced due to siltation. Silt or the concentration of suspended matter was greatest near the bottom and appears to have been the major factor reducing the growth of *H. multirugosus* held 1 m above the bottom at both stations in my study.

Foe (1978) observed that growth of *H. multirugosus* was controlled by concentrations of chlorophyll-*a* within the temperature range 16-20°C. He found that growth was adversely affected by temperatures greater than 20° C. The results of the stepwise multiple regression analysis in my study show that temperature, and concentrations of chlorophyll-*a* and particulate organic carbon, as measured in the field, may not have had an effect on the growth of the rock scallop. Apparently, the animals were able to adjust their metabolisms to the natural fluctuations in the field. However, the results of the stepwise multiple regression are not conclusive and it is recommended that further studies be initiated to find the factor or factors which influence the growth of *H. multirugosus* in the field.

The rock scallops grown in the ocean appear to have developed heavier adductor muscles than did those in Quivira Basin (Table 1). This difference appears to be due to the production of deeper shells by rock scallops in the ocean. However, the difference could be due to an artifact of sampling or some unknown environmental stimulus.

The ideal stocking density for *H. multirugosus* appears to be between 5 and 10 animals/0.1 m^2 after one year of growth (Table 4). Animals stocked at densities of 20/0.1 m^2 exhibited a reduction in growth after six months of observations (Monical, 1980). *A. irradians* exhibited reduced growth at densities greater than 25/0.1 m^2 (Dug-

TABLE 4. *Mean and 95% confidence interval for the mean (95% CI) of shell diameter, shell depth and the wet weights of total body, shell, soft body, and adductor muscle for rock scallops,* Hinnites multirugosus, *sacrificed at the beginning of the study (SRC; n=20) and those grown at densities of 5 (n=9), 10 (n=10), and 20 (n=38) animals/0.1m². The data from the scallops that were sacrificed initially are the approximate beginning measurements for the groups examined for one year. Groups in a given box are not significantly different from each other ($p > 0.05$) but are significantly different from those in other connected boxes ($p < 0.05$) based upon the results of the Newman-Keuls tests.*

SHELL DIAMETER (mm)			
Group	Mean	±	95% CI
SRC	23.03	±	1.55
20/0.1 m²	75.87	±	2.93
10/0.1 m²	92.13	±	5.84
5/0.1 m²	93.28	±	5.30

SHELL DEPTH (mm)			
Group	Mean	±	95% CI
SRC	7.06	±	0.48
10/0.1 m²	24.17	±	1.59
20/0.1 m²	24.87	±	1.28
5/0.1 m²	27.32	±	2.20

TOTAL BODY WEIGHT (g)			
Group	Mean	±	95% CI
SRC	1.76	±	0.31
20/0.1 m²	101.18	±	9.68
10/0.1 m²	129.93	±	20.58
5/0.1 m²	147.29	±	11.70

SHELL WEIGHT (g)			
Group	Mean	±	95% CI
SRC	0.90	±	0.17
20/0.1 m²	67.13	±	6.02
10/0.1 m²	83.55	±	11.93
5/0.1 m²	95.29	±	8.08

SOFT BODY WEIGHT (g)			
Group	Mean	±	95% CI
SRC	0.52	±	0.10
20/0.1 m²	23.05	±	2.97
10/0.1 m²	29.46	±	5.98
5/0.1 m²	35.70	±	5.22

ADDUCTOR MUSCLE WEIGHT (g)			
Group	Mean	±	95% CI
SRC	0.12	±	0.02
20/0.1 m²	8.93	±	1.12
10/0.1 m²	12.47	±	2.25
5/0.1 m²	14.44	±	1.74

gan, 1973). He concluded that an increase in competition for food resulted in decreased growth of the bay scallop. Competition for food plus growing space appeared to be the limiting factors of growth for *H. multirugosus* in my study.

The results of this study show that *H. multirugosus* is capable of optimal growth in one of the outermost basins in Mission Bay and in the uppermost 15 m layer of the ocean. This species had a mean growth rate of approximately 6 cm in shell diameter per year at both the bay and open ocean stations in this study. Leighton and Phleger (1977) also observed a mean growth rate of 6 cm per year in Quivira Basin. Leighton (1979) has measured similar growth rates at a depth of 30 m off La Jolla, California. It appears that this animal is capable of the same growth shown in this study at a depth of 30 m.

The ocean appears to be a slightly better location than the bay for scallops because of greater adductor muscle growth. The adductor muscle is the part of the scallop which is of commercial importance. The ocean also offers a relatively stable environment in comparison with Quivira Basin. Unusual environmental fluctuations could affect growth adversely, although these were not experienced during the course of this one year study.

Growth characteristics observed in this study suggest that another, larger study should be initiated to test the feasibility of culturing the purple-hinge rock scallop. Techniques used for culturing oysters and other scallops can be applied or modified for this animal since *H. multirugosus* can grow equally well attached to a substrate or in the free-living state (personal observation).

LITERATURE CITED

Duggan, W.F. 1973. Growth and survival of the bay scallop, *Argopecten irradians*, at various locations in the water column and at various densities. Proc. Natl. Shellfish Assoc. 63:68-71.

Foe, C. 1978. Effect of temperature and food on the purple-hinge rock scallop, *Hinnites multirugosus* (Gale) in Southern California. Master's Thesis, San Diego State University, 69 pp.

Keen, A.M. 1937. An abridged check list and bibliography of Northwest marine mollusca. Stanford University, California. 84 pp [3].

Leighton, D.L. and C.F. Phleger. 1977. The purple-hinge rock scallop, a new candidate for marine mariculture. Proc. World Mariculture Soc. 8th Ann. Mtg. 8:457-459.

Leighton, D.L. 1979. A growth profile for the rock scallop, *Hinnites multirugosus,* held at several depths off La Jolla, California. Mar. Bio. 51:229-232.

Monical, J.B. 1980. Comparative studies on growth of the purple-hinge rock scallop, *Hinnites multirugosus* (Gale) in Southern California. Master's Thesis, San Diego State University.

Mullin, M.M. and Evans, P.M. 1974. The use of a deep tank in plankton ecology 2. Efficiency of a planktonic food chain. Limnol. and Oceanogr. 19:902-911.

Nie, N.H., C.H. Hull, J.G. Jenkins, K. Steinbrienner, and D.H. Bent. 1970. Statistical Package for Social Sciences. McGraw Hill, N.Y. 675 pp.

Sokal, R.R. and F.J. Rohlf. 1969. Biometry; Principles and Practice of Statistics in Biological Research. W.H. Freeman and Co., San Francisco, California. 776 pp.

Strickland, J.D.H. and T.R. Parsons. 1965. A practical handbook of seawater analysis. Bull. Fish. Res. Board Can. No. 125. Ottawa. Quans Priner. 311 pp.

Tukey, J.W. 1953. Some selected quick and easy methods of statistical analysis. Trans. New York Acad. Sci. Ser. II, 16(2):88-97.

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REPRODUCTIVE CYCLE OF *MERCENARIA MERCENARIA* IN A SOUTH CAROLINA ESTUARY[1,2]

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ABSTRACT

Hatchery seed of Mercenaria mercenaria *planted at two tidal locations and at three clam densities were sampled over a three year period. Gonadal development was determined histologically and related to clam size and experimental treatments. Male gametogenesis preceded female development and a 9.5:1 male to female sex ratio occurred during the first year of the experiment. Equal numbers of males and females were approached in the second year and maintained through the third year of growth. Changes in percent lumen; in percent lumen filled with ovocytes or spermatocytes, spermatids and spermatozoa; and the number and size of ovocytes were used to delineate spawning and tissue regeneration periods. Spawning extended for approximately six months with peaks in May and June and in September and October. Spawning was followed by rapid regeneration of gonadal tissue.*

Shell length, tissue wet weight and internal shell volume varied significantly between sexes and developmental stages of clams. Females were longer, weighed more and had more space within the shell than male clams. No histological differences were detected between clams from different densities or tidal locations. Age, size and sex relationships to gonadal development are presented with a discussion of seasonal gonadal changes.

INTRODUCTION

A number of studies have dealt with the reproductive biology of *Mercenaria mercenaria* (Linnaeus 1758) in natural populations. Loosanoff (1936, 1937a) conducted the first detailed histological study of gonadal development and sexual phases in *M. mercenaria*. The reproductive cycle of *M. mercenaria* is known for Long Island Sound (Loosanoff, 1937b), Delaware Bay (Keck, Maurer and Lind, 1975) and Core Sound, North Carolina (Porter, 1964); but little data have been presented for clams from more southern waters. Also, the reproductive biology of clams in extensive culture is poorly known.

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A study of the gonadal cycle of *M. mercenaria* was undertaken as part of a larger project to investigate the feasibility of clam culture in South Carolina. In recent reports, Eldridge, Waltz, Gracy and Hunt (1976) demonstrated clams could be reared to commercial size in South Carolina in two years; Whetstone and Eversole (1978) reported *Panopeus herbstii* as an important predator of cultured seed clams; and Eldridge, Eversole and Whetstone (1979) correlated reduced growth with increased clam density and proposed a clam mariculture strategy for South Carolina. The present study was designed to determine the onset of sexual maturity and describe the reproductive cycle of cultured clams. Additional objectives were to determine if increased clam density affected gonadal development and if any size differences occurred between sexes and developmental stages.

MATERIALS AND METHODS

Seed clams ($\bar{x}$ = 13 mm shell length (anterioposterior axis), SL) obtained from Coastal Zone Resources Corporation of North Carolina were planted in trays (118 × 61 ×14 cm) in May 1975. Twenty trays (10 intertidal, 10 subtidal) were placed in an estuarine area near Clark Sound, South Carolina. At each tidal location, four trays were planted at 290 clams m^{-2} and three trays at 869 clams m^{-2} and 1,159 m^{-2}, and maintained throughout the experimental period. Details of the sampling methods used to determine clam growth and mortality, characteristics of the sampling site and trays, and tray density maintenance procedures were outlined by Eldridge et al. (1979) and Whetstone and Eversole (1978).

Clams were sampled 21 times from May 1975 to May 1978. Clams were sampled monthly through 1975 and quarterly thereafter. Clams from each density level and tidal location were sacrificed, and preserved in 10% buffered formalin. Subsamples, usually 15 clams representative of the size distribution of clams for a given sampling date, were selected for histological preparation. Clams selected were measured for SL, tissue wet weight (TWW) and internal shell volume (ISV) before gonadal tissue was excised. Gonadal tissue was dissected from the mid-lateral portion of the visceral mass, dehydrated in an alcohol series, cleared in xylene and embedded in paraplast. Sections were cut at 10 μm, stained with a modification of Harris' hematoxylin and counterstained with eosin Y.

Qualitative and quantitative criteria were used to describe the seasonal gametogenic cycle of *M. mercenaria*. Gonads were qualitatively classified into one of five developmental stages: undifferentiated, male active, male ripe and spawning, female active, and female ripe and spawning. Porter (1964) and Keck et al. (1975) used 14 and 10 developmental stages respectively to describe the gametogenic cycle of *M. mercenaria*. Number of stages was condensed because of occasional difficulty in staging gonads to such stages as male early active and male active, and because of inherent variability encountered within a single gonad. The five developmental stages were distinguished by the following characteristics:

Undifferentiated (Fig. 1a)

During this stage gonadal tissue was undergoing follicular differentiation. Follicles were usually compressed in clams during 1975. Undifferentiated clams from 1976-1978 had expanded follicles suggesting adult clams had spawned. Follicles contained numerous undifferentiated cells, but no recognizable primary or secondary gametogonia. A few primary bisexual gonads as described by Loosanoff (1937a) found early in 1975 were included in this stage.

Active male (Fig. 1b)

In active males the follicle wall was expanded and the lumen contained spermatocytes. Spermatogonia were attached to the follicle wall, forming a thin layer between the follicle wall and spermatocytes. Spermatids and a few spermatozoa were observed in the lumen of late active males. Follicles were smaller in this stage than those in ripe and spawning males.

Ripe and spawning male (Fig. 1c)

In the ripe and spawning male stage, follicle areas were filled with dense radiating bands of spermatids and spermatozoa. Follicle walls were often compressed and obscured by the large number of spermatocytes and spermatids. In staging ripe and spawning males, ripe follicles were observed with dense bands of eosinophilic spermatozoa and basophilic spermatids surround-

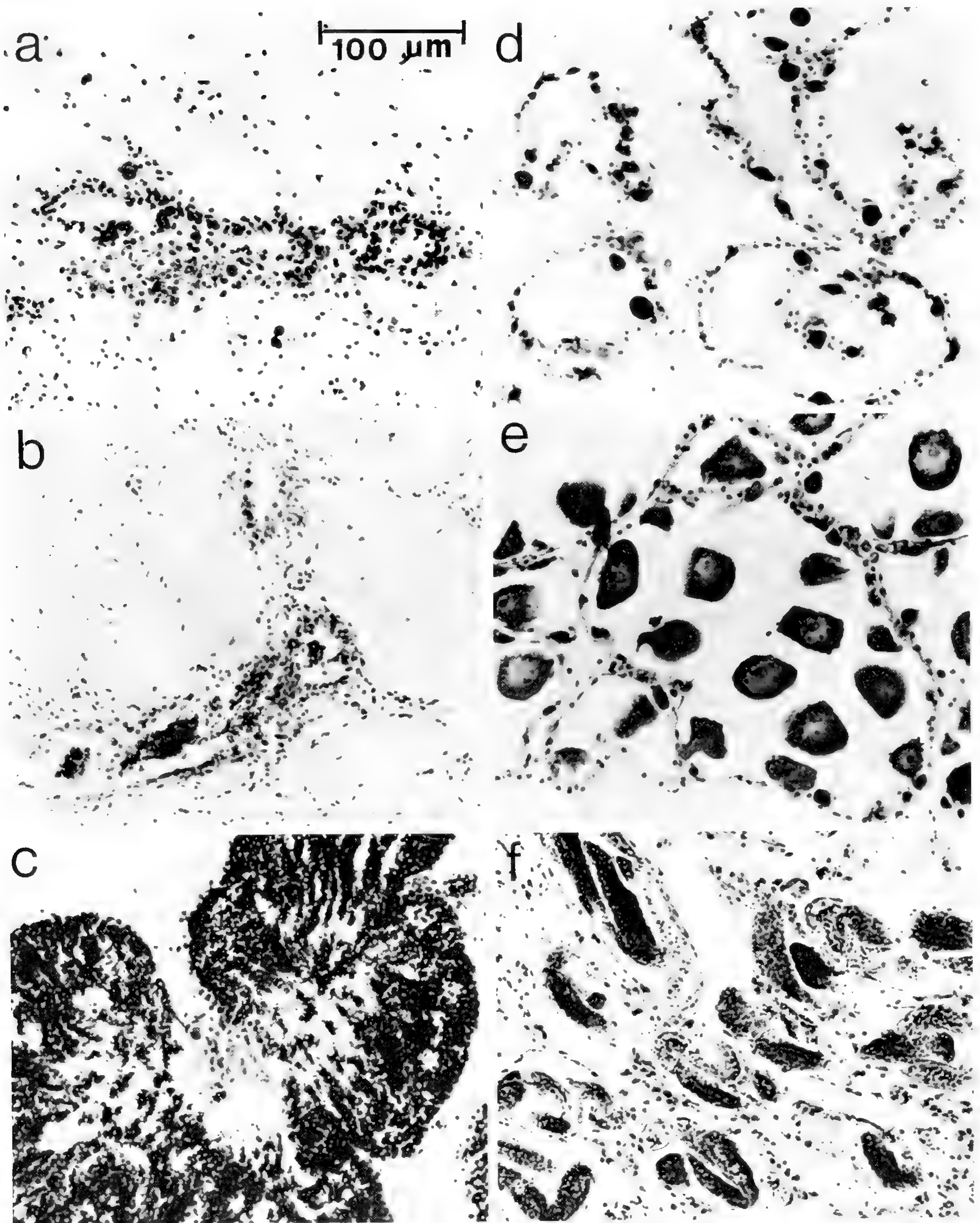

FIGURE 1. *Gonad tissue sections of* Mercenaria mercenaria *from Clark Sound, South Carolina. Plates include: a.) tissue in the undifferentiated stage; b.) male active; c.) male ripe and spawning; d.) female active; e.) female ripe and spawning; and f.) a gonad infested with digenetic trematodes.*

ing a thick layer of spermatocytes; partially spawned follicles had varying amounts of the central lumen filled with primary and secondary sex cells; and spawned out, distended follicles were found with few spermatocytes, spermatids and spermatozoa.

Active female (Fig. 1d)

In active females numerous small ovocytes (<40 μm) were attached to the thickened follicle wall. Central lumen area was empty except for occasional large ovovytes (⩾ 40 μm) which did not possess the large basophilic nucleus characteristic of mature ovocytes.

Ripe and spawning female (Fig. 1e)

Ripe and spawning female follicles contained numerous large ovocytes, usually 40-64 μm in diameter. Many of the ovocytes were free of the follicle wall. Ovocytes characteristically had a large basophilic nucleus. Spawned and partially spawned follicles had varying degrees of empty lumens and ovocytes attached to the follicle wall.

Magnified images of female and male gonads were projected onto graph paper to quantify the seasonal gametogenic cycle. Gonad area occupied by lumen, that area within the follicle wall, was traced, cut out, and run through a Li-Cor portable area meter to determine percent lumen within a standard area (254 μm^2) of gonad. Ovocytes were counted and diameters measured in each 254 μm^2 sample of female gonad. Percent lumen filled with ovocytes was determined by dividing the sum of the calculated areas of ovocytes by lumen area. Ovocytes were assumed to be circular for area calculations. Percent lumen of male gonad filled with spermatocytes, radiating bands of spermatids and spermatozoa was determined by measuring areas in the lumen not occupied by primary and secondary sex cells and substracting this area from the lumen area. These quantitative methods combined with more classical qualitative methods of describing gametogenic activity were used to document seasonal changes in reproductive cycle and gonadal differences between density levels and tidal locations.

Sex ratios were tested against 1:1 ratio with chi-square tests (Steel and Torrie, 1960). Values for size and gonadal condition were calculated and compared with analysis of covariance and t-tests using Statistical Analysis System (SAS-76) developed by Barr, Goodnight, Sall and Helwig (1976).

RESULTS AND DISCUSSION

A total of 304 gonads were sectioned and 303 were placed in one of five developmental stages. One gonad was heavily infested with digenetic trematodes and appeared completely castrated (Fig. 1f).

Histological examination of gonads revealed the same basic cellular components involved in gametogenesis of *M. mercenaria* as previously described by Keck et al. (1975), Loosanoff (1937a,b) and Porter (1964). Follicle and partition cells were present, primarily in female clams, but not to the degree suggested by Keck et al. (1975) or Porter (1964). Follicle cells were reported to serve functions in nutrition or phagocytosis (Ansell, 1961; Porter, 1964; Ropes and Stickney, 1965) or in expansion of developing follicles (Keck et al., 1975). Porter (1964) suggested partition cells as sites for cytological destruction of ovocytes while Ansell (1961) proposed that ovocytes were carried over in partition cells until the following year.

Developmental stages and sex of 303 sectioned clams for the three-year period are summarized in Figure 2. During 1975, 73% of the clams were undifferentiated. A few undifferentiated clams early in 1975 appeared to have primary bisexual gonads (Loosanoff, 1937a). Otherwise, undifferentiated clams have low levels of gametogenesis, a stage

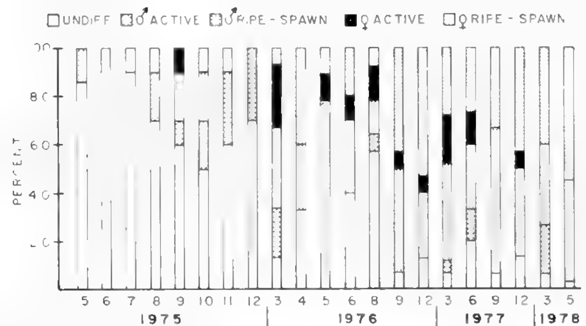

FIGURE 2. *Developmental stages and sex of* Mercenaria mercenaria *from Clark Sound, South Carolina. The length of each shaded area represents the percentage frequency of clams in each developmental stage and sex from May 1975 to May 1978.*

between spawned out and active development. Due to difficulties in accurately determining sex during the undifferentiated stage, observed sex ratios were computed with definite male and female stages as defined by Loosanoff (1937a,b). Males outnumbered females 9.5 to 1 in 1975. Chi-square test performed against 1:1 sex ratio was significant ($P<0.05$) (Steel and Torrie, 1960). After passing through a protandric phase gametogenic development appeared to proceed faster for those clams developing into definite males than females. Females were not detected until September 1975. Loosanoff (1937a) reported that transformation to female after completion of the protandric phase required radical changes in the gonad and more time. Greater trophic cost of femaleness may also increase time necessary to develop into females (Russell-Hunter and McMahon, 1975). Males and females approached equal proportions in 1976; and sex ratios were not significantly different from 1:1 in 1976, 1977 and 1978. Most active males in 1975 were in the process of transforming into definite males for the first time, with the possible exception of some active males found in October, November and December. Increased percentages of active males and undifferentiated clams and decreased percentages of ripe and spawning males in fall 1975 indicated spawning and regeneration of gonadal tissue.

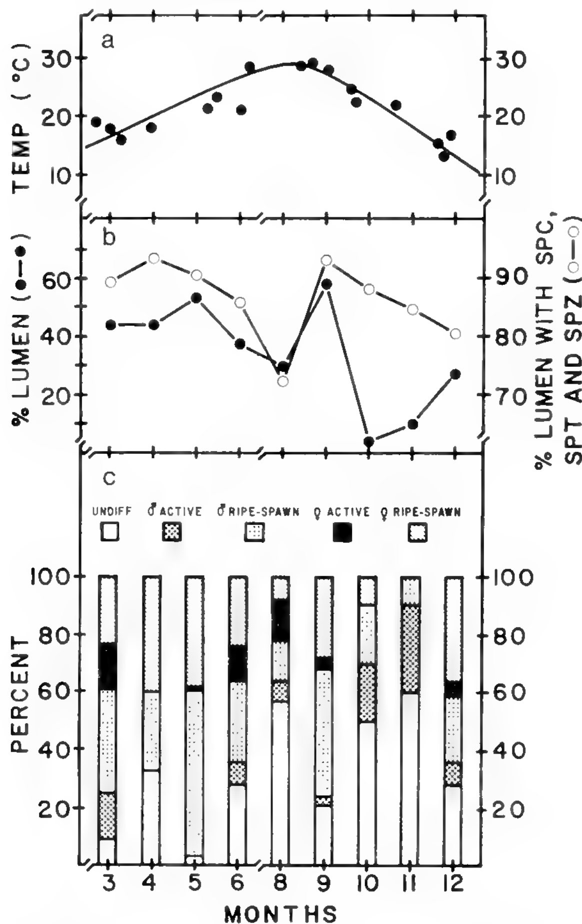

FIGURE 3. *Composite of the developmental stages and sex of* Mercenaria mercenaria *in relation to water temperature and quantitative condition of male gonad. a.) Closed circles represent water temperatures at Clark Sound, South Carolina at low tide from May 1975 to May 1978, and the hand fitted line approximates unmeasured temperatures. b.) Composite of quantitative condition of male gonads represents monthly means of percent lumen (closed circles) and percent lumen with spermatocytes (SPC), spermatids (SPT) and spermatozoa (SPZ) (open circles) from September 1975 to May 1978. c.) The length of shaded areas represents monthly composites of percentage frequencies of clams in each developmental stage and sex from September 1975 to May 1978.*

A composite of qualitative observations from September 1975 through May 1978 is presented in Figure 3c. The first appearance of female clams, an active female, was observed in September 1975. Spawning period started in May and June and continued through October. The spawning period was similar to that reported for clams from Delaware Bay (Keck et al., 1975) and North Carolina waters (Porter, 1964), but longer than that reported by Loosanoff (1937a) for clams from Long Island Sound. May collections were made at the end of the month and the large proportion (96%) of ripe and spawning clams reflected reproductive activities during late May and early June. A decline in June in the percentage of ripe and spawning clams (52%) with a corresponding increase in undifferentiated and active gonads indicated spawning had taken place and gonads were beginning to regenerate. Porter (1964) reported regeneration of gonadal tissue immediately following a June spawning in North Carolina. The percentage (20%) of ripe and spawning clams continued to decline through August as the percentage of undifferentiated and

active clams increased. Gametogenic activity over summer and early September resulted in an accumulation of gonads in the ripe and spawning condition. Spawning occurred in late September and October when percentages of ripe and spawning clams declined and undifferentiated clams increased. Percentage increases in ripe and spawning clams in December, March and April collections indicated regeneration occurred after the fall spawning and continued in spring. Percentage of undifferentiated clams declined when redevelopment occurred.

Quantitative data for males (Fig. 3b) and females (Fig. 4a,b) reflect a developmental trend similar to the qualitative data (Fig. 3c). Female development in spring (March and April) was

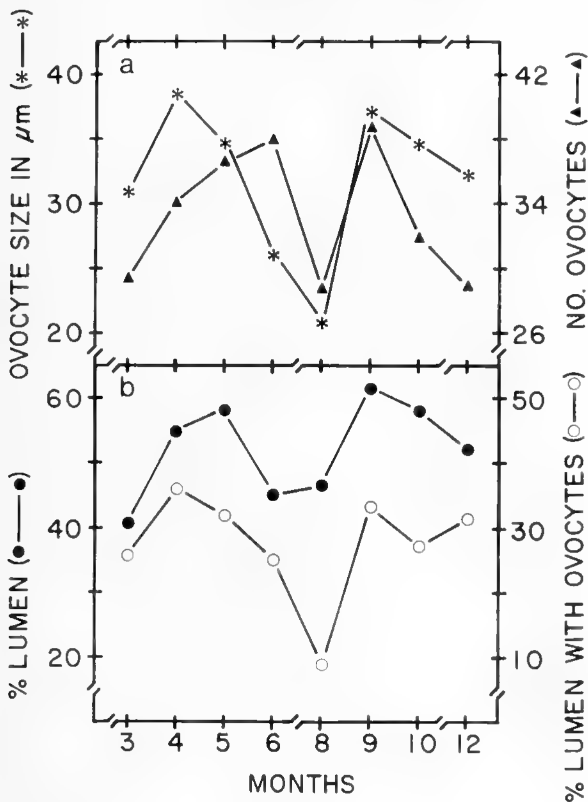

FIGURE 4. *Composite of the quantitative condition of female gonads of* Mercenaria mercenaria *from Clark Sound, South Carolina from September 1975 to May 1978. The location of points represents monthly means in a.) ovocyte size (μm) (asterisk) and number of ovocytes (closed triangles) and in b.) percent lumen (closed circles) and percent lumen with ovocytes (open circles).*

marked by increases in percent lumen, percent lumen filled with ovocytes, and number and size of ovocytes. Spawning activity was represented by decreases in May and June of ovocyte size and percent lumen filled with ovocytes. Changes in quantitative data through August indicated continued spawning and female gonadal regeneration limited to expansion of follicle lumen. In September, female gonads had regenerated and exhibited increased size and number of ovocytes, percent lumen and percent lumen filled with ovocytes. Quantitative condition of female gonads declined in October indicating a second spawning peak. The majority of female gonad regeneration after this peak occurred later (March and April) than that indicated by the qualitative data (Fig. 3c).

Spawning in males was represented by declines in spring (May and June) and fall (September and October) of percent lumen and percent lumen filled with spermatocytes, spermatids and spermatozoa. Quantitative data for the male (Fig. 3b) coincided with data for the female reproductive cycle (Fig. 4a,b). Decreases in the percent lumen filled with spermatocytes, spermatids and spermatozoa were related to increases in areas within the lumen that were spawned out in ripe and spawning males or incompletely filled with spermatocytes in active males. Regeneration of male gonadal tissue was marked by increases in percent lumen and percent lumen filled with spermatocytes, spermatids and spermatozoa in August through September. A dramatic decline was noticed also at this time in the amount of lumen area not occupied by spermatocytes. Some of the regeneration of male gonadal tissue occurred after the fall spawning peak and continued in spring. Male regeneration preceded female redevelopment in fall and more closely agrees with the qualitative data shown in Figure 3c. As noted previously, egg production may require greater energetic expenditures than sperm production and may increase the time necessary for redevelopment of female gonadal tissue after spawning.

Results (Fig. 3,4) indicate that *M. mercenaria* have two breeding peaks per year in Clark Sound, South Carolina. In North Carolina, Porter (1964) reported a bimodal reproductive pattern and a very similar breeding period. Unimodal breeding

patterns were observed in Delaware Bay (Keck et al., 1975) and in Long Island Sound (Loosanoff, 1937b). A similar pattern of changing reproductive strategies along a latitudinal gradient has been noted for *Mya arenaria* (Pfitzenmeyer, 1962; Ropes and Stickney, 1965; Shaw, 1962, 1965). The breeding season of *M. mercenaria* changes with respect to latitude; going further south it becomes prolonged and contains synchronized polymodal breeding patterns. This phenomenon has been observed in other temperate marine invertebrates (Giese, 1959).

Spawning occurred over a water temperature range similar to that observed in North Carolina by Porter (1964). Spawning appeared to start in May when water temperatures rose above 20-23 °C and continued into October until temperatures fell below 20-23 °C (Fig. 3a).

Figure 5 shows the mean SL of sectioned clams by month and by sex (i.e. undifferentiated, male, and female). Differences in SL were observed between sexes. Females appeared larger than males and undifferentiated clams, and males appeared larger than undifferentiated clams. Similar differences in tissue wet weight (TWW) and internal shell volume (ISV) were observed between undifferentiated, male and female clams. Analysis of covariance confirmed that SL, TWW and ISV were significantly different ($P<0.05$) between sexes through 1977 and 1978.

Ansell (1961) Fraser and Smith (1928), Quayle (1952), and Weymouth, McMillan and Rich (1931) found no significant size difference between male and female clams. Sexual dimorphism in bivalves is rare; and occurs less frequently than in gastropods. Sexual dimorphism observed as larger, longer-lived females in gastropods has been hypothesized to be linked to higher trophic cost of femaleness by Russell-Hunter and McMahon (1975).

Histogram percentages of SL of clams in each developmental stage by date are illustrated in Figure 6. Undifferentiated clams occurred more frequently among the smaller size classes. Also, male and female active clams were more abundant in smaller size classes than ripe and spawning male and female clams. Analysis of covariance revealed significant differences ($P<0.05$) in SL, TWW and ISV between active and ripe and spawning clams within each sex. These differences would be expected if those clams in a cohort which initially grew faster also developed faster resulting in an accumulation of larger clams in the more mature

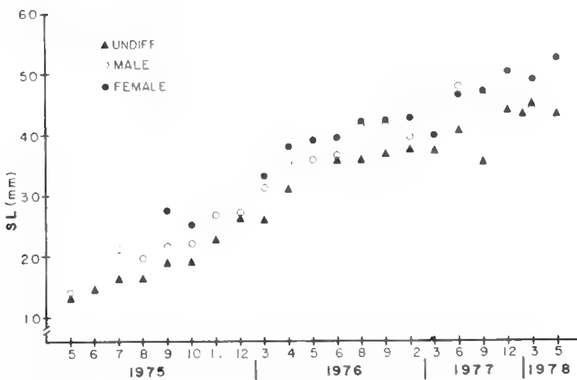

FIGURE 5. *Monthly mean shell length (SL) of undifferentiated, male and female* Mercenaria mercenaria *from Clark Sound, South Carolina.*

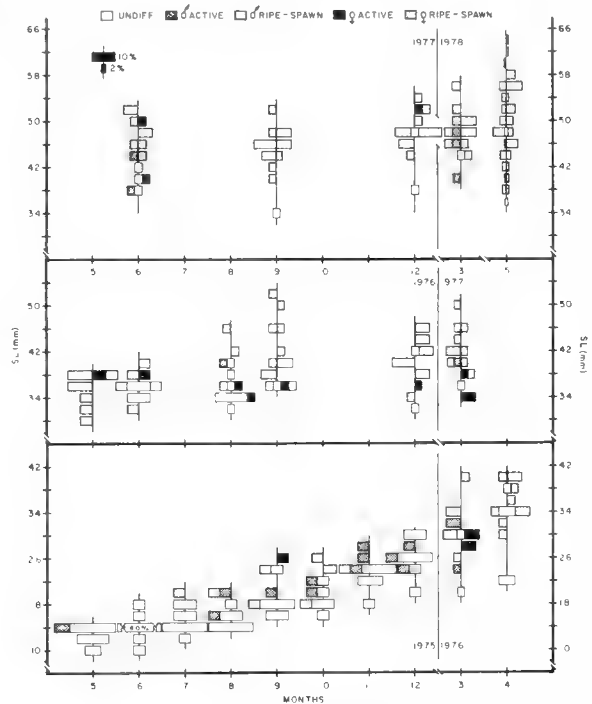

FIGURE 6. *Shell lengths (SL) of reproductive stages of* Mercenaria mercenaria *from Clark Sound, South Carolina. The length of histograms represents frequency percentages of each clam size in each sex and developmental stage from May 1975 to May 1978.*

sexual stages. As clams in this cohort continued to grow and entered subsequent breeding periods, these size differences should become less apparent. In relation to this, no statistical differences in size (SL, TWW and ISV) were detected between active and ripe and spawning clams in 1977 and 1978. Thus, male and female clams in this cohort appeared to have passed a size threshold ('minimum size') for development and spawning, and appeared to have initiated a synchronous reproductive cycle with appropriate environmental cues. In contrast, significant size differences observed between male and female clams over the latter part of the study (1977 and 1978) probably resulted from factors more closely involved in the maintenance of sexual dimorphism. Mechanisms suggested as explanations for significant size differences between developmental stages and sexes should not be considered as mutually exclusive because some combination of them could account for size differences observed between sexual categories such as male active and female ripe and spawning clams, especially during the early part of the experiment (1975 and 1976).

No significant differences in SL, TWW and ISV were detected with analysis of covariance when sexual stages were compared between density levels and tidal locations. Also, sex ratios of clams did not significantly deviate from 1:1 ratio among treatments. Therefore, size differences observed between sexes and developmental stages cannot be accounted for by density and tidal location effects.

Peterson (1978) showed that amount of gonadal tissue in two suspension-feeding bivalves was significantly reduced by increases in population density. In the present study, there was insufficient information to determine if increased density influenced *M. mercenaria* in a similar way. However, histological evidence indicated that gametogenesis was not significantly affected by increased density because no statistical differences were revealed when quantitative data were compared between treatments.

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LITERATURE CITED

Ansell, A.D. 1961. Reproduction, growth and mortality of *Venus striatula* (da Costa) in Kames Bay, Millport. J. Mar. Biol. Assoc. U.K. **41**:191-215.

Barr, A.J., J.H. Goodnight, J.P. Sall and J.T. Helwig. 1976. A Users Guide to SAS-76. SAS Institute Inc., Raleigh, N.C. 329 pp.

Eldridge, P.J., A.G. Eversole, and J.M. Whetstone. 1979. Comparative survival and growth rates of hard clams, *Mercenaria mercenaria*, planted in trays subtidally and intertidally at varying densities in a South Carolina estuary. Proc. Natl. Shellfish. Assoc. **60**:30-39.

Eldridge, P.J., W. Waltz, R.C. Gracy and H.H. Hunt. 1976. Growth and mortality rates of hatchery seed clams, *Mercenaria mercenaria*, in protected trays in waters of South Carolina. Proc. Natl. Shellfish. Assoc. **66**:13-20.

Fraser, C.M. and C.M. Smith. 1928. Notes on the ecology of the butter clam *Saxidomus giganteus* (Deshayes). Trans. Roy. Soc. Can. B. **22**:271-284.

Giese, A.C. 1959. Comparative physiology: Annual reproductive cycles of marine invertebrates. Ann. Rev. Physiol. **21**:547-576.

Keck, R.T., D. Maurer and H. Lind. 1975. A comparative study of the hard clam gonad developmental cycle. Biol. Bull. **148**:243-258.

Loosanoff, V.L. 1936. Sexual phases in the quohog. Science **83**: 287-288.

Loosanoff, V.L. 1937a. Development of the primary gonad and sexual phases in *Venus mercenaria* Linnaeus. Biol. Bull. **72**:389-405.

Loosanoff, V.L. 1937b. Seasonal gonadal changes of adult clams, *Venus mercenaria* (L.). Biol. Bull. **72**:406-416.

Peterson, C.H. 1978. The effects of varying density on growth, reproduction, and survivorship of 2 infaunal, suspension-feeding bivalves. Bull. Ecol. Soc. **59**:57.

Pfitzemeyer, H.T. 1962. Periods of spawning and

setting of the soft-shelled clam, *Mya arenaria*, at Solomons, Maryland. Chesapeake Sci. **3**:114-120.

Porter, H.J. 1964. Seasonal gonadal changes of adult clams, *Mercenaria mercenaria* (L.) in North Carolina. Proc. Natl. Shellfish. Assoc. **55**:35-52.

Quayle, D.B. 1952. The rate of growth of *Venerupis pullastra* (Montagu) at Millport, Scotland. Proc. Roy. Soc. Edinb. B. **64**:384-406.

Ropes, J.W. and A.P. Stickney. 1965. Reproductive cycle of *Mya arenaria* in New England. Biol. Bull. **128**:315-327.

Russell-Hunter, W.D. and R.F. McMahon. 1975. An anomalous sex-ratio in the subtidal marine snail, *Lacuna vincta* Turton, near Woods Hole. Nautilus **89**:14-16.

Shaw, W.N. 1962. Seasonal changes in female soft-shell clams, *Mya arenaria*, in the Tred Avon River, Maryland. Proc. Natl. Shellfish. Assoc. **53**:121-132.

Shaw, W.N. 1965. Seasonal changes in the male soft-shell clam, *Mya arenaria*, in Maryland. U. S. Fish Wildlf. Serv., Spec. Sci. Rept., Fish. No. 508, 5 pp.

Steel, R.G.D. and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc. New York. 481 pp.

Weymouth, F.W., H.C. McMillan and W. H. Rich. 1931. Latitude and relative growth in the razor clam — *Siliqua patula*. J. Exp. Biol. **8**:228-249.

Whetstone, J.M. and A.G. Eversole. 1978. Predation in hard clams, *Mercenaria mercenaria*, by mud crabs, *Panopeus herbstii*. Proc. Natl. Shellfish. Assoc. **58**:42-48.

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ANNUAL GONADAL CYCLE IN THE CARPET-SHELL CLAM *VENERUPIS DECUSSATA* IN VENICE LAGOON, ITALY

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ABSTRACT

The reproductive cycle of Venerupis (Tapes) decussata *in the Venice Lagoon during 1978 was determined by monthly examination of gonads. From October 1977 to the end of February 1978 no gametogenesis took place. Gametocytes began to appear in March, and by May fully ripe eggs and sperm were present. Spawning occurred between late August and the beginning of September, and on 2 October gonads were void of gametes. By December the gonads had entered the resting phase. There is only one gametogenic cycle per year and the various phases seem to be closely related to temperature, actual spawning taking place during the period of temperature maxima.*

INTRODUCTION

Among the wide variety of bivalves consumed in Italy the carpet-shell clam, *Venerupis (Tapes) decussata*, is one of the most relished. Overfishing has greatly depleted or completely destroyed the natural beds and the resulting scarcity has brought the retail price in the shell to more than $8/kg. In recent years the daily catch of a professional fisherman in the Venice Lagoon has been only 10 to 15 kg. This high commercial value and the adaptability of the species to easily managed bottom types warrant research into the possibility of culturing *V. decussata* in Italy.

The purpose of this paper is to describe the carpet-shell clam's gonadal cycle in Venice Lagoon in view of acquiring basic knowledge necessary for research on its controlled reproduction and culture.

METHODS

Monthly samples were taken through most of 1977 and all of 1978. Due to their scarcity the animals were not collected directly in nature but were furnished by a dealer who pools the daily catches from the Lagoon. Twenty samples, averaging 16 animals each, were fixed in modified Davidson's fixative (Shaw & Battle, 1957). The size of the clams ranged from 1.5 to 5 cm. Histological sections were examined microscopically and the state of the gonads were classified following the scheme of Shaw (1964). An attempt was made to correlate the phases of the gonadal cycle with the physical and chemical parameters of the Lagoon in order to identify which external stimuli might regulate reproduction in the carpet-shell clam.

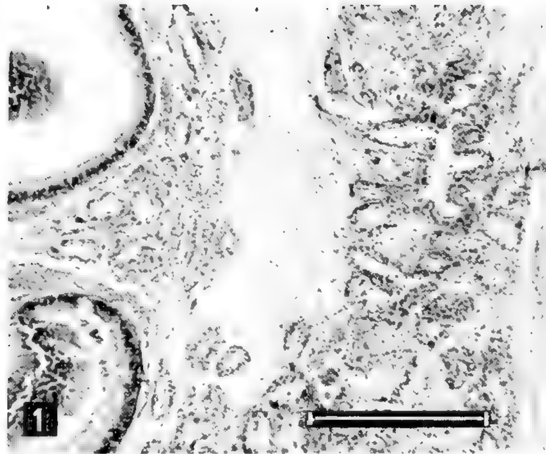
RESULTS

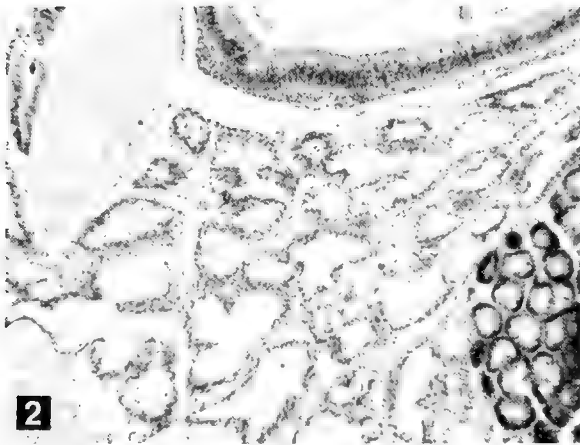
Inactive stage

Clams collected on 5 October 1977 and on 2 October 1978 clearly showed that spawning had been completed. Most animals on this date were spawned out while a few, especially males, still held a residuum of gametes. Full resting condition was attained in December when the recognizable gonadal tissue was reduced to a minimum (Figure 1); yet, it was still possible to find a few individuals with some eggs or sperm.

Developmental stage

Gametocytes were distinguishable at the end of February, and by the end of March males and





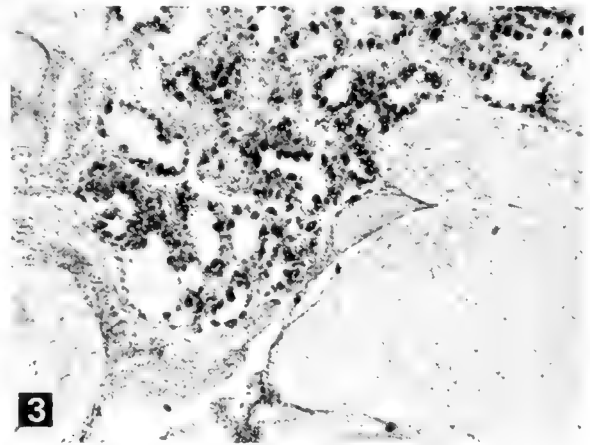


FIGURE 1. Winter or inactive stage. Clam showing reduced gonadal tissue with no sign of gametogenetic activity. February 1978. The bar represents 500 μ; all other figures to the same scale.

FIGURE 2. Developmental stage. Male clam with young spermatocytes. March 1978.

FIGURE 3. Developmental stage. Female clam with young ovocytes. March 1978.

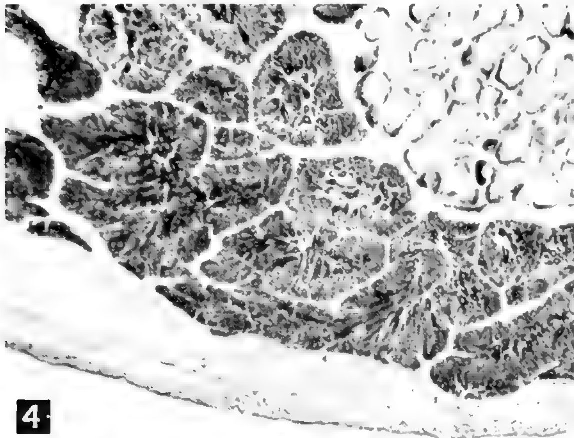
females were clearly recognizable (Figures 2 and 3). The gonad tubules had penetrated a considerable portion of the visceral mass and had well developed lumina. At the beginning of May eggs and sperm appeared to have reached maturity.

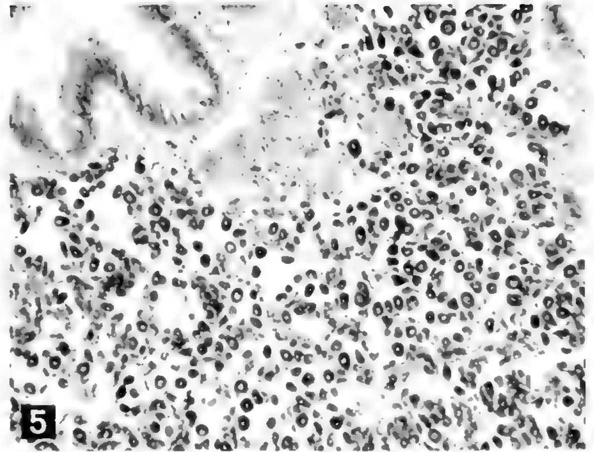
Mature stage

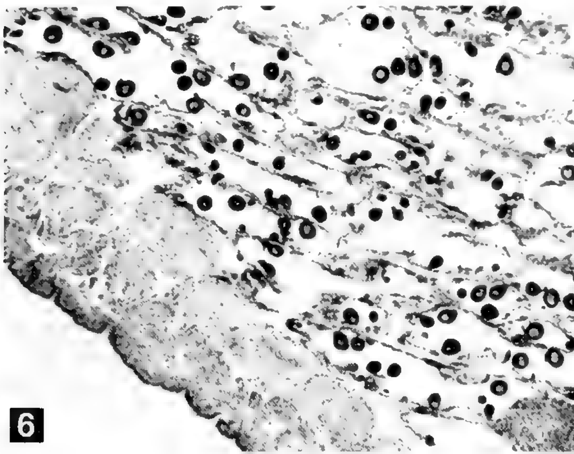
From May onwards *V. decussata* held apparently ripe gametes (Figures 4 and 5). The gonads occupy most of the visceral mass but do not extend into the gills or the mantle. Although spawning probably never occurs earlier than mid-July, histologically the gametes appear as well developed in May as they do in August. The tubules are fully distended, with stalked eggs protruding into the lumina in the females, while in the males they are filled by masses of sperm.

Partially spawned stage

In 1978 signs of spawning were evident in animals collected in September. The eggs were less closely packed in the tubules and voids indicated where they had been shed. By the middle of September spawning appeared to be nearly completed with gonads containing only a few eggs (Figure 6). The males, however, held a relatively large store of gametes in this period (Figure 7), in contrast with the condition of the females. In 1977 spawning had commenced somewhat earlier, prior to the first week of August.







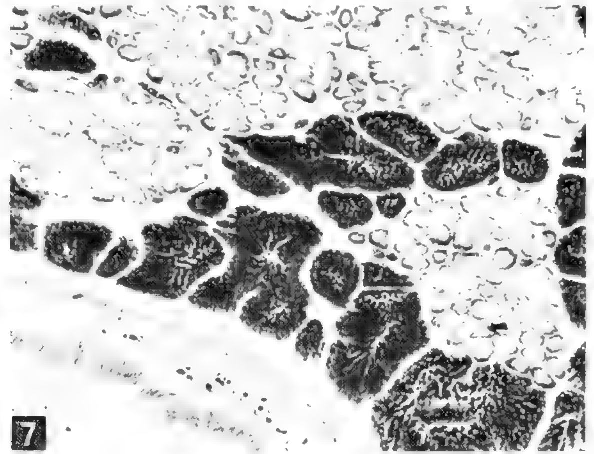


FIGURE 4. Mature stage. Male clam with ripe sperm. May 1978.

FIGURE 5. Mature stage. Female clam with ripe eggs. May 1978.

FIGURE 6. Partially spawned stage. Female clam showing empty spaces in the germinal tubules where eggs have been shed. August 1978.

FIGURE 7. Male clam showing good quantities of sperm late in the season. September 1978.

Completely spawned stage

Clams taken on 2 October showed the lumen of the tubules void of gametes. It was observed that some animals in this spawned-out condition (Figure 8) were to be found right into November and December while in other animals the gonadal tissue had assumed, in the same period, an inactive condition. No degradation of residual eggs was evident but this was possibly due to the rather long interval between the samples.

DISCUSSION

The various phases of the reproductive cycle in *V. decussata* closely coincide with those of the temperature cycle. The temperature regime (Figure 9) might be divided into four periods: a warm period from June to August when the water is above 20°C, a cold period from November to March when the temperature generally stays below 10°C, and two transition periods, one from April to May when there is a rise of 10 °C and one from September to November when there is a drop of 10°C. During the winter period the gonad is inactive. In Spring the rise in temperature, which is a gradual in March but rapid in April and May, probably stimulates initiation of gamete

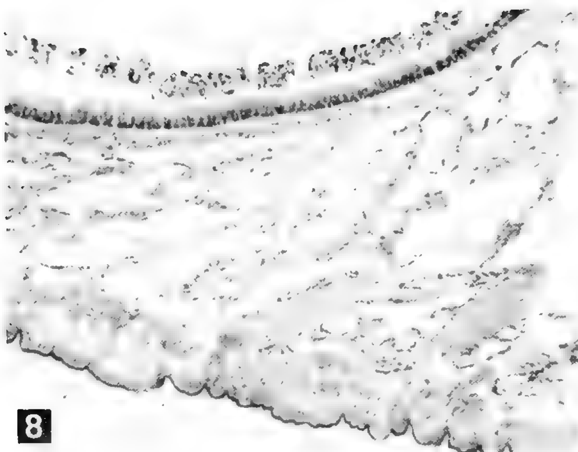


FIGURE 8. Completely spawned stage. Spawned-out clam showing empty germinal tubules with no trace of gametes. December 1978.

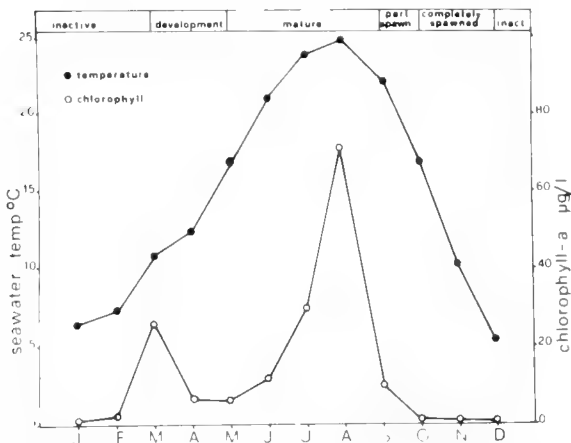


FIGURE 9. Water temperature and phytoplankton levels (expressed in µg/1 of chlorophyll-a) in Venice Lagoon during 1978 and on upper margin various stages of the gonadal cycle of Venerupis decussata.

formation. From May onwards the animals have apparently ripe gametes; however, since the species requires considerably higher temperatures (25°C+) for spawning to be stimulated, discharge of gametes is restricted to the warmest period of the year, which in Venice Lagoon occurs in mid- and late summer (Brunetti & Canzonier, 1973; Brunetti, Menin, & Canzonier, 1977). It was experimentally observed that the minimal temperature requirement for the discharge of gametes is 28°C, which is not usually attained by the major water masses in the Lagoon, but is reached only in the shallow intertidal areas. This high temperature requirement is obviously a restricting factor and limits the species to only one cycle of gonadal development per year in the Lagoon. The above observations correspond well to those of Vilela (1949) regarding *Venerupis decussata* in Faro Lagoon (Southern Portugal) where the temperature cycle and the gonadal cycle of the species are similar to those in Venice Lagoon and show the same relationship. Comparing with other well-known estuarine clams interesting differences arise, however. *Mercenaria mercenaria* (Loosanoff, 1937), *Mya arenaria* (Shaw, 1964), and *Venerupis japonica* (Holland & Chew, 1974) show lower temperature requirements in their reproductive cycle. With these species gametogenesis recommences right after summer spawning; thus it is possible to find gametocytes in winter, which is never the case in the carpet-shell clam. Spawning occurs much earlier and lasts longer, with usually two peak periods in warmer waters, whereas *Venerupis decussata* spawns only once and in a very restricted period.

The only other parameter of the Lagoon that might show some correlation with the cycle of gametogenesis in the carpet-shell clam is phytoplankton abundance which normally exhibits a sharp increase in early spring and maintains a fair level of abundance through September or October (Brunetti, Menin, & Canzonier, 1977). It is entirely probable that food supply operates synergystically with temperature in controlling the gonadal cycle.

In the specimens observed there was no indication of hermaphroditism or of sex inversion.

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REFERENCES

Brunetti, R. & W.J. Canzonier. 1973. Physico-chemical observations on the waters of the southern basin of the Laguna Veneta from 1971 to 1973. Atti Ist. Veneto Sci. Lett. Arti **131**:503-523.

Brunetti, R., F. Menin, & W.J. Canzonier. 1977. Physico-chemical parameters of the water of the lower basin of the Laguna Veneta for 1973-1974. Riv. Idrobiol. **16**(½):173-198.

Coe, W.R. & H.J. Turner. 1938. Development of the gonads and gametes in the soft-shell clam (*Mya arenaria*). J. Morph. **62**(1):91-111.

Holland, D.A. & K.K. Chew. 1974. Reproductive cycle of the Manila clam (*Venerupis japonica*), from Hood Canal, Washington. Proc. Natl. Shellfish. Assoc. **64**:53-58.

Loosanoff, V.L. 1937. Seasonal gonadal changes of adult clams, *Venus mercenaria* (L.). Biol. Bull. **72**:406-416.

Shaw, W.N. 1964. Seasonal gonadal changes in female soft-shell clams, *Mya arenaria*, in the Tred Avon river, Maryland. Proc. Nation. Shellfish. Assoc. **53**:121-132.

Shaw, W.N. & H.I. Battle. 1957. The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin). Can. J. Zool. **35**:325-347.

Vilela, H. 1949. Quelques données sommaires sur l'écologie de *Tapes decussatus* (L.). Conseil Permanent Int. pour l'Exploration de la Mer, Rapp. et Proc. Verb. des Re'un. **128**:60-62.

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COMPARISON OF RECENT AND PAST PATTERNS OF OYSTER SETTLEMENT AND SEASONAL FOULING IN BROAD CREEK AND TRED AVON RIVER, MARYLAND[1,2]

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ABSTRACT

Settlement of oyster spat, barnacles, encrusting bryozoans and some additional invertebrates was studied for three summers (1977-1979) in Broad Creek and Tred Avon River, tributaries of the Choptank River, Maryland. In 1977, settlement substrate included asbestos-cement plates and oyster shell. Patterns of settlement periodicity and intensity, and larval "preference" for upper or under surfaces were similar for both substrates so only plates were used in 1978 and 1979. Results were compared with a similar study in these two tributaries by W. N. Shaw in 1961 to 1966. In contrast to his findings, oysters settled predominantly on upper surfaces, perhaps as a result of increased turbidity or decreased light penetration associated with greater land erosion or elevated primary productivity in the intervening years. Average numbers of oyster spat were lower than in 1961 to 1966, perhaps as a result of lower salinity levels. As Shaw had found, barnacles and bryozoan colonies settled predominantly on under surfaces. Numbers of hooked mussels settling were lower than in the past. As before, Broad Creek had a higher incidence of oyster settlement than did Tred Avon River which continued at its former low level.

INTRODUCTION

Over the last three decades, there has been a general decline in numbers of oyster spat, *Crassostrea virginica* (Gmelin), settling successfully and surviving in Maryland's portion of Chesapeake Bay (Krantz and Meritt, 1977). To understand why this has been occurring, we have been studying gametogenesis in central Chesapeake Bay, and advective and dispersive characteristics of two tributaries of the Choptank River, i.e., Broad Creek and Tred Avon River. A supplementary study during three summers (1977-1979) has involved monitoring settlement of oyster spat and associated invertebrate organisms in the two Choptank River tributaries in order to determine occurrence and intensity of settlement.

A similar study in these two tributaries was performed from 1961 to 1966 by Shaw (1967, 1969). We report here the results of our study and compare them with those of Shaw (1967, 1969). Our

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results supplement Shaw's findings but also provide evidence of important differences between environmental conditions and invertebrate settlement patterns as they were then (1961 to 1966) and are now (1977 to 1979).

MATERIALS AND METHODS

Broad Creek and Tred Avon River (Figure 1) have been described by Shaw (1969) who found them to be similar in temperature, salinity and dissolved oxygen. Broad Creek has been reported to be an area of usually successful spat settlement, whereas Tred Avon River usually experiences poor or negligible oyster settlement (Beaven, 1955; Shaw, 1969; Krantz and Meritt, 1977).

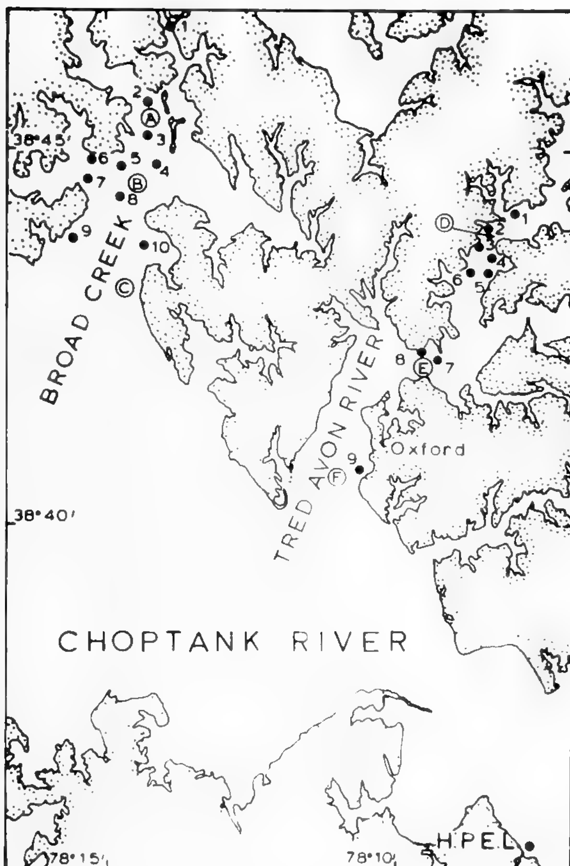

FIGURE 1. *Location of the study area. Positions of spat-plate holders are indicated by numbers. Letters A to F locate the stations used during the 1978 chlorophyll* a *study. H.P.E.L. locates the Horn Point Environmental Laboratories.*

Over the summers of 1977 to 1979, the following stations were established: Broad Creek - Stations 1, 2, 4, 5, 8 and 9 in 1977; Stations 2, 3, 4, 5, 8 and 10 in 1978; Stations 2 to 8, and 10 in 1979; Tred Avon River - Stations 4 and 9 in 1977; Stations 1, 3, 5, 8 and 9 in 1978; Stations 1 to 3 and 5 to 9 in 1979. Stations were added as the study program expanded or were dropped because they were difficult to get to or because channel markers which were the point of attachment for some of the spat-collecting material were removed by ice.

At each station, a spat-plate holder with two horizontal asbestos-cement plates (each measuring about 12 cm square and both held about 2.5 cm apart) was submerged, with both plates replaced approximately weekly with clean plates (Shaw, 1969). In our study, the top plate was designated Plate A and the bottom Plate B. Before initial use the plates were conditioned by holding them in flowing Choptank River water for two weeks. After examination, each plate was scrubbed thoroughly with a wire brush and stored dry in haphazard order in a slotted rack before being used again. Generally, the same plates were used in all three years (more being added each year as more stations were added or as plates were lost in the field). They were not specifically returned to the same station after counts of fouling organisms had been made; each plate had an equal chance of being taken from the rack and used at any station at any time. The plate holders were tied to docks or channel markers and were held 10 to 20 cm above the bottom in depths ranging from 1 to 3 m depending on the station.

In 1977 only, a bag of shell was placed next to the spat-plate holder at each station and changed weekly. The bags were made of plastic-coated crab pot mesh, with a mesh size of about 5.5 cm in the long dimension and about 3.5 cm perpendicular to the long axis. Inside each bag, six scrubbed oyster shells were placed with the concave (inside) surface up and six with the concave surface down. The bags were suspended horizontally, about 10-20 cm above the bottom. Shells of roughly comparable size were used at all times.

On the center of both sides (top, bottom) of each plate, all oysters and a variety of invertebrate species were counted within a grid covering 85 cm^2. Numbers were then corrected to numbers 100

cm⁻² for comparison with Shaw's results (1969). Numbers of individual barnacles and of individual colonies of bryozoans were counted but not identified to species. Average weekly abundances in each tributary were determined for top or bottom of plates A or B by dividing the total count of oysters, etc., on each surface by the number of plates examined. For the oyster shell, counts were made of the settled organisms on each concave surface. These counts were used for semi- quantitative comparison with the data from the plates because of the difficulty in accurate determination of the surface areas involved. The raw data on animal abundance, including organisms not dealt with in this paper, are contained in a data report (Kennedy, Smawley and Boettger, 1979).

When the plates were collected at each station, bottom or mid-depth temperature and salinity readings were made. In 1978 and 1979, Secchi disc measurements were made on each station.

From May to August 1978, measurements of chlorophyll *a* concentrations were made on water samples collected with a plastic 6-liter Van Dorn sampler at the surface at three stations along the axis of each tributary (Figure 1). Broad Creek samples were collected in the morning whereas Tred Avon River samples were collected later in mid-morning or early afternoon. Samples were filtered through glass-fiber filters and chilled or frozen in tightly sealed petri dishes kept in the dark. Chlorophyll *a* concentrations were determined using a fluorometer following the methods of Strickland and Parsons (1968).

RESULTS

Water Quality

Average temperature, salinity and Secchi disc measurements for the two tributaries are plotted on Figure 2. Temperatures were generally similar in both areas with Tred Avon River being slightly warmer than Broad Creek. In 1977, temperatures reached the highest levels over the three-year study, attaining 29.9° C in Broad Creek and 30.0° C in Tred Avon River in mid-August. Temperatures fell thereafter through September. In 1978, temperatures remained in the upper 20's from mid-June to early September. In 1979, temperatures rose rapidly from below 20° C in late May to the upper 20's in early August. A cold spell dropped temperatures to the low 20's in mid-

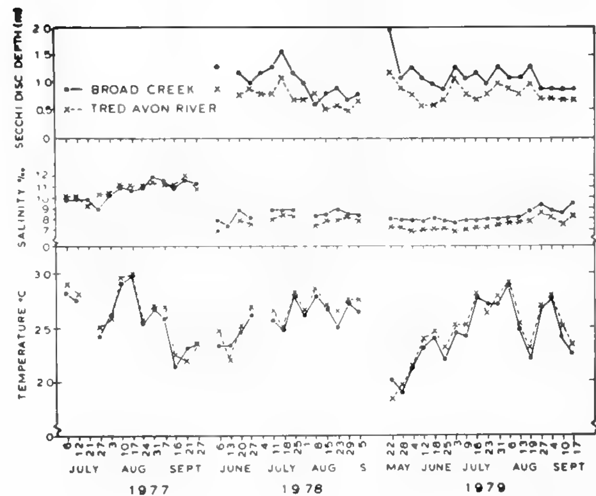

FIGURE 2. *Average weekly values for temperature, salinity and Secchi disc depths in Broad Creek and Tred Avon River during the summers of 1977 to 1979.*

August but they increased in late August, dropping again in September.

Salinity was similar in both locations in 1977 but was slightly lower in Tred Avon River in 1978 and 1979 compared with Broad Creek (Figure 2). Salinities were highest (range: about 9 to 12 ‰) in 1977, declining in 1978 (range: about 7 to 9 ‰) and 1979 (range: about 7 to 9.5 ‰).

Secchi disc readings (a measure of water transparency) were consistently lower in Tred Avon River in both 1978 and 1979 (Figure 2). Surface chlorophyll *a* values (averaged for the three stations in each tributary) were higher in Tred Avon River compared with Broad Creek (Figure 3). Values showed much variability, especially in Tred Avon River, but the differences between the two locations are clear. In both areas, values generally increased from May through August.

Scrutiny of Assumptions

Oyster shell was not used after 1977. The flat surfaces of asbestos-cement plates allow quantitative estimation of abundances per unit area (Butler, 1955). This is difficult on the irregular surfaces of shells. The plates are less attractive to oyster larvae than shells, but peaks and patterns of setting are similar (Shaw, 1967; Drinnan and

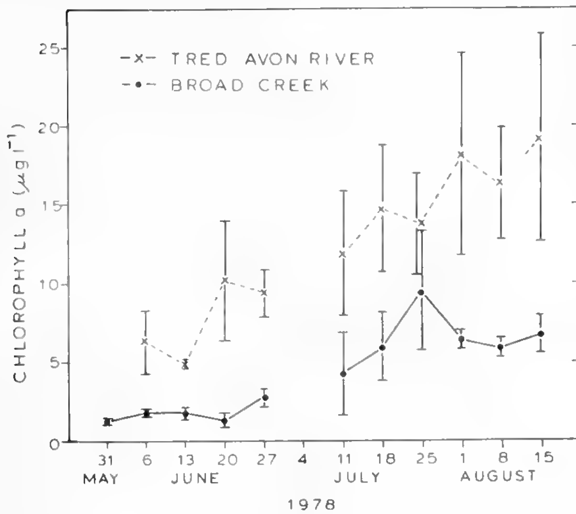

FIGURE 3. *Weekly values for surface chlorophyll a (averaged for three stations in each tributary) in Broad Creek and Tred Avon River in summer 1978. Vertical bars represent one standard deviation on each side of the mean. Values are μg liter⁻¹.*

Stallworthy, 1979c). We found that oyster settlement peaks generally occurred in the same week for both plates and shell (Kennedy, Smawley and Boettger, 1979). Comparisons of spat abundances per unit area between both substrates were not possible because of the problems of measuring shell surface area. However, settlement intensity among stations was compared for the two substrates by determining total numbers of spat settling on each substrate over the summer (1977) at each station. The six stations were then ranked in decreasing numbers of spat as follows:

Station Number:	1	2	4	5	8	9
Rank (shell)	5	1	3	2	4	6
Rank (plates)	6	1	3	2	4	5

Settlement intensity was generally similar for both substrates except at the stations with fewest larvae settling over the summer (Stations 1 and 9). Thus, we dropped the use of shell and added more stations.

In presenting abundance data (below), numbers of organisms have been combined for all stations within a tributary. A check was made of inter- station variability in settlement intensities in Broad Creek, using the four stations (2, 4, 5, 8) common to all three years of the study. Because oyster settlement was poor in 1978, only 1977 and 1979 data were used. In 1977, a total of 3320 spat were counted compared with 1529 in 1979. The percentage of the total settling at each of the four stations was as follows:

Station Number:	2	4	5	8
1977	48%	18%	19%	15%
1979	20%	20%	34%	26%

At each station, spat abundances varied yearly with, for example, Station 2 ranking first in 1977 and last in 1979, and Station 8 moving from last in 1977 to second in 1979. Similarly, for barnacles and bryozoans, none of the four stations maintained its rank from year to year. For example, for barnacles, Station 2 ranked 1 in 1977, 3 in 1978, and 2 in 1979 whereas Station 8 was 4 in 1977 and 1978 and 1 in 1979 (see also Cory, 1967, for inter-station variability in barnacle settlement).

I concluded that no one station should be more or less a center of abundance than another, given the variability noted above, so, for comparative purposes, each tributary was treated as a whole.

Oyster Settlement

Figure 4 presents data on oyster spat abundance. Values are averages (number per 100 cm²)

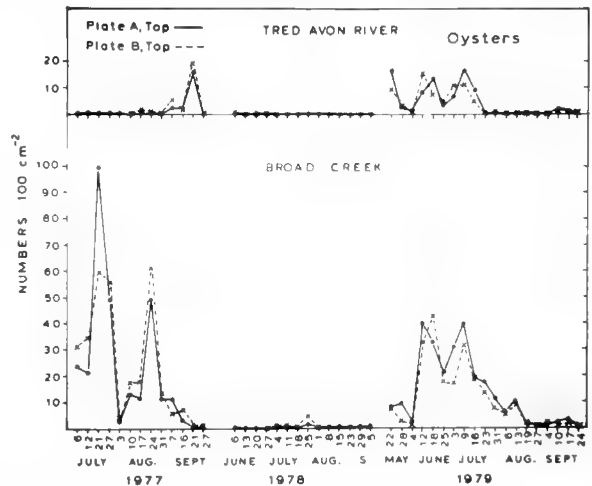

FIGURE 4. *Weekly settlement of oyster spat on the upper surface of horizontal asbestos-cement plates held in Broad Creek and Tred Avon River. Values are the average number of individuals per plate (corrected to 100 cm² of surface area).*

for the top surfaces of Plates A and B from both tributaries. Average values for bottom surfaces were much lower (maximum value (a) in Broad Creek: 27 per 100 cm^2 in 1977; < 1 in 1978; 9 in 1979; (b) in Tred Avon River: < 1 in 1977 and 1978; 4 in 1979) and are not presented. For both areas, 1978 was a very poor year with almost no settlement occurring. In Broad Creek, two peaks of settlement occurred in 1977, in late July and late August. In 1978, a small peak of settlement occurred in early August. In 1979, there were two peaks again, in mid-June and early July, with few spat being found thereafter. In Tred Avon River in 1977, numbers of oysters settling were very low until a small peak of settlement occurred in late September, which was later than settlement occurred in Broad Creek. In 1978, almost no spat were noted on plates in Tred Avon River during the period of study. Research ended after the first week in September so it is not known if a late September peak occurred in 1978 as it had in 1977. Spat were most abundant in Tred Avon River in 1979, with three peaks of abundance in late May, mid-June, and early July, after which settlement was negligible. The peaks in mid-June and early July coincided between both tributaries, unlike the situation in 1977 when settlement was later in Tred Avon River. In general, average numbers of oyster spat were greater in Broad Creek in all three summers compared with Tred Avon River.

Barnacle Settlement

Figure 5 presents data on barnacles. We expect that these were mostly *Balanus improvisus* Darwin but we did not determine species because of the very small size of the newly settled individuals. Unlike oyster spat, barnacles settled in much greater numbers on bottom surfaces of both plates. Maximum average numbers settling on top surfaces were (a) Broad Creek: 11 per 100 cm^2 in 1977; 3 in 1978; 18 in 1979; (b) Tred Avon River: 8 in 1977 and 1978; 15 in 1979. Therefore, only the bottom surfaces are considered here. In 1977, two peaks occurred during the period of study in Broad Creek in early July and early September. In 1978, numbers were low in Broad Creek during the period of study, with a small peak in early August. In 1979, barnacles had two large peaks of abundance, in late May (the highest numbers recorded in Broad Creek over the three summers) and late July, with a small peak in mid-June. In Tred Avon River in 1977, a peak of barnacle settlement occurred in early September (as in Broad Creek) but there was no peak earlier in the summer. Barnacle numbers increased again in late September (as they did to a lesser extent in Broad Creek). Barnacle settlement was much greater in Tred Avon River than in Broad Creek in 1978. The major peak of abundance occurred in mid-June with minor peaks in mid-July (Plate B) or early August (Plate A). In 1979, as in Broad Creek, the greatest barnacle abundance occurred in late May, followed by a small episode of settlement in early June and another peak in late July. In general, Tred Avon River averaged more barnacles settling over the three summers than did Broad Creek.

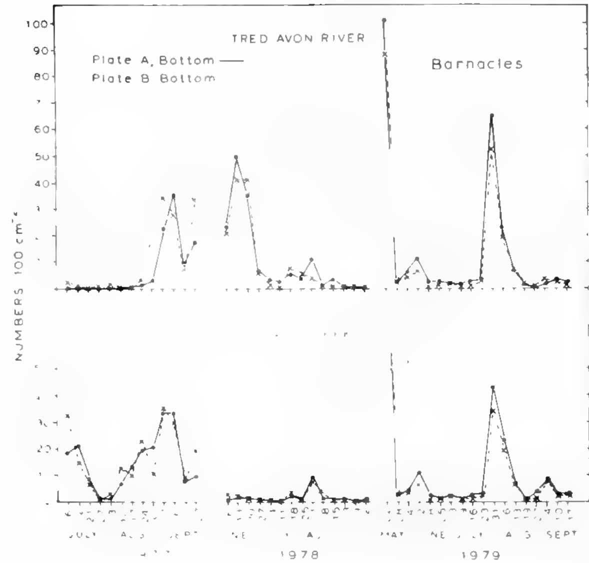

FIGURE 5. *Weekly settlement of barnacles. As Figure 4.*

Bryozoan Settlement

Figure 6 presents data on encrusting bryozoan colonies. We did not determine the species but they were probably *Conopeum tenuissimum* (Canu) (=*Electra crustulenta*) and perhaps *Membranipora tenuis* Desor. As was the case with the barnacles, bryozoans settled predominantly on the bottoms of the two plates. Maximum average

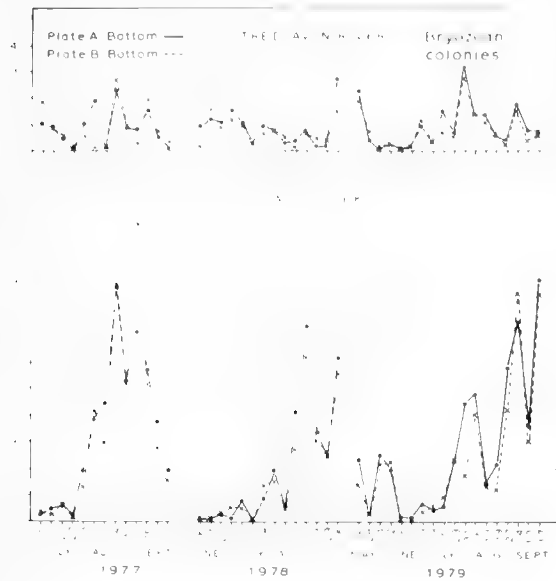

FIGURE 6. *Weekly settlement of colonies of encrusting bryozoans. As Figure 4.*

numbers of colonies on top surfaces in (a) Broad Creek were: 80 per 100 cm² in 1977 (a year with high numbers in both tributaries. However, numbers were still less than those from bottom surfaces); 25 in 1978; 22 in 1979; (b) Tred Avon River: 23 in 1977; 7 in 1978; 4 in 1979. In Broad Creek in 1977, peaks of colony numbers occurred in late August and early September. In 1978, a peak occurred in mid-August, with numbers increasing again in early September. In 1979 numbers were moderate in late May, a small peak occurred in early June, a larger peak occurred in early August, with abundance increasing through mid-September when the study ended. In Tred Avon River, a variety of similar-sized peaks of moderate abundance occurred in all three summers. The greatest peaks of settlement occurred in late August 1977, early September 1978 and late July 1979. In general, average numbers of bryozoan colonies were greater in Broad Creek than Tred Avon River.

Miscellaneous Species

Numbers of spat of the hooked mussel, *Ischadium recurvum* (Rafinesque) were low in both tributaries in all three summers. Total numbers on Broad Creek plates were 305 in 1977, 60 in 1978 and 6 in 1979. Approximate numbers per plate were 2.0 in 1977, 0.4 in 1978 and 0.02 in 1979. In Tred Avon River, total numbers on the plates were 103, 191 and 30 for 1977, 1978 and 1979 respectively. The approximate numbers per plate were 2.4 in 1977, 1.4 in 1978 and 0.1 in 1979. A sessile ciliate protozoan, *Folliculina* sp., became common in late July through early September in both tributaries each summer. Mud tubes built by the amphipod *Corophium* sp. and the annelid *Polydora* sp. were encountered every week. In 1978, the sea anemone, *Diadumene leucolena* (Verrill) appeared on the plates in July with numbers increasing to mid- or late August, declining thereafter.

Comparison of Settlement Surfaces

Table 1 presents data on the percentage of animals settling on the upper or lower surfaces of the plates. There were no important differences noted between the two tributaries. For oysters, settling on the upper surface of both plates predominated with percentages ranging between 78% and 99% (the value of 50% for 1978 in Tred Avon River derived from results involving 6 spat). For barnacles and bryozoans, settlement occurred predominantly on the lower surfaces of the plates, with percentages ranging between 81% to 93% for barnacles and 57% to 94% for bryozoans. For hooked mussels, the selected surface varied each year. Oddly, the mussels settled predominantly on the lower surfaces in both tributaries in 1977, on the upper surfaces in 1978 and generally equally or slightly "preferring" the lower surface in 1979.

In 1977, comparisons of settlement on concave oyster shell surfaces revealed that 61% of the oyster spat were found on the shell facing upward whereas 69% of barnacles, 72% of bryozoans and 66% of hooked mussels settled on downward-facing shell. These patterns were similar to those reported for these organisms settling on plates (Table 1).

Table 1 also contains data on the average numbers of individuals or colonies settling per plate deployed each summer (*a* in Table 1). In calculating these values for barnacles in 1979, the data collected in May were not included as May is generally an important month for barnacle settlement and was not surveyed in 1977 and 1978. For

TABLE 1. *Percentage of animals settling on upper or lower surfaces (as indicated) of Plates A and B during the summers, 1977 to 1979. n= total number of individuals or colonies settling;* a*=n÷ total number of plates deployed each summer (note that in 1979 the value for barnacles represents* a *calculated after elimination of the data for May 1979).*

Organism		Broad Creek			Tred Avon River		
		1977	1978	1979	1977	1978	1979
Oysters	%	78	79	84	99	50	84
(upper)	n	3817	49	3599	77	6	1209
	a	25.1	0.3	12.8	1.8	0.04	4.3
Barnacles	%	85	81	92	89	93	91
(lower)	n	2353	242	3078	328	1263	3693
	a	15.5	1.5	6.3	7.8	9.0	8.9
Bryozoans	%	68	85	88	57	94	92
(lower)	n	6531	3141	7514	548	956	2319
	a	43.0	19.3	26.6	13.0	6.8	8.3
Hooked	%	74	9	50	78	31	56
Mussels	n	305	60	6	103	191	30
(lower)	*a*	2.0	0.4	0.02	2.5	1.4	0.1

oysters, barnacles and bryozoans, average numbers declined from 1977 to 1978 and rose again in 1979 (except for barnacles in Tred Avon River). For hooked mussels however, average values declined from 1977 through 1978 to 1979.

Finally, when comparisons were made of percentages of oyster spat, barnacles and bryozoans settling on their "preferred" surface of Plate A versus Plate B and of percentages settling on both sides of Plate A versus both sides of Plate B, no obvious patterns emerged. I concluded that there was no clear "preference" for settling on the upper surface (oyster) or lower surface (barnacles, bryozoans) of Plate A rather than the comparable surface of Plate B. Similarly, there was no clear "preference" for one plate (both sides combined) over the other.

DISCUSSION

Water Quality

Temperature values in 1977 to 1979 resembled those recorded in Tred Avon River in 1963 (Hanks, 1964) and in Broad Creek in 1963 to 1965 (range of 17.8 to 29.0 C, Shaw, 1967).

Salinity in both tributaries (especially in 1978 and 1979) was low compared with values recorded by others for this geographical area. In the lower Choptank, along a stretch of river near the mouths of the two tributaries, salinity values in 1946 varied from a range of 7.0 to 8.7 ‰ during the week of June 15, rising to a range of 11.3 to 12.6 ‰ during the week of September 15 (Engle, 1948). Hanks (Figure 2, 1964) reported a salinity range of about 10 ‰ (June 1963) to about 15 ‰ (September 1963) in Tred Avon River. Shaw (Figure 2, 1967) recorded summer (June to September) salinity measurements in Broad Creek of about 11.5 to 17.5 ‰ (1963); 10 to 14.5 ‰ (1964); and 11 to 14 ‰ (1965). Low salinities can inhibit gametogenesis and Butler (1949) noticed such an inhibition in oysters from upper Chesapeake Bay exposed to salinities below 6 ‰ during the summer. In 1977 and 1978, when salinities ranged from 7 to 9.5 ‰, oysters in Broad Creek and Tred Avon River did mature and spawn in a fashion similar to oysters in higher salinity regions of central Chesapeake Bay (Kennedy, unpublished data). However, it is not clear what effects low

salinity has on the success of spat settlement although it is usually assumed that animals living at or near one extreme of their tolerance range are under stress.

Our surface chlorophyll *a* measurements for Tred Avon River were higher than those reported for the river in 1963 (Figure 2, Hanks, 1964). Then the range of values over the summer was about 2 to 7 μg liter^{-1} compared with average values of about 4.9 to 19.2 μg liter^{-1} in 1978 (Figure 3). The 1978 values for Broad Creek were lower than those for Tred Avon River and these differences were reflected in differences in transparency between both tributaries (Figure 2). Perhaps the differences between the tributaries are due to input of nutrients from the communities of Oxford and Easton into the Tred Avon River. Broad Creek has no such concentrated populations along its banks. It may be that the increase in chlorophyll *a* concentrations from 1963 to the present in Tred Avon River are due to increases in the population of the region and associated waste disposal, or to changes in farming practices.

Settlement Patterns

Oyster settlement is variable in intensity and locations (Beaven, 1951; Loosanoff and Nomejko, 1956; Drinnan and Stallworthy, 1979b). For example, Drinnan and Stallworthy (1979a) note differences in intensity of spat settlement between shells on different arms of a spat collector. Nelson (1953) records that, of two wire bags of shell placed 4 paces apart on a tidal flat, one was covered with oyster spat whereas the other had almost none. Such differences must be kept in mind in assessing the results of spat settlement (and fouling) studies. For this reason, the 1977 results for Tred Avon River should be considered with care as they derive from only 2 sites in the river. Nevertheless, for this study, general patterns of settlement are obvious and can be compared with Shaw (1967, 1969) and others.

For oysters, the 1977 to 1979 results are in agreement with the earlier results of Shaw in that spat settlement in Broad Creek remains higher than that in Tred Avon River (Shaw, 1969). However, there are differences in average settlement between the two periods of study. Shaw counted spat on the under surface of the upper plate and the upper surface of the bottom plate (1969). He then determined the average number of spat per plate. I made the same determination and the data are presented in Table 2 for comparison between the two periods of study. In Broad Creek in 1977, average values resembled those of 1962, 1963 and 1966. However, values were lower in 1978 and 1979. In Tred Avon River, average set in 1978 was very low but average numbers in 1977 and 1979 were comparable to or slightly greater than the 1961 to 1966 values.

These differences between the tributaries are corroborated by field data collected by Dr. G. Krantz of Horn Point Environmental Laboratories (personal communication). As part of an intensive survey of spat settlement on central Chesapeake Bay oyster bars, he has counted spat per bushel on Deep Neck bar in Broad Creek (near Station 8, Figure 1) and Double Mills bar in Tred Avon River (near Station 3, Figure 1). His data for 1977 to 1979 are as follows:

	Deep Neck	Double Mills
1977	272	2
1978	2	0
1979	6	0

The differences between tributaries and among years are obvious.

TABLE 2. *Average number of oyster spat settling on the bottom of Plate A and the top of Plate B for two study periods in Broad Creek and Tred Avon River. Data for 1961 to 1966 taken from Shaw (1969).*

Location	1961	1962	1963	1964	1965	1966	1977	1978	1979
Broad Creek	—	12.4	11.6	33.4	37.7	13.1	14.2	0.4	7.9
Tred Avon River	0.1	0.6	1.1	0.8	8.0	0.7	1.2	0.04	2.4

The fact that oyster larvae settled preferentially on the upper surfaces of the collecting plates (and of oyster shell in 1977) in the present study is of interest because it has generally been noted that larvae of *Crassostrea virginica* settle under conditions of reduced light intensity (Nelson, 1953; Chestnut, 1968; Ritchie and Menzel, 1969; Shaw, Arnold and Stallworthy, 1970) and that settling is more intense on under surfaces compared to upper surfaces (Sieling, 1951; Medcof, 1955) although Butler (1955) has reported to the contrary. Sieling (1951) performed experiments in St. Marys River, Chesapeake Bay, using scrubbed oyster shells held horizontally near the bottom (120-150 cm deep) for seven days in early July. Two sets of shells were placed concave side up and two sets were placed concave side down. The under surface of the cultch was settled on by 77% to 85% of the spat. Shaw (1969) reported no clear difference in oyster settlement between upper and lower surfaces in his study. Settlement was heavier on the lower surface in 6 out of 11 comparisons. It may be that the difference in settlement behavior between Sieling's results and those of Shaw (1969) and this report are due to changes in water clarity in Chesapeake Bay. Nelson (1953) indicated that in 1952, oyster larvae at Cape May, New Jersey, showed a preponderance of settling on the upper versus lower side of test shells, unlike the situation in earlier years. He observed that larvae exposed to light would crawl to the edge of test shells, move to the dark underside, and attach. He speculated that the change in settlement in 1952 was related to increased diatom and dinoflagellate concentrations near Cape May which led to decreased light transmission (a Secchi disc disappeared at 0.6 to 1 m depth). He felt that the decreased light intensity may have been such that stimulation of the larvae to crawl into a darker area to set did not occur.

Although data on turbidity changes in Chesapeake Bay are scarce, it appears that conditions have been becoming more turbid. This was the claim of Newcombe in reviewing historical descriptions of the Bay (1950, 1952) although he provided no quantitative data. Roberts and Pierce (1976) discussed the increased erosion and resulting increase in sediment load into the upper Patuxent estuary, a tributary of Chesapeake Bay and perhaps a microcosm of the Bay in general. Much of this increased sediment load is attributable to a change from farming activity to recent urban-related construction activity which accompanies population increase. I speculate that, with increased sediment load and increased nutrient input which results in increased primary production, it may be that light penetration has decreased to the extent that oyster larvae are not now stimulated to settle on under surfaces but will settle on upper surfaces. Thus Shaw (1969) may have had mixed results because conditions of water clarity and light transmission were intermediate between those in 1950 when Sieling (1951) performed his experiment and those prevailing in 1977 to 1979 (present study).

If a switch in intensity of oyster settlement on upper surfaces has occurred in central Chesapeake Bay, it may have some survival value for oysters. Other researchers report heavier barnacle settlement on under surfaces compared with upper surfaces (Sieling, 1951; Manning, 1953; Butler, 1955; Shaw, 1967). In St. Marys River, Manning (1953) noted that the presence of barnacles apparently inhibited oyster settlement. Cultch that was heavily fouled by barnacles caught only about 25% as many spat as did cultch that was relatively free of barnacles. Steinberg and Kennedy (1979) report that *Balanus improvisus* depleted numbers of oyster larvae in experimental containers and that at least one individual barnacle had fed on oyster larvae. Thus, settlement on upper surfaces that are lightly fouled by barnacles may result in higher survival in comparison with that on under surfaces with many barnacles. Butler (1955) felt that the presence of barnacles on under surfaces would deter oyster settlement because the cirral activity of barnacles would cause oyster larvae to close their shells and fall away from the surface, something which would be less of a problem on upper surfaces as the larvae could land on the surface and proceed to settle. However, in our study, clean plates were used each week. Thus the under surface was free of barnacles to begin with and, even in periods of heavy barnacle settlement, given the small size of barnacles on first settling, most of the surface area would be available to spat. Unless barnacles produce an inhibitory chemical material, the bottom of the plates should

have been acceptable to the spat, yet the top surface received most of the set.

Barnacles and encrusting bryozoans had spring and late summer or fall periods of heavy settlement (Figures 5 and 6) in agreement with studies elsewhere in Chesapeake Bay (Manning, 1953; Calder and Brehmer, 1967; Cory, 1967). It is not clear what effect bryozoans might have on oyster spat settlement. Osburn (1944) felt that colonies would cover cultch to such an extent that oyster larvae would not find free space for attachment. Manning (1953) found no apparent effect of light to moderate bryozoan fouling on oyster settlement. The subject remains open for investigation.

The number of hooked mussels reported by Shaw (1967) is higher than that reported here (Table 1). Figure 7 in Shaw (1967) shows weekly peaks of settlement of up to 50 mussels per plate. It seems that mussel recruitment was in a decline during 1977 to 1979 whereas it was generally stable in 1963 to 1965. The reason for the decline is not apparent.

Resource Management

In relation to resource management, Shaw (1967, 1969) suggested that shell be placed in Broad Creek during the first week in July to collect seed oysters which could then be moved to Tred Avon River to grow. This recommendation would have been generally suitable for 1977 and 1979 because oyster spat settlement reached a peak in July. However, the June settlement in 1979 would have been missed and shell planting would have been ineffective in 1978 when spat settlement apparently was negligible in Broad Creek. This does not negate Shaw's recommendation but does indicate the need for monitoring spawning and larval development each year in order to predict if and when settlement will occur (Lindsay, Westley and Sayce, 1959; Quayle, 1969).

Finally, because of the continuously demonstrated unsuitability of Tred Avon River as an area of successful spat settlement, it seems unnecessary to place oyster shell in the river to serve as cultch, as has been done in the past.

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LITERATURE CITED

Beaven, G.F. 1951. Recent observations on the season and pattern of oyster setting in the middle Chesapeake area. Proc. Natl. Shellfish. Assoc. (1950):53-59.

Beaven, G.F. 1955. Various aspects of oyster setting in Maryland. Proc. Natl. Shellfish. Assoc. **45**:29-37.

Butler, P.A. 1949. Gametogenesis in the oyster under conditions of depressed salinity. Biol. Bull. **96**:263-269.

Butler, P.A. 1955. Selective setting of oyster larvae on artificial cultch. Proc. Natl. Shellfish. Assoc. **45**:95-105.

Calder, D.R. and M.L. Brehmer. 1967. Seasonal occurrence of epifauna on test panels in Hampton Roads, Virginia. Int. J. Oceanol. Limnol. **1**:149-164.

Chestnut, A.F. 1968. Setting behavior of oyster larvae. In: Proceedings of the Oyster Culture Workshop, July 11-13, 1967. T.L. Linton, ed. Contribution No. 6, Marine Fisheries Division, Brunswick, Ga. pp. 32-34.

Cory, R.L. 1967. Epifauna of the Patuxent River Estuary, Maryland, for 1963 and 1964. Chesapeake Sci. **8**:71-89.

Drinnan, R.E. and W.B. Stallworthy. 1979a. Oyster larval populations and assessment of spatfall, Bideford River, P.E.I., 1959. Fisheries and Environment Canada, Fisheries and Marine Service Tech. Rep. No. 793. 16 pp.

Drinnan, R.E. and W.B. Stallworthy. 1979b. Oyster larval populations and assessment of spatfall, Bideford River, P.E.I., 1961. Fisheries and Environment Canada, Fisheries and Marine Service Tech. Rep. No. 795. 19 pp.

Drinnan, R.E. and W.B. Stallworthy. 1979c. Oyster larval populations and assessment of spatfall, Bideford River, P.E.I., 1963 and 1964. Fisheries and Environment Canada, Fisheries and Marine Service Tech. Rep. No. 797. 19 pp.

Engle, J.B. 1948. Distribution of setting guides the Maryland oyster program. Proc. Natl. Shellfish. Assoc. (1947):16-20.

Hanks, R.W. 1964. Environmental features of the laboratory site; the Tred Avon River. In: The Bureau of Commercial Fisheries Biological Laboratory, Oxford, Maryland: Program and Perspectives, C.J. Sindermann, ed. U.S. Fish. Wildl. Serv., Circular 200:18-23.

Kennedy, V.S., D.L. Smawley and J.A. Boettger. 1979. Final report on a study of oyster settlement and fouling in Broad Creek and Tred Avon River, Maryland, during the summers of 1977 to 1979. University of Maryland, Center for Environmental and Estuarine Studies. Ref. No. 79-194 HPEL.

Krantz, G.E. and D.W. Meritt. 1977. An analysis of trends in oyster spat set in the Maryland portion of the Chesapeake Bay. Proc. Natl. Shellfish. Assoc. **67**:53-59.

Lindsay, C., R.E. Westley and C.S. Sayce. 1959. Prediction of oyster setting in the state of Washington. Proc. Natl. Shellfish. Assoc. **48**:59-70.

Loosanoff, V.L. and C.A. Nomejko. 1956. Relative intensity of oyster setting in different years in the same areas of Long Island Sound. Biol. Bull. **111**:387-392.

Manning, J.H. 1953. Setting of oyster larvae and survival of spat in the St. Mary's River, Maryland, in relation to fouling of cultch. Proc. Natl. Shellfish. Assoc. (1952):74-78.

Medcof, J.C. 1955. Day and night characteristics of spatfall and of behavior of oyster larvae. J. Fish. Res. Bd. Canada. **12**:270-286.

Nelson, T.C. 1953. Some observations on the migrations and setting of oyster larvae. Proc. Natl. Shellfish. Assoc. (1952):99-104.

Newcombe, C.L. 1950. Treasures in troubled waters: the plight of the oyster. The Scientific Monthly. **70**(2):1-6.

Newcombe, C.L. 1952. '"Sullage" in the East. The Land. **11**:59-61.

Osburn, R.C. 1944. A survey of the bryozoa of Chesapeake Bay. Chesapeake Biological Laboratory Publ. No. 63.

Quayle, D.B. 1969. Pacific Oyster Culture in British Columbia. Fish. Res. Bd. Canada, Bulletin No. **169**:1-193.

Ritchie, T.P. and R.W. Menzel. 1969. Influence of light on larval settlement of American oysters. Proc. Natl. Shellfish. Assoc. **59**:116-120.

Roberts, W.P. and J.W. Pierce. 1976. Deposition in upper Patuxent estuary, Maryland, 1968-1969. Est. Coast. Mar. Sci. **4**:267-280.

Shaw, R., D.C. Arnold and W.B. Stallworthy. 1970. Effects of light on spat settlement of the American oyster (*Crassostrea virginica*). J. Fish. Res. Bd. Canada. **27**:743-748.

Shaw, W.N. 1967. Seasonal fouling and oyster setting on asbestos plates in Broad Creek, Talbot County, Maryland, 1963-65. Chesapeake Sci. **8**:228-236.

Shaw, W.N. 1969. Oyster setting in two adjacent tributaries of Chesapeake Bay. Trans. Amer. Fish. Soc. **98**:309-314.

Sieling, F.W. 1951. Influence of seasoning and position of oyster shells on oyster setting. Proc. Natl. Shellfish. Assoc. (1950):57-61.

Steinberg, P.D. and V.S. Kennedy. 1979. Predation upon *Crassostrea virginica* (Gmelin) larvae by two invertebrate species common to Chesapeake Bay oyster bars. The Veliger **22**:78-84.

Strickland, J.D.H. and T.R. Parsons. 1968. A Practical Handbook of Seawater Analysis. Fish. Res. Bd. Canada, Bulletin No. **167**:1-310.

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FOOD OF *PANDALUS BOREALIS, PANDALUS HYPSINOTUS* AND *PANDALUS GONIURUS* (PANDALIDAE, DECAPODA) FROM LOWER COOK INLET, ALASKA[1]

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ABSTRACT

Stomach contents of three commercially important species of shrimp from Lower Cook Inlet, Alaska, were investigated: Pandalus borealis, Pandalus hypsinotus, *and* Pandalus goniurus. *The frequency of occurrence method was used to analyze the stomach contents of more than 195 individuals of each species. Twenty-eight food categories were observed in* P. borealis *with Crustacea, Polychaeta, and diatoms the most common. Nineteen categories were observed in* P. hypsinotus *and 22 categories were observed in* P. goniurus *with Crustacea, Polychaeta, and Bivalvia the most common in both of these species. A high frequency of occurrence of sediment and unidentifiable organic matter was also observed for each species. Stomach contents typically contained 60% dry weight sediment, 66% dry weight sediment, and 62% dry weight sediment for* P. borealis, P. hypsinotus, *and* P. goniurus, *respectively. This study suggests that these shrimps feed predominantly on the benthos and not in the water column. They appear to be both active predators of infaunal invertebrates as well as foragers ingesting large amounts of detritus and sediment.*

INTRODUCTION

The pink shrimp, *Pandalus borealis*, the coonstripe shrimp, *Pandalus hypsinotus*, and the humpy shrimp, *Pandalus goniurus*, are commercially harvested in Lower Cook Inlet, Alaska. The Kachemak Bay area constitutes the principal region in the Inlet for commercial catches of these crustaceans. Average annual catches for each species from 1973 to the present are 1,000,000 kilograms for *P. borealis*; 79,000 kilograms for *P. hypsinotus*; and 1,000,000 kilograms for *P. goniurus*. These seasonal landings have a commercial value of slightly over one-half million dollars annually (Al Davis, Alaska Department of Fish and Game, Personal Communication). Yet, except for this paper, little information is available on the diet and feeding habits of these shrimps (Barr, 1970a; Crow, 1977). Interest in the possible impacts of oil and gas exploitation in Alaskan waters has led to a series of studies that were included in the Outer Continental Shelf Environmental Assessment Program (OCSEAP) (Feder et al., 1978). This paper presents data on the food of the above three species of pandalid shrimps that were collected as part of OCSEAP-related studies (Feder et al., 1978; Paul et al., 1979; Feder and Paul, in press).

METHODS

Pink, coonstripe, and humpy shrimps were col-

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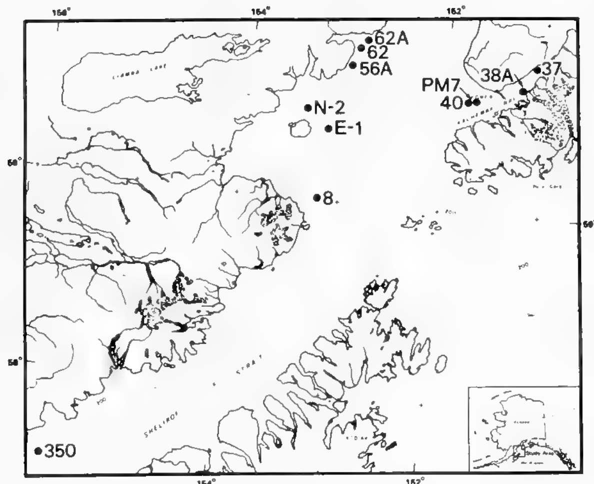

FIGURE 1. Lower Cook Inlet, Alaska, and stations sampled for pandalid shrimp.

lected during six cruises: November, 1977; March, 1978; May, 1978; June, 1978; July, 1978; August, 1978. Collections were made with trawls in Lower Cook Inlet (Figure 1). Specimens were fixed in 10% buffered formalin for examination in the laboratory. The frequency of occurrence method was employed in the following manner. Stomach contents were first observed using a Wild dissection scope at 60 magnifications, and whole animals or large fragments were identified to the lowest taxon possible. A subsample of the contents was placed on a slide and observed at 100X with a compound microscope. The latter method facilitates identification of diatoms, polychaete setae, and small fragments. Percent frequency of occurrence was computed based on the number of stomachs with contents. Identification of organisms captured by additional sampling at each station with dredges, grabs, and fine mesh nets facilitated identification of stomach contents.

Analysis of the gut sediment was performed on a dry weight basis. A number of stomach contents of each species from selected stations was dried at 60°C, weighed, digested with 10% potassium hydroxide, and treated with concentrated hydrochloric acid to eliminate shell and carapace fragments. The sample was redried and weighed. The sediment component was then calculated as the initial dry weight of contents divided by the final dry weight and expressed as a percentage. A control with known dry weights of sand and tissue was used to evaluate this method. The control evaluation showed the sediment analysis technique to be accurate to within 2%, with the error resulting in underestimation of sediment content. Sediment weight determined by this method is, therefore, conservative since carbonates naturally associated with the sediment are eliminated (see also Holme and McIntyre, 1971).

RESULTS

Pandalus borealis

A total of 233 pink shrimp stomachs were examined from Lower Cook Inlet; 82% (192 individuals) contained food (Table 1). The three most frequently observed items, based on the number of feeding shrimps, were unidentified Crustacea, frequency of occurrence 57%; unidentified Polychaeta, 26%; and diatoms, 20%. Other crustaceans identified to lower taxa were Decapoda, 6%; and Ostracoda, 4%. Other polychaetes included Spionidae, 6%; Nephtyidae, 3%; and *Lumbrineris* sp., 3%; the centric diatom *Melosira sulcata* and naviculoid diatoms were frequently observed. Other food items included Bivalvia, 19%; with *Nucula tenuis* observed in an additional 7% and *Nuculana* sp. in 4% of the stomachs with food. Foraminifera were observed in 17% of the stomachs; Teleostei remains in 6%, and plant detritus in 3%. Pink shrimp stomachs typically contained a variety of items. For example, the stomach of one shrimp, carapace length 19 mm, contained one whole *Nuculana* sp., numerous crustacean fragments, four Foraminifera, unidentifiable fibers, numerous *Melosira sulcata*, and unidentifiable spines. Unidentified organic matter was frequently observed, 45% frequency of occurrence, and sediment was common, 54%. In addition, sediment constituted 60% of the dry weight of stomach samples so analyzed (Table 4). Fifteen additional food categories were infrequently observed in pink shrimp specimens (Table 1).

Percent frequency of occurrence data for pink shrimp from station 37 (7/78), were examined for differences between males and females. Sediment, all Crustacea, unidentified organic matter, combined Polychaeta, and all Mollusca were used as the five most frequent food categories for the purposes of comparison between the sexes. No significant difference was observed ($X^2 = 4.01$, $p > .1$).

TABLE 1 *Food of* Pandalus borealis *taken from Cook Inlet, Alaska*

Station	Date	Depth (m)	No. stomachs examined	No. with contents	Diatoms	Plant material	Foraminifera	Porifera	Anthozoa	Nematoda	Polychaeta	Polynoidae	Syllidae	Nephtyidae	*Lumbrineris* sp.	Spionidae	Maldanidae	Sabellidae	Bivalvia	*Nucula tenuis*	*Nuculana* sp.	*Yoldia* sp.	Crustacea	Ostracoda	Harpacticoid copepods	Cumacea	Decapoda	Brachyura	Echinodermata	Chaetognatha	Teleostei	Unid. organic material	Sediment
350	11/77	210	50	22			5				4				1	1	1		2				6		1	1		1			1	2	11
PMEL 7	7/78	84	19	17	7		6				6				1	1			9	1	2		15				1				1	11	17
5	3/78	170	21	18	1		2				7				1	1			1	1			13				1					8	12
37	11/77	42	43	42	2	2	13	1	1	1	13		1				1	1	21	10	6	1	33	8	1		3			2	9	16	22
37	5/78	60	50	47	23	2	2				5			3		2							4				5					41	
37	7/78	52	50	46	5	2	5				15	3		2	2	7		1	4	1			38						1		1	8	41
Total frequency of occurrence			233	192	38	6	33	1	1	1	50	3	1	5	5	12	2	2	37	13	8	1	109	8	2	1	11	1	1	2	12	86	103
% frequency of occurrence	(Feeding shrimp)			100	20	3	17	.5	5	.5	26	2	.5	3	3	6	1	1	19	7	4	.5	57	4	1	.5	6	.5	.5	1	6	45	54
% frequency of occurrence	(Total examined)		100	82	16	3	4	.4	4	.4	21	1	.4	2	2	5	1	1	16	6	3	.4	47	3	1	.4	5	.4	.4	1	5	37	44

TABLE 2 *Food of* Pandalus hypsinotus *taken from Cook Inlet, Alaska*

Station	Date	Depth (m)	No. stomachs examined	No. with contents	Diatoms	Plant material	Foraminifera	Porifera	Hydrozoa	Polychaeta	Polynoidae	Nephtyidae	Spionidae	*Disoma multisetosum*	Maldanidae	Terebellidae	Sabellidae	Bivalvia	*Nucula tenuis*	*Nuculana* sp.	Crustacea	Harpacticoid copepods	Decapoda	Echinodermata	Teleostei	Unid. organic material	Sediment
56A	3/78	31	20	18	2				3	16	4				1	1			6		9		4			11	15
40	7/78	56	18	12						1									2					1		5	9
38A	3/78	55	18	9	1	1				3											3			1		5	4
37	11/77	42	50	43	1	1	6		1	16	5	2	1	2	6	2		9	16		24	3	4	3	7	11	24
37	3/78	50	32	25	1	2	2			14	2	1			1		1	1	6	1	17			1	5	18	16
37	5/78	60	7	5																	4		1		1	1	5
37	7/78	52	50	45	5	5	1	12		26	4	4	9	16	1		2	7	4		38		1			5	38
Total frequency of occurrence			195	157	10	9	9	12	4	76	15	7	10	18	9	3	3	17	34	1	95	3	10	6	13	56	111
% frequency of occurrence	(Feeding shrimp)			100	6	6	6	8	3	48	10	4	6	11	6	2	2	11	22	.6	61	2	6	4	8	36	71
% frequency of occurrence	(Total examined)		100	80	5	5	5	6	2	39	8	4	5	9	5	2	2	9	17	.5	49	2	5	3	7	29	57

TABLE 3 *Food of* Pandalus goniurus *taken from Cook Inlet, Alaska*

Station	Date	Depth (m)	No. stomachs examined	No. with contents	Diatoms	Plant material	Foraminifera	Polychaeta	Polynoidae	*Lumbrineris* sp.	*Disoma multisetosum*	Maldanidae	Ampharetidae	Terebellidae	Sabellidae	Serpulidae	Gastropoda	Bivalvia	*Nucula tenuis*	*Yoldia* sp.	Crustacea	Ostracoda	Amphipoda	Decapoda	Echinodermata	Teleostei	Unid. organic material	Sediment
E-1	3/78	49	48	43	2	1	6	8			3	1	4	2	1	1	1	2	2		19		2	5		8	12	32
37	3/78	50	48	42	1	7	2	4								2			1		19					4	25	30
N-2	3/78	27	50	30		1	1											4	2	1	3	6			1	1	14	27
62	3/78	26	41	30		2		4									1				7						13	16
62A	3/78	19	30	29	3		4	7	1	2		5			1		3	7	3		10		3	2		1	12	27
62A #2	3/78	19	24	23	4	2	7	3		1		3					1	3	4		9		1	10	1		13	22
Total frequency of occurrence			241	197	10	13	20	26	1	3	3	9	4	2	2	3	6	16	12	1	67	6	6	17	2	14	89	154
% frequency of occurrence	(Feeding shrimp)			100	5	7	10	13	.5	2	2	5	2	1	1	2	3	8	6	.5	34	3	3	9	1	7	45	78
% frequency of occurrence	(Total examined)		100	82	4	5	8	11	.4	1	1	4	2	1	1	1	2	7	5	.4	28	2	2	7	1	6	37	64

TABLE 4 *Total dry weight of stomach contents and percentage of dried stomach contents composed of sediment.*

Animal	Station	Date	No. stomach contents	Total dry wt. stomach contents (g)	Total dry wt. sediments in stomachs after KOH, KC1 digestion treatment	% of dried stomach contents sediment
Pandalus goniurus	62	3/78	18	.525	.320	61
Pandalus goniurus	8	6/78	52	.775	.494	64
Pandalus hypsinotus	PMEL 7	7/78	3	.123	.108	88
Pandalus hypsinotus	40	7/78	12	.407	.250	61
Pandalus hypsinotus	38A	3/78	8	.121	.062	51
Pandalus hypsinotus	37	7/78	50	1.152	.827	72
Pandalus borealis	PMEL 7	8/78	37	.428	.267	62
Pandalus borealis	37	8/78	25	.285	.164	58
Control (Sand .565 g Tissue .215 g)	—	—	—	.780	.556	—

Pandalus hypsinotus

One hundred ninety-five coonstripe shrimp stomachs were examined; 80%, (157 individuals) contained food (Table 2). The three most frequently observed foods, based on the number of feeding shrimp, were unidentified Crustacea, 61% frequency of occurrence; unidentified Polychaeta, 48%; and unidentified Bivalvia, 11%. Other crustaceans identified to a lower taxon included Decapoda, 6%. Additional polychaetes included *Disoma multisetosum*, 11%; Polynoidae, 10%; and Spionidae, 6%. An additional bivalve, *Nucula tenuis*, was observed in 22% of the stomachs. Other food items included Teleostei, 8%; Porifera, 8%; diatoms, 6%; plant material, 6%; and Foraminifera, 6%. Eight other food categories were infrequently observed (Table 2). Individual coonstripe shrimp typically contained a variety of organisms. For example, the stomach of one shrimp, carapace length 22.5 mm, contained an intact *Nucula tenuis* (2 mm in length), numerous crustacean fragments, and broken polychaete setae. Another individual, carapace length 32 mm, contained 17 intact *Nucula tenuis* (2-4 mm), terebellid polychaete setae, naviculoid diatoms, and unidentified tissue.

Unidentifiable organic matter was frequently observed, 36% frequency of occurrence, and sediment was common, 71%. In addition, sediment averaged 65% of the dry weight of stomach contents so analyzed (Table 4).

Percent frequency of occurrence data for coonstripe shrimp from station 56A (3/78) were examined for differences between males and females. Combined Polychaeta, sediment, all Crustacea, unidentified organic matter, and all Mollusca were used as the five most frequent food categories for the purpose of comparison between the sexes. No significant difference was observed ($X^2 = 4.06$, $p > .1$).

Pandalus goniurus

Two hundred forty-one humpy shrimp stomachs were examined; 82% (197 individuals) contained food (Table 3). The three most frequently observed food items, based on the number of feeding shrimp, were unidentifiable Crustacea, 34% frequency of occurrence; unidentifiable Polychaeta, 13%; and unidentifiable Bivalvia, 8%. Additionally, decapods, ostracods, and amphipods were observed in 9%, 3%, and 3%, respectively, of the stomachs. Other polychaetes in-

cluded Maldanidae, 5%. The clam *Nucula tenuis* was also observed in 6% of the specimens. Other food items included Foraminifera, 10%; Teleostei, 7%; plant material, 7%; and diatoms, 5%. Ten other food categories were infrequently observed (Table 3). Humpy shrimp typically fed on a variety of organisms. For example, one shrimp, carapace length 13.3 mm, contained amphipod pieces, decapod fragments, two foraminiferans, and shell fragments of *Nucula tenuis* and gastropods.

Unidentifiable organic matter and sediment were observed in 45% and 78% of the stomachs, respectively. In addition, sediment constituted 62% of the dry weight of the stomach contents so analyzed (Table 4).

Percent frequency of occurrence data for humpy shrimp from station E-1 (3/78) were examined for differences between males and females. Sediment, all Crustacea, unidentified organic matter, combined Polychaeta, and Teleostei were used as the five most frequent food categories for the purposes of comparison between the sexes. No significant difference was observed ($X^2 = 4.26$, $p > .1$).

DISCUSSION

Results of the present investigation suggest that the three pandalid shrimp species examined are opportunistic foragers or food generalists. The stomachs examined showed that the three species utilized a total of 32 food categories with the most common food items reflecting the foods most available (see Feder et al., 1978). No major differences in the most frequently observed food categories of the three shrimp species were observed, i.e., they all fed primarily on Crustacea, Polychaeta, Bivalvia, and diatoms. Also, the variety of organisms and the type of remains observed indicated active predation.

Additionally, this study demonstrates that these three species of pandalid shrimps in Lower Cook Inlet feed primarily on the bottom, and suggests that they do a considerable amount of sediment sorting for small prey and detritus. In contrast, Barr (1970b) reported *Pandalus borealis* in Kachemak Bay, Alaska, most frequently on zooplankton in the water column. Crow (1977) reported that the principal food of pandalid shrimps in Kachemak Bay was detritus and diatoms. The present report suggests that active predation on infaunal invertebrates is a common mode of feeding. It is possible that sediment and detritus are ingested inadvertently with prey. The importance of detritus and bacterial carbon associated with the sediment as an additional carbon source for shrimp is unknown. It is thought to be significant for some detrital-feeding organisms (Fenchel and Jørgensen, 1977; Kofoed, 1975; Moriarty, 1976; Rieper, 1978). The results of this study indicate that sediment and detritus constitute a significant portion of the stomach contents of the shrimps examined. Hence, the importance of detrital and bacterial carbon as food for pandalid shrimps needs to be investigated.

ACKNOWLEDGEMENTS

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LITERATURE CITED

Barr, L. 1970a. Alaska's Fishery Resources. The Shrimps. U.S. Fish. Wildl. Serv. Fish. Leaflet 631, 10 pp.

Barr, L. 1970b. Diel vertical migration of *Pandalus borealis* in Kachemak Bay, Alaska. J. Fish. Res. Bd. Can. **27**(4):669-676.

Crow, J.H. 1977. Food habits of shrimp in Kachemak Bay, Alaska. In: Environmental Studies of Kachemak Bay and Lower Cook Inlet, B. Trasky Flagg and D.C. Burbank (eds.). Alaska Dept. Fish and Game.

Feder, H.M., M. Hoberg, A.J. Paul, J. McDonald, G. Matheke and J. Rose. 1978. Distribution, abundance, community structure, and trophic relationships of the nearshore benthos of the Kodiak Shelf, Cook Inlet, Northeast Gulf of Alaska, and the Bering Sea. Annual Report. In:

Environmental Assessment of the Alaska Continental Shelf. Annual Reports of Principal Investigators for the Year Ending March 1978. Vol. IV. Receptors-Fish. Littoral, Benthos 416-730 pp.

Feder, H.M. and A.J. Paul. In press. Food of the king crab, *Paralithodes camtschatica*, and the dungeness crab, *Cancer magister*, in Cook Inlet, Alaska. Proc. Natl. Shellfish Assoc. 70:

Fenchel, T.M. and B.B. Jørgensen. 1977. Detritus Food Chains of Aquatic Ecosystems: The Role of Bacteria. In: Advances in Microbial Ecology, Volume I, M. Alexander ed. Plenum Press, New York.

Holme, N.A. and A.D. McIntyre, editors. 1971. Methods for the Study of Marine Benthos; Publications for The International Biological Program. Oxford Backewell Scientific Publications, London, 334 p.

Kofoed, L.H. 1975. The feeding biology of *Hydrobia ventrosa* (Montagu). II. Allocation of the Components of the Carbon-Budget and the Significance of the Secretion of Dissolved Organic Material. J. Exp. Mar. Biol. Ecol., **19**:243-256.

Moriarty, D.J.W. 1976. Quantitative studies on bacteria and algae in the food of the mullet *Mugil cephalus* L. and the prawn *Metapenaeus bennettae* (Rasek and Dall). J. Exp. Mar. Biol. Ecol. **22**:131-143.

Paul, A.J., H.M. Feder and S.J. Jewett. 1979. Food of the snow crab *Chionoecetes bairdi* Rathbun 1924, from Cook Inlet, Alaska. Crustaceana, Suppl. **5**:62-68.

Rieper, M. 1978. Bacteria as food for marine harpacticoid copepods. Marine Biology **45**:337-345.

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BIOLOGICAL AND TECHNOLOGICAL STUDIES ON THE AQUACULTURE OF YEARLING SURF CLAMS PART I: AQUACULTURAL PRODUCTION

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ABSTRACT

The surf clam, Spisula solidissima *(Dillwyn), has been raised from egg to a potentially marketable size of 55 mm in length in less than one year. Widely accepted techniques of larval culture were utilized to rear clams to metamorphosis in about 21 days. Flowing seawater culture systems have been developed to raise post-set clams. The duration of this phase of culture depends upon whether ambient temperature is raised and supplemental algal food is added. Under optimal conditions, post-set clams can grow to a size of 18 mm in 6 weeks. In May 1978, clams of this size were planted in a sand substratum within fiberglass tanks of a pumped raceway system. By September the clams averaged 55 mm in length. Growth in the raceway system is dependent upon ambient levels of naturally occurring phytoplankton, the sole source of nutrition. Data indicate a strong correlation between available phytoplankton and growth. Preliminary economic analysis indicates that the major operating expenses of the pumped raceway system are the costs of electric power and labor.*

INTRODUCTION

Intensive aquaculture, for bivalve species, requires that methodology be devised to rear the animals through each phase of their life history. By integrating gametogenic conditioning, spawning, larval culture, intermediate growth, and final grow-out, a species can be considered for intensive controlled culture.

In this study, an effort has been made to synthesize methodologies into a workable culture scheme for the surf clam, *Spisula solidissima* (Dillwyn). Although little information has been reported specifically dealing with the culture of the surf clam, there is much information on the culture of other bivalves which can be applied.

Gametogenesis and larval culture of bivalves have been widely studied (Loosanoff and Davis, 1963; Walne, 1966; Culliney et al., 1975). Generally reliable methodology has been extensively utilized in commercial aquaculture situations (Glude, 1977). The existing body of knowledge lends itself, with minor modifications, to the larval culture of a large number of bivalve species.

In bivalve rearing, the period of growth from metamorphosis to several millimeters in length is a difficult link in the culture process, due primarily to the fouling of young clams by epizootic organisms. Growth through this stage has been considered as a continuation of culture in the hatchery with some degree of environmental control.

Walne (1966) and Breese and Malouf (1975) describe methodology in which recent oyster spat are grown in tanks of filtered seawater which receive cultured algae. Castagna and Kraeuter (1977) have grown *Mercenaria mercenaria* by allowing them to set on fiberglassed wooden tables and then maintaining a flow of unaltered raw seawater.

Culture of bivalves larger than several millimeters has been demonstrated, utilizing varied approaches. These include containment and protection in the natural or semi-natural environment (Carriker, 1959; Shaw, 1968; Matthiessen, 1969; Menzel et al., 1976; Castagna and Kraeuter, 1977), rearing in a land-based system where pumped seawater provides naturally occurring phytoplankton as the sole nutrition source (Malouf and Breese, 1977), and in a land-based system where cultured algae provide nutrition (Ryther et al., 1972; Baab et al., 1973; Lucas, 1976; Epifanio et al., 1976).

The aim of this study is to design a culture system for the surf clam by utilizing and developing methodology which promises the highest yield at the lowest cost. This paper offers an integrated methodology for raising the surf clam from egg to a potentially marketable size of 55 mm in less than one year. Emphasis is on presenting a broad overview of research that demonstrates the biological feasibility of aquaculture for this species.

Some economic considerations are also presented for surf clam aquaculture. The costs of the final grow-out phase are identified since this is probably the most expensive portion of the culture process. These data are offered as a preliminary estimate of costs. It is beyond the scope of this work to speculate about commercial feasibility.

GAMETOGENESIS, SPAWNING, AND EGG DEVELOPMENT

The natural habitat of the surf clam is an oceanic one. When held in an estuarine environment, as at Milford Laboratory, this species is subjected to lower salinities and a wider range of temperatures than in the ocean. The salinity of seawater at the laboratory averages about 26 parts per thousand (ppt) with only slight tidal and seasonal variation. Temperatures range annually from 0°C to 26°C. These conditions have had no apparent adverse effect on the gametogenesis, spawning, and egg development of the surf clam.

In nature, surf clam populations normally undergo gametogenesis and spawning in the late spring and again in early fall (Ropes, 1968). In our laboratory, precocious gonad development may be induced in mid-winter by keeping adult surf clams at 15°C to 20°C for about 2 weeks. Success in inducing gametogenesis in mid-winter depends upon the occurrence of seasonal diatomal blooms, providing a nutritional source. Attempts to induce gametogenesis out of synchrony with nature, by providing only cultured algae for nutrition, have been unsuccessful. It is speculated that the quantities of cultured algae available were insufficient to effect ripening.

The methods of Loosanoff and Davis (1963) have been followed for spawning clams and culturing their fertilized eggs.

Spawning is generally accomplished by thermal stimulation. The spawning temperature threshold varies with the temperature at which the broodstock are maintained. Ripened clams held at 20°C often spawn at about 28°C, while ripened clams held at 10°C often spawn at 22°C. A sperm suspension obtained from a stripped male may be used as an additional spawning stimulus.

In the present study, experiments have illustrated that if parent clams undergo gametogenesis at estuarine conditions, their embryos have the highest rate of development at an ambient salinity of about 26 ppt and a temperature of 20°C.

LARVAL CULTURE

Standing water larval culture in 10-1 plastic buckets with daily changes has been effective in rearing surf clams through metamorphosis. Clams are screened on increasing sizes of Nitex[R] mesh screens as they grow to remove debris and slow-growing clams from the cultures. Larval cultures are fed a mixture of about 10^5 cells of *Monochrysis* and *Isochrysis* per milliliter of culture.

The highest survival and growth rates occur when larvae are reared at an ambient salinity of about 26 ppt and 20°C. Surf clams have been raised to metamorphosis consistently at densities up to 15 larvae per milliliter of culture. The average length of the larval period for clams cultured at a density of 15/ml and at 20°C is 21

days. Clams generally reach a size of 280 μ in length at metamorphosis. No byssal attachment has been observed although a byssus gland has been seen histologically (Chanley and Andrews, 1971). The post-set clams, therefore, do not adhere to culture vessels.

INTERMEDIATE GROW-OUT OF POST LARVAE

During this phase of culture, clams grow from a length of 280 μ to about 15 mm. In this study, attempts to culture post-set surf clams in standing water have been largely unsuccessful because many epizootic fouling organisms, such as *Vorticella* and *Zoothamnium*, attach to young clams, severely inhibiting their growth. Consistently higher survival rates have been recorded in two flowing culture systems where fouling is far less severe.

In both systems, clams are retained on screens fabricated from 10" PVC pipe and Nitex[R] mesh in a once-through flowing seawater system. The screens are kept partially submerged in an outer container which maintains the water level above the clams. Seawater flows into and through the screen containing the clams and then into the outer container to the drain. Flow rates are maintained at 750 ml per minute. Experiments have shown that this flow rate consistently supported the growth of varying biomasses of clams and introduced no apparent limiting factors. The first system described utilizes unfiltered raw seawater, while the second uses seawater filtered to 10 μ.

The unfiltered system is similar to one described by Castagna and Kraeuter (1977), in that the sole source of nutrition is naturally occurring phytoplankton in the seawater. During periods of the year when phytoplankton is abundant, up to 10,000 recent set have been reared to an average length of 3 mm in 3 weeks on a 10"-diameter screen. As the clams grow, the mesh sizes of the screens are increased to permit better water exchange. Clams have been raised from 3 mm to a length of 15 mm in 3 weeks with a final density of 2000 clams per 10" screen. The density was reduced to insure an adequate supply of available nutrition.

The second approach is similar; however, seawater is filtered to 10 μ, using cloth-wound cartridge filters so that larger planktonic forms and organic debris are removed. A cultured algae mixture of *Dunaliella*, *Chlorella*, and *Phaeodactylum* is introduced into the system as a nutrition source. This system offers the advantages of less siltation and biofouling in the culture containers and control of the level of nutrition. The 10 μ filters do not remove all nannoplankton and growth has been recorded when no cultured algae were added. The rate of growth, however, has equalled that of the unfiltered system only when cultured algae were supplemented. The filtered seawater system is of most value in rearing clams up to 5 mm. The nutritional requirements of large numbers of clams greater than 5 mm are considerable and the quantity of algae that must be produced increases markedly. Clams greater than 5 mm are then moved to the raw seawater system.

FINAL GROW-OUT

The grow-out facility at Milford Laboratory is a land-based, pumped raceway system. It consists of three rows of eighteen, 10 m × 1.3 m × 1 m, tanks. The tanks are fabricated of opaque black fiberglass, which prevents growth of macroalgae. Three 7.5-horsepower pumps service the tanks at flow rates up to 50 1/min/tank. Seawater is pumped directly from Milford Harbor and then discharged after passage through the tanks. Tanks containing surf clams are filled with sand, 10 cm deep. Experiments have shown that growth of surf clams greater than 25 mm is greatly enhanced by the presence of the substratum. The raceway system is operated only from May through October when temperatures permit growth.

Clams as small as 2 mm have been planted and grown successfully in the raceways. To achieve a final size of 55 mm in one growing season, however, the initial size of the clams must be about 18 mm in length. This was demonstrated in 1978, when surf clams of an initial average length of 18 mm were planted at the beginning of the growing season in May in tanks at densities of 100 and 500 per square meter. By September 15 both groups of clams had grown to an average length of 55 mm. Mortality was minimal over the course of the growing season in those at $100/m^2$, but clams planted at a density of $500/m^2$ showed a 26% mortality.

Throughout the growing season, growth rates vary significantly with the level of phytoplankton abundance. These levels are estimated by measuring the level of fluorescence of *in vivo* seawater samples (Lorenzen, 1966; Kirby-Smith and Barber, 1974). The weekly averages of *in vivo* fluorescence indicate seasonal trends (Figure 1).

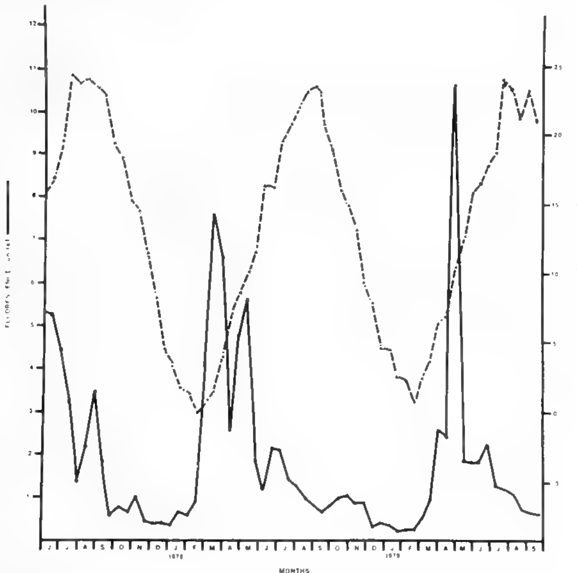

FIGURE 1. *Seasonal cycles of temperature and* in vivo *fluorescence for Milford, Connecticut, 1977-1979. Weekly averages are plotted from data collected daily. Broken line — temperature; solid line —* in vivo *fluorescence.*

Growth of clams in the raceway system relates to the phytoplankton levels over the growing season. Figure 2 illustrates the percent change in length/day (K × 100) for three size classes of surf clams and the weekly average of daily *in vivo* fluorescence data from June through September, 1977. The coefficient K is determined by the equation:

$$K = \frac{\ln L_2 - \ln L_1}{T_2 - T_1}$$

where L_2 is the final length; L_1, the initial length; and $T_2 - T_1$, the interval over which the clams were measured (in this experiment 14 days).

Experiments have shown the relationship between the flow rate in a given tank and clam growth. Slower flow rates introduce less phytoplankton, therefore, limiting growth. Fluorometric data of incoming and outgoing seawater from individual tanks indicate this phenomenon.

In the raceway system, temperature appears to have less effect on growth of clams than phytoplankton availability. In a laboratory experiment, groups of 30 mm clams received ambient seawater at temperatures of 14°, 18°, 22°, and 25°C. All groups had comparable rates of growth. Malouf and Breese (1977) have hypothesized that when phytoplankton levels are moderate, growth does not relate directly to temperature.

OPERATING COSTS OF GROW-OUT FACILITY

The main operating expenses of the pumped raceway system are the costs of electric power and labor. An attempt is made here to estimate those factors and indicate the yield of clams in a given tank. Data are presented for one set of conditions which are by no means optimal.

Groups of 1000 clams were raised in individual tanks for a 5-month growing season. At a rate of 5¢/kwh for electricity it cost $70/tank to pump 50 1/min for 5 months. The tanks required about one hour of labor per week for maintenance and cleaning. At a wage rate of $3.50/hour, this cost would total $70/tank for the season. With a combined cost of $140 for power and labor per tank, the cost of operating the grow-out system to raise 1000 clams is then 14¢/clam. It should be noted that this expenditure does not represent the optimal yield per tank or the most efficient use of pumping time and space. Since the initial biomass is much smaller than the final, clams can be maintained at higher densities throughout the early months of the growing season, thus saving pumping and labor expenses. The potential economy of scale may also serve to reduce production costs.

DISCUSSION

Larval culture and intermediate grow-out of surf clams have been generally reliable over 3 years of experimentation. Occasionally, complete mortality of a larval culture will occur, probably due to bacterial disease. Disease does not appear to be a culture problem after metamorphosis.

The land-based pumped raceway system offers

the culturist some degree of control over the culture environment; yet, it is still dependent upon natural productivity. The problem of predation, encountered in a field situation, is eliminated and the recovery of clams at harvest is higher in a land-based system. The production expense of the raceway is probably lower than a more technical system, especially where cultured algae are the sole nutrition source.

Two possibilities exist for extending the growing season in the raceways. During the late winter months, ambient seawater temperatures of less than 10°C are too low to support growth, even though natural phytoplankton levels are high. Addition of heat to the system might add several months to the growing season. Since cost is a factor, a waste-heat source might be considered. Another way to lengthen the growing season might be through large-scale algal production, as demonstrated by Ryther et al. (1972). In midsummer and in the fall, growth drops dramatically, due to low phytoplankton levels (Figure 2).

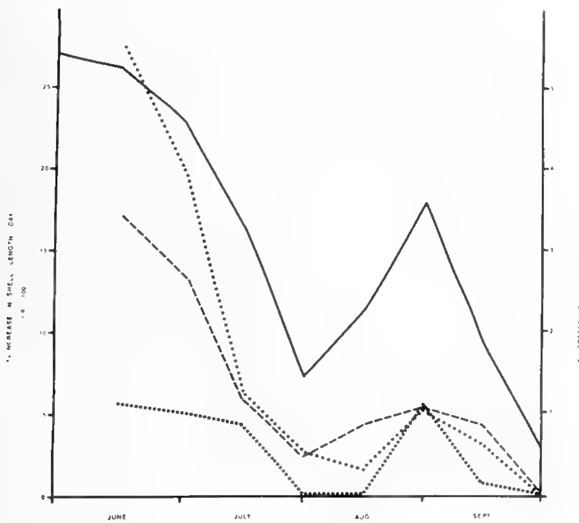

FIGURE 2. *Weekly averages of* in vivo *fluorescence compared to growth rates of three size classes of surf clams. Solid line — in vivo fluorescence; broken lines — % change in length/-day; dotted line — size class 1 (10.2 mm, initial length); long dash — size class 2 (24.3 mm, initial length); short dash — size class 3 (36.8 mm, initial length).*

Periodic use of cultured algae might produce a uniform level of growth during the season.

The rapid growth rate of young surf clams is an essential element in the potential aquacultural success of this system. The growth rate of clams in the raceway can equal that of clams in nature (Ropes et al., 1967). By rearing seed clams in the hatchery throughout the late winter and early spring, clams planted in the raceways in late spring have a 3-month head start over natural populations. The amount of growth after one growing season, therefore, can exceed that of one year in nature. In an optimal culture sequence (Figure 3), marketable 55-mm clams could be raised in 8 months.

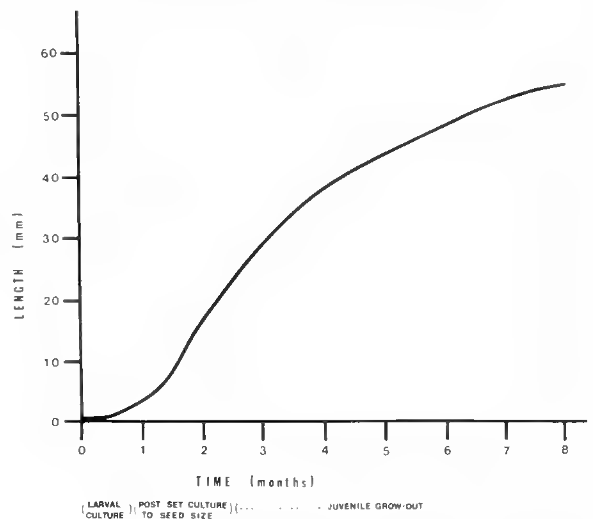

FIGURE 3. *Hypothetical, optimal culture sequence for surf clam, indicating size vs phase of culture.*

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Note: Reference to trade names does not imply endorsement by the National Marine Fisheries Services, NOAA.

LITERATURE CITED

Baab, J.S., G.L. Hamm, K.C. Haines, A. Chu and O.A. Roels. 1973. Shellfish mariculture in an artificial upwelling system. Proc. Nat. Shellfish. Assoc. **63**:63-67.

Breese, W.P. and R.E. Malouf. 1975. Hatchery manual for the Pacific oyster. Oregon State University Sea Grant Publication No. ORESU-H-75-002.

Carriker, M.R. 1959. The role of physical and biological factors in the culture of *Crassostrea* and *Mercenaria* in a salt water pond. Ecol. Monogr. **29**(3):219-266.

Castagna, M. and J.N. Kraeuter. 1977. *Mercenaria* culture using stone aggregate for progection. Proc. Nat. Shellfish. Assoc. **67**:1-6.

Chanley, P. and J.D. Andrews. 1971. Aids for identification of bivalve larvae of Virginia. Malacologia **11**(1):45-119.

Culliney, J.L., P.J. Boyle and R.D. Turner. 1975. New approaches and techniques for studying bivalve larvae. In: W.L. Smith and M.H. Chanley (editors), Culture of Marine Invertebrate Animals. Plenum Publishing Corp., New York, pp. 257-271.

Epifanio, C.E., C. Mootz Logan and C. Turk. 1976. Culture of six species of bivalves in a recirculating seawater system. In: G. Persoone and E. Jaspers (editors), Proc. 10th European Symposium on Marine Biology, Vol. 1. Inst. Mar. Sci. Res., Bredene, pp. 97-108.

Glude, J.B. 1977. NOAA Aquaculture Plan. U.S. Dep. Commerce, U.S. Govt. Printing Office, Washington, D.C.

Kirby-Smith, W.W. and R.T. Barber. 1974. Suspension-feeding aquaculture systems: effects of phytoplankton concentration and temperature on the growth of the bay scallop. Aquaculture **3**:135-145.

Loosanoff, V.L. and H.C. Davis. 1963. Rearing of bivalve mollusks. In: F.S. Russell (editor), Advances in Marine Biology, Vol. 1. Academic Press, London, pp. 1-136.

Lorenzen, C.J. 1966. A method for the continuous measurement of *in vivo* chlorophyll concentration. Deep-Sea Res. **13**:223-227.

Lucas, A. 1976. A new type of nursery for rearing bivalve postlarvae: construction, equipment and preliminary results. In: G. Persoone and E. Jaspers (editors), Proc. 10th European Symposium on Marine Biology, Vol. 1. Inst. Mar. Sci. Res., Bredene, pp. 257-269.

Malouf, R.E. and W.P. Breese. 1977. Seasonal changes in the effects of temperature and water flow rate on the growth of juvenile Pacific oysters, *Crassostrea gigas* (Thunberg). Aquaculture **12**:1-13.

Matthiessen, G.C. 1969. Seed oyster production in a salt pond. In: H.W. Youngken (editor), Proc. 2nd Conf. Food and Drugs from the Sea. Mar. Tech. Soc., Washington, D.C., pp. 87-91.

Menzel, R.W., E.W. Cake, M.L. Haines, R.E. Martin and L.A. Olsen. 1976. Clam mariculture in northwest Florida: field study on predation. Proc. Nat. Shellfish. Assoc. **65**:59-62.

Ropes, J.W. 1968. Reproductive cycle of the surf clam, *Spisula solidissima*, in offshore New Jersey. Biol. Bull. **135**:349-365.

Ropes, J.W., R.M. Yancey and A.S. Merrill. 1967. The growth of juvenile surf clams at Chincoteague Inlet, Virginia. (Abstract). Proc. Nat. Shellfish. Assoc. **57**:5.

Ryther, J.H., W.M. Dunstan, K.R. Tenore and J.E. Huguenin. 1972. Controlled eutrophication-increasing food production from the sea by recycling human wastes. BioScience **22**(3):144-152.

Shaw, W.N. 1968. Farming oysters in artificial ponds — its problems and possibilities. (Abstract). Proc. Nat. Shellfish. Assoc. **58**:9.

Walne, P.R. 1966. Experiments in the large scale culture of the larvae of *Ostrea edulis* L. Min. Agric., Fish. Food, Fish. Invests., Series 2, **24**(4):1-53.

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BIOLOGICAL AND TECHNOLOGICAL STUDIES ON THE AQUACULTURE OF YEARLING SURF CLAMS. PART II: TECHNOLOGICAL STUDIES ON UTILIZATION

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ABSTRACT

Surf clams, grown in an aquaculture system, to a size of 50-60 mm in less than one year, were investigated as a new potential source of clams to meet the steadily growing U.S. demand for all types of clams. They were organoleptically comparable as steamers, fried, and on the half-shell with both hard clams (Mercenaria mercenaria) *and soft-shell clams* (Mya arenaria) *and were frozen in three product forms for one year with no organoleptically detectable deterioration. Compositional data indicate that these small surf clams are an excellent source of low fat, high protein seafood.*

INTRODUCTION

The commercial surf clam *(Spisula solidissima)* industry is based on large clams which are used for clam strips, chowders, minced clams, and other specialty clam products. Demand for surf clams and other clams such as the soft clam and the hard clam has steadily increased. In recent years, prices for all types of clams have increased dramatically due to consumer demand and a general decline in resources resulting from overharvesting and environmental problems.

For several years, the Northeast Fisheries Center (NEFC) Milford Laboratory has been carrying out basic biological research on the spawning, growth rates, and food requirements of surf clams. One result of these studies has shown that under the proper conditions, surf clams can be grown to 55 mm size in less than one year. (Goldberg, 1980).

Under the present conditions in the clam industry, new sources of clams are needed, and a small surf clam grown in sufficient quantities in aquaculture systems could prove to be economically feasible. Since most bivalves presently grown in aquaculture systems require 3 to 5 growing seasons, a cultured surf clam grown to a marketable size of 55 mm annually or even semi-annually represents an attractive species for aquaculture. To determine the commercial feasibility, the questions to be answered are those relating to product form, meat yield, processing technology, and consumer acceptance.

MATERIALS AND METHODS

Live clams grown at Milford were shipped to the Gloucester Lab on ice in insulated containers for the utilization studies. Determinations were made for meat yield, organoleptic acceptance, chemical composition, and storage stability for both shell stocks and shucked meats.

Meat Yield

Meat yield was determined for both raw

shucked and "hot dipped" clams. For the raw shucked yield, clams were hand shucked carefully, and the entire shell contents (meat and liquor) were weighed. The meats were drained for 2 minutes on a screen, and the weights of shell, meat, and liquor were taken. The drained meats were then washed in fresh water (~13°C) for 2 minutes, drained, and weighed again.

Considering the costs of hand shucking small clams and the fact that "hot dipping" to facilitate shucking is standard practice, a simulated "hot dipping" procedure was tested. The clams were immersed in 82°C water for 3 minutes, hand shucked, and the meats were washed and drained as described above.

Organoleptic Acceptance

Yearling clams were tested in various product forms using a 12-member laboratory taste panel. The panelists were served samples of surf clams, steamed, fried, and raw on the half shell. The samples were graded for appearance (A), odor (O), flavor (F), and texture (T) on a nine-point scale (9 — excellent, 1 — inedible).

After establishing the clams' overall acceptability, the panelists participated in a difference/preference test designed to evaluate surf clams in comparison to traditional clam species prepared in typical product forms. This was done to determine where the new clams could compete in the marketplace. Thus, surf clams were compared to cherrystones *(Mercenaria mercenaria)* raw on the half shell (Figure 1), and to softshell clams *(Mya arenaria)* steamed and also fried (Figure 2).

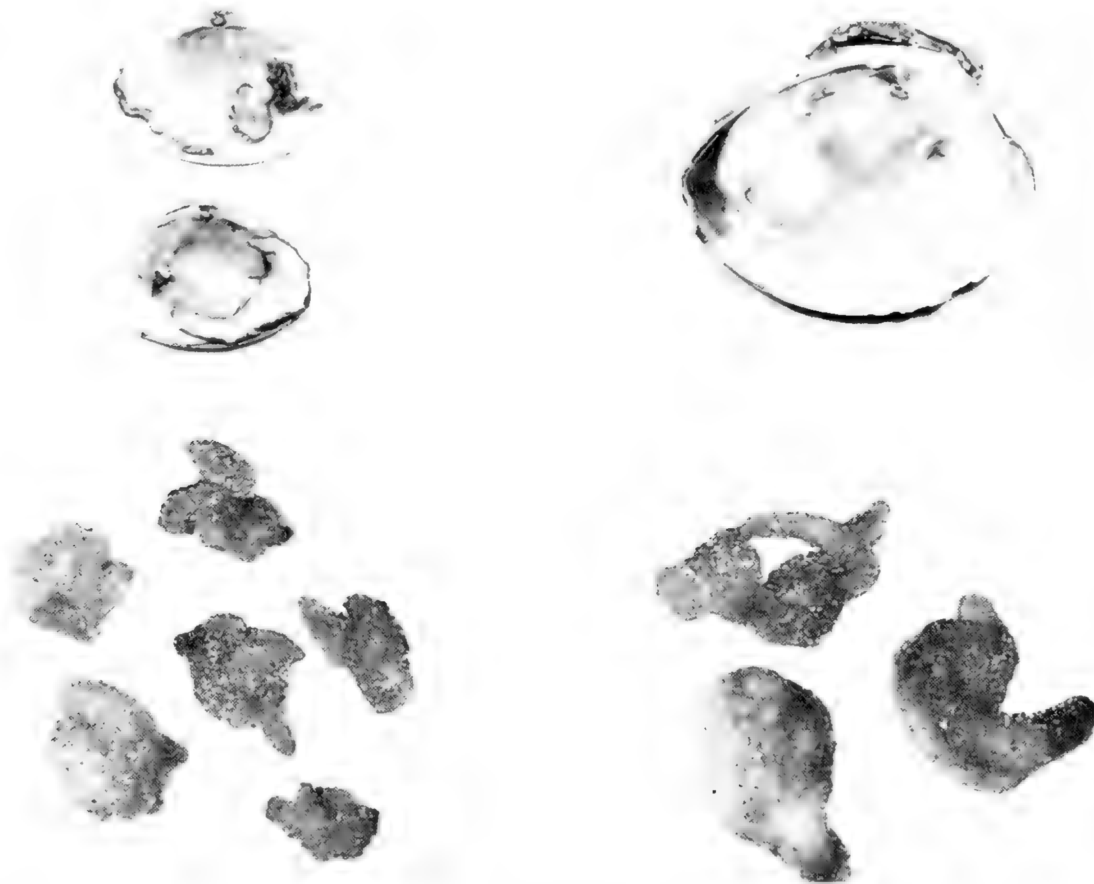

FIGURE 1. Spisula solidissima *(left) and* Mercenaria mercenaria *(right) served live, on the half shell.*

FIGURE 2. Spisula solidissima *(left) and* Mya arenaria *(right) served breaded and fried.*

Storage Stability

Live clams received from Milford were divided into three lots for frozen storage studies. One lot was vacuum packaged "in shell" in plastic bags and quick frozen at −30°C for 24 hours. The other two lots were shucked, one lot raw and the other after a 3 minute hot-dip in 82°C water, packed in pint polyethylene containers, covered with broth, and quick frozen at −30°C for 24 hours. All frozen samples were then removed to −20°C for long- term storage.

Taste tests were conducted monthly to assess changes in appearance (A), odor (O), flavor (F), and texture (T) during frozen storage. A reference sample of live surf clams was used as a control sample. Twelve judges familiar with clam products comprised a panel. Samples frozen in the shell were served steamed versus live, steamed surf clams. The raw shucked and hot-dipped shucked surf clam meats were served fried versus fresh, fried surf clam meats. Sensory data were analyzed by analysis of variance.

The sample units used for chemical analysis contained a known initial weight of clams. The sample containers were placed in water at ambient temperature until the sample was thawed. Drip loss was determined by evenly distributing the thawed clams onto a No. 8 sieve inclined about 20°. After 2 minutes, the clams were weighed, and this weight compared against the initial packed weight.

The drained sample was blended in a VirTis homogenizer until homogeneous for use in chemical analyses. Moisture content was determined by drying approximately 10 g of sample to constant weight at 100°C. Total ash was determined by heating dried samples to constant weight at 525°C (AOAC, 1975). Lipid content was determined on two 25 g samples according to Bligh and Dyer (1959). Total protein was determined on three 400 mg samples by a micro-Kjeldahl method described by the American Instrument Company (1959).

Extractable protein (XP) was determined by blending three 4 g samples in a VirTis homogenizer with 50 ml of 0.5 ionic strength buffered KCl for 2 minutes. The extractant was made according to Connell (1958). The samples were centrifuged for 30 minutes at 1800 G at 0°C. Three ml of supernatant were used in the micro-Kjeldahl determination for protein content. Sample variation was tested as being significant at ≤ 0.01, and points of difference were detected by Duncan's (1955) multiple range test.

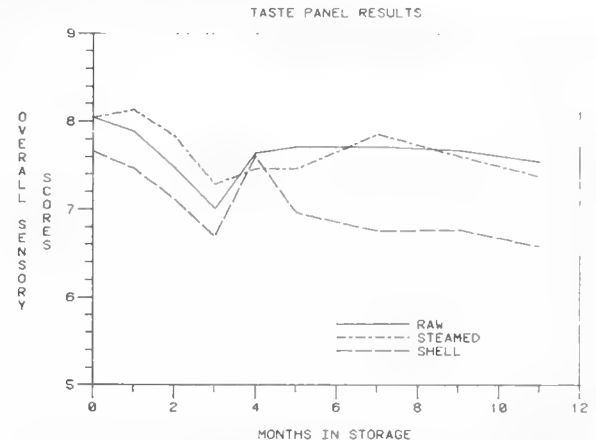

FIGURE 3. *Overall scores from surf clam taste tests using averages of appearance, odor, flavor, and texture scores. Scores indicate high acceptability for raw, shucked (raw), hot-dipped shucked (steamed), and whole frozen in-shell surf clams (shell).*

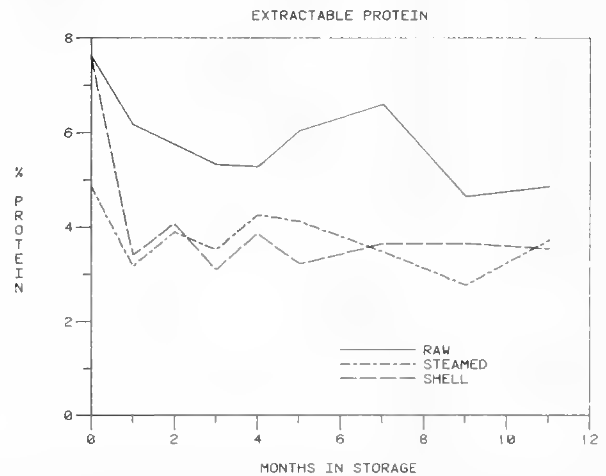

FIGURE 4. *Extractable protein over storage for raw, shucked (raw), hot-dipped, shucked (steamed) and whole, frozen in-shell surf clams (shell).*

RESULTS AND DISCUSSION

Meat yields were 20 percent on hot-dipped shucked clams, 25 percent on raw shucked clams. Proximate composition of the surf clam meats was

as follows: 15.1 percent protein, 0.7 percent fat, 3.5 percent ash, and 80.6 percent moisture. By way of comparison, softshell clams and cherrystones have 18 percent and 11 percent protein, respectively.

Taste test scores on fresh yearling surf clams for the three product forms of steamed, on the half-shell, and fried were excellent to very good, a numerical rating of 8.5. Panelists declared moderate differences between steamed surf clams and soft-shell "steamers," preferring the latter because of a slightly tougher texture encountered in the surf clam. Moderate differences were also found between surf clams and cherrystones served raw on the half-shell; the majority of panelists preferring the surf clams. Surf clams and soft-shell clams were comparable when served breaded and fried. The overall taste test scores, which are the average of the A, O, F, and T scores, never fell below 6.8 during one year of frozen storage (Figure 3); and no sample was rated significantly different from the fresh control samples.

The chemical composition analyses indicated that the surf clams have excellent storage stability. Only the hot-dipped shucked meats had drip loss averaging about 4 percent. The raw meats had a slight weight gain, and the frozen in-shell clams, when shucked, resulted in a 31 percent meat yield, a substantial gain in yield over the live, raw shucked clams. Fat and ash remained constant over storage. All clams gained in moisture the first month of storage, and thereafter remained constant. Extractable protein (Figure 4) showed a significant decrease from 7.5 percent to 5 percent due to denaturation from hot-dipping at zero time, and another decrease from 7.5 percent to 3.5 percent due to denaturation from freezing for the in-shell clams during the first month of storage.

The decrease in extractable protein after one month for both shucked meats is accounted for by the moisture gains and does not indicate denaturation during frozen storage.

SUMMARY

These preliminary studies indicate that cultured surf clams may have commercial potential. Laboratory results show that the organoleptic acceptance was very good, and the average meat yields were comparable to other clams.

The yearling clams were highly acceptable in a variety of product forms: steamed, fried, and on the half-shell. All these were competitive organoleptically with the more familiar clam species. The clam meats were frozen successfully for one year in three modes, shucked raw, shucked hot-dipped, and in-shell with little or no loss in organoleptic quality.

Chemical analyses during the frozen storage period indicate that the surf clam meats represent an excellent clam product for freezing exhibiting minimal drip loss as opposed to other clam species.

Extensive market testing and economic studies are still necessary to confirm the economic feasibility of a cultured yearling surf clam. However, results have shown a general acceptance and an indication that these clams could potentially compete with the hard clam or the soft clam in the marketplace.

LITERATURE CITED

American Instrument Company. 1959. Aminco Reprint No. 104, The determination of nitrogen by the Kjeldahl procedure including digestion, distillation, and titration. Distributed by American Instrument Company, Silver Spring, MD 20910.

AOAC. 1975. Official Methods of Analysis, W. Horowitz, ed. Association of Official Analytical Chemists, Washington, D.C.

Bligh, E.G., and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**:911-917.

Connell, J.J. 1958. Studies on the proteins of fish skeletal muscle. 5. Molecular weight and shape of cod fibrillar proteins. Biochem. J. **70**:81-91.

Duncan, D.B. 1955. New multiple range and multiple F tests. Biometrics **11**:1-42.

Goldberg, R. 1980. Biological and technological studies on the aquaculture of yearling surf clams. Part I: Aquacultural production. Proc. Nat. Shellfish. Assoc. **70**(1):55-60.

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FUNCTIONAL ANATOMY, HISTOLOGY AND ULTRASTRUCTURE OF THE SOFT TISSUES OF THE LARVAL AMERICAN OYSTER, *CRASSOSTREA VIRGINICA*

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ABSTRACT

Anatomical, histological, ultrastructural and related functional aspects of the soft tissues of larval Crassostrea virginica *(Gmelin) from the prodissoconch I through the prodissoconch II stage were examined. The visceral cavity is limited ventrally by the velum, posteriorly by the foot and a thin cellular membrane, dorsally and laterally by the mantle and anteriorly by a membrane connecting the left and right mantle lobes. The primary ciliary ring of the velum is retained by a grooved peripheral lobe whose cytological specialization facilitates the food collecting function. The foot is a complex, rapidly changing structure located both in the visceral and mantle cavities. It contains the byssal gland and paired byssal ducts as well as several presumably rudimentary tissues. The digestive system consists primarily of simple or stratified epithelia. The specialized cells of each digestive system organ are bounded on their visceral cavity aspect by an attenuated enveloping cell layer, presumably of mesodermal origin. The digestive gland consists of absorbtive, secretory, and undifferentiated cells. The striated velar, pedal and oral retractor muscles insert dorsally on the mantle at specialized desmosomal processes. Phagocytic cells, and other free cells in the visceral cavity appeared in the earliest prodissoconch I stage larvae examined. A previously undescribed non-phagocytic free cell, with highly specialized cytoplasmic components was also found in the larvae. Gill rudiment development was evident well before the foot was large enough to protrude from the mantle cavity. This rudiment consists of folds growing out from the mantle lobes and connected across the mantle cavity by a cellular bridge. Other rudimentary tissues were found near the posterior adductor muscle and are presumably precursors of components of the vascular system. The new findings are integrated with previous literature to develop a detailed synthesis of the three dimensional relationships of all organ systems.*

INTRODUCTION

Although adult bivalve molluscs have been extensively studied, the larvae of these species have received relatively little attention. Renewed interest in larval oysters has resulted in several important works (e.g. Carriker and Palmer, 1979; Waller, in press) dealing with basic structures in these animals. The recent emphasis on the culture of larval oysters and related problems has also been responsible for some of the interest in this area (Leibovitz et al., 1978; Elston, 1979a; Elston, 1979b; Elston, 1980). The general purpose of this paper is to further add to our basic knowledge of these early stages by studying the functional

anatomy, histology and ultrastructure of the soft tissues of the larval American oyster, *Crassostrea virginica* (Gmelin). Comparison of the results herein with those of some of the papers cited above suggests that most of these findings apply also to the larvae of the closely related *Crassostrea gigas*.

The recent reports of basic larval oyster structure include a detailed ultrastructural study of morphogenesis of the valves of *Crassostrea virginica* (Carriker and Palmer, 1979). Waller (in press) also using the scanning electron microscope (SEM), reported on shell ultrastructure and some soft tissue features of the larval European oyster, *Ostrea edulis*. Those two papers, and Galtsoff's (1964) classic work, review most of the literature on larval oyster anatomy. Three dimensional representations of *C. virginica* available in the literature (e.g. Prytherch, 1934; Galtsoff, 1964) were drawn from whole larvae and lack the detail obtainable using histological and ultrastructural methods. Stafford's (1913) paper is the only one which defines some histological structures in larval *Crassostrea virginica*. Additional studies, which include greater detail are those of Horst (1884), Yonge (1926), Erdmann (1935), Millar (1955), Hickman and Gruffydd (1971) and Cranfield (1973, 1974), on structure and function in larval *O. edulis*. Cole (1938) and Hickman and Gruffydd (1971) studied some aspects of metamorphosis of larval *O. edulis*. Review of the literature on soft tissue anatomy of larval *Crassostrea* sp. reveals that while there are isolated, sometimes conflicting, reports on various aspects of larval structure, there is no detailed study available which examines all of the tissue types and synthesizes the basic anatomical relationships. The purpose of this paper is, therefore, not only to present substantial new functional, anatomical, histological and ultrastructural information on larval *C. virginica* (Gmelin) but also to integrate this information with previous findings.

This report deals with the structure and development of soft tissue organ systems in planktonic larvae. This includes the prodissoconch I and II larvae (P-I and P-II, see Carriker and Palmer, 1979, for definitions) and early pediveliger larvae. These larvae exhibit most of the major organ systems or rudiments thereof, but are relatively less complex than the advanced pediveliger stage which has begun to settle. This paper, then, provides both a detailed reference for those culturing and studying larval oysters and a clear point of departure for further ultrastructural, histochemical and developmental studies.

MATERIALS AND METHODS

Ten batches of larval *Crassostrea virginica* from 24 hours to 21 days post-fertilization (averaging 57 μm to 290 μm in shell height) were obtained from a commercial hatchery on Long Island, New York. These consisted of planktonic P-I and P-II larvae and early pediveliger larvae whose foot was not well enough developed to protrude from the mantle cavity. Selected batches were exposed to an India ink solution (approx. 0.5 ml Higgins India ink in 1 liter of seawater) for 4 to 12 hours before fixation in order to study digestive and phagocytic function. All larvae were examined with interference contrast microscopy before fixation. Prior to fixation, the larvae were chilled to between 5°C and 10°C, anesthetized for 2 to 4 minutes by pipetting two drops of diethyl ether on a 5 ml suspension of oysters. Fixation for whole mounts, histological sections and scanning electron microscopy (SEM) was accomplished by withdrawing the ether-sea-water mixture and adding 5 ml of chilled Karnovsky's type fixative (2% paraformaldehyde added to Cloney's (1972) fixative) to approximately 0.25 ml of concentrated suspension of larvae. Fixation was allowed to continue for 4 to 16 hours. For preparation of whole mounts, specimens were dehydrated in an ethanol series (including a staining step of 2% eosin in 70% ethanol for 45 minutes), transferred through xylene, infiltrated with a commercial mounting medium and mounted on glass slides. For histological examination, larvae were decalcified in 10% formic acid, adjusted to pH 4.5 with sodium citrate, dehydrated through an ethylene glycol, ethanol, propanol, butanol series and embedded in glycol methacrylate according to the method of Fedder and O'Brien (1968). Sections were cut with a glass knife at a thickness of 1 μm to 2 μm. Sections were stained with: (1) 2% aqueous eosin and Harris' hematoxylin, (2) the Feulgen reaction with picro-methyl blue counterstain (Farley, 1968), (3) Ziehl's carbol fuchsin (Thompson, 1966) followed by differentiation in acetic formalin solution and

Heidenhain's aniline blue-orange G acetic stain (Thompson, 1966) or (4) Azure A and eosin at pH 4.5 (Thompson, 1966).

Samples for scanning electron microscopy were dehydrated through an acetone series, critical point dried using CO_2 as a transitional fluid, and coated with palladium-gold in an evaporative coater with a rotating planetary stage.

Samples were fixed for 4 to 6 hours for transmission electron microscopy following the anesthetization procedure using Cloney's (1972) fixative. Samples were then either: (1) postfixed in 2% osmium tetroxide in 0.2 M Millonigs phosphate buffer and decalcified in the formic acid solution or (2) postfixed in 2% osmium tetroxide in 1.25% sodium bicarbonate and decalcified by chelation in 5% EDTA (pH 7.4) as described by Bonar and Hadfield (1974). All samples were then dehydrated in an ethanol series and embedded in Spurr's (1969) resin. Thin sections on uncoated grids were stained with 2% uranyl acetate and 1% lead citrate.

The three dimensional representations were prepared by first tracing projected enlargements of whole mounts (see Figure 41) to obtain proper organ proportion and location. These drawings were then supplemented with observations made with the scanning electron microscope and with reconstructions made from serial histological sections. Most descriptions were made from specimens with extended vela and viscera; the orientation of major organs in the retracted velar state was also noted.

Descriptive terminology for tissues is introduced in those cases where appropriate terms were not available in the literature.

RESULTS

Tissues of the oyster larva were grouped into the following six functional organ systems for purposes of discussion: (1) organs limiting and enclosing the visceral cavity including the velum, mantle and associated membranes; (2) the foot; (3) the digestive system; (4) musculature; (5) free cells of the visceral cavity; (6) rudimentary organs (including presumptive components of the vascular, excretory and nervous systems). Figure 1 shows the relationship of shell structure to anatomical planes of sectional and directional orientation.

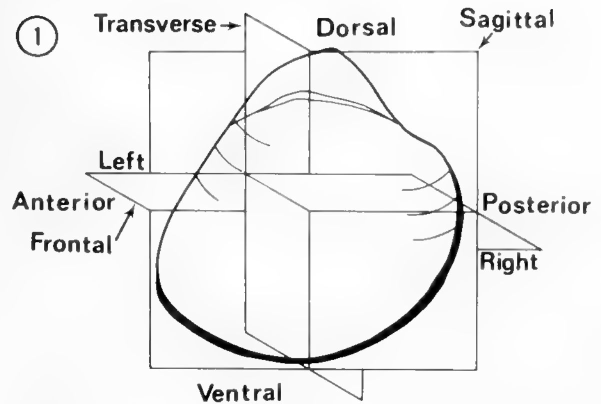


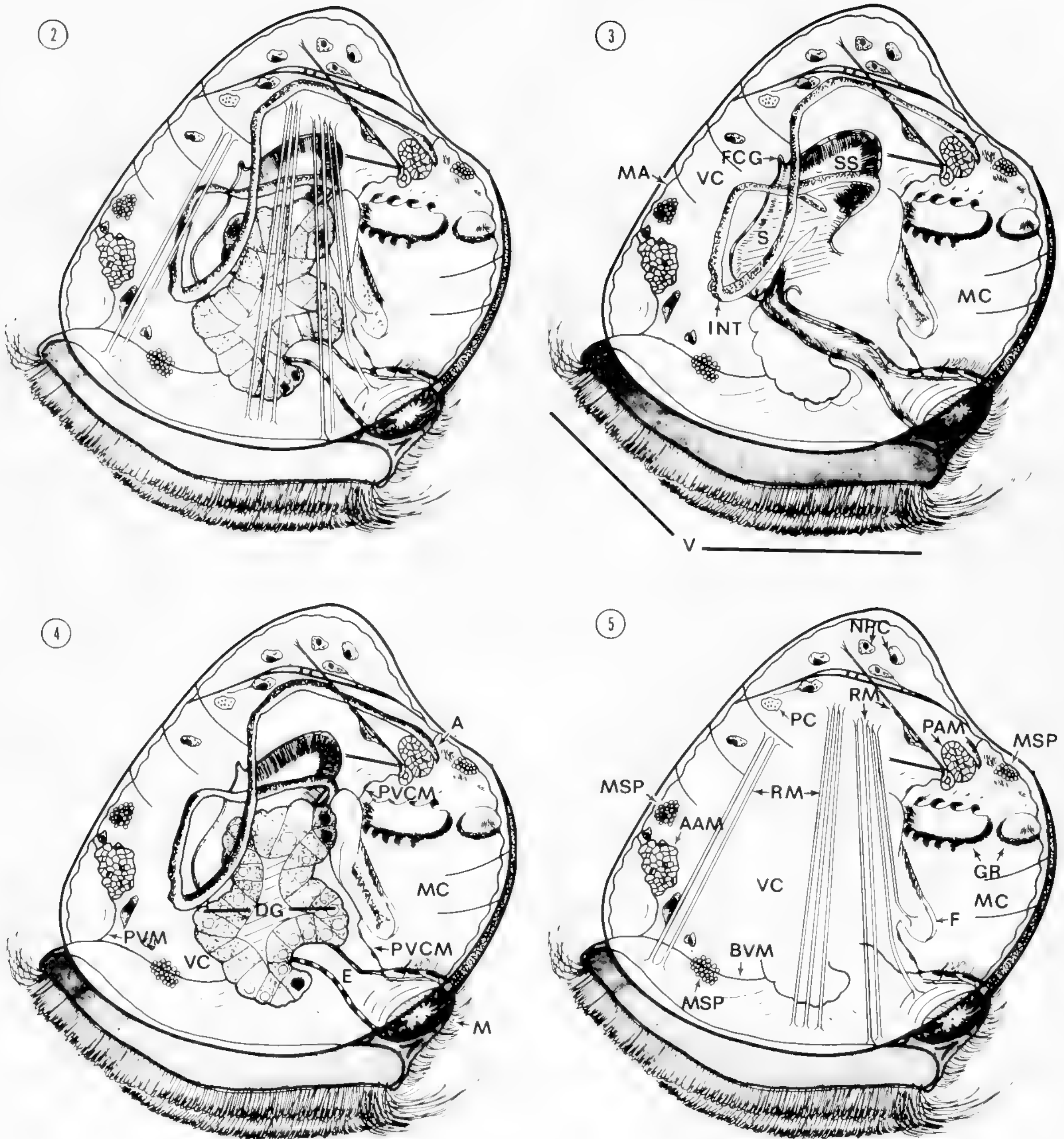
FIGURE 1. *Diagrammatic representation of larval oyster shell viewed from the left showing directional orientation and anatomical planes of section.*

Figures 2 through 5 demonstrate the relationship of shell to soft tissues.

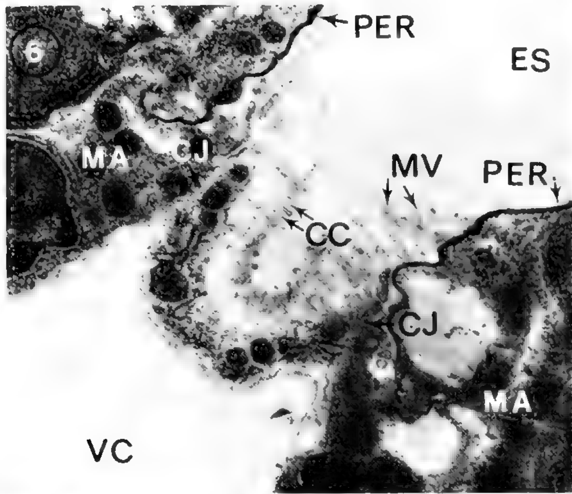
Organs defining the visceral cavity.

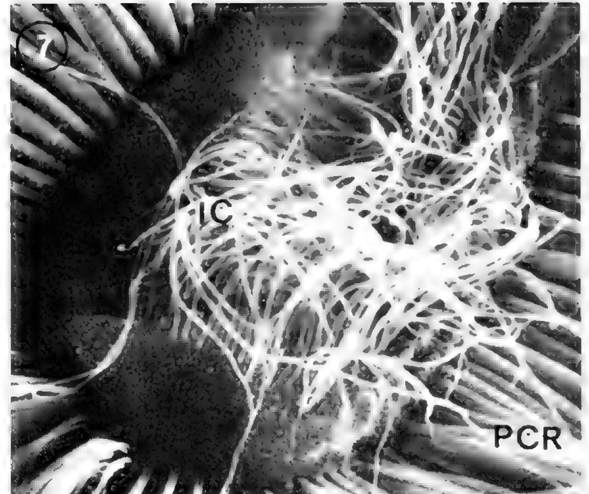
The visceral cavity (Figs. 2-5) is an enclosed fluid filled chamber containing the digestive organs, musculature and free cells. It is limited laterally and dorsally by the mantle and ventrally by the velum (Figs. 2-5). A thin cellular membrane joins the mantle and velum (Figs. 16, 17). The anterior aspect of the visceral cavity, dorsal to the anterior adductor muscle, is limited by a connecting membrane between the right and left lobes of the mantle (Fig. 6). The posterior visceral cavity membrane ("the bottom of the mantle cavity" of Erdmann, 1935; "the body wall" of Ansell, 1962) defines that aspect of the chamber (Figs. 2-5, 21). The mouth and, in later stage P-II larvae, the foot protrude through the posterior visceral cavity membrane. All of the visceral cavity organs are bounded by a thin enveloping cell layer (Fig. 30). Thus, the enveloping cell layer (see below, 3. Digestive System) in continuity with the membranes defined above forms the wall of the visceral cavity. Presumably, this layer originates from embryonic mesoderm and represents the lining of the coelomic cavity (Fig. 30).

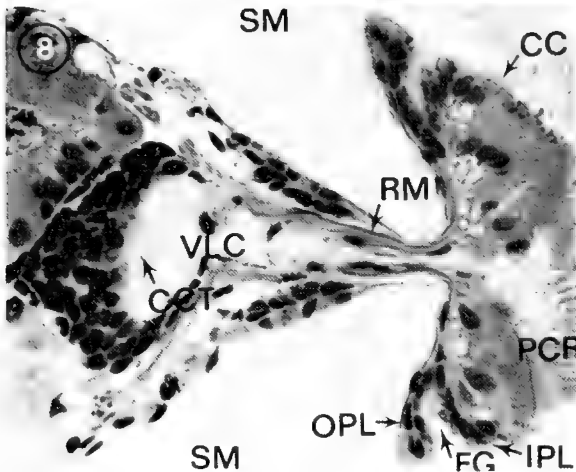
These organs, whose external surfaces form all of the nondigestive epithelia of the larval oyster, are covered externally by a prominant cell coat layer (see also Elston, 1980, Figs. 6, 7, 8, 20, 29) similar to that described in, for example, *Lymnaea*



FIGURES 2, 3, 4, 5. *Diagrammatic representation of prodissoconch II larvae viewed from the left showing complete larvae (Fig. 2), digestive system without (Fig. 3) and with (Fig. 4) digestive gland and with digestive tract removed (Fig. 5). Note that retractor muscles represent only the left-hand groups of the symmetrical set. A, anus; AAM, anterior adductor muscle; BVM, basal velar membrane; DG, digestive gland; E, esophagus; F, foot; FCG, fecal groove; GR, gill rudiment; INT, intestine; M, mouth; MA, mantle edge; MC, mantle cavity; MSP, mantle secretory product; NPC, nonphagocytic free cells; PAM, posterior adductor muscle; PC, phagocytic cells; PVCM, posterior visceral cavity membrane; PVM, peripheral velar membrane; RM, retractor muscle; S, stomach; SS, style sac; V, velum; VC, visceral cavity.*







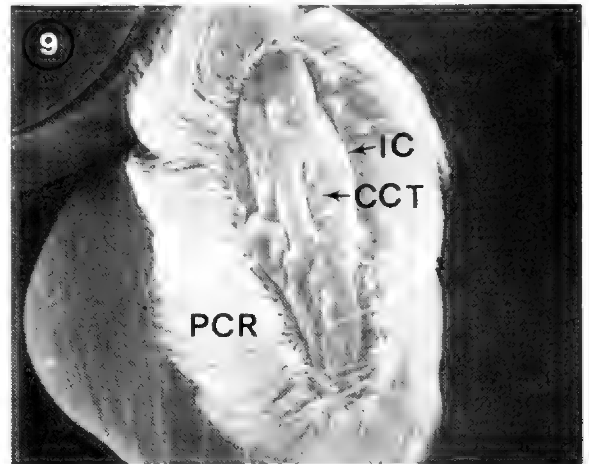


FIGURE 6. *Transmission electron micrograph, frontal section through anterior peripheral valve junction showing connecting membrane between left and right mantle lobes (MA), elaboration of periostracum (PER), cell coat (CC) and microvilli (MV). Note the prominant cell junction (CJ), the visceral cavity (VC) and the external space (ES). 9000×, decalcified by chelation, pH 7.4.*

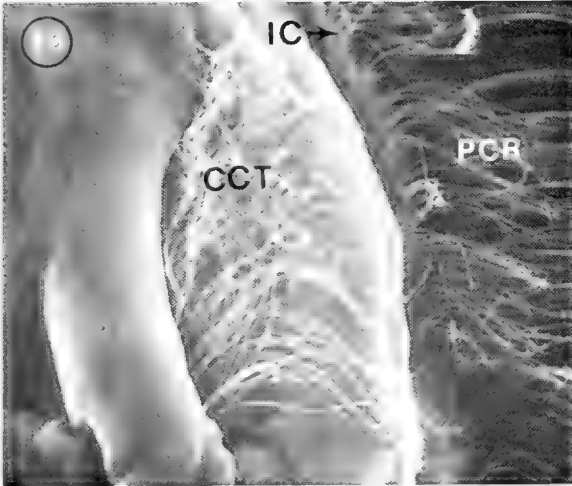
FIGURE 7. *Scanning electron micrograph of velar surface within principal ciliary ring (PCR) showing inner ciliary ring (IC) surrounding the cavity of the velar cup (see Fig. 8). Note the granular texture of the cell coat between the two ciliary rings. 1700×.*

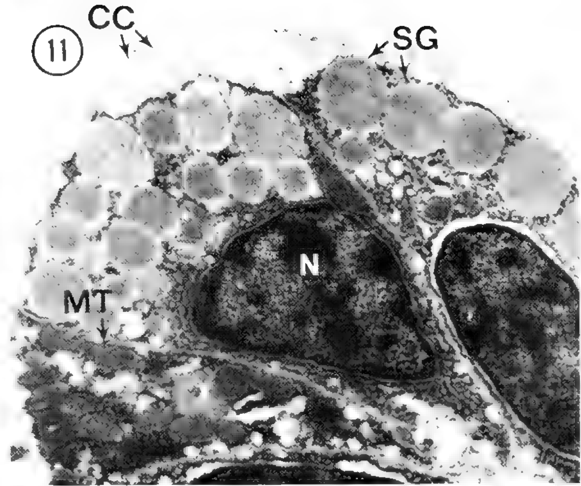
FIGURE 8. *Transverse histological section through the velum showing the inner (IPL) and outer (OPL) peripheral lobes and food groove (FG), the principle ciliary ring (PCR), the cavity of the velar cup (VLC). Note the central ciliary (CCT), the retractor muscles (RM), the cell coat (CC) and the space representing shell matrix (SM). 600×, Feulgen, picromethyl blue stain.*

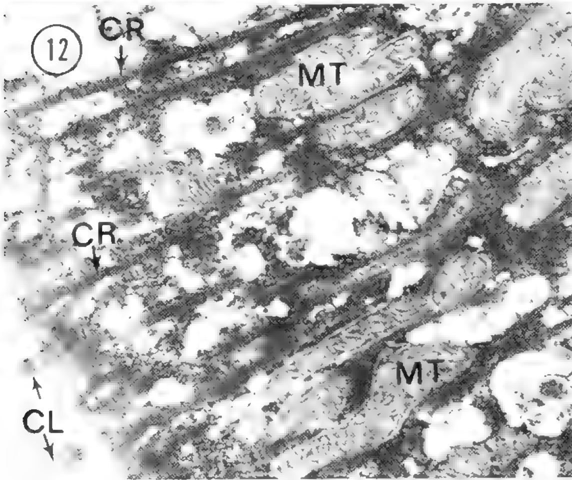
FIGURE 9. *Scanning electron micrograph of prodissoconch II larvae show extended velum with principal ciliary ring (PCR), inner ciliary ring (IC) and central ciliary tuft (CCT). 350×.*

stagnalis (Kniprath, 1977). This layer consists of a uniform extracellular sheet supported by the apical aspect of the microvilli.

The velum (Figs. 2-5, 7-10) is a cupshaped organ attached by the velar retractors and the peripheral velar membrane. Its position, and the relative







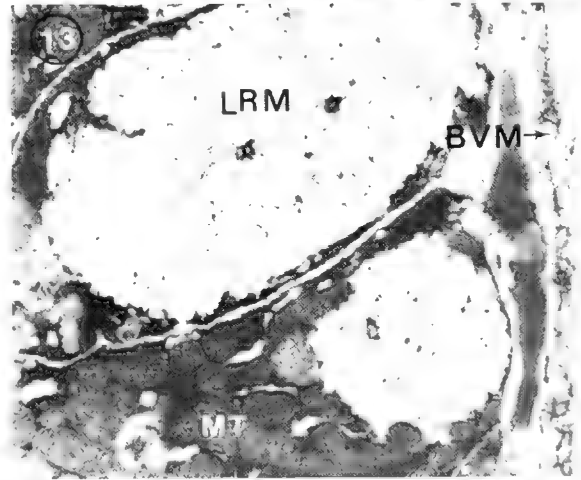


FIGURE 10. *Higher magnification scanning electron micrograph of a portion of Fig. 9 showing the same features. 1700×.*

FIGURE 11. *Transmission electron micrograph through the outer peripheral lobe of the velum showing protruding secretion granules (SG), cell coat (CC). N, nuclei, MT, mitochondria. 4800×, decalcified at pH 4.5.*

FIGURE 12. *Transmission electron micrograph of the apical portion of a principal ciliary ring cell. Note the characteristic longitudinal orientation of mitochondria (MT) along the ciliary rootlets (CR). CL, cilia. 9000×; decalcified by chelation, pH 7.4.*

FIGURE 13. *Transmission electron micrograph of the basal portion of a principle ciliary ring cells showing mitochondria (MT), electron lucent reticulated material (LRM) and the underlying basal velar membrane (BVM). 6500×, decalcified by chelation, pH 7.4.*

position of the basal velar membrane, differs from that reported for *Ostrea edulis* by Erdmann (1935). This could result from either the type of anesthesia used before fixation or from actual species differences.

The margin of the velum consists of an outer peripheral lobe of sparsely ciliated, flattened to cuboidal cells with prominant nuclei (Fig. 8). Protruding secretion granules of medium electron density are present in the apical portions of these cells (Fig. 11). The inner peripheral lobe of the velum consists of more columnar, sparsely ciliated cells which merge with, underlie, and form a retaining cup for the ring of highly specialized,

densely ciliated velar columnar cells (Fig. 8). The relatively sparse ciliation of the outer peripheral lobe and adjacent part of the inner peripheral lobe were termed adoral and postoral cilia by Erdmann (1935) for *Ostrea edulis*. A food groove is formed between these lobes (Fig. 8). The highly specialized cells forming the principal ciliary ring (Fig. 8) (preoral cilia of Erdmann, 1935) exhibit dense bundles of cilia (Figs. 7, 9, 10) which are continuous with deep cytoplasmic extensions or rootlets as shown in part of Galtsoff (1964). Erdmann's (1935) designation of the preoral cilia does not seem to be a functionally or morphologically appropriate name; thus, the term principal ciliary ring was adopted here. The cells are packed with elongated mitochondria which tend to be oriented longitudinally along the rootlets (Fig. 12). Basal aggregations of reticulated electron lucent material are often present (Fig. 13).

In early veliger larvae, the outer peripheral lobe of the velum is less well developed and the specialized densely ciliated velar cells tend to be cuboidal with more prominant nuclei than in older larvae.

Medially, the specialized velar ciliated cells end abruptly and are bordered by a continuation of the underlying inner peripheral lobe of the velum (cephalic disk, Horst, 1884; apical plate, Meissenheimer, 1901). The cells of this lobe form a thin layer as they extend medially and form the base of the velar cup or basal velar membrane (Fig. 8). This sparsely ciliated cell layer may exhibit prominant cytoplasmic protuberances into the cavity of the velar cup (Fig. 8). A ring of cilia from these cells forms an inner ciliary ring within the principal or preoral ciliary ring of the velum (Fig. 7 and 10); this ciliary band was not shown for *O. edulis* by Erdmann (1935).

The central basal portion of the velar cup is occupied by a ciliated zone (Figs. 8, 9, 10) corresponding in location to the "Scheitelorgan" of *O. edulis* (Erdmann, 1935). Although the underlying cells have been designated as the cerebral ganglion in other bivalve larvae (Meisenheimer, 1901; Erdmann, 1935), they appear to be a collection of undifferentiated cells (Fig. 8) in larvae at the stage represented in Figure 2. Ultrastructural examination demonstrates that these cells have a high nucleus to cytoplasmic ration with no apparent signs of specialization (Fig. 14).

In the retracted velum the apices of the principal

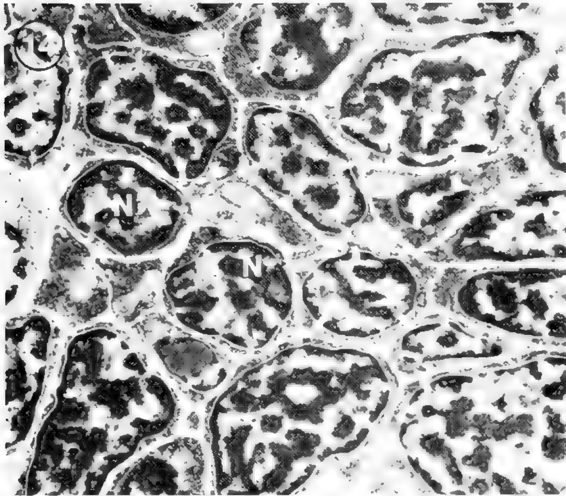


FIGURE 14. *Transmission electron micrograph of undifferentiated cells underlying the central ciliary tuft at the base of the velar cup; N, nucleus. 4500×, decalcified at pH 4.5.*

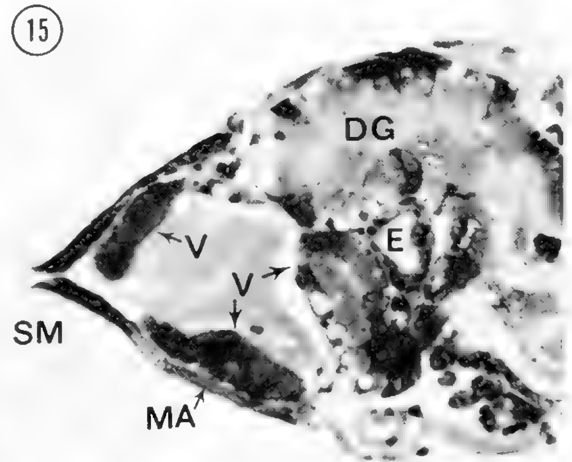


FIGURE 15. *Transverse histological section of prodissoconch II larvae showing compaction of visceral organs when velum (V) is retracted. DG, digestive gland; E, esophagus; MA, mantle; SM, shell matrix. 400×, trichrome.*

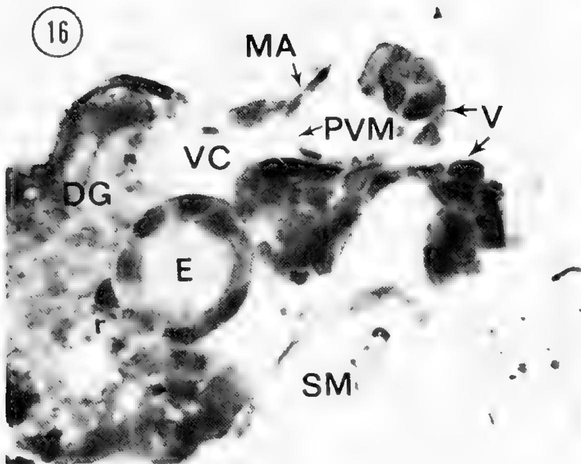


FIGURE 16. *Transverse histological section of prodissoconch I larvae showing peripheral velar membrane (PVM); velum (V), mantle (MA); esophagus (E); digestive gland (DG); shell matrix (SM); visceral cavity (VC). 2100×, hematoxylin and eosin.*

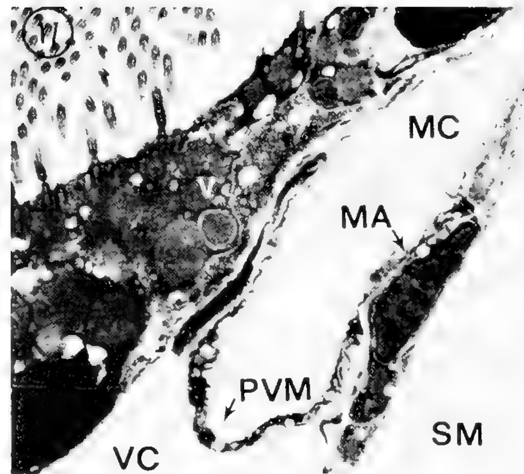


FIGURE 17. *Transmission electron micrograph of prodissoconch II larvae showing peripheral velar membrane (PVM) connecting the velum (V) and mantle (MA). Note the visceral (VC) and mantle cavities (MC). SM, shell matrix. 3000×, decalcified by chelation, pH 7.4.*

ciliated ring cells are oriented dorsally; their cilia fold at the base of the velar cup and continue in a ventral direction toward the shell margins (Fig. 15).

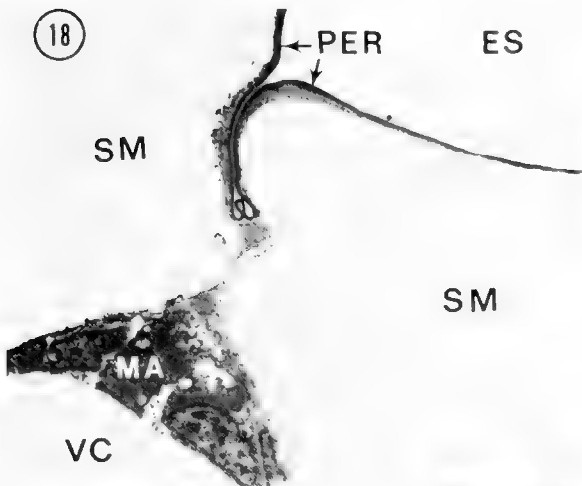
The velum attaches to the mantle laterally with the peripheral velar membrane which is a continuation of the outer peripheral lobe of the velum (Figs. 16, 17). This membrane terminates at and encloses the anterior adductor muscle on the anterior aspect (Fig. 4). Since this connecting membrane, of single cell thickness, usually lies between and closely appressed to the velum and mantle, it is often not apparent in histological sections.

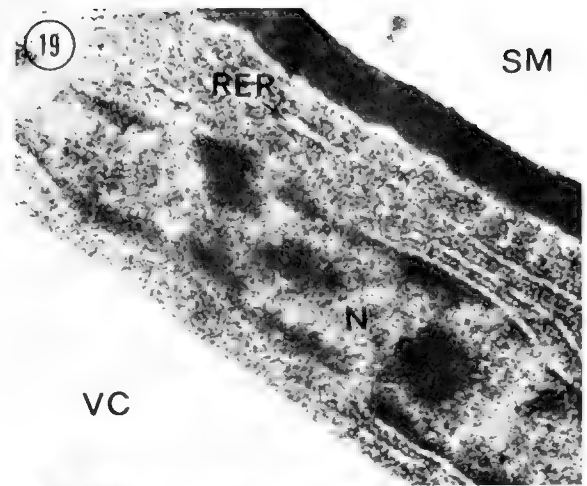
The mantle is a paired organ, each part consisting of a thin sheet of tissue adjacent to the medial aspect of its corresponding valve. The mantle forms an external, or epithelial surface where it laterally and dorsally limits the visceral cavity. The thickened peripheral zone of the mantle, two to four cells in thickness, consists of two folds ("folds" used in preference to "lobes" as proposed by Yonge, 1957). From between these two folds, the newly elaborated layer of electron dense periostracum emerges (Fig. 6). The mantle becomes an extremely attenuated layer of single cell thickness where it forms part of the visceral cavity, but thickens in the dorsal hinge region. In this area the thickened mantle lobes merge and secrete the periostracum which forms a continuous layer between the right and left valves of the shell (Fig. 18) (See Carriker and Palmer, 1979 for discussion of larval ligament). The cells of the mantle are typically flattened and contain dense granular cytoplasm with abundant profiles of rough endoplasmic reticulum (RER) (Fig. 19), even within the visceral cavity.

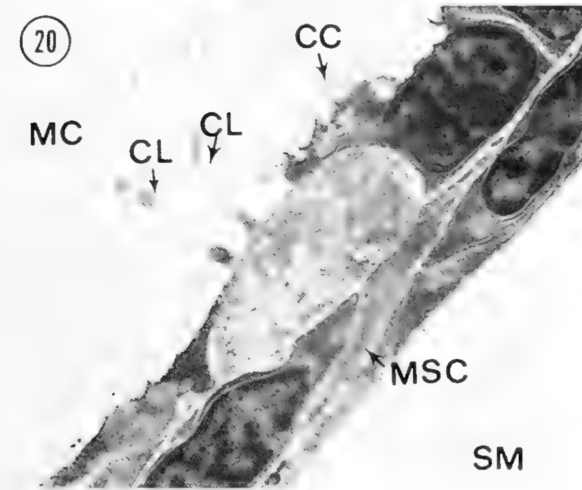
Dense ciliary tufts occur on the mantle near the anus (described in *O. edulis* by Waller, in press) and at the peripheral mantle-gill junction. Lesser tufts and single cilia are diffusely distributed on the epithelial surface of the mantle (Fig. 20). In P-II larvae, three zones of avidly eosinophilic refractile granules occur at specific sites in each lobe of the mantle. These are located near the anus, near the anterior adductor muscle and at the ventral aspect (Figs. 2-5).

The mantle is confluent with the posterior visceral cavity membrane and the gill rudiment (Figs. 2-5). This membrane (Figs. 2-5, 21, 32, 57) attaches to the mouth ventrally, the right and left mantle lobes laterally, the posterior adductor muscle ventrally, and the foot centrally.

In live larvae the extended velum moves food







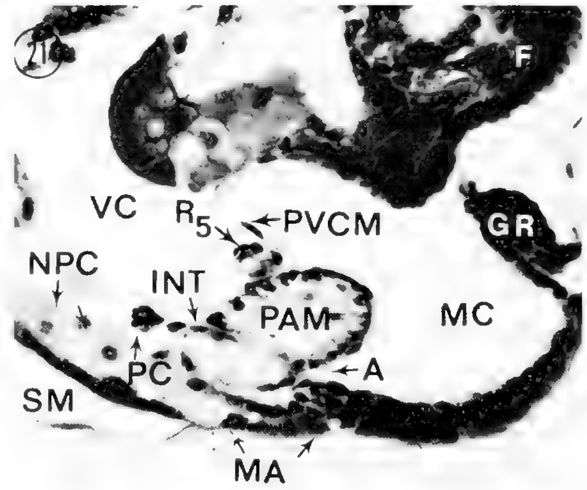


FIGURE 18. *Transmission electron micrograph of transverse section through umbo region of prodissoconch II larvae showing the continuity of the looped periostracum (PER) between the left and right valves represented by the areas of shell matrix (SM). Note the mantle (MA), the visceral cavity (VC) and external space (ES). 4000×, decalcified at pH 4.5.*

FIGURE 19. *Transmission electron micrograph of mantle within visceral cavity (VC) showing adjacent dense and light portions of shell matrix (SM). Note the rough endoplasmic reticulum (RER) and nucleus (N). 18,000×, decalcified by chelation, pH 7.4.*

FIGURE 20. *Transmission electron micrograph of ciliary tuft cell in mantle cavity (MC) portion of mantle. Note the cilia (CL), cell coat (CC), muscle cell (MSC) and shell matrix (SM). 10,000×, decalcified at pH 4.5.*

FIGURE 21. *Saggital histological section through dorsal region showing posterior adductor muscle (PAM), intestine (INT), which enters the mantle cavity (MC) between the posterior adductor muscle (PAM) and the mantle (MA) forming the anus (A). Note the posterior visceral cavity membrane (PVCM), visceral cavity (VC), phagocytic cell (PC), non-phagocytic free visceral cavity cell (NPC), foot (F), gill rudiment (GR) and shell matrix (SM).* R_5 *indicates a rudimentary tissue (see text). Dorsal is at the bottom of the figure. 900×, trichrome.*

particles around its periphery toward the mouth, as noted in *Ostrea edulis* by Yonge (1926). Ciliary movement is reduced, but not altogether stopped, when the velum and viscera are retracted, and the process of food movement by the velum continues. Velar and visceral retraction also consist of dorsal and posterior displacement of the organs, resulting in a reduction of mantle cavity volume.

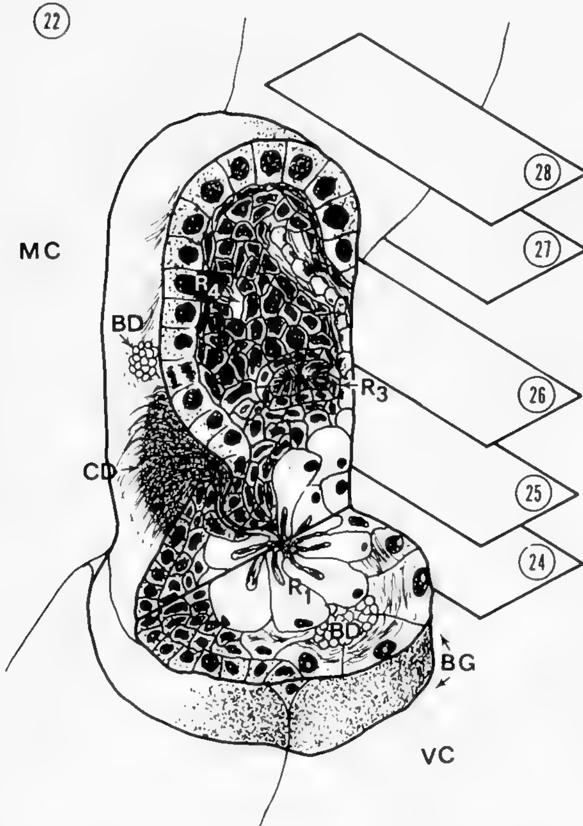


FIGURE 22. *Diagrammatic representation of the foot of planktonic prodissoconch II larvae with midsaggital and frontal cutaway to show internal structures. The dorsal connection to the gill rudiment is not shown. The numbered rectangular planes represent the levels of histological sections shown in Figs. 24-28.* R_1-R_4, *rudimentary structures, see text for discussion. BD, byssal duct; BG, byssal gland; CD, ciliated duct; MC, mantle cavity; PCVM, posterior visceral cavity membrane; RM, retractor muscles; VC, visceral cavity. Note that the dorsal aspect is at the bottom of the drawing.*

Foot

The foot, discovered by Stafford (1905), appears early in the P-II stage as a thickening in the posterior visceral cavity membrane; it increases in size and complexity throughout the larval period. The foot lies primarily in the mantle cavity but its base lies within the visceral cavity (Figs. 2-5, 22 23). It is suspended by its attachment to the posterior visceral cavity membrane and, in P-II larvae by the paired pedal retractor muscles. The description which follows was made from advanced planktonic P-II larvae.

Earlier authors (e.g. Erdmann, 1935; Cole, 1938) ascribed functions and corresponding names to structures of the foot but, as noted by Cranfield (1973) in his detailed study of the foot of *O. edulis*, these designations are based purely on morphological grounds, and as such should be regarded as tentative. The following description,

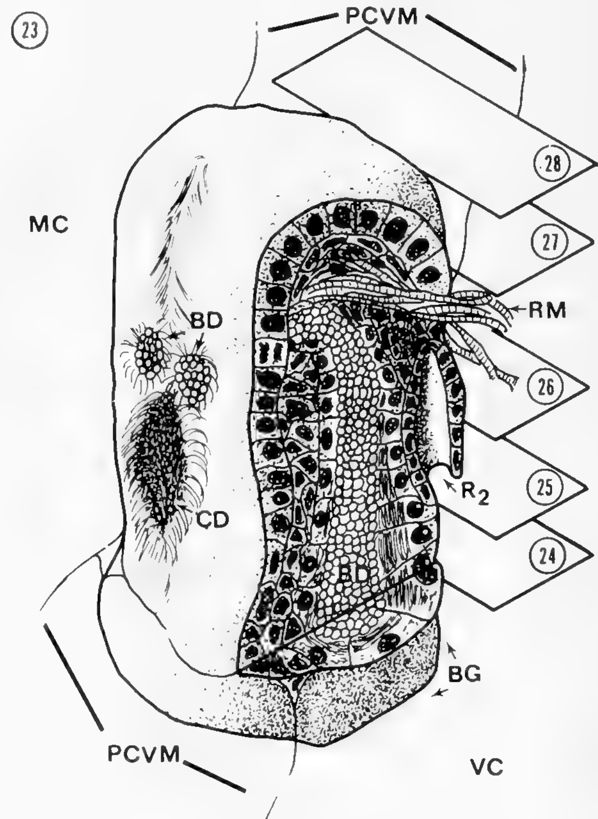
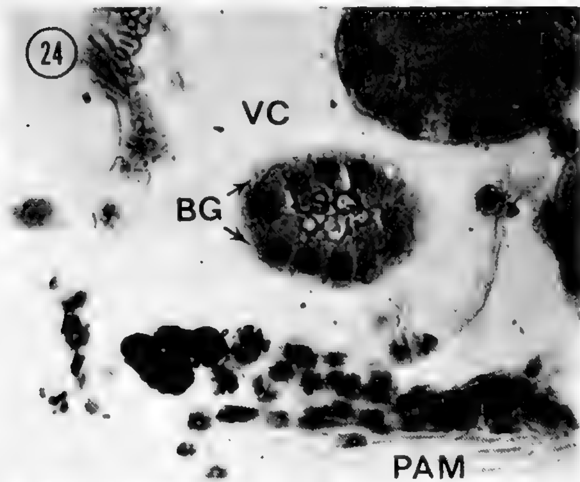
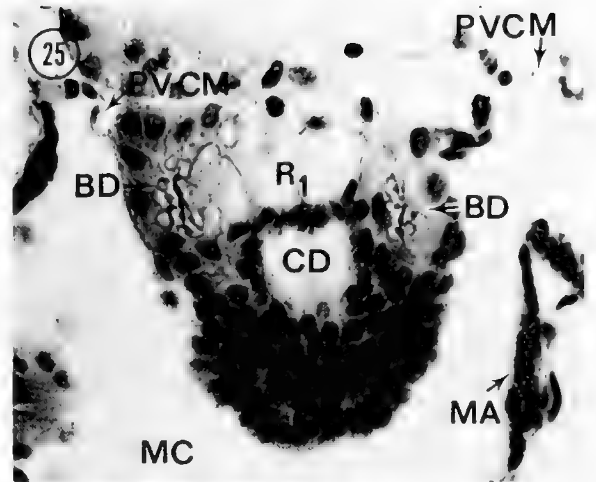
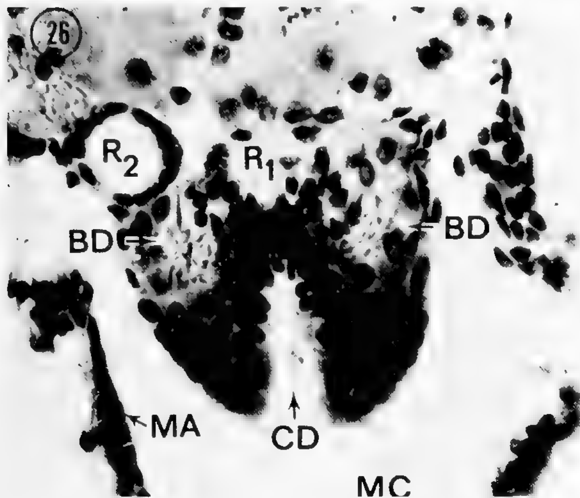


FIGURE 23. *Similar to Figure 22, but with parasaggital cutaway.*







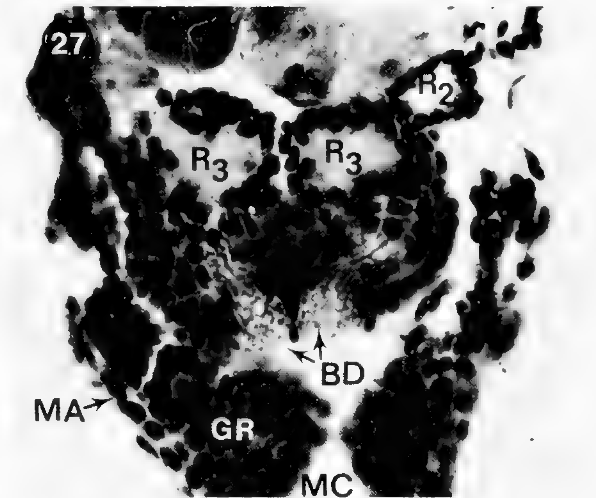


FIGURE 24. *Frontal histological section of base of the foot (see Figs. 22, 23) within the visceral cavity (VC) showing the byssal gland (BG) and central collection of secretory granules (SG). Note the digestive gland (DG); PAM, posterior adductor muscle. 1200×, Feulgen, picromethyl blue stain.*

FIGURE 25. *Frontal histological section of foot (see Figs. 22, 23) showing the ciliated duct (CD) of R_1, the byssal ducts (BD) filled with secretion granules, the thickened epithelium at the foot-gill rudiment junction, the posterior visceral cavity membrane (PVCM), the mantle (MA) and mantle cavity (MC). 1200×, Feulgen picromethyl blue stain.*

FIGURE 26. *Frontal histological section of foot (see Figs. 22, 23) showing the opening of the ciliated duct (CD) of R_1, the tubular structure of R_2, the byssal ducts (BD) with secretion granules, the mantle (MA) and mantle cavity (MC). 1200×, Feulgen picromethyl blue stain.*

FIGURE 27. *Frontal histological section of the foot (see Figs. 22, 23) showing the ciliated cavities of R_3 (the structure designated as the pedal ganglion by Erdmann, 1935), the tubular structures of R_2, the openings of the byssal ducts (BD) into the mantle cavity (MC). Note also the mantle (MA) and the attached gill rudiment (GR). 1200×, Feulgen picromethyl blue stain.*

is intentionally conservative in ascribing functional names to the structures observed.

The byssal gland and ducts are, however, easily identified. The base, or dorsal aspect of the foot, within the visceral cavity is formed by the secretory cells of the byssal gland (Figs. 22, 23,

24). These cells form a central mass of byssal secretion which bifurcates into the paired left and right ducts (previously noted by Prytherch, 1934) of the byssal gland. These ducts are lined throughout most of their length with secretory-type cells morphologically similar to those of the byssal gland proper (Figs. 23-27). The byssus ducts open independently to the mantle cavity on the posterior aspect of the foot, ventral to the ciliated furrow described below (Figs. 23, 27). The byssal secretion is characteristically granular, refractile and avidly eosinophilic. It appears well before the foot is fully developed, even at duct openings, and at about the same time that primordial gills become visible in the whole, live larvae. Byssal secretory cells contain abundant profiles of rough endoplasmic reticulum basal to the forming secretory granules, suggesting a proteinaceous component of the secretory substance.

A distinctive structure lies near the base of the foot, ventral to the core of byssal secretory cells and between the left and right ducts of the byssus gland. Its cells are the only cells of the larva staining blue with the picromethyl blue procedure used; the nuclei are small and relatively inapparent. (Cells of this structure are designated R_1 in Figs. 22 and 25). Finger-like processes from a central core of these cells converge on a cavity which is continuous with a ciliated duct opening into a furrow on the posterior aspect near the heel, or dorsal-most aspect within the mantle cavity, of the foot (Fig. 22, 25, 26). Other similar cells continue from the base of the foot up the mid-anterior aspect and deflect into the central part of the foot near the entry of the pedal retractors (Figs. 22, 28). Similar cells are observed laterally in this region and all appear to be enclosed in a thin fibrous sheath, suggesting a nervous function.

Paired blind tubules (labeled R_2 in Figs. 23, 26, 27, 28) begin as furrows on the anterior lateral aspects of the foot; similar structures were designated as statocysts in *Ostrea edulis* by Erdmann (1935). These tubules are lined by a flattened epithelium (Figs. 23, 26) and contain multiple refractile concretions. In the *Crassostrea virginica* larvae examined, the tubules do not appear to communicate with the mantle cavity. Ventral to the termination of these tubules, paired pedal retractor muscles enter the foot from the visceral cavity (Figs. 23, 28). This region of the foot also

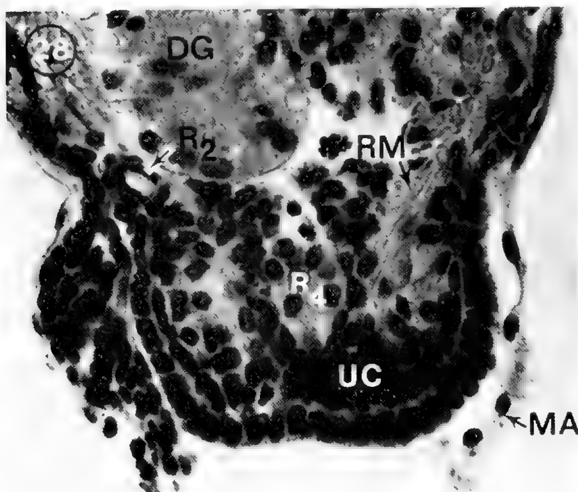


FIGURE 28. *Frontal histological section of the foot (see Figs. 22, 23) showing the pedal retractor muscles (RM), undifferentiated cells (UC), R_4, the tubular R_2. Note also the mantle (MA) and the digestive gland (DG). 1200×, Feulgen picromethyl blue.*

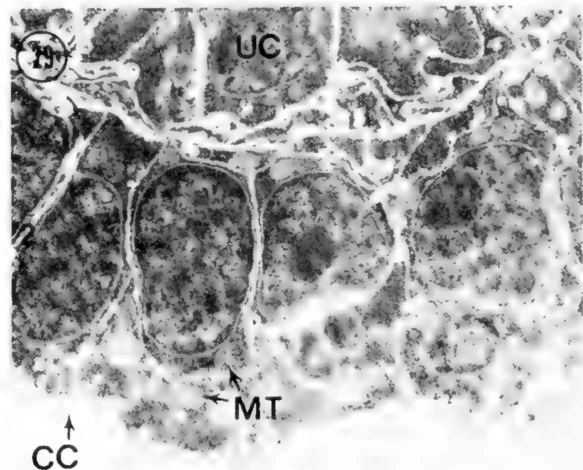


FIGURE 29. *Transmission electron micrograph of foot epithelium covering that part of the foot within the mantle cavity. Note the cuboidal character of these large cells, the apical mitochondria (MT), the cell coat (CC) and the underlying undifferentiated cells (UC). 4800×, decalcified at pH 4.5.*

contains a ciliated cavity (labelled R_3 in Figs. 22, 27) which is elongated from left to right and extends into short paired lateral tubular structures ending blindly toward the ventral tip of the foot. This structure may represent the pedal ganglion as suggested by Erdmann (1935) for *O. edulis*.

The interior of the foot contains other areas of undifferentiated cells of unknown fate (Figs. 22, 23). These include a small central, blind tubular structure ventral to the openings of the byssal ducts (labelled R_4 in Figs. 22, 28).

All epithelial tissue of the foot which contacts the mantle cavity consist of a cuboidal epithelium with prominant nuclei (Figs. 22, 23, 25-28, 29). The epithelium is underlain by elongated, but undifferentiated cells, possibly corresponding to the musculature described by Cranfield (1973) for *Ostrea edulis*. The foot of P-II larvae appears to be ciliated primarily along its posterior median axis. The portion of the foot within the visceral cavity is covered by a thin enveloping cell layer like that found on the visceral cavity aspect of the digestive system organs.

Digestive System

The organs of the digestive system of *Crassostrea virginica* larvae consist of the mouth, esophagus, stomach, style sac, digestive gland, and intestine (Figs. 2-4), arranged similarly to the same organs in *Ostrea edulis* (Yonge, 1926; Erdmann, 1935; Millar, 1955). The digestive system organs are generally organized as either single cell layer or stratified epithelium bounded by a thin enveloping cell layer on the visceral cavity aspect of the digestive organs (Fig. 30). The digestive epithelia show a cell coat structure which is similar to, but distinct from, that of the non-digestive epithelia. Except for the digestive gland and gastric shield the cell coat appears as a diffuse mucinous precipitation matrix below the apical aspects of the microvilli (Fig. 31). In the digestive gland, the mucinous secretions appear to be continuous across the lumen.

When the velum is extended, the mouth is located at the ventral posterior aspect (Figs. 2-5), as figured by Prytherch (1934) and Galtsoff (1964). It

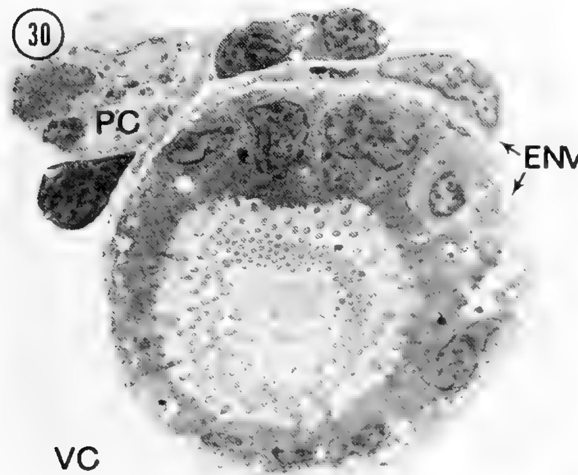


FIGURE 30. *Transmission electron micrograph of transverse section through intestine showing the enveloping cell layer (ENV). Note the phagocytic cell (PC) and other free cells within the visceral cavity (VC). 3600×, decalcified by chelation, pH 7.4.*

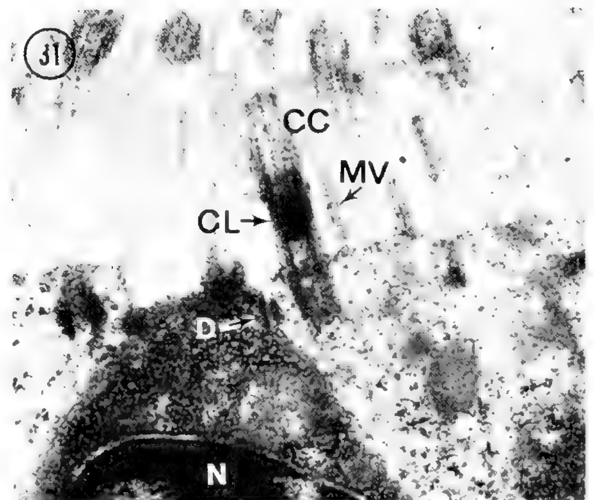


FIGURE 31. *Transmission electron micrograph of intestinal epithelium and thick cell coat (CC) supported by microvilli, (MV). D, desmosome; N, nucleus; CL, cilia. 18,000×, decalcified at pH 4.5.*

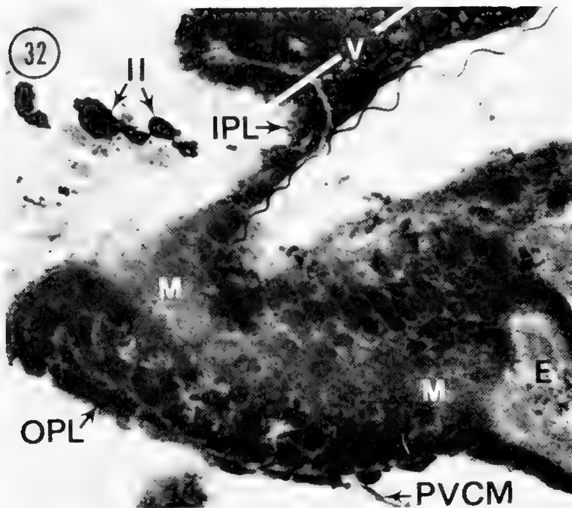


FIGURE 32. *Saggital histological section showing the relationship of the mouth (M) to the inner (IPL) and outer (OPL) peripheral lobes of the velum (V). Note the sharp demarcation between the mouth and esophagus (E). PVCM, posterior visceral cavity membrane; II, India ink particles. 800×, hematoxylin and eosin.*

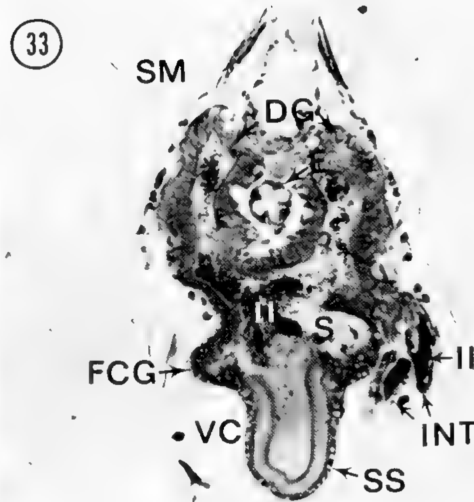
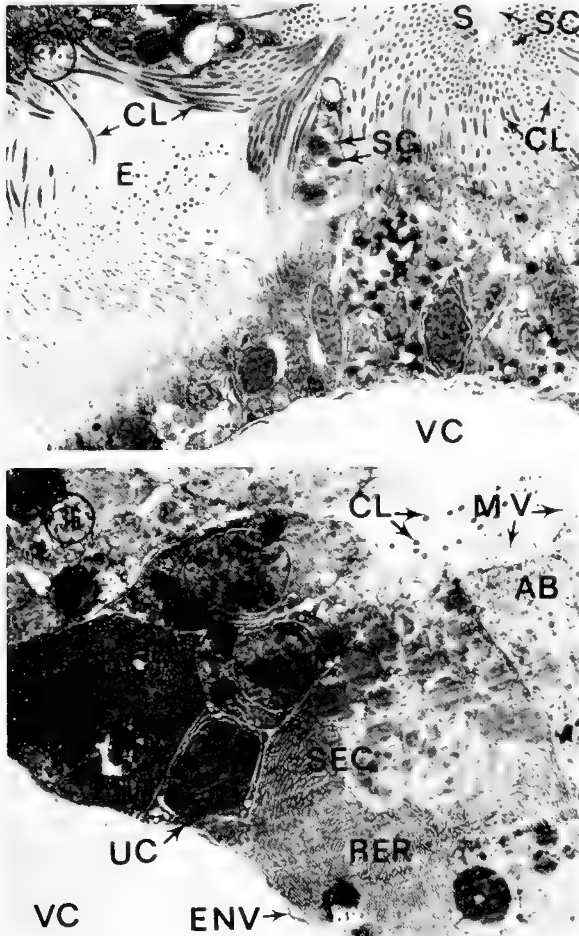


FIGURE 33. *Transverse histological section through whole larva showing components of the digestive system. DG, digestive gland; E, esophagus; FCG, fecal groove; II, India ink particles; INT, intestine; SS, style sac; SM, shell matrix; VC, visceral cavity. Note that only the basal, dorsal portion of the stomach (S) appears in the section. 400×, hematoxylin and eosin.*

merges laterally with the outer peripheral lobe of the velum and ventrally with the inner peripheral lobe (Fig. 32). It thus forms a funnel which is continuous with the food groove of the velum; this general arrangement was noted in *Crassostrea virginica* by Stafford (1913) but he did not report tissue and cytological details. The lumen of the mouth is formed by heavily ciliated cuboidal epithelium with prominant nuclei. The junction of the mouth with the esophagus is sharply demarcated (Fig. 32).

The esophagus (Figs. 2-5) is a densely ciliated tube. Its cross sectional appearance tends to be stellate in the retracted state (Fig. 33) and round in the extended state (Fig. 16). Its cells are flattened to columnar, depending on the degree of velar extension, and contain prominant basal nuclei and abundant mitochondria. At the mouth-esophagus junction, the wall of the esophagus folds and thus forms an expanded ciliated cavity (Fig. 32). A cellular constriction, probably corresponding to the "ventral ridge of the gastric shield" noted by Millar (1955) in *Ostrea edulis*, identifies the junction of the esophagus with the stomach (Fig. 34). The cells of this constriction contain abundant secretion granules; through the aperature of this junction cilia of esophageal cells pass into the stomach. This aperature apparently serves, in part, to prevent the dense mucus secretion and food particles in the stomach from entering the esophagus (Fig. 34), as well as acting as a control point for food particles entering the stomach.

The central organ of the digestive system, the stomach, is a highly distensible, bell-shaped organ (Fig. 3). The stomach joins the esophagus at its ventral apex, the style sac at its flared dorsal base and the digestive gland lobes posteriorly near its dorsal base (Fig. 33). The stomach joins the intestine through a spiralling extension of its basal or fecal groove (Figs. 3, 33, 41, 42). The stomach wall is primarily a pseudostratified columnar ciliated epithelium interspersed with goblet cells (Fig. 34). Near portions of its base, however, the epithelium is simple and non-ciliated. Stellate, electron lucent figures are sometimes found in the stomach (Fig. 34) and are identical to those apparently originating in the digestive gland (see discussion of digestive gland below). A portion of the anterior and lateral luminal aspect of the stomach wall is covered by the gastric shield as



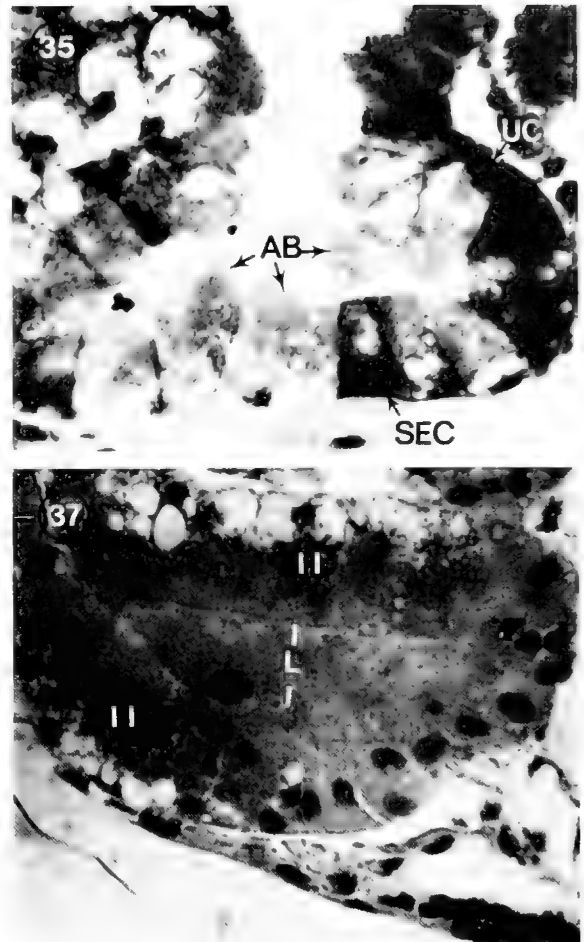


FIGURE 34. *Transmission electron micrograph of the esophagus (E)-stomach (S) junction. Note the secretion granules (SG) within the funnel-shaped cellular constriction forming the junction which maintains the dense mucus environment of the stomach. CL, cilia; VC, visceral cavity. Note the stellate crystals (SC) within the stomach. 2800×, decalcified at pH 4.5.*

FIGURE 35. *Histological section of digestive gland lobe showing absorbtive cells (AB), secretory cells (SEC) and undifferentiated cells (UC). 1200×, trichrome.*

FIGURE 36. *Transmission electron micrograph of the digestive gland showing absorbtive cells (AB), secretory cells (SEC) and a basal undifferentiated cell (UC). Note the long microvilli (MV), the cilia (CL) in the lumen of the gland and the enveloping cell layer (ENV) on the visceral cavity (VC) aspect. RER, rough endoplasmic reticulum. 4500×, decalcified at pH 4.5.*

FIGURE 37. *Histological section of digestive gland lobe showing absorption of India ink granules (II) within absorptive cells. Note the basal vacuoles within these cells. L, lumen of digestive gland. 1200×, Feulgen picromethyl blue stain.*

described for *Ostrea edulis* by Erdmann (1935) and Millar (1955). The gastric shield appears in thin sections as long intertwining microvilli embedded in a dense mucinous precipitation matrix (Fig. 40).

The digestive gland is an H-shaped organ; its paired lobes communicate through the central bar of the H with the stomach (Fig. 33). The more prominant lobes of the digestive gland extend ventrally around the esophagus in P-II larvae (Fig. 33)

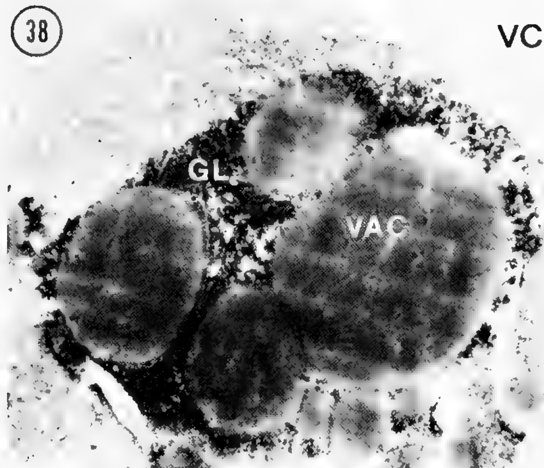


FIGURE 38. *Transmission electron micrograph of vacuoles (VAC) in basal portion of absorptive cell of the digestive gland. Note the glycogen-like material (GL) surrounding the vacuoles. VC, visceral cavity. 10,000×, decalcified at pH 4.5.*

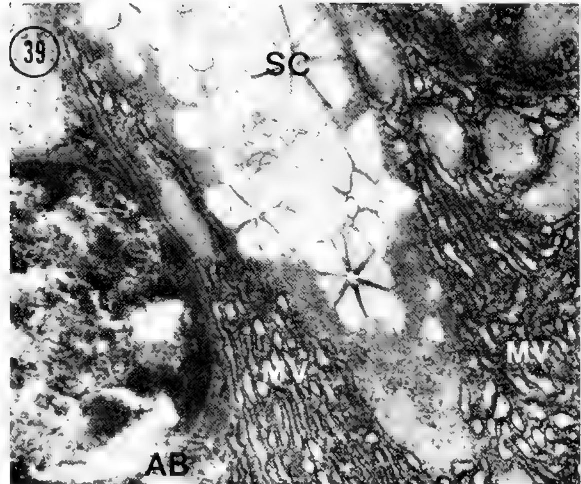


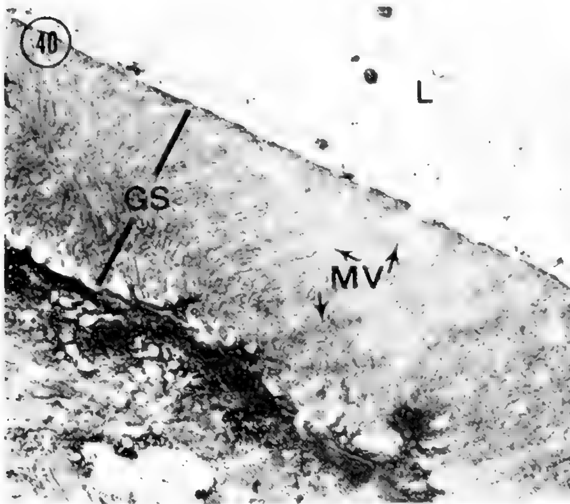
FIGURE 39. *Transmission electron micrograph of a microvillous (MV) cleft between absorptive cells (AB) of the digestive gland containing stellate crystals (SC). Note the vacuole in the adjacent absorptive cell containing elongate crystalline material. 13,000×, decalcified at pH 4.5.*

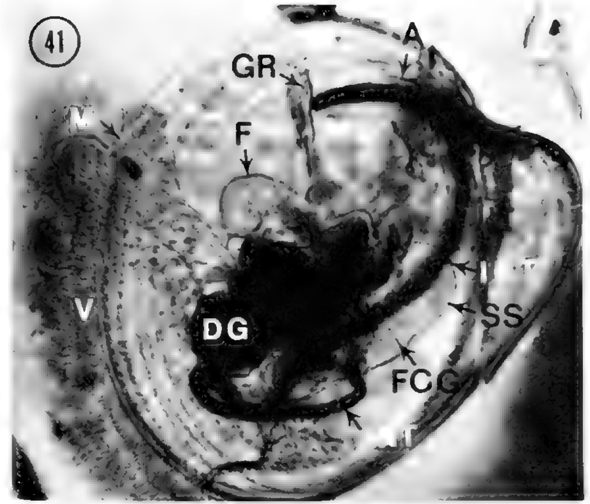
as described for *Ostrea edulis* by Yonge (1926). In P-I larvae the digestive gland lobes are very short extensions from the stomach and do not extend ventrally around the esophagus (Fig. 16). The length of the gland develops by proliferation of undifferentiated cells at the tips of lobes where the epithelium tends to be stratified (Figs. 35, 36).

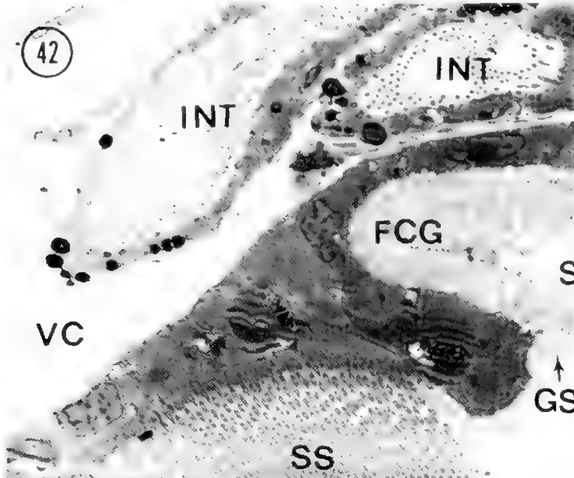
In addition to undifferentiated cells, the digestive gland contains primarily large absorbtive cells interspersed with less numerous secretory cells (Fig. 35). Ingested India ink particles were observed histologically in the absorbtive cells. Sequential sampling, following exposure to India ink, demonstrated the movement of these particles from the luminal to the basal aspect of the absorptive cells (Fig. 37). These cells may contain histologically clear but moderately electron dense vacuoles suggestive of lipid, which increase in size toward the cell base (Figs. 35-38). Ultrastructurally, the vacuoles are surrounded by dense granular material characteristic of glycogen (Fig. 38). The remaining cytoplasm consists of a variety of dense granular material and vacuoles, often containing elongated crystalline type material (Fig. 39). Histologically, the cytoplasmic granules are variably clear, refractile, or show a range of tinctorial qualities. Vacuolar protuberances may be observed on the basal surfaces of these cells. The absorbtive cells display long widely spaced microvilli on their luminal aspect (Fig. 36).

The secretory cells of the digestive gland stain intensely with basic stains (Fig. 35). These cells show typical ultrastructural features of protein secreting cells including abundant rough endoplasmic reticulum, Golgi complexes and apical condenstation vacuoles (Fig. 36). Stellate crystals are found in deep microvillar clefts between absorptive cells as well as in the lumen of the digestive gland (Fig. 39). Cilia were observed in the lumen of the gland (Fig. 36) but their origin on digestive gland cells was not observed. When the velum is extended, lumina of the digestive gland lobes are patent; the lumina are relatively inapparent when the velum is retracted, although the characteristic pulsations may continue.

The relatively rigid style sac protrudes dorsally from the fecal groove of the stomach on the left hand aspect of the midsaggital plane (Figs. 2-4, 33). The style sac is a deep, cupshaped organ consisting of pseudostratified, densely ciliated cells (Figs. 33, 41, 42, 43). This prominant dense ciliation of uniform length is the most characteristic







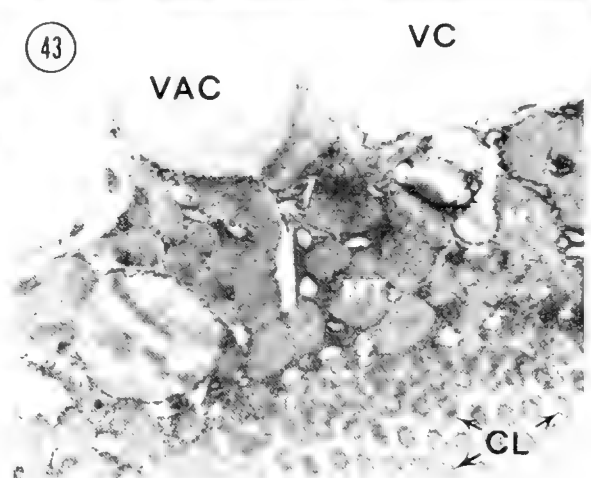


FIGURE 40. *Transmission electron micrograph showing gastric shield (GS) of the stomach. It consists of a dense intertwined mat of microvilli (MV) and mucoid secretion. L, lumen of stomach. 10,000×, decalcified at pH 4.5.*

FIGURE 41. *Photomicrograph of whole mount of prodissoconch II larva viewed from the right. The digestive tract is outlined with India ink. A, anus; DG, digestive gland; FCG, fecal groove; F, foot; GR, gill rudiment; M, mouth; MC, mantle cavity; INT, intestine; SS, style sac; V, velum; VC, visceral cavity. 400×.*

FIGURE 42. *Transmission electron micrograph of the fecal groove (FCG) formed by the ridge between the stomach (S) and style sac (SS). Shows the anterior aspect of these organs. Note the gastric shield (GS) and the intestine (INT) with prominant osmiophilic vacuoles. VC, visceral cavity. 2400×, decalcified by chelation, pH 7.4.*

FIGURE 43. *Transmission electron micrograph of style sac wall showing the abundant globose mitochondria (MT), dense ciliation (CL) on the luminal aspect and the protruding vacuole (VAC) on the visceral cavity (VC) aspect. 7800×, decalcified at pH 4.5.*

feature of the style sac in both living and sectioned specimens (Figs. 33, 41, 42). This ciliated mat of the style sac, whose luminal border is avidly eosinophilic, ends abruptly at the stomach-style sac junction which is defined by a cellular ridge (Figs. 33, 42). A crystalline style was not observed in any of the larval specimens examined. The time of formation of the crystalline style may be

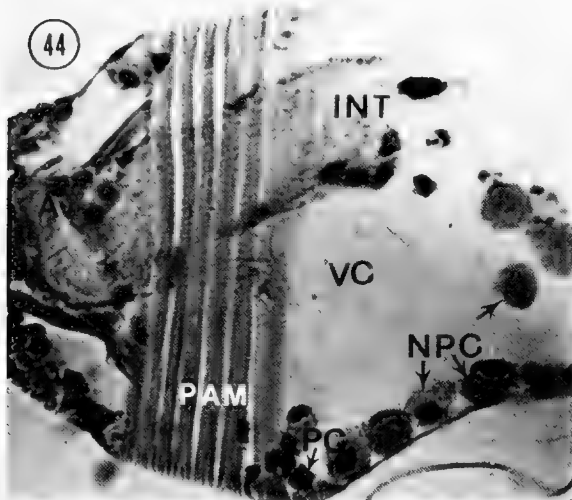


FIGURE 44. *Frontal histological section showing the longitudinal extension of the posterior adductor muscle (PAM) between the two valves. Note the intestine (INT), anus (A), nonphagocytic free cells (NPC) and phagocytes (PC) within the visceral cavity (VC). 1000×, Feulgen, picromethyl blue.*

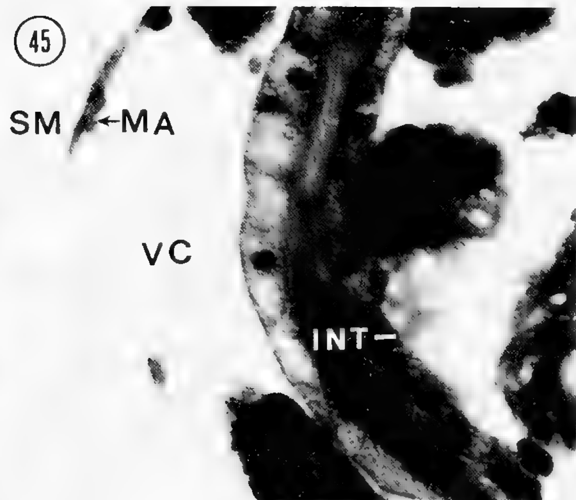


FIGURE 45. *Histological section through intestine (INT) showing abundant clear vacuoles within intestinal cells. MA, mantle; SM, shell matrix; VC, visceral cavity. 1000×, trichrome.*

variable in the larva. Ultrastructurally, the cells of the style sac variably contain large electron lucent and moderately electron dense vacuoles (Fig. 43). Densely packed, globose mitochondria indicating the high level of energy consumption of the style sac occupy much of the cell volume, but are separated by a fine dark granular cytoplasm (Fig. 43). Vacuolar protuberances, similar to those of the digestive gland, are observed on the visceral cavity aspect of the style sac cells (Fig. 43).

The intestine begins as an extension of the fecal groove at the base of the stomach (Figs. 3, 33, 42). A fecal pellet of compacted India ink was observed forming in this groove in live larvae. The groove deepens as it passes through the stomach wall, forming the intestine. The intestine thus begins parallel with the frontal plan and on the right hand aspect of the mid-saggital plane. It then turns dorsally and curves 180 degrees around the style sac, proceeding on the left-hand aspect of the mid-saggital plane (as noted by Stafford, 1913) toward the anterior ventral aspect of the visceral cavity (Figs. 2-4). In P-II larvae the intestine then proceeds to a point approximately between the ventral tips of the digestive gland lobes and the anterior adductor muscle. There it makes another 180 degree turn and proceeds in a posterior dorsal direction to the anus which is located between the posterior adductor muscle and the mantle lobes (Fig. 2-4, 21, 44). The length of the anterior ventral loop is considerably shorter in P-I larvae, consisting in the early veliger of a nearly straight tube originating on the developing style sac.

The ciliated intestinal cells are flattened to cuboidal. Their shape may be variable at any one point due to the eccentricity of the lumen (Fig. 30). The lumen may be round in cross section or irregular, due to cellular pleats. The cells display prominant basal nuclei and a dense granular cytoplasm. Aggregations of reticulated electron lucent material may be observed in the cytoplasm.

The cells of the intestine, style sac, digestive gland, stomach, and to a lesser degree the esophagus, show a variable degree of vacuolation. For example, in some specimens, the observation of histologically clear but electron dense or osmiophilic vacuoles (suggesting lipid) in the intestine and style sac may be the most prominant cytological feature of those organs (Figs. 42, 45). In other specimens, much less vacuolation is pre-

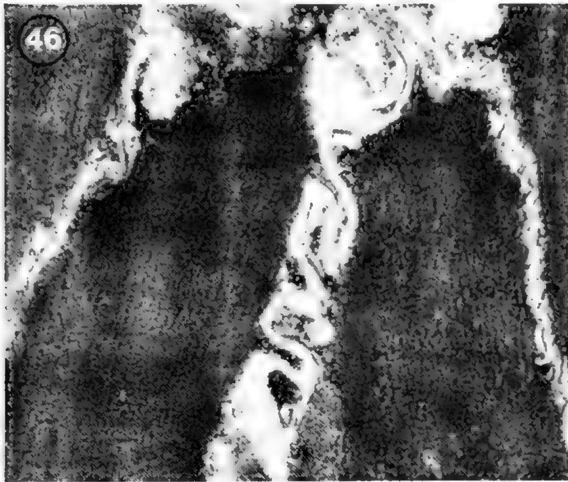


FIGURE 46. *Transmission electron micrograph of adductor muscle cells showing cytoplasmic interdigitations between cells. 30,000×, decalcified at pH 4.5.*

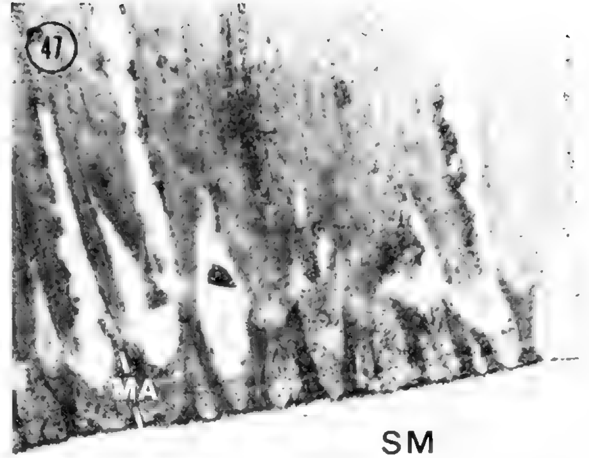


FIGURE 47. *Transmission electron micrograph showing attachment of the posterior adductor muscle to the shell, effected through the specialized myoepithelial cells of the mantle (MA). Note the dense fibrillar bundles within these cells. SM, shell matrix. 11,000×.*

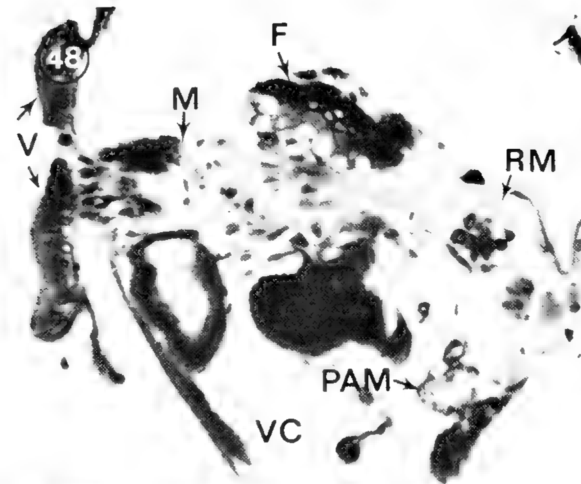
sent. Frequently, the region of the intestine near its origin is the most vacuolated portion of that organ.

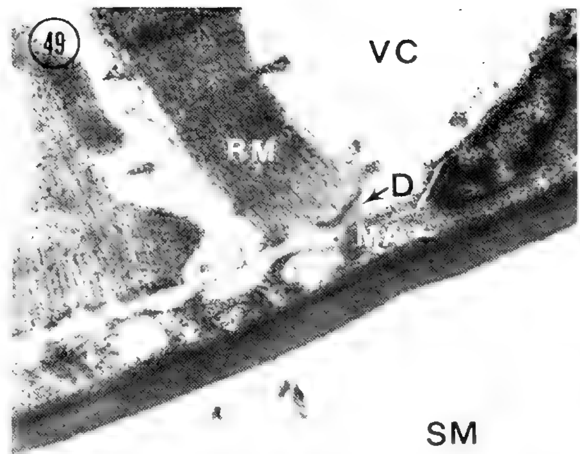
Observation of live larvae reveals some mechanisms of food cycling through the gut, which are similar to those described by Millar (1955) for *Ostrea edulis.* Food particles can be temporarily sequestered within the esophagus. Capture of these particles and their continuous movement through the digestive tract seems to be facilitated by the mucus secretions of the velum and esophagus. Ciliary motion of the style sac creates a continuous rapid swirl of food particles in that organ and in the dorsal portion of the stomach. Currents thus created tend to drive dissolved material (e.g. yellow pigments of algal cells) and fine particulates ventrally into the digestive gland. Apparently, a grinding action created by the rapid swirling of whole and macerated food particles in the style sac and stomach results in mechanical breakdown of ingested material. The simultaneous formation and initial movement of the fecal pellet in the basal groove of the stomach is also a result of the rapid swirling action created by the style sac cilia. This action continues when the velum is retracted. A rapid, spasmodic and continuous expansion and contraction of the digestive gland lobes forces nutrient material into and out of that organ.

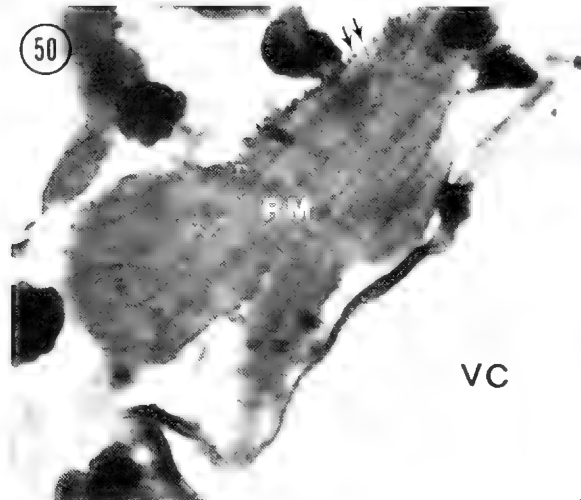
Musculature

The organized musculature of *Crassostrea virginica* larvae, described in part by Stafford (1913) and Galtsoff (1964) consists of the two adductor muscles and four paired groups of striated retractor muscles (Figs. 2, 5).

The anterior adductor develops first in early larvae but by the end of the P-I phase the adductors are of nearly equal size. In larvae measuring 102 μm in shell height each adductor consisted of about 8 muscle fibers or cells as observed in histological transverse section. By comparison, larvae measuring 263 μm in shell height displayed between 35 to 40 fibers in each adductor muscle (Fig. 21). These usually run in a straight course between the valves (Fig. 44), but in some specimens, run in a spiral course. The muscle cells exhibit peripheral nuclei and cytoplasmic interdigitations with adjacent fibers (Fig. 46). At the surface of the valves, fibers split into several components and terminate on an intermediate layer of modified mantle tissue. (Figs. 44, 47). Within this layer of myoepithelial tissue, dense bundles of fibrils travel from the shell to the junctional aspects (Fig. 47).







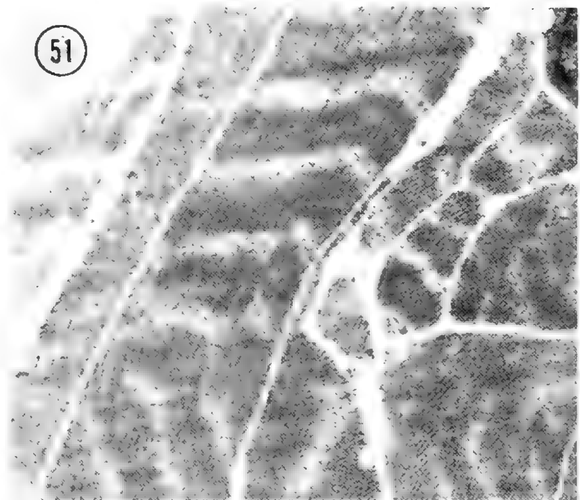


FIGURE 48. *Histological section showing the attachment of one of the pair of posteriormost bundle to retractor muscles (RM) to the foot (F), mouth (M) and velum (V). PAM, posterior adductor muscle; VC, visceral cavity. 300×, trichrome.*

FIGURE 49. *Transmission electron micrograph showing the attachment to the retractor muscles (RM) to the attenuated mantle (MA). Note the dense hemidesmosomes (D). SM, shell matrix. 23,000×, decalcified at pH 4.5.*

FIGURE 50. *Histological section showing striations (arrows) of a retractor muscle (RM). VC, visceral cavity. 1200×, trichrome.*

FIGURE 51. *Transmission electron micrograph showing striations, composed of alternating light and dark bands, of retractor muscles. 8000×, decalcified at pH 4.5.*

Resorption of the anterior adductor muscle occurs at a later stage than those studied here.

The retractor muscles of the P-II larvae studied here consist of four paired groups, all of which insert dorsally on the mantle (Figs. 2,5). Each left or right component of a given pair inserts on its respective mantle lobe. One of the two largest paired groups, having the most posterior dorsal insertion, consists of pedal, oral and velar retractor fibers (Fig. 5, 8, 23, 28, 48). The second large paired group of retractors inserts dorsally close to the first large group but its ventral insertion is primarily on the velum (Figs. 2,5). A third, but smaller, paired group of velar retractors inserts dorsally, but anterior to the first two paired groups. Finally, the fourth paired group, figured

by Galtsoff (1964) and consisting of a single muscle fiber in each group, extends from its dorsal insertion to the posterior visceral cavity membrane (Figs. 2, 5).

The retractor muscles insert dorsally on attenuated areas of mantle tissue at desmosomal processes (Fig. 49). These processes are characterized by a distinctive electron density at the terminal aspect of the retractors. Similar ventral attachments to specific organs can be observed. The fibers of the retractors are striated (Figs. 50, 51) as noted by Galtsoff (1964), and exhibit fine longitudinal fibrils within the dark bands (Fig. 52). The interfibrillar space is packed with electron dense granules in the dark bands. This granularity is absent in the light bands, across which the longitudinal fibrils appear to be continuous (Fig. 52). Nuclei are peripheral.

In addition to these organized muscle groups, isolated fibers and groups of fibers are evident in the mantle (Fig. 20) and in the presumptive rudiments of heart and of gill tissue.

Free cells of the visceral cavity.

The cells described here are found throughout the visceral cavity but may also be observed within channels of the rudimentary vascular system. Two prominant cell types are observed during the P-I and -II phases: (1) phagocytic cells with intracytoplasmic granules and ameboid processes; (2) non-phagocytic cells with few ameboid processes and abundant tubular processes resembling smooth endoplasmic reticulum. In addition, other cells with intermediate characteristics are also observed.

The phagocytic cells attach to all surfaces of the visceral cavity (Figs. 44, 53). Their cytoplasm tends to be acidophilic and they contain spherical electron lucent granules and/or membrane bound vacuoles of phagocytized material such as India ink (Figs. 54, 55).

The relatively large, cuboidal cells tend to accumulate in the umbo region of the visceral cavity (Figs. 44, 56). These nonphagocytic cells contain abundant profiles of smooth endoplasmic reticulum (Fig. 56).

A detailed consideration of functional and morphological aspects of both cell types will be available in another paper (Elston, in press).

Rudimentary organs

The most prominent rudimentary tissue consists

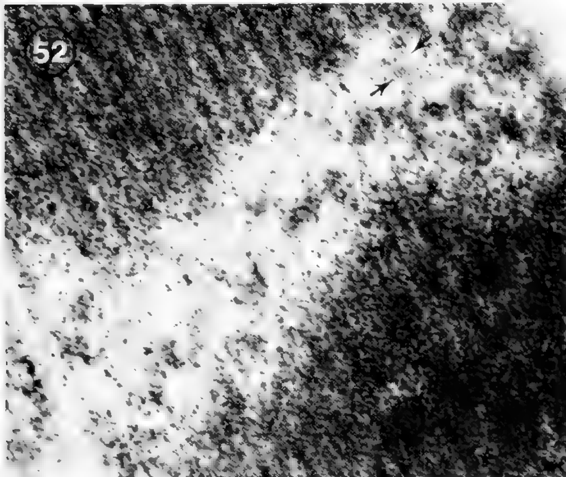


FIGURE 52. *Transmission electron micrograph of retractor muscle showing the longitudinal fibrils (arrows) which are continuous across the light bands. Note the dense granular matrix surrounding these fibrils in the dark bands. 64,000×, decalcified by chelation, pH 7.4.*

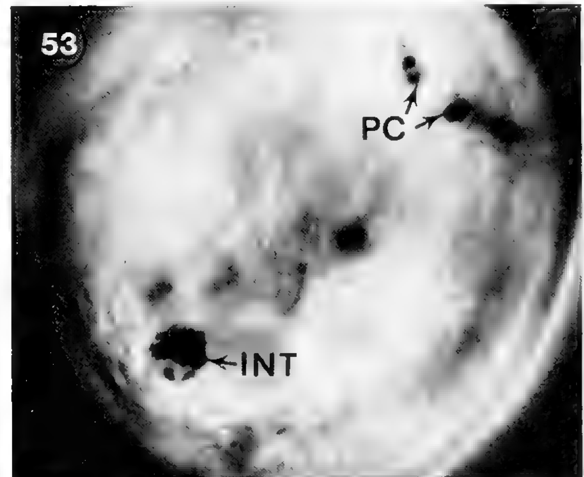
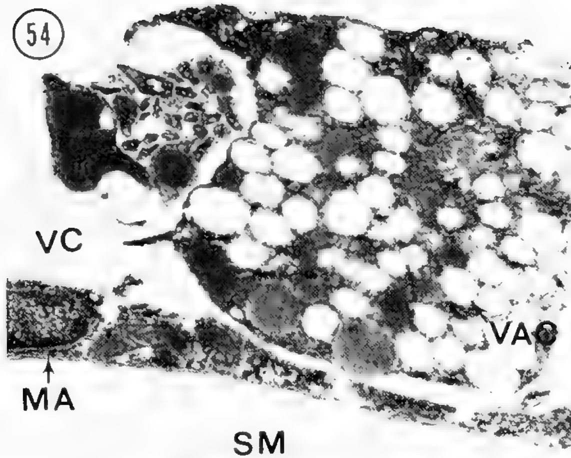
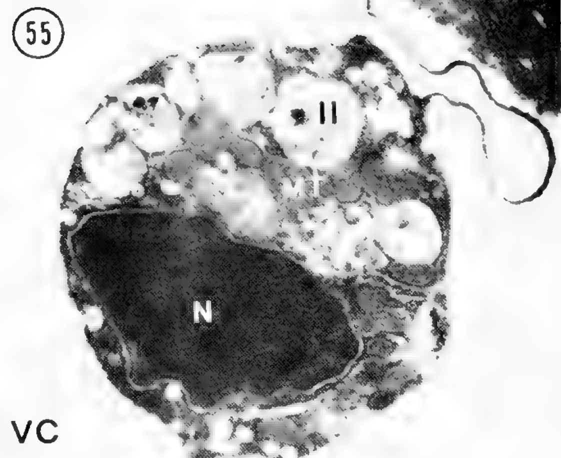
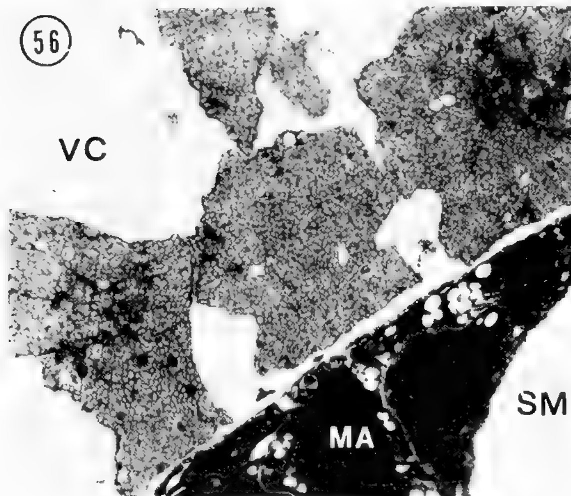


FIGURE 53. *Photomicrograph of whole larval oyster showing uptake of India ink particles by phagocytic cells (PC) within the visceral cavity. Note also the India ink within the intestine (INT). 400×.*







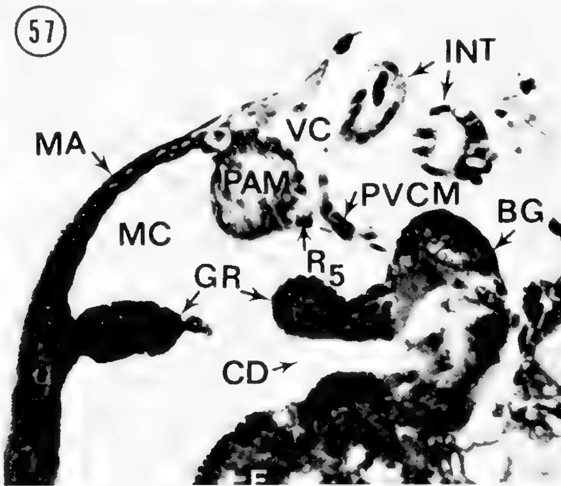


FIGURE 54. *Transmission electron micrograph of phagocytic cell engulfing cellular debris. Note the membrane bound vacuoles (VAC). MA, mantle; SM, shell matrix; VC, visceral cavity. 9000×, decalcified at pH 7.4.*

FIGURE 55. *Transmission electron micrograph of phagocytic cell within the visceral cavity (VC) showing India ink (II) particles within membrane bound vacuoles. N, nucleus; MT, mitochondria. 10,000×, decalcified at pH 4.5.*

FIGURE 56. *Transmission electron micrograph of non-phagocytic free cells of the visceral cavity (VC) showing the abundant intracellular smooth endoplasmic reticulum. MA, thickened mantle of umbo region; SM, shell matrix. 4000×, decalcified at pH 4.5.*

FIGURE 57. *Parasaggital histological section through posterior dorsal aspect of prodissoconch II larvae showing the ciliated duct (CD) of the foot (F) and associated gill rudiment (GR). BG, byssal gland; INT, intestine containing India ink particles; MA, mantle; MC, mantle cavity; PAM, posterior adductor muscle; PVCM, posterior visceral cavity membrane; R_5, rudiment associated with posterior adductor muscle (see test for discussion); VC, visceral cavity. Dorsal is at the top of the figure. 650×, trichrome.*

of the paired gill plates or demibranchs. This tissue is readily identified as gill tissue by its characteristic morphology and location and by the early study of Meissenheimer (1901) who followed the tissue through metamorphosis. Waller (in press) also described this tissue on *Ostrea edulis.*

These gill plates arise laterally as folds of tissue from each mantle lobe and project into the mantle cavity (Figs. 2-5). The gill folds, consisting of typical undifferentiated tissue, extend nearly parallel with the frontal plane from the peripheral mantle to the base of the foot (Figs. 41, 57). As the gill develops in P-II larvae a blind ciliated cavity forms from gill tissue near the base of the foot ("Kiemenhohle" in *Ostrea edulis*, Erdmann, 1935). The paired folds join each other across the mantle cavity during the later stages of development (Figs. 2-5, 58) as described in *Ostrea edulis* by Waller (in press). The rudimentary gill filaments project as nubbins from the paired gill folds into the mantle cavity. Luminal spaces within primordial gill tissues, as well as other vascular tissues including parts of the mantle may contain wandering ameboid cells. The continuity of such vascular channels is difficult to determine in larvae due to small or nonexistent lumina.

A tubular structure, the presumptive forerunner of adult vascular tissue, lies adjacent to and along the axis of the posterior adductor muscle (Figs. 21, 57, 59, labelled R_5). The tube consists of cells with prominant nuclei which extend finger-like processes into the lumen. It is separated from the space of the visceral cavity by the thin enveloping cell layer (Fig. 59). The diameter of this tubular structure is smallest in the midsaggital plane and increases towards its junctures with the right and left mantle lobes. The lumen of this structure appears to be continuous at the mantle with both the gill primordia and an additional pair of thick walled tubular structures lying adjacent to the mantle, (Fig. 59, labelled R_6) posterior adductor muscle and its associated rudiment.

DISCUSSION

The existence and functional significance of the visceral cavity have not been fully appreciated in previous studies. Some previous authors have diagrammed this space in bivalve larvae (Horst, 1884; Lillie, 1895; Meissenheimer, 1901; Erdmann, 1935) but others (Stafford, 1913; Roughley, 1933; Prytherch, 1934; Galtsoff, 1964) either were not clear with regard to its existence or fail to identify it.

The thin posterior visceral cavity membrane, basal velar membrane and especially the peripheral velar membrane, which is adjacent to the circulating currents of the velum, are likely to be important respiratory surfaces in the larva.

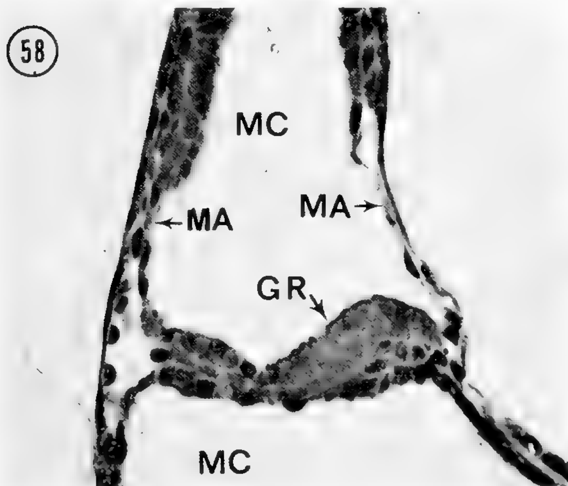


FIGURE 58. *Frontal histological section showing the gill rudiment (GR) which is continuous between the right and left mantle (MA) lobes. MC, mantle cavity. 300×, hematoxylin and eosin.*

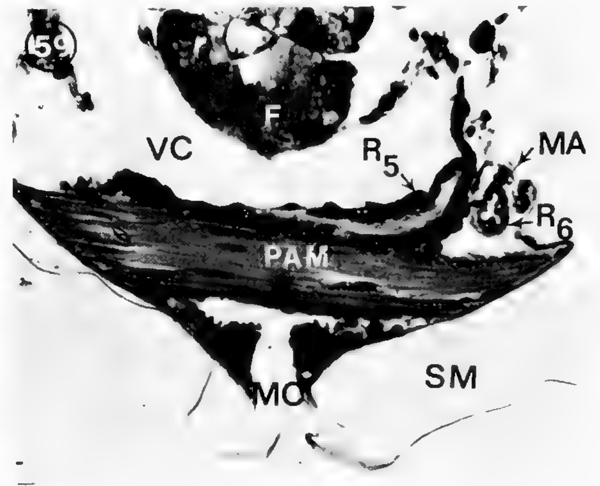


FIGURE 59. *Transverse histological section through dorsal aspect of larva showing two rudimentary tissues (R_5, R_6)associated with the posterior adductor muscle (PAM). F, foot, MA, mantle; MC, mantle cavity; SM, shell matrix; VC, visceral cavity. 650×, azure E and eosin.*

These membranes must also possess the distensibility required for rapid extension and retraction of the velum and visceral mass. In the retracted state, the mantle cavity is nearly non-existent and the visceral cavity consists of only small spaces between the tightly packed organs. In the extended state, however, visceral cavity membranes form the open and spacious mantle and visceral cavities. The volumetric space of the fluid filled visceral cavity must be maintained by displacement of the mantle cavity and digestive organ lumina during visceral retraction. Thus, it must consist of the spaces between organs in the visceral cavity. The accumulation of free cells in the umbonal regions (especially of the left valve) probably results from mechanical action of the retracting organs. The style sac, oriented on the left-hand side of the saggital plane, rests during retraction in the left umbonal region but leaves a relatively large clear space there where the free cells reside. The fate of the visceral cavity in metamorphosis is not clear and calls for further investigation.

The velum has been incorrectly described as attached to the body by a broad stalk (Prytherch, 1934); rather it is attached to the mantle with retractor muscles and the peripheral velar membrane. The central ciliated zone of the velum described here for *Crassostrea virginica* corresponds to the "Scheitelorgan" of *Ostrea edulis* (Erdmann, 1935) and was mentioned by Galtsoff (1964) for *Crassostrea virginica*. These and other authors have suggested a sensory function for this organ. This is a likely function but the underlying cells in the P-II larvae examined in this study appeared to be undifferentiated. While it is likely that further investigation will reveal at least rudiments of peripheral nerves and ganglia, the phenomenon of "neuroid transmission" (Prenerve, cell to cell transmission of impulses) suggested by Carter (1926) for early veliger larvae may well explain the lack of a discernible nervous system.

The food collecting function of the peripheral velum has long been recognized (Yonge, 1926; Erdmann, 1935) but the grooved conformation of the peripheral lobe and its ultrastructural specialization have not been described.

The reticulated electron lucent material at the base of the highly ciliated velar cells likely represents accumulations of energy rich material since these cells must consume large quantities of energy. Its ultrastructural appearance, however, does not conform to the typical appearance of glycogen or lipids.

The membranous attachment of the left and right mantle lobes on the anterior aspect of the larva as described here does not occur in the adult. If, however, metamorphosis proceeds as described by Galtsoff (1964) (i.e. counter-clockwise when viewed from the right) the anterior mantle connection of the larva corresponds approximately to the dorsal-posterior area of mantle connection seen in the adult. This would assume that the mantle rotates during the reorganization process; a seemingly unlikely event considering the peripheral association of the mantle and periostracum. The distinct junctions between the mantle and connecting membranes suggest, alternatively, that it is a temporary structure. The elaboration of periostracum by the peripheral mantle has been described in detail for *Ostrea edulis* larvae by Cranfield (1974). The marginal mantle was incorrectly stated to be free by Stafford (1913). The three zones of granules in each mantle lobe of prodissoconch II larvae may expand peripherally (see Galtsoff, 1964, figure of *Crassostrea virginica* pediveliger) and function during attachment of the pediveliger (see Cranfield, 1974). The existence of mantle cells filled with rough endoplasmic reticulum in the non-peripheral and visceral cavity mantle suggests their secretory role in contrast to Cranfield's (1974) designation of these cells as non-secretory in *O. edulis* larvae.

The transmission electron microscope observation of the looped, continuous periostracum in the hinge region of prodissoconch II larvae confirms the observations of Carriker and Palmer (1979) based on scanning electron microscopy.

Cranfield's (1973) detailed histochemical and ultrastructural study of the foot of the pediveliger of *Ostrea edulis* demonstrated four groups of glands; the base of the foot was occupied by one group, opening into the single byssal duct. The relative structural simplicity of the foot of P-II *Crassostrea virginica* larvae reported here is due largely to the stage of development of specimens studied, the methods of study and possibly, to real species differences.

The central structure near the heel of the foot opening by a single ciliated duct (designated R_1) seems to correspond to the byssal gland and duct identified by Cranfield (1973) and Erdmann (1935) for *Ostrea edulis*. The preliminary observations made in this study, however, suggested no evidence of the presence of secretory material either intra- or extracellularly; in addition, these highly differentiated cells with apical processes and cilia, small basal nuclei and fibrous sheaths, are more like nerve cells as seen at the light microscope level. Prytherch (1934) did not specifically identify this structure but did correctly recognize the paired byssal ducts with their distinctive secretory material. He incorrectly thought that the byssal gland was not part of the foot. These paired ducts and glands roughly correspond to the "C1" complex designated by Cranfield (1973) in *O. edulis*, but he states that this complex consists of long cells emptying ventrally through two very short ducts. Further developmental studies utilizing histochemical and ultrastructural techniques will be required to identify the function of these structures in *Crassostrea virginica* larvae.

The numerous subepidermal glands identified in *O. edulis* pediveligers by Cranfield (1973) are not found in the P-II stage larvae examined in this study. The identity and fate of the other structures noted in the foot in this study should also be regarded as tentative until further studies are made, rather than identified by analogy with similar structures in *O. edulis*.

The digestive system of both larval and adult *Ostrea edulis* has been extensively studied (Yonge, 1926; Erdmann, 1935; Owen, 1955; Millar, 1955). Galtsoff (1964) presented a detailed study of the digestive tract of adult *Crassostrea virginica*. Prytherch (1934), reported the most accurate account of the larval digestive system in *C. virginica* but did not distinguish between the stomach and style sac.

The sphincter-like structure at the esophagus-stomach junction has not been previously described in any bivalve larva. The pigmentation of the esophagus, reported by Yonge (1926) for *Ostrea edulis*, was not observed in this study. In that classic study, Yonge demonstrated the absorption of iron-saccarate by digestive gland cells and its transfer to phagocytes in the larva. Yonge also noted a layer of connective tissue surrounding the digestive gland. Millar (1955), likewise studying *O. edulis* larvae, believed that this was a muscular layer, and was responsible for rhythmic contractions of the digestive gland. A similar layer was observed on all organs of the digestive system in this study but ultrastructural examination demonstrated neither muscular nor connective tissue differentiation. Thus, the mechanism of digestive gland pulsation remains unresolved. Phagocytic cells containing India ink were commonly observed on digestive organ surfaces demonstrating the same process in *Crassostrea virginica* larvae as described by Yonge (1926) for *Ostrea edulis*. In addition, this study has presented morphological evidence for the mechanism of exocytosis-endocytosis in energy transfer. The osmiophilic vacuoles surrounded by coarse electron dense granules at the base of the absorptive cells of the digestive gland suggest lipid vacuoles surrounded by glycogen. The significance of the stellate crystals in clefts between absorptive cells, and in the lumina of digestive organs is not clear, but their presence points to an accessory function for the absorptive cells. Alternatively, these stellate forms may originate from cells basal to the absorptive cells. Secretory cells of the digestive gland, clearly demonstrated here, have apparently not been reported in larval bivalves. Yonge (1926) figured "Crypts of young cells" in the larval digestive gland. Ansell (1962) reported similar cells, designated "fc" in the larval digestive gland of *Venus striatula*. These clearly correspond to the undifferentiated cells of the digestive gland reported here. Millar (1955) notes "ciliated cells" in the digestive gland of *O. edulis* larvae. Yonge (1926) also believed the cells of the larval digestive gland were ciliated. Although cilia were observed in the digestive gland lumina in this study, their origin in digestive gland cells or rootlets within the cells could not be found. This indicates that these may be cilia which extend into the gland from the stomach. Millar's (1955) evidence for the ciliation of digestive gland cells in *O. edulis* larvae is based on observations of live larvae. The origin of the relatively sparse ciliation of the digestive gland observed here merits further study.

It seems likely that food movement is facilitated by visceral extension and retraction. Although digestive gland pulsations are primarily responsible for nutrient movement into the gland, this process may be augmented by the rotating current created by style sac cilia and absorption of materials by the digestive gland cells which probably tends to pull soluble material into the gland, as suggested for adult *Ostrea edulis* by Owen (1955). The absence of a crystalline style in sectioned preparations could result from the decalcification process. Its presence in live larvae is not easily determined without staining (see Yonge, 1926) and no attempt was made here to determine its time of appearance or its prevalence. It should be noted that the crystalline style is an unstable structure in adult oysters.

Galtsoff (1964) states that oyster adductor muscle is nonstriated but other authors (Hanson and Lowy, 1961) suggest that the translucent adductor of adult *Crassostrea angulata* is more properly considered striated. Preliminary observations of muscle ultrastructure made in this study do confirm Galtsoff's (1964) report that clearly striated larval retractor muscle is a specialized tissue differing from adult muscle. In addition, the present study shows the differing mode of shell insertion of the two muscle types. Muscle attachment has been studied in some detail in gastropods (e.g., Tompa and Watabe, 1976; Bonar, 1978) and is generally found to be effected through hemidesmosomes and specialized myoepithelial cells between the muscle and shell. The filamentous electron density of the myoepithelial cells between shell and adductor muscle observed here may represent a similar structural arrangement. In contrast myoepithelial cells underlying retractor muscles do not exhibit the electron density, but rather are attenuated relative to adjacent areas not underlying muscle.

Cranfield (1973) described anterior, posterior and cruciform pedal retractor muscles in *Ostrea edulis* pediveligers. The contrasting simplicity of pedal musculature described in this study for *Crassostrea virginica* may reflect the earlier developmental stages studied. Hickman and Gruffydd (1971) state that retractor attachment is primarily to the right valve in *O. edulis* pediveligers. Prytherch (1934), however, correctly stated that in *C. virginica* equal numbers of retractors pass on both the right and left aspects of the viscera and attach to the respective valves. It is evident that Prytherch was not referring to individual muscle fibers when he stated that the fully developed larva possessed 14 muscle fibers. The observation of oral retractor muscles made in this study is apparently the first such report; it should be noted that the distinction between the adjacent and continuous oral and velar tissue is not well defined.

The character of blood cells in adult bivalves is the subject of much study (see Mix, 1976 for review). The present study has revealed several important new points regarding ameboid cells in larval oysters: cells with phagocytic capacity appear in the P-I stage or earlier. A differentiated free cell, not reported in adult oysters, appears in the larva. The suggested secretory function of this cell and its potential role in modifying the visceral cavity fluids merits further attention. As well, the tissue source of ameboid cells and their ultimate fate, not identified here, deserve investigation.

Previous authors studying bivalve larvae (Meissenheimer, 1901; Erdmann, 1935) have stated that a visceral ganglion, rudimentary heart and pericardium and rudimentary or functioning excretory organ (pronephros) lie near the posterior adductor muscle. Both authors describe a primordial kidney ("urniere") between the foot and digestive gland. Stafford (1913) reported the presence of a visceral ganglion adjacent to the posterior adductor muscle in *Crassostrea virginica* larvae. The tubular structure observed in this study in that location showed no evidence of nervous tissue differentiation. Its structure, its apparent continuity with adjacent lateral structures, and its location with respect to adult organs suggest that this tubular structure is more likely to become part of the vascular system. The adjacent, lateral thick-walled tubule observed in this study is suggestive of rudimentary ventricle of the heart because of its location and morphology.

The designation of rudimentary gill tissue is more certain and has been similarly reported by previous authors such as Stafford (1913), Prytherch (1934), Erdmann (1935), Galtsoff (1964) and Waller (in press). Each fold of gill tissue is reported to exhibit eight papilla in the pediveliger; in addition most authors agree that the left gill fold develops at a faster rate than the right. In con-

trast to other authors, Hickman and Gruffydd (1971) reported that they did not observe gill rudiments in pediveligers of *O. edulis*.

In summary, this report has presented new details regarding the structure and function of the visceral cavity, the foot, the digestive system, the free cells of the visceral cavity, the musculature and rudimentary tissues in larval *Crassostrea virginica*. These findings demonstrate more complex adaptive specializations at the larval stage than previously understood. Possible homologies between *C. virginica* larvae and other bivalve larvae, as well as adult bivalves, present enticing problems with potential phylogenetic significance. For example, the fate of the visceral cavity in the adult, the relationship of the intestine to the visceral cavity and the development of the foot represent fruitful areas for future studies. It is also now important to study the development and metamorphosis of individual organ systems of the larva with appropriate ultrastructural and histochemical methods.

This study should also aid those attempting to culture larval bivalves. Increased demand for oysters and declining natural spawn in some areas (McHugh and Williams, 1976) has resulted in the refinement of a complex hatchery technology for rearing larval oysters (e.g. see Loosanoff and Davis, 1963; Breese and Malouf, 1976). However, serious problems in hatchery operation, most notably those related to disease outbreaks, still occur. A thorough understanding of the oyster larva by hatchery operators is their most valuable tool for the early recognition of disease.

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LITERATURE CITED

Ansell, A.D. 1962. The functional morphology of the larva, and the post-larval development of *Venus striatula* (Da Costa). J. Marine Biological Association of the United Kingdom. **42**:419-443.

Bonar, D.B. 1978. Fine structure of muscle insertions on the larval shell and operculum of the nudibranch *Phestilla sibogae* (Mollusca: Gastropods) before and during metamorphosis. Tissue and Cell **10**:143-152.

Bonar, D.B. and M.G. Hadfield. 1974. Metamorphosis of the marine gastropod *Phestila sibogae* Bergh (Nudibranchia: Aeolidacea). I. Light and electron microscopic analysis of larval and metamorphic stages. J. Marine Biol. Ecol. **16**:227-255.

Breese, W.P. and R.E. Malouf. 1975. Hatchery manual for the Pacific oyster. Oregon State University Sea Grant College Program. Pub. No. ORESU-H-75-002. 23p.

Carriker, M.R. and R.E. Palmer. 1979. Ultrastructural morphogenesis of prodissoconch and early dissoconch valves of the oyster *Crassostrea virginica*. Proc. Nat. Shellfish. Assoc. **69**:103-128.

Carter, G.S. 1926. On the nervous control of the velar cilia of the nudibranch veliger. J. Exper. Biol. **4**:1-26.

Cloney, R.A. 1972. Cytoplasmic filaments and morphogenesis: effects of cytochalasin B on contractile epidermal cells. Z. Zellforsch. mikrosk. Anat. Bd. **132.** S. 167-216.

Cole, H.A. 1938. The fate of the larval organs in the metamorphosis of *Ostrea edulis*. J. Mar. Biol. Assoc. **22**:469-484.

Cranfield, H.J. 1973. A study of the morphology, ultrastructure, and histochemistry of the foot of the pediveliger of *Ostrea edulis*. Marine Biol. **22**:187-202.

Cranfield, H.J. 1974. Observation on the morphology of the mantle folds of the pediveliger of *Ostrea edulis* L. and their function during settlement. J. Marine Biol. Assoc. U.K. **54**:1-12.

Elston, R. 1979a. Virus-like particles associated with lesions in larval Pacific oysters, *Crassostrea gigas*. J. Invertebr. Pathol. **33**:71-74.

Elston, R. 1979b. Economically important larval bivalve diseases and their control. Revista Italia di Piscicoltura e Ittiopatologia **14**(2):47-54.

Elston, R. 1980. New ultrastructural aspects of a serious disease of hatchery reared larval oysters, *Crassostrea gigas*. J. Fish Dis. **3**:1-10.

Elston, R. in press. Functional morphology of the coelomocytes of the larval oysters (*Crassostrea virginica* and *Crassostrea gigas*). J. Mar. Biol. Assoc. U.K.

Erdmann, W. 1935. Untersuchungen uber die Lebensgeschichte der Auster. Nr. 5. Uber die Entwicklung und die Anatomie der "ansatzreifen" Larve von *Ostrea edulis* mit Bermerkungen uber die Lebensgeschichte der Auster. Wiss. Meeresunters., Abt. Helgoland. Bd. **19**:1-25.

Farley, C.A. 1968. Probable neoplastic disease of the hematopoietic system in oysters, *Crassostrea virginica* and *Crassostrea gigas*. *In* Neoplasms and Related Disorders of Invertebrate and Lower Vertebrate Animals. pp. 541-555. National Cancer Institute Monograph. 31. U.S. Government Printing Office, Washington, D.C.

Fedder, N. and T.P. O'Brien. 1968. Plant technique: some principles and new methods. Amer. J. Botany **55**:123-142.

Galtsoff, P.S. 1964. The American oyster. U.S. Fisheries Bull. Fish and Wild. Serv. **64**:1-480.

Hanson, J. and J. Lowy. 1961. The structure of the muscle fibers in the translucent part of the adductor of the oyster *Crassostrea angulata*. Proc. Roy. Soc. Lond. B **154**:173-196.

Hickman, R.W. and L.L.D. Gruffydd. 1971. The histology of the larva of *Ostrea edulis* during metamorphosis. In: Fourth European Marine Biology Symposium 1969. pp. 281-294. Cambridge Univ. Press.

Horst, R. 1884. The development of the oyster *(Ostrea edulis* L.) Rep. of the Commissioner for 1884, U.S. Comm. on Fish and Fisheries, Part XII, pp. 891-911.

Kniprath, E. 1977. Zur ontogenese des schalenfeldes von *Lymnaea stagnalis*. Wilhelm Rous's Archives of Developmental Biology. **181**:11-30.

Leibovitz, L., R. Elston, V.P. Lipovsky and J. Donaldson. 1978. A new disease of larval Pacific oysters *(Crassostrea gigas)*. Proc. Ninth Ann. Meet. World Maricult. Soc. pp. 603-615.

Lillie, F.R. 1895. The embryology of the Unionidae. J. Morphol. **10**:1-100.

Loosanoff, V.L. and H.C. Davis. 1963. Rearing of bivalve molluscs. Advances Marine Biol. 1:2-136.

McHugh, J.L. and A.D. Williams. 1976. Historical statistics of the fisheries of the New York bight area. New York Sea Grant Inst. NYSSGP-RS-76-013. Albany, New York.

Meissenheimer, J. 1901. Entwicklungsgeschichte von *Dreissensia polymorpha* Pall. Z. Wissensch. Zool. **69**:1-137.

Millar, R.H. 1955. Notes on the mechanism of food movement in the gut of the larval oyster, *Ostrea edulis*. Quart. J. Micros. Sci. **96**:539-544.

Mix, M.C. 1976. A general model for leukocyte cell renewal in bivalve molluscs. Marine Fish. Rev. **38**(10):37-41.

Owen, G. 1955. Observations on the stomach and digestive diverticula of the Lamellibranchia. I. Anisomyaria and Eulamellabrachia. Quart. J. Miscoscop. Sci. **96**:517-537.

Prytherch, H.F. 1934. The role of copper in the setting, metamorphosis, and distribution of the American oyster, *Ostrea virginica*. Ecol. Monogr. **4**:47-109.

Roughley, T.C. 1933. The life history of the Australian oyster *(Ostrea commercialis)*. Proc. Linn. Soc. New South Wales. **58**:279-333.

Spurr, A.R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastructure Research **26**:31-43.

Stafford, J. 1905. On the larva and spat of the Canadian oyster. Amer. Naturalist. **39**:41-44.

Stafford, J. 1913. The Canadian Oyster. Comm. of Conserv., Canada. Mortimer Co. Ltd. Ottawa.

Thompson, S.W. 1966. Selected histochemical

and histopathological methods. 1639 p. Charles C. Thomas, Springfield, Illinois.

Tompa, A.S. and N. Watabe. 1976. Ultrastructural investigation of the mechanism of muscle attachment to the gastropod shell. J. Morphology **149**:339-352.

Waller, T.R. In press. Functional morphology and development of veliger larvae of the European oyster, *Ostrea edulis* Linne. Smithsonian Contributions to Zoology. No. 328.

Yonge, C.M. 1926. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. J. Mar. Biol. Assoc. **14**:295-386.

Yonge, C.M. 1957. Mantle fusion in the Lamellibranchia. Publicazioni della Stazione Zoologica di Napoli **29**:151-171.

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INFECTIONS OF *TYLOCEPHALUM* METACESTODES IN COMMERCIAL OYSTERS AND THREE PREDACEOUS GASTROPODS OF THE EASTERN GULF OF MEXICO

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ABSTRACT

Acudate glando-procercoids (metacestodes) of Tylocephalum *sp.* sensu *Sparks (1963*b*) (Cestoda; Cephalobothriidae) are reported from the American oyster,* Crassostrea virginica *(Gmelin) (Bivalvia; Ostriedae), and three molluscivorous gastropods in the eastern Gulf of Mexico: the lightning whelk,* Busycon contrarium *(Conrad) (Gastropoda; Melongenidae), the apple murex,* Murex pomum *Gmelin (Muricidae), and the southern oyster drill,* Thais haemastoma canaliculata *(Gray) (Muricidae). Sixty of 138 oysters (43%) from 12 of 17 localities, 79 of 90 whelks (88%) from 14 of 15 localities, 32 of 33 murexes (97%) from 6 of 6 localities, and 23 of 53 drills (43%) from 5 of 8 localities harbored encysted* Tylocephalum *metacestodes. These predaceous gastropods may acquire* Tylocephalum *from oysters and other infected bivalves. No pathological conditions were observed in oysters or their predators.*

INTRODUCTION

Acudate glando-procercoids of one or more unidentified species of *Tylocephalum* (Figure 1) are common parasites of marine mollusks of the eastern Gulf of Mexico and at least two species of *Tylocephalum* occur in molluscivorous, myliobatid stingrays in the Gulf. Cake (1975, 1976, 1978) found metacestodes in 49 of 92 species of Gulf coast mollusks including the American oyster, *Crassostrea virginica,* the lightning whelk, *Busycon contrarium,* the apple murex, *Murex pomum,* and the southern oyster drill, *Thais haemastoma canaliculata.* Burton (1963) described metacestodes of *Tylocephalum* from oysters from Apalachicola Bay, Florida, that had been transplanted into Chincoteague Bay, Virginia, and Quick (1971) reported their presence in oysters at several other localities along the west coast of Florida. Sparks (1963*a*, 1963*b*) and Cheng (1966) reported *Tylocephalum* in *C. virginica* from Hawaii.

Sakaguchi (1973) reported metacestodes of an unidentified species of *Tylocephalum* from four gastropods including two predaceous muricids, *Chicoreus asianus* Kuroda and *Reishia (= Thais) clavigera* (Küster), and from six bivalves including

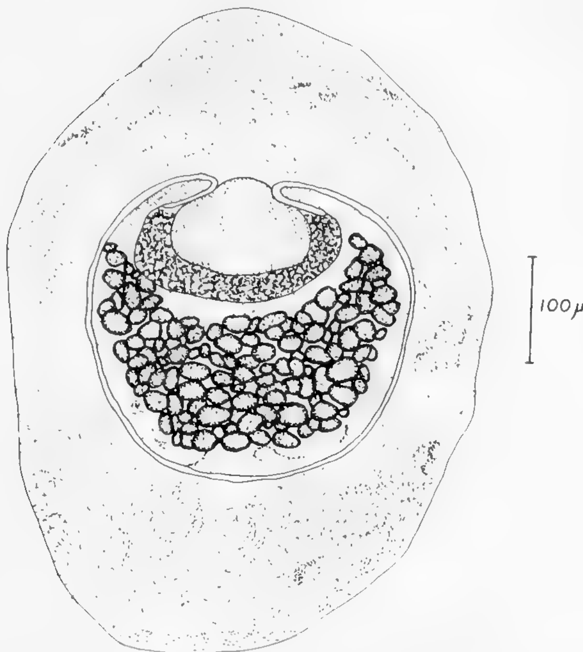

FIGURE 1. *Encysted, acudate glando-procercoid of* Tylocephalum *sp. from the stomach wall of* Crassostrea virginica *(Gmelin).*

the giant Pacific oyster, *Crassostrea gigas* (Thunberg), and the pearl oyster, *Pinctada fucata* (Gould) in Tanabe Bay, Wakayama prefecture, Japan. Sakaguchi "successfully" infected the molluscivorous cat shark ("Nekozame"), *Heterodontus japonicus* (Domeril), with *Tylocephalum* metacestodes from pearl oysters. The metacestodes excysted in the shark's stomach within 24 hours and migrated to the spiral valve within seven days after infection. They remained in the spiral valve but exhibited no maturation during the brief experiment.

Sakaguchi (1973) collected infected oysters and muricid drills from Tanabe Bay, Wakayama prefecture, but did not indicate if they occupied the same habitat. He reported, however, that all of the oysters and muricids examined from that bay were infected with *Tylocephalum* sp.

Tylocephalum metacestodes also infect other edible bivalves and predaceous gastropods. Cake (1976, 1978) found *Tylocephalum* sp. in a total of 33 bivalves (27 "edible") and 16 gastropods (13 predaceous) in the eastern Gulf of Mexico (see also Sakagucki, 1973; and Table 1).

Encysted metacestodes (Figure 1) are generally restricted to the vesicular connective tissue of the digestive gland and the stomach wall of infected mollusks, but infrequently occur in the foot, gills and labial palps of pelecypods, while those in gastropods are generally restricted to the digestive gland (Cake, 1975). Cheng (1966) reported what he believed were infective, ciliated "coracidia" (with penetration glands) intimately associated with gill surfaces and in the stomach of *Tylocephalum*-infected oysters. Wolf (1976) also reported the presence of *Tylocephalum* "coracidia" in the labial palp region of Sydney rock oysters, *Saccostrea (= Crassostrea) commercialis* (Iredale and Roughley), from northern New South Wales and southern Queensland, Australia.

Linton (1916) described adults of *T. marsupium* from the spotted eagle ray, *Aetobatis narinari* (Euphrasen), at Dry Tortugas, Florida, and Tom Mattis (GCRL, Parasitology Section, personal communication) reported adults of *T. pingue* Linton from the cow-nosed ray, *Rhinoptera bonasus* (Mitchill), in East Bay, Panama City, Florida, and from Mississippi Sound. Both of those ray species are well-known mollusk predators (Bigelow and Schroeder, 1953) and *R. bonasus* is a well-known predator of *C. virginica* (Merriner and Smith, 1979).

The predator-prey relationship between the lightning whelk and oysters is well documented (Magelhaes, 1948; Menzel and Nichy, 1958; and Paine, 1962) as is the relationship between the apple murex and oysters (Menzel and Nichy, 1958) and between the southern oyster drill and oysters (Butler, 1953; Chapman, 1958; Menzel et al., 1966; Gunter, 1968; McGraw and Gunter, 1972).

Bivalves and molluscivorous gastropods probably serve as intermediate or paratenic hosts in the life cycle of *Tylocephalum* sp. (Jameson, 1912; Sakaguchi, 1973; Cake, 1975). This report presents infection data that suggest, circumstantially, a transfer of metacestodes from oysters to their predatory gastropods.

The descriptive metacestode term, acudate glando-procercoid, used herein follows the convention of Freeman (1973).

MATERIALS AND METHODS

Oysters and predaceous gastropods were collected from intertidal and shallow, subtidal

TABLE 1. *Synoptic review of bivalve hosts of* Tylocephalum *spp.*

SPECIES	BIVALVE HOST	LOCATION	LOCALITY	REFERENCE
Tylocephalum ludificans Jameson	Pinctada (= *Margaritifera*) *vulgaris* (Schumacher)	Visceral mass, and elsewhere, encysted	Gulf of Mannar and Tricomalee, Sri Lanka (Ceylon)	Herdman & Hornell, 1903 Southwell, 1924 Willey, 1909
T. margaritifera Seurat	*P.* (= *M.*) *margaritifera cumingi* (Reeve)	Throughout body, encysted	Gambier Islands	Seurat, 1906
T. minus Jameson	*P. vulgaris*	Throughout body, encysted	Gulf of Mannar, Sri Lanka (Ceylon)	Shipley & Hornell, 1904
Tylocephalum sp.	*Arca rhombea* Born	Digestive gland, encysted	Sambore River, Sri Lanka (Ceylon)	Willey, 1907
Tylocephalum sp.	*Argopecten* (= *Aequipecten*) *irradians* (Say)	Stomach walls and gills, encysted	Beaufort, North Carolina	Gutsell, 1930
Tylocephalum sp.	*Chamys nobilis* (Reeve)	Digestive gland, encysted	Tanabe Bay, Wakayama pref., Japan	Sakaguchi, 1973
Tylocephalum sp.	*Crassostrea gigas* (Thunberg)	Gills, encysted	Coasts of Hong Kong and Peoples' Republic of China	Cheng, 1975
Tyocephalum sp.	*C. gigas*	Digestive gland, encysted	Tanabe Bay, Wakayama pref., Japan	Sakaguchi, 1973
Tylocephalum sp.	*C. madrasensis* (Preston)	Gills, encysted	Mulki, State of Karnataka, India	Stephen, 1978
Tylocephalum sp.	*C. virginica* (Gmelin)	(Unknown)	(Unknown)	Tennet, 1906

TABLE 1. *Continued*

Tylocephalum sp.	*C. virginica*	Gills, gut epithelium, palps, encysted	Apalachicola Bay, Florida	Burton, 1963
Tylocephalum sp.	*C. Virginica*	Digestive diverticula, encysted	West Loch, Pearl Harbor, Hawaii	Sparks, 1963 *a*, *b*
Tylocephalum sp.	*C. virginica*	Gills and stomach (coracidia); gut wall, digestive gland, encysted	West Loch, Pearl Harbor, Hawaii	Cheng, 1966
Tylocephalum sp.	*C. virginica*	Connective, leydig tissues, encysted	Choctawhatchee and Tampa Bays, Florida	Quick, 1971
Tylocephalum sp.	*C. virginica.*	(Unknown)	Coasts of Georgia and North Carolina	Sindermann and Rosenfield, 1968
Tylocephalum sp. (= *Monobothrium)*	*Meleagrina occa* Reeve & *M. irradians* Reeve	Digestive gland	Nossi-Be, Madagascar	Seurat, 1906
Tylocephalum sp.	*Mytilus edulis* Linné	Digestive gland, encysted	Tanabe Bay, Wakayama pref., Japan	Sakaguchi, 1973
Tyocephalum sp.	*Placuna placenta* Linné	Digestive gland, encysted	Lake Tampalakaman, Tuticorin, Sri Lanka (Ceylon)	Hornell, 1905
Tylocephalum sp.	*P. placenta*	Digestive gland, encysted	Sambore River and Lake Tamblegam, Sri Lanka (Ceylon)	Willey, 1907
Tylocephalum sp.	*Pinctada fucata* (Gould)	Digestive gland, encysted	Tanabe Bay, Wakayama pref., Ago Bay, Mie pref., Japan	Sakaguchi, 1973
Tylocephalum sp.	*P. vulgaris*	(Unknown)	Eniwetok Atoll	Cheng & Rifkin, 1970

TABLE 1. *Continued*

Tylocephalum sp.	*Pinna bicolor* Gmelin (= *P. attenuata* Reeve)	Digestive gland, encysted	Tanabe Bay, Wakayama pref., Japan	Sakaguchi, 1973
Tylocephalum sp.	*Saccostrea* (= *Crassostrea*) *commercalis* (Iredale & Roughley)	Gills and palps, (coracidia); digestive gland, encysted	Northern New South Wales & southern Queensland, Australia	Wolf, 1976
Tylocephalum sp.	*S.* (= *C.*) *echinata* (Quoy & Gaimard)	Digestive diverticula, encysted	Northern Territory, Australia	Wolf, 1976
Tylocephalum sp.	*Spondylus barbatus* Reeve	Digestive gland, encysted	Tanabe Bay, Wakayama pref., Japan	Sakaguchi, 1973
Tylocephalum sp.	*Tapes philippinarum* (= *T. semidescusata*) (Adams & Reeve)	Digestive diverticula, encysted	Kaneohe Bay, Oahu, Hawaii	Cheng & Rifkin, 1968
Tylocephalum sp.	*Venus* spp.	Digestive gland, encysted	Sambore River, Sri Lanka (Ceylon)	Willey, 1907

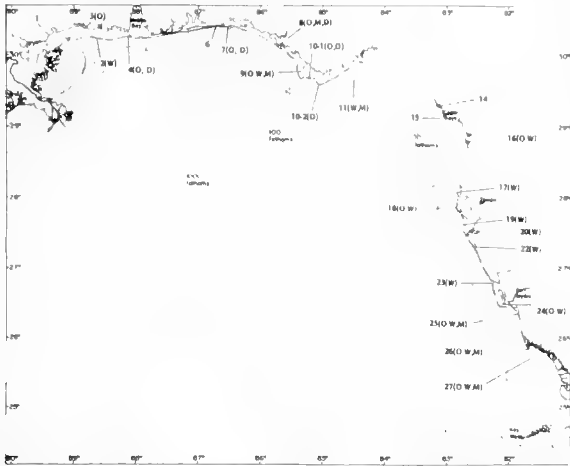

FIGURE 2. *Gulf of Mexico localities where* Tylocephalum *metacestodes occurred in oysters (O), lightning whelks (W), apple murexes (M), and oyster drills (D).*

habitats at 24 localities between St. Louis Bay, Mississippi, and the Ten Thousand Islands of south Florida (Figure 2), and maintained alive in Styrofoam® tanks of aerated seawater until examined. During necropsy the following tissues and locations were examined with the aid of a dissecting microscope: (in oysters) gills, labial palps, stomach and stomach wall, and digestive diverticula; (in gastropods) lumen of stomach and digestive gland (exterior).

Accurate quantification of infection intensities (number of metacestodes per host) in oysters and predaceous gastropods was not practical during this investigation. Because of the small size of the *Tylocephalum* metacestodes (length, 144 to 340 μ) and the nature of the digestive gland tissues, exact counts would have required serial sectioning and light microscopic examination of those tissues and locations where metacestodes would occur if the mollusk was infected. Intensities were based, therefore, on the examination of fresh tissues and we believe that the infection data reported herein are valid for comparison of the four hosts under consideration.

RESULTS

Infected oysters occurred at 12 of 17 localities throughout the eastern Gulf of Mexico, where 60 of 138 oysters (44%) contained an average of 15.8 metacestodes. Infected lightning whelks occurred at 14 of 15 localities, all but two of which were east of the Apalachicola River, where 79 of 90 whelks (88%) contained an average of 10.3 metacestodes. Infected apple murexes occurred at 6 of 6 localities, three each in the northeastern and southeastern Gulf, where 32 of 33 murexes (97%) contained an average of 22.1 metacestodes. Infected oyster drills occurred at 5 of 8 localities, all but one of which were west of the Apalachicola River, where 23 of 53 drills (43%) contained an average of 14.0 metacestodes. The infection data are summarized in Table 2.

Oysters and drills were collected from the same oyster reef habitat at four localities west of the Apalachicola River (Stations 4, 6, 7 and 10-1; Figure 2). Both species were infected with *Tylocephalum* at Station 4, 6 and 10-1; neither were infected at Station 7. Infected oysters and whelks were collected from the same reef habitat at Station 18. Infected oysters, whelks, and murexes were collected from the same reef habitat at four localities in southern Florida (Stations 24, 25, 26 and 27).

The relative incidence (percent infected) and intensity (number per host) of infections of *Tylocephalum* in oysters and predaceous gastropods were similar at most stations, especially where the mollusks occurred together in the same reef habitat. At those localities where oysters were uninfected, but whelks were infected (Stations 22 and 23), the two species did not occupy the same habitat. The whelks were foraging on other bivalves, many of which were infected with *Tylocephalum* sp. (Cake, 1976).

Of the three gastropods, *Murex pomum* exhibited the highest infection incidence (97%) and intensity (22.1 metacestodes per murex). The infection incidences and intensities for oysters and drills were similar (44 vs. 43% and 15.8 vs. 14.0 metacestodes per mollusk, respectively) but the data base was limited. All predatory gastropods examined were infected at six localities where they were foraging on infected oysters (Stations 10-1, 18, 24, 25, 26, and 27).

All infected mollusks appeared to be otherwise healthy (i.e., none were weak or moribund, or exhibited significant loss of body volume and weight). In no case was the digestive tract blocked by massive infections as was observed by one of us (EWC) in the case of encysted plerocercoids of

TABLE 2. *Infection data summary from four molluscan hosts of* Tylocephalum *sp.* (sensu *sparks*) *in the eastern Gulf of Mexico.*

STA NO	LOCALITIES	*CRASSOSTREA VIRGINICA*	*BUSYCON CONTRARIUM*	*MUREX POMUM*	*THAIS HAEMASTOMA CANALICULATA*	REMARKS
1	Bay St. Louis, MS	0/10				
2	Horn Island, MS		2/5; 15*			
3	Deer Island & Davis Bayou, MS	1/15; 1				
4	Dauphin Island, AL	1/15; 1			4/4; 65	*C.& T.* together
6	Santa Rosa Sound, Navarre, FL	0/10			0/5	*C. & T.* together
7	Choctawhatchee Bay, Destin, FL	10/10; 128			4/10; 34	*C. & T.* together
8	Grand Lagoon, St. Andrew Bay (entrance), FL	10/10; 32		10/10; 97	2/10; 4	
9	Eagle Harbor & Black's Island, St. Joseph Bay, FL	5/10; 18	5/6; 22	4/5;7		
10-1	Indian Lagoon, Apalachicola Bay, FL	10/10; 570			10/10; 163	*C.& T.* together
10-2	Cape St. George, FL				3/3; 55	
11	Alligator Harbor & St. Teresa Beach, FL		20/23; 108	3/3; 115	0/10	
14	Suwannee River (entrance), FL	0/3				
15	Seahorse Key, Cedar Key, FL		0/1			
16	Crystal River (power plant canals), FL	2/5; 5	2/3; 4		0/1	
17	North Anclote Key, FL		5/6; 45			
18	Clearwater (Pass), FL	4/6; 14	3/3; 15			*C. & B.* together
19	Mullet and Mandelaine Keys, Boca Cicga Bay, FL		3/3; 75			
20	Snead Island, Tampa Bay, FL		1/1; 1			
22	Big Sarasota (Bay) Pass, FL	0/5	2/3; 35			

TABLE 2. *Continued*

23	Gasparilla Sound, Placida, FL	0/5	4/4, 60			
24	York Island, Pine Island Sound, FL	3/5; 3	10/10; 97			*C.* & *B.* together
25	Estero Bay & Big Carlos Pass, FL	3/5; 55	7/7; 135	10/10; 410		*C.*, *B.* & *M* together
26	Cape Romano Shoals, FL	3/4; 57	10/10; 110	2/2; 30		*C.*, *B.* & *M.* together
27	Indian Key & Chokoloskee Bay (Everglades City), FL	8/10; 66	5/5; 90	3/3; 47		*C.*, *B.* & *M.* together
	TOTALS	60/138; 950*	79/90; 812*	32/33; 706*	23/53; 321*	
	INCIDENCE OF INFECTION (%)	43.5	87.8	97.0	43.4	
	MEAN INFECTION INTENSITY (# / Infected Host)	15.8	10.3	22.1	14.0	
	MAXIMUM INFECTION INTENSITY	125+	25+	75+	50+	
	MOLLUSK SIZE RANGE (mm)	50-110	73-290	43-80	40-70	

* [# Infected Hosts / # Mollusks Examined (at that station); # *Metacestodes*]

Parachristianella sp. *sensu* Cake (1976) which completely blocked the intestine of a heavily infected sunray venus clam, *Macrocallista nimbosa* (Lightfoot).

DISCUSSION

We believe that predaceous gastropods become infected with metacestodes of *Tylocephalum* by ingesting infected oyster tissues. We are unaware of any biochemical, physical, or physiological factors that would prevent the transfer of infective metacestodes from oysters to gastropods during feeding. The metacestodes are small enough to be ingested without destruction during the rasping of the gastropod's radula. The gastropod's gut environment is probably suitable physiologically for at least a short time since tetraphyllidean plerocercoids are common gut parasites of molluscivorous gastropods in the Gulf of Mexico (including *B. contrarium, M. pomum,* and *T.h. canaliculata*) (Wardle, 1974; Cake, 1976). Once liberated from the oyster tissues in the gastropod gut, the metacestodes could migrate to other regions including the digestive gland via the diverticula. Since the cyst that surrounds the metacestodes in both the oyster and the gastropod is of host-origin, the metacestodes are probably subject to reencystment in the gastropod host. The presence of metacestodes in the digestive gland of the gastropod, instead of in the lumen of the gut (or gut epithelium as in the oyster), indicates definite site-selection in the gastropod; however, Wardle (1974) found similar metacestodes free in the gut of *T. haemastoma* (= *T.h. canaliculata*). The presence of metacestodes in the digestive tract of predaceous gastropods also suggests that the infection occurred during feeding rather than from a penetration of the external surface by the metacestodes or earlier infective stages.

Cheng (1966) and Wolf (1976) observed small, ciliated, multicellular organisms in the gut and associated with the gills of oysters that were infected with encysted metacestodes of *Tylocephalum.* They concluded that the organisms were "coracidia" (free-swimming stages which hatch from aquatic cestode eggs and which are precursors of metacestodes). The possibility that the infected gastropods in this investigation acquired infections of *Tylocephalum* via gill-penetration of "coracidia" as suggested by Cheng (1966) was not considered plausible primarily because no coracidial stage has yet been demonstrated in the ontogeny of any known lecanicephalidean cestode. Cheng's "coracidia" were five to seven times larger than those known from species of marine pseudophyllidean and tetrarhynchidean cestodes. Cheng's "coracidia" also lacked the larval hooks that are typical of the coracidia of other marine cestodes, and the ciliated epithelium illustrated by Cheng appears to be part of an epidermis rather than a ciliated embryophore that surrounds the oncosphere of a true coracidium (Rybicka, 1966). Cheng's observations notwithstanding, no penetration glands have been observed in marine coracidia (Rybicka, 1966; Tom Mattis, GCRL Parasitology Section, personal communication). Cheng's "coracidia" are probably not the precursors of the metacestodes of *Tylocephalum,* but are perhaps advanced metazoan larvae of another oyster symbiont.

If no coracidium exists in the ontogeny of *Tylocephalum,* filter-feeding mollusks such as *C. virginica* may become infected by ingesting planktonic or demersal eggs that contain oncospheres. We cannot, however, entirely eliminate the possibility that oysters may ingest the remains of small, infected, first intermediate hosts such as copepods. That pathway appears unlikely, however, since oysters "select" food particles within the size range of approximately 1 to 10 μ. The smallest eggs of most marine cestodes are somewhat larger than 10 μ but may be ingested infrequently. The physical action of the oyster's gastric mill and the biochemical action of the gut enzymes may permit the oncosphere to escape from the egg and penetrate the stomach wall. The ingested-egg pathway may also account for the *Tylocephalum* infections that Cake (1976) observed in three species of filter-feeding gastropods of the genus *Crepidula* in the eastern Gulf of Mexico.

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LITERATURE CITED

Bigelow, H.B., and W.C. Schroeder. 1953. Fishes of the western North Atlantic: II. Sawfishes, guitarfishes, skates and rays. Mem. Sears. Found. Mar. Res. (Yale Univ., New Haven, Conn.); no. **1**:1-514.

Burton, R.W. 1963. Report on a cestode microparasite of the oyster, *Crassostrea virginica*, in Florida. Shellfish Mortality Conference. Jan. 29-30, 1963. Oxford, Maryland. 6 p. (Mimeo.)

Butler, P.A. 1953. The southern oyster drill. Proc. Natl. Shellfish. Assoc. **44**:67-75.

Cake, E.W., Jr. 1975. Larval and postlarval cestode parasites of shallow-water, benthic mollusks of the Gulf of Mexico from the Florida Keys to the Mississippi Sound. Tallahassee, FL: Florida State University. Available from: University Microfilms, Ann Arbor, MI; Publ. no. 75-15,488. 434 p. Dissertation. (Diss. Abstr. **36**:40-41B).

Cake, E.W., Jr. 1976. A key to larval cestodes of shallow-water, benthic mollusks of the northern Gulf of Mexico. Helminthol. Soc. Wash., Proc. **43**(2):160-171.

Cake, E.W., Jr. 1978. Larval cestode parasites of edible mollusks of the northeastern Gulf of Mexico. Gulf Res. Repts. **6**(1):1-8.

Chapman, C.R. 1958. Oyster drill *(Thais haemastoma)* predation in Mississippi. Proc. Natl. Shellfish. Assoc. **49**:87-97.

Cheng, T.C. 1966. The coracidium of the cestode *Tylocephalum* and the migration and fate of this parasite in the American oyster, *Crassostrea virginica*. Trans. Amer. Microscop. Soc. **85**:246-255.

Cheng, T.C. 1975. New geographic records for *Tylocephalum* metacestodes. J. Invertebr. Pathol. **26**:395-396.

Cheng, T.C., and E. Rifkin. 1968. The occurrence and resorption of *Tylocephalum* in the clam, *Tapes semidecussata*. J. Invertebr. Pathol. **10**:65-69.

Cheng, T.C., and E. Rifkin, 1970. Cellular reactions in marine molluscs in response to helminth parasitism. Snieszko, S.F., ed. A symposium on diseases of fish and shellfishes. Washington, D.C.: Amer. Fish. Soc. Spec. Publ. no. 5:443-496.

Freeman, R.S. 1973. Ontogeny of cestodes and its bearing on their phylogeny and systematics. In: Advances in Parasitology, B. Dawes, ed. Academic Press, N.Y. vol. **11**:481-557.

Gunter, G. 1968. Some factors concerning the drilling apparatus and the feeding and predation of prosobranchiate gastropods especially on other molluscs. Proceedings of Symposium on Mollusca. Mar. Biol. Assoc. India. **I**:370-378.

Gutsell, J.S. 1930. Natural history of the bay scallop. Bull. U.S. Bureau Fish. **46**:569-631.

Herdman, W.A. and J. Hornell. 1903. Note on pearl-formation in the Ceylon pearl oyster. British Assoc. Rept., Southport. 695 p.

Hornell, J. 1905. Report on the *Placuna placenta* pearl fishery of Lake Tampalakaman. Ceylon Sessional Papers. 14 p.

Jameson, H.L. 1912. Studies on pearl-oysters and pearls. I. The structure of the shell and pearls of the Ceylon pearl-oyster *(Margaritifera vulgaris* Schumacher): with an examination of the cestode theory of pearl production. Zool. Soc. London, Proc. **11**:260-358.

Linton, E. 1916. Notes on two cestodes from the spotted sting ray. J. Parasitol. **3**:34-38.

Magelhaes, H. 1948. An ecological study of snails on the genus *Busycon* at Beaufort, North Carolina. Ecol. Monogr. **18**:377-409.

McGraw, K.A., and G. Gunter. 1972. Observations on killing of the Virginia oyster by the Gulf oyster borer, *Thais haemastoma*, with evidence for a paralytic secretion. Proc. Natl. Shellfish. Assoc. **62**:95-97.

Menzel, R.W., and F.E. Nichy. 1958. Studies on the distribution and feeding habits of some oyster predators in Alligator Harbor, Florida. Bull. Mar. Sci. Gulf Carib. **8**(2):125-145.

Menzel, R.W., N.C. Hulings, and R.R. Hathaway. 1966. Oyster abundance in Apalachicola Bay, Florida, in relation to biotic

associations influenced by salinity and other factors. Gulf Res. Repts. **2**(2):73-96.

Merriner, J.V., and J.W. Smith. 1979. A report to the oyster industry of Virginia on the biology and management of the cownose ray *(Rhinoptera bonasus* Mitchill) in lower Chesapeake Bay. Va. Inst. Mar. Sci. Spec. Rept. Applied Mar. Sci. and Ocean Engin. no. **216**:33 p.

Paine, R.T. 1962. Ecological diversification in sympatric gastropods of the genus *Busycon.* Evolution **16**:515-523.

Quick, J.A., Jr. 1971. Pathological and parasitological effects of elevated temperatures on oysters with emphasis on *Labyrinthomyxa marina.* In: A preliminary investigation: the effect of elevated temperatures of the American oyster, *Crassostrea virginica* (Gmelin), J.A. Quick, ed. Fla. Dept. Nat. Res. Prof. Paper Ser. no. **15**:105-171.

Rybicka, Krystyna. 1966. Embryogenesis in cestodes. In: Advances in parasitology, B. Dawes, ed. Academic Press, N.Y. vol. **4**:107-186.

Sakaguchi, 1973. A larval tapeworm, *Tylocephalum,* in marine molluscan shellfishes. Bull. Nansei Reg. Fish. Res. Lab. **6**:1-9.

Seurat, L.G. 1906. Sur un cestode parasite des huîtres déterminant la production des perles fines aux îles Gambier. C.R. Hebd. Séanc. Acad. Sci., Paris **142**:801-803.

Shipley, A.E., and J. Hornell. 1904. The parasites of the pearl oyster. Herdman, W.A., ed. Report to the Government of Ceylon on the pearl oyster fisheries of the Gulf of Manaar. Royal Soc. Lond. part 2:77-106.

Sindermann, C.J., and A. Rosenfield. 1968. Principal diseases of commercially important marine bivalve Mollusca and Crustacea. Fish. Bull. **66**(2):335-385.

Southwell, T. 1924. The pearl-inducing worm in the Ceylon pearl oyster. Ann. Trop. Med. Parasitol. **18**:37-53.

Sparks, A.K. 1963*a*. Survey of the oyster potential of Hawaii. Hawaii Div. Fish and Game. 44 p.

Sparks, A.K. 1963*b*. Infection of *Crassostrea virginica* (Gmelin) from Hawaii with a larval tapeworm, *Tylocephalum.* J. Insect Pathol. **5**(3):284-288.

Stephen, D. 1978. First record of the larval cestode *Tylocephalum* from the Indian backwater oyster, *Crassostrea madrasensis.* J. Invertebr. Pathol. **32**:110-111.

Tennent, D.H. 1906. A study of the life-history of *Bucephalus haimeanus,* a parasite of the oyster. Quart. J. Microscop. Sci. N.S. **49**(4):635-690.

Wardle, W.J. 1974. A survey of the occurrence, distribution and incidence of infection of helminth parasites of marine and estuarine Mollusca from Galveston, Texas. College Station, TX, Texas A&M University. Available from: University Microfilms, Ann Arbor, MI; Publ. no. 74-21,235. 346 p. Dissertation. [Diss. Abstr. **35**(4B):1516.]

Willey, A. 1907. Report on the window-pane oyster *(Placuna placenta,* "Muttuchchippi") in the backwaters of eastern province (June 1907). Spolia Zeylanica **5**(17):33-56.

Willey, A. 1909. Report of the marine biologist for 1908. Ceylon Administration Reports for 1908, Colombo, p. G. 1.

Wolf, P.H. 1976. Occurrence of larval stages of *Tylocephalum* (Cestoda: Lecanicephaloidea) in two oyster species from Northern Australia. J. Invertebr. Pathol. **27**:129-131.

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SURVIVAL OF *MYA ARENARIA* LARVAE (MOLLUSCA: BIVALVIA) EXPOSED TO CHLORINE-PRODUCED OXIDANTS[1]

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ABSTRACT

Survival of two larval stages of the soft clam, Mya arenaria L., *exposed to chlorination was studied in flowing estuarine water. Straight-hinge veliger larvae were exposed to 0.2, 0.3, 0.4 and 0.5 ppm chlorine-produced oxidants (CPO) for 2, 4, 8 and 16 h and setting larvae were exposed to the same CPO concentrations for 4, 16, 24, 48, 72 and 96 h. All experiments were performed twice. For both larval stages, there was a direct relationship of mortality with increasing CPO concentration and exposure time. Setting larvae were more tolerant of CPO than were the younger straight-hinge larvae. Increasing numbers of setting larvae left their microscope-slide substrate with increasing CPO concentration and time. Clams which left the substrate suffered higher mortality than those which remained attached. It is not clear if the detaching clams had an avoidance response to CPO or if they died and were subsequently sloughed off. Both stages of clam larvae were more sensitive to CPO than were similar stages of oyster larvae tested in our laboratory.*

INTRODUCTION

Chlorine is used as a disinfectant of effluents of sewage treatment plants and as a biocide to combat fouling of condenser tubes in steam-electric power plants (Whitehouse, 1975; Brungs, 1976; Coughlan and Whitehouse, 1977). As larger facilities are built, they require larger bodies of water to accommodate increased effluent. Modern facilities are being located beside estuaries and the sea. Meroplankton may be circulated through power plant cooling systems, coming into close contact with chlorine. Also, meroplankton near outfalls of both types of utilities can be affected by chlorine.

Early life-history stages of the soft clam, *Mya arenaria* L. are meroplanktonic and thus may be affected by chlorination. Holmes (1970a, b) found that strength and secretion of byssus threads of mussels *(Mytilus edulis)* are adversely affected by chlorination, preventing mussels from attaching to cooling system surfaces. Because *M. arenaria* also uses byssal attachment when setting, it is important to determine its tolerance to chlorine-produced oxidants (CPO) and to investigate whether chlorination would affect setting of this commercially important species.

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We report here the survival of straight-hinge and pediveliger larvae of *M. arenaria* under different conditions of CPO concentration and time.

MATERIAL AND METHODS

Clams used in our research came from a population in the Patuxent estuary in Chesapeake Bay. Ripe clams were held for 1 or 2 days in flowing seawater at 17 C prior to spawning. In fall 1976, clams spawned spontaneously in the laboratory and provided straight-hinge veliger larvae and setting pediveliger larvae which were used in a preliminary experiment. In spring 1977, spawning was induced by raising water temperature to a maximum of 27 C and by injecting the gonad with 1 ml 0.1N ammonium hydroxide (Stickney, 1964). Clams were isolated as soon as they began to spawn and a sample of spawn was examined under the microscope to identify eggs or sperm. Males and females were then kept separate until fertilization. Spawn of several males and females was pooled to provide genetic diversity (Calabrese and Davis, 1970). Cultures were reared as described by Hidu et al. (1969). Experimental larvae were selected at two stages of development: straight-hinge veligers (36-48 hours old) and pediveligers (18 days old).

Apparatus

The bioassay apparatus has been described by Morgan and Prince (1977) and Roosenburg et al. (1980). Briefly, the system delivered a controlled flow of chlorinated bay water with four different CPO concentrations, plus one unchlorinated control. Each of four different stock solutions made up from a saturated calcium hypochlorite solution was fed by a four-way peristaltic pump into a mixing chamber that also received a measured flow of 1 μm core-filtered saltwater. A standpipe in the mixing chamber allowed the chlorinated saltwater to run into a distribution tank that fed the experimental chambers with the desired CPO concentration. Controls received water through a similar series of tanks without the addition of chlorine.

In the experimental chambers, larvae were held in 8.5 cm wide x 20 cm high glass cylinders with 30 μm mesh nylon screen bottoms. These were placed in 14 cm high one-liter beakers. Experimental and control saltwater ran into these cages, passed through the screen bottom into the beaker, and ran over its edge.

Procedure

Based on the results of our preliminary experiment, the following procedure was established. Experimental temperatures ranged between 17 to 20 C and salinities were about 13 o/oo. For straight-hinge larvae, a replicated experiment tested larvae for 2, 4, 8, and 16 hours in 0.2, 0.3, 0.4 and 0.5 mg CPO liter^{-1}. A sample of larvae was drawn from the stock culture and concentrated to a suspension of about 75 larvae ml^{-1}. Each experimental chamber received 4 ml of the suspension which resulted in about 300 larvae per chamber.

At least one unpreserved sample exposed to high CPO concentration was counted immediately after each exposure period to determine the appearance of live and killed larvae. Also, one control was counted and compared with the stock culture to check for possible effects of the experimental apparatus on survival. All other larvae were preserved in 1% buffered formalin.

When pediveliger larvae in the stock culture were in a swim-crawl phase prior to settlement, they were concentrated into a suspension of 500+ml^{-1} and decanted into a fiberglass pan of 20 cm x 55 cm x 75 cm whose entire bottom was covered with microscope slides. The pan was then filled with 1 μm filtered seawater. This environment induced attachment of the larvae. Slides were removed from the tank when they were covered by a sufficient number of larvae. Each slide was numbered and wiped clean except for an area of about 4 cm^2 which contained an average of 162 attached larvae. The slides were then put in a similar pan with very slowly flowing 1 μm filtered saltwater to await the start of the experiment.

Experiments on setting larvae investigated not only tolerance to CPO but also larval behavior noted in the preliminary experiment, i.e., larval departure from the microscope-slide substrate in conditions of increased CPO concentration and time. Numbered slides containing the attached larvae were transferred from their waiting tank into the control and experimental chambers at the start of the experiment. A replicated experiment tested

larvae for 4, 16, 24, 48, 72, and 96 hours at CPO concentrations of 0.2, 0.3, 0.4, and 0.5 ppm. At the end of the exposure period, the cages were removed from their beakers. Larvae remaining on each slide and those in each cage (i.e., detached larvae) were counted immediately without preservation. Criteria for death in setting larvae (and in straight-hinge larvae), included lack of internal movement and disrupted internal organization.

Data on survival, mortality, and departure from the slides were converted to percentages. Control mortalities were generally low, so no correction was made for them. Multiple regression analyses of percentage mortality and percentage remaining attached on CPO concentration and exposure time were calculated (Roosenburg et al., 1980). First and second order terms for main effects (CPO concentration and time) and their interactions were examined. For straight-hinge larvae, variables with $F \geq 4.13$ ($P = 0.05$, *df* 1, 37) were entered in the final equation. For pediveliger larvae, $F \geq 4.00$ ($P = 0.05$, *df* 1, 57). Davis and Calabrese (1964) indicated that experiments with bivalve larvae generally have an accuracy of $\pm 10\%$, therefore differences of less than 20% mortality between treatments may have limited meaning.

RESULTS

For both larval stages, there was a direct relationship of mortality with increasing CPO concentration and exposure time (a pattern found also in the preliminary experiment). Straight-hinge larvae were more sensitive to CPO than were pediveliger larvae over the same time exposure (Tables 1 and 2).

For both larval stages, predictive equations for percentage mortality (Y) were as follows (C = CPO concentration; T = Time in hours):

A. Straight-hinge larvae

$$Y = 11.2815 + 9.8033CT - 0.4297C^2T^2$$

Coefficient of determination = 92.0%

B. Pediveliger larvae

$$Y = 10.9091 + 3.3976CT - 0.0426C^2T^2$$

Coefficient of determination = 92.6%

Resulting values of Y are in transformed form and must be converted to untransformed values (Sokal and Rohlf, 1969). Figures 1 and 2 were prepared using these equations. The final empirical models appeared to be statistically satisfactory (Table 3).

Tables 4 and 5 present the percentage mortalities of pediveligers that became detached from the slides and those remaining attached. Percentage mortalities were calculated from the pooled data of the replicated experiments. The increasing numbers of clams leaving the substrate with increasing CPO concentration and increasing time

TABLE 1. *Percentage mortality of straight-hinge larvae of* Mya arenaria *under different conditions of chlorine-produced oxidant (CPO) concentrations and time. Control values represent no exposure to CPO. A dash indicates no data available.*

		CPO (ppm)			
Time (h)	Control	0.2	0.3	0.4	0.5
2	3.0	1.6	2.0	6.1	23.2
2	4.1	6.1	12.9	4.2	15.7
4	3.8	2.2	8.7	30.6	31.0
4	3.3	-	13.0	14.4	29.3
8	5.3	14.4	29.2	41.9	47.9
8	10.0	15.6	30.9	41.0	51.6
16	11.0	39.9	56.8	70.4	75.7
16	7.1	43.1	45.7	65.4	82.1

TABLE 2. *Percentage mortality of pediveligers of* Mya arenaria *under different conditions of chlorine-produced oxidant (CPO) concentrations and time. Control values represent no exposure to CPO. A dash indicates no data available.*

		CPO (ppm)			
Time (h)	Control	0.2	0.3	0.4	0.5
4	1.8	3.6	1.9	3.2	2.6
4	1.8	1.6	8.6	5.8	7.2
16	6.3	15.2	25.9	18.6	16.8
16	-	18.0	26.0	19.3	16.2
24	3.5	41.5	29.1	45.6	64.2
24	7.9	12.5	48.0	47.2	70.8
48	2.4	62.9	62.0	65.5	67.3
48	2.0	72.6	60.9	62.7	85.2
72	2.6	71.5	74.9	90.5	96.5
72	2.2	76.8	71.1	91.8	84.6
96	2.6	82.0	84.3	92.2	98.7
96	3.9	89.1	88.2	95.5	98.5

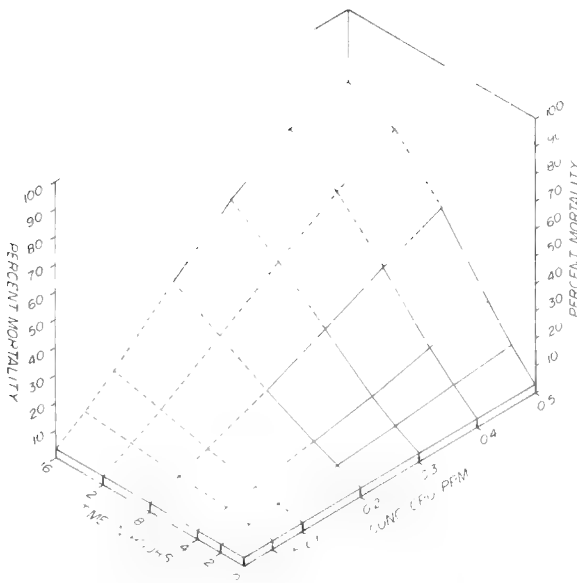

FIGURE 1. Mya arenaria *straight-hinge larvae. Response surface generated from multiple regression analysis of percentage mortality on CPO concentration and time. Dashed lines indicate regions of extrapolation or interpolation.*

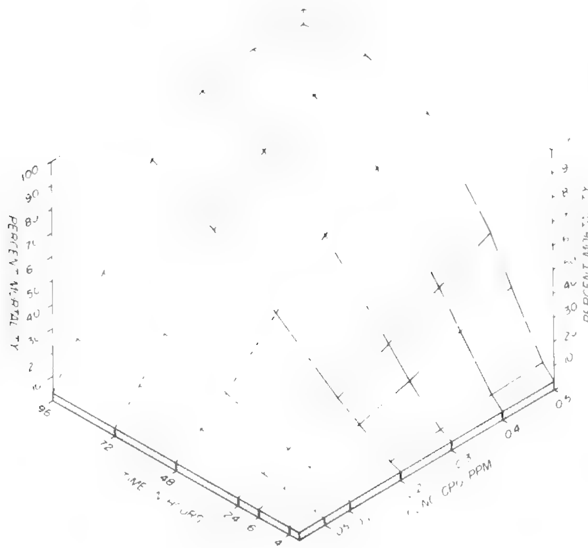

FIGURE 2. Mya arenaria *pediveliger larvae. Response surface as in Figure 1.*

TABLE 3. *Analysis of variance of multiple regression of percentage mortality on chlorine-produced oxidant concentrations and time for straight-hinge and pediveliger larvae of* Mya arenaria. *df = degrees of freedom; MS = Mean Square.*

Source of variation	Straight-Hinge Larvae		Pediveliger Larvae	
	df	MS	df	MS
Regression	2	1.369[a]	2	5.124[a]
Residual	36	0.007	56	0.015

[a]Significant at the 0.001 level.

are evident (Table 4 and Figure 3). Figure 3 was prepared from the predictive equation for calculating percentage of pediveligers remaining on the substrate (Y):

$$Y = 75.8252 - 4.5607CT + 0.0688\,C^2T^2$$

Coefficient of determination = 93.2%

Again, values of Y must be converted to untransformed values (Sokal and Rohlf, 1969).

The analysis of variance was as follows with the final empirical model apparently statistically satisfactory (see Table 3 for meaning of abbreviations):

Source of variation	df	MS
Regression	2	6.789[a]
Residual	56	0.037

Clams that had left the substrate suffered much greater mortality than did those which remained attached (Tables 4 and 5). This was also true for control animals (Table 4). Even though very few control animals became detached (numbers increased with time), those which did detach suffered greater mortality (15 to 82%) than did those which remained attached (1 to 4%).

Figure 4 presents the LC50 (50% mortality) values for both larval stages. These values were calculated using equations A and B. Approximate values of LC50 for straight-hinge larvae were 0.35 ppm CPO at 12 hours and 0.27 ppm CPO at 16 hours. For pediveligers, approximate LC50 values were 0.5 ppm at 24 hours, 0.25 ppm at 48 hours, 0.165 ppm at 72 hours and 0.125 ppm CPO at 96 hours.

TABLE 4. *Percentage mortality of setting* Mya arenaria *which detached from slides and were retrieved from experimental cages. Number in parentheses is total number of clams from two replicate experiments.*

Time (h)	CPO (ppm)				
	Control	0.2	0.3	0.4	0.5
4	50.0 (4)	15.8 (19)	23.1 (13)	9.4 (32)	15.0 (60)
16	82.3 (6)	27.5 (131)	51.7 (149)	58.9 (107)	50.7 (75)
24	37.5 (8)	63.2 (128)	69.5 (154)	74.3 (136)	87.6 (259)
48	40.5 (15)	68.4 (278)	67.4 (190)	77.0 (204)	83.9 (242)
72	15.4 (26)	75.2 (315)	75.8 (429)	92.6 (309)	98.0 (251)
96	36.0 (25)	86.5 (297)	86.2 (429)	93.8 (289)	93.2 (294)

TABLE 5. *Percentage mortality of setting* Mya arenaria *which remained attached to slides. Number in parentheses is total number of clams from two replicate experiments.*

Time (h)	CPO (ppm)				
	Control	0.2	0.3	0.4	0.5
4	1.5 (599)	1.8 (329)	5.7 (194)	4.1 (268)	2.6 (340)
16	3.8 (184)	7.8 (166)	1.9 (159)	2.7 (260)	6.6 (259)
24	2.8 (425)	1.9 (103)	1.6 (123)	10.2 (118)	9.6 (83)
48	0.8 (387)	0.0 (6)	9.1 (22)	0.0 (40)	5.9 (34)
72	1.5 (395)	0.0 (4)	7.7 (13)	0.0 (2)	0.0 (24)
96	0.6 (319)	50.0 (6)	62.5 (8)	0.0 (1)	0.0 (2)

DISCUSSION AND CONCLUSIONS

The patterns of increasing mortality with increased CPO concentrations and time and of greater tolerance with increasing age paralleled findings on the oyster *Crassostrea virginica* (Roosenburg et al., 1980). A comparison of the oyster data with the soft clam data indicates that the clam larvae were more sensitive to CPO than were oyster larvae. Data for straight-hinge larvae of oysters were sampled at different exposure times than were straight-hinge clam larvae which makes comparison difficult, but, at 8 hours and 0.3 ppm CPO, about 6.5% of oyster larvae died compared with about 30% of clam larvae (Tables 1 in Roosenburg et al., 1980 and in this report). At 16 hours, clam larvae suffered about 42% mortality in 0.2 ppm CPO and about 51% mortality in 0.3 ppm CPO whereas at 24 hours oyster larvae mortality was about 26% in 0.2 ppm CPO and about 29% in 0.3 ppm CPO. For setting larvae, a comparison of Tables 2 in Roosenburg et al., 1980 and this report reveals the greater sensitivity of clam pediveligers to similar CPO concentrations when compared with oyster pediveligers.

The increasing tendency to leave the substrate with increasing CPO concentration and time indicates that chlorine may cause soft clams to detach from the substrate. The departure of setting larvae from the slides and the associated mortalities correlate with the findings of Holmes (1970a, b) on *Mytilus edulis*. It is not clear whether the detaching clams would survive upon reaching water with reduced or no chlorine content. In our experiments, the few clams which detached in the controls suffered relatively high mortalities (Table 4) compared with low mortalities in control clams which remained attached (Table 5), thus indicating that detachment and incipient mortality are somehow closely associated. Holmes (1970b) indicates that chlorine deterior-

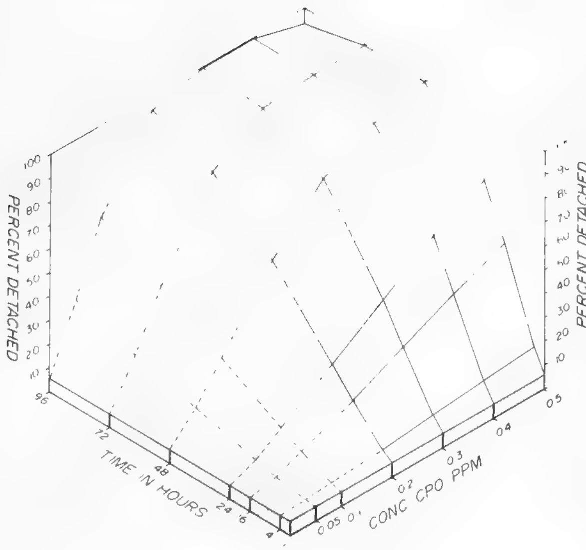

FIGURE 3. Mya arenaria *pediveliger larvae. Response surface generated from multiple regression analysis of percentage of larvae remaining on slide substrate on CPO concentration and time. Values represent percentage of animals detaching from slides. Dashed lines indicate regions of extrapolation.*

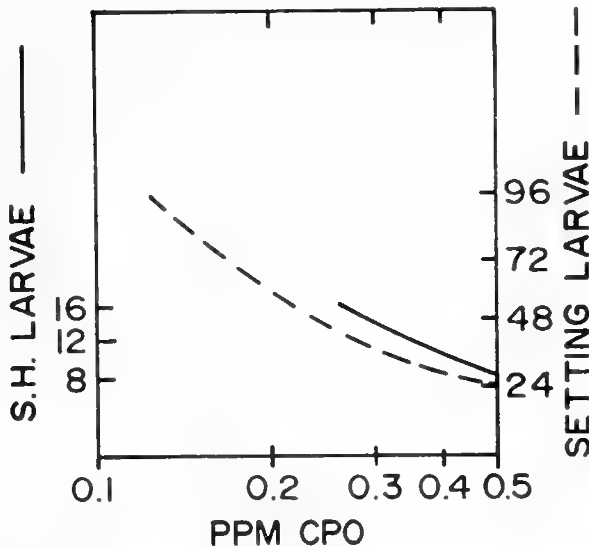

FIGURE 4. Mya arenaria. *LC50 curves for two larval stages generated from predictive equations of percentage mortality under different conditions of CPO concentration and time. Note change in time scale for the two larval stages.*

ates mussel byssus threads, although he does not believe that this is the primary reason that mussels detach from the substrate. It is not known whether the dead *M. arenaria* had left the slides as an avoidance response to unfavorable conditions or died on the slides and subsequently sloughed off into the water. The ability to detach from the initial settlement substrate upon exposure to chlorinated water and to move or be carried to a more suitable habitat would have obvious survival benefit if the larvae were able to tolerate chlorine exposure.

ACKNOWLEDGEMENTS

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LITERATURE CITED

Brungs, W.A. 1976. Effects of wastewater and cooling water chlorination on aquatic life. EPA-600/3-76-098, USEPA, Environmental Research Laboratory, Duluth, MN, USA 55804. 52 pp.

Calabrese, A. and H.C. Davis. 1970. Tolerances and requirements of embryos and larvae of bivalve molluscs. Helgolander wiss. Meeresunters. 20:553-564.

Coughlan, T. and J.W. Whitehouse. 1977. Aspects of chlorine utilization in the United Kingdom. Chesapeake Sci. **18**:102-111.

Davis, H.C. and A. Calabrese. 1964. Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *C. virginica.* Fishery Bull. 63:643-655.

Hidu, H., K.G. Drobeck, E.A. Dunnington, Jr., W.H. Roosenburg and R.L. Beckett. 1969. Oyster Hatcheries for the Chesapeake Bay Region. N.R.I. Special Report No. 2, Contrib. no. 382, Natural Resources Institute, University of Maryland, Solomons, MD 20688. 18 pp.

Holmes, N.J. 1970a. Marine fouling in power stations. Mar. Poll. Bull. 1:105-106.

Holmes, N.J. 1970b. The effects of chlorination on mussels. Central Electricity Research Laboratories Report No. RD/L/R 1672. 31 pp.

Morgan, R.P., II and R.D. Prince. 1977. Chlorine toxicity to eggs and larvae of five Chesapeake Bay fishes. Trans. Am. Fish. Soc. 106:380-385.

Roosenburg, W.H., J.C. Rhoderick, R.M. Block, V.S. Kennedy, S.R. Gullans, S.M. Vreenegoor, A. Rosenkranz, and C. Collette. 1980. Effects of chlorine-produced oxidants on survival of larvae of the oyster *Crassostrea virginica.* Marine Ecology Progress Series (in press).

Sokal, R.R. and F.J. Rohlf. 1969. Biometry. San Francisco: W.H. Freeman. 776 pp.

Stickney, A.P. 1964. Salinity, temperature and food requirements of softshell clam larvae in laboratory culture. Ecology 45:283-291.

Whitehouse, J.W. 1975. Chlorination of cooling water: a review of literature on the effects of chlorine on aquatic organisms. Central Electricity Research Laboratories Memorandum No. RD/L/M 496. 22 pp.

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INFLUENCE OF NEUTRAL LIPID ON SEASONAL VARIATION OF TOTAL LIPID IN OYSTERS, *CRASSOSTREA VIRGINICA*[1]

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ABSTRACT

Oysters (Crassostrea virginica) *were sampled from a wild population in Malpeque Bay in Ellerslie, P.E.I. every month from October 1973 to December 1974. Total tissue lipid, sterol content, fatty acid composition and percent meat weight were shown to follow a seasonal cycle. Lipid, sterol and meat weight decreased over the winter, increased from spring to midsummer, declined sharply when oysters spawned and increased from later summer to late fall. The percent polar lipid in the tissue remained relatively constant year round; the variation in lipid content resulted largely from changes in neutral lipid. Immediately after spawning there was a significant reduction in the ω3 polyunsaturated fatty acids (PUFA), particularly 20:5ω3 and 22:6ω3, and 16:1ω7 in the oyster neutral lipid. It is possible that these fatty acids play an important role in embryonic and larval development and sterol metabolism. The role of lipids in oyster nutrition is discussed.*

INTRODUCTION

Seasonal variation in flesh weight and biochemical composition of bivalves is a function of the complex interactions of food availability and temperature with growth and reproductive processes (Ansell and Trevallion, 1967). The reproductive cycle has been shown to have a major effect on lipid and carbohydrate content of many bivalves (Loosanoff, 1942; Engle, 1950; Walne, 1970; Calzolari et al., 1971; Stancher et al., 1971; Ansell, 1972; 1974 a,b,c; Dare and Edwards, 1975). Although there is some information on the fatty acid composition of the various lipid classes of oysters, *Crassostrea virginica* (Watanabe and Ackman, 1974; Swift, 1977), little information is available on the effects of seasonal variation on classes of fatty acids. The purpose of this study was to provide information on natural seasonal variations in lipids of oysters *(C. virginica)*.

MATERIALS AND METHODS

Oysters 7-15 cm long were taken from an estuary of Malpeque Bay near Ellerslie, Prince Edward Island. Ten animals were sampled every month from October 1973 to December 1974. Additional animals were held in the laboratory in unfiltered, ambient seawater to provide samples during the winter months (December to March) when the estuary was ice-covered.

[1] This paper was part of the research done by D.J. Trider in partial fulfillment of the requirements for the MSc degree in Biology at Dalhousie University, Halifax, Nova Scotia. Research was supported by a Fisheries Research Board of Canada Grant in aid of research.

Lipid was extracted from each oyster using the Bligh and Dyer (1959) method. Samples (50-100 mg) of total lipid were eluted with chloroform followed by methanol from columns (20 mm ID) packed with 5g acid washed Florisil[R] (Caroll, 1963) to separate neutral and polar fractions. Methyl esters of neutral and polar lipids were prepared by transesterification with 10% boron trifluoride in anhydrous methanol (Mehlenbacher et al., 1965). Fatty acid methyl esters (3 animals for each of 4 monthly samples, Feb., Apr., July, December 1974) were analyzed by gas chromatography on an open tubular (capillary) column, coated with butanediol-succinate (BDS) operated at 170°C with a pressure of 40 psig. helium in a Perkin Elmer model 900 chromatograph fitted with a hydrogen flame detector (Ackman et al., 1967). Peak areas were measured with a mechanical integrator fitted on a Honeywell 1 mv recorder. The fatty acids were tentatively identified by log plots of relative retention times (Ackman, 1972), by comparison with the data of Ackman et al. (1967) and Watanabe and Ackman (1974) and by comparison with retention times of fatty acids of reference cod liver oil methyl esters. The sterol content of the neutral lipid was determined by the perchloric acid-phosphoric acid-ferric chloride procedure of Momose et al. (1963).

RESULTS

The total lipid content of individual oyster meats ranged from 0.5 to 2 percent of the wet meat weights (Figure 1). There was an increase in percent of lipid in meats in the fall of 1973, followed by a decline over the winter to a minimum value in February 1974. The lipid content increased in the spring of 1974 as the water temperature rose. A sudden decrease in lipid was observed in July coinciding with spawning. The lipid content then rose again in the fall of 1974 and decreased as winter approached. Mean lipid levels were lower in the fall of 1974 than 1973.

The basic biochemistry involved is clearer when the polar and neutral lipids are measured separately (Figure 2). The polar lipids were constant at approximately 0.5% of live weight of meats, while the neutral lipid changed dramatically over the seasons. Especially significant was the drop in

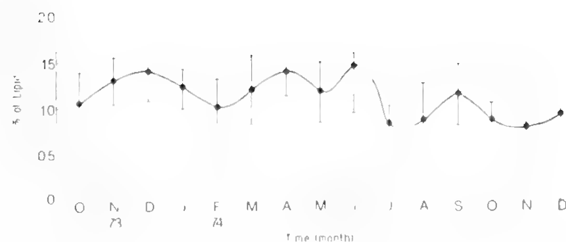

FIGURE 1. *Variation of total oyster meat lipis over a fifteen month period. The lipid is expressed as a percentage of the total wet meat weight. Each point is the average of 10 individuals. (mean ± SD).*

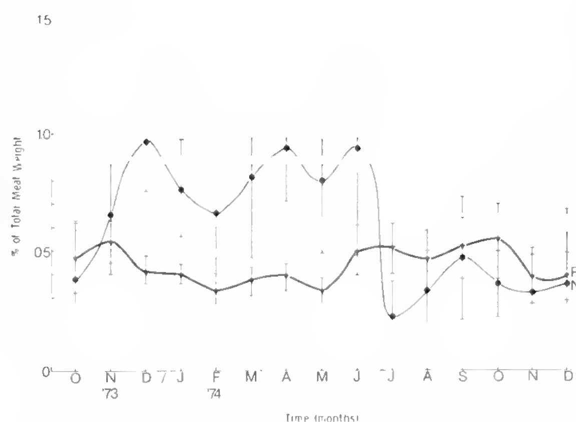

FIGURE 2. *Changes in the neutral (N) and polar (P) lipid fraction of the total oyster meat lipid over a fifteen month period. The values are expressed as percents of the total wet meat weight. Each point is the average of 10 individual animals. (mean ± SD).*

FIGURE 3. *Seasonal variation of total oyster meat sterol as shown by samples from a natural population over a fifteen month period. Each point is the average of 3 individual animals (mean ± SD).*

neutral lipid in July coincident with the oysters' spawning.

The sterol levels showed only minor fluctuations throughout the annual cycle (Figure 3). The low sterol levels in July and August with small sample standard deviations, however, probably indicates an incorporation of relatively high levels of sterols into gametes.

While the fatty acids of the polar lipids were constant and similar to those reported by Watanabe and Ackman (1977), the seasonal fluctuation is reflected in the fatty acid composition of the neutral lipid (Table 1). The relative amounts of

TABLE 1. *Changes in fatty acid composition of the neutral lipid fraction sampled from oysters taken from Malpeque Bay near Ellerslie, P.E.I. (Data expressed as uncorrected area percent.)*

Fatty acid	Relative Retention Time	February	April	July	December
10.0		.1	.16	—	.1
12:0	.112	.02	—	.1	tr
13:0	.163	—	—	—	—
iso 14:0	.198	.05	—	.1	.1
14:0	.238	5.3	4.7	5.3	5.3
br. chains 15:0	.259	—		—	—
iso 15:0	.291	.3	.3	.4	.3
anteiso 15:0	.309	.1	.1	.2	.1
15:0	.341	1.0	.8	1.6	.9
iso 16:0	.414	.2	—	.3	—
16:0	.486	23.0	23.1	38.0	25.1
iso 17:0	.591	1.0	1.0	1.1	1.1
anteiso 17.0	.614	.5	.5	.8	.4
17:0	.696	1.5	1.6	3.0	1.6
iso 18:0	.841	.4	.4	.3	.3
18:0	1.0	2.1	1.9	3.8	2.5
19:0	1.42	.4	.3	.1	.2
20:0	2.05	.1	.07	.2	.1
ΣSaturates		36.7	34.93	55.0	38.10
15.1?	.393	.2	.2	—	—
16:1ω9	.531		1.3	.4	1.1
16:1ω7	.546	5.8	3.8	.6	5.7
16:1ω5	.577	.5	.4	.4	.3
17:1ω8	.756	.3	.4	.5	.4
18:1?	1.03	2.4	2.6	3.6	2.8
18:1ω9	1.08	3.5	3.6	8.5	4.2
18:1ω7	1.11	6.2	5.1	7.6	5.1
18:1ω5	1.15	.6	.6	.7	.6
19:1?	1.54	.1	.1	.5	.1
20:1ω11	2.14	1.9	1.9	2.5	2.2
20:1ω9	2.22	.3	.2	.3	.5
20:1ω7	2.36	.2	.2	.3	.2
20:1ω5	2.41	.2	.6	.2	.1
22:1ω1	4.44	tr	.1	.1	.1

TABLE 1. *Continued*

ΣMonounsaturates		23.2	21.1	26.2	23.4
16:2ω4	.678	.1	.1	.1	.1
16:3ω6	.737	.2	.3	.4	.2
16:3ω4	.799	.2	.3	.2	.2
16:3ω3	.882	.9	1.4	.2	1.4
16:4ω3	.930	.1	.1	.1	tr
18:2ω6	1.30	2.3	2.4	1.9	2.3
18:2?	—	—	—	.1	tr
18:3ω6	1.39	.1	.1	.1	tr
18:3ω3	1.66	1.9	2.0	1.9	2.1
18:4ω3	1.88	1.9	3.4	1.1	2.0
20:2NMID	2.25	2.4	2.1	3.0	2.3
20:2NMID	2.34	3.8	4.5	3.2	5.2
20:2ω9	2.46	.4	.3	.3	.3
20:2ω6	2.61	.2	.2	.2	.2
20:3ω9	2.66	.1	.1	.1	.1
20:3ω6	2.81	.2	.2	.1	.3
20:4ω6	3.06	1.8	1.7	.6	1.7
20:3ω3	3.24	.1	.1	tr	.1
20:4ω3	3.61	.4	.4	.1	.5
20:5ω3	3.95	9.8	10.1	1.3	5.6
22:2NMID	4.54	.5	.5	.5	.6
22:2NMID	4.64	2.4	2.5	1.9	2.3
22:2ω6	4.90	.1	.1	.1	.1
21:5ω2	5.54	.3	.3	.1	.1
21:6ω2	5.83	.4	.4	.1	.2
22:4ω6	6.33	.1	.1	tr	.1
22:5ω6	6.95	.4	.4	tr	.3
22·5ω3	8.04	.5	.6	tr	.4
22:6ω3	8.94	8.7	8.9	.5	7.6
ΣPolyunsaturates		40.30	43.60	18.0	36.0
ω3	fatty acids	24.3	27.0	5.2	19.7
ω6	fatty acids	5.4	5.5	3.4	5.2

*non methylene interrupted dienes

saturated and monounsaturated fatty acids were greatest in July. The total polyunsaturated fatty acid (PUFA) content was at its lowest in July after the oysters spawned. There was, in fact, a ten fold drop in the levels of 16:1ω7, 20:5ω3 and 22:6ω3 fatty acids with minor changes in the non-methylene interrupted diens (NMID) and 20:4ω6 fatty acid.

The seasonal changes in percent meat weight followed patterns similar to the neutral lipid (Figure 4). The increase in May reflects the rise in water temperature and resumption of feeding and the presence of enlarged, ripened gonads. This corresponds to a slight drop in percent total lipid. The decline in July coincided with spawning as evidenced by shrunken, watery gonads and reduced total lipid levels.

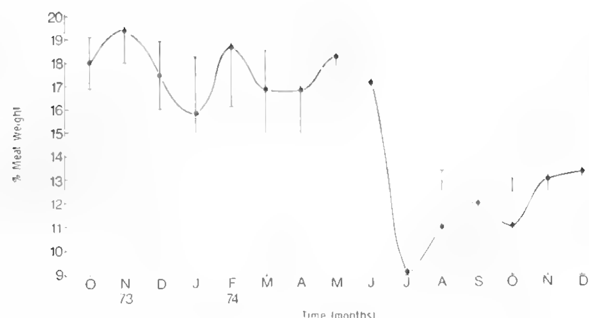

FIGURE 4. *Variation of oyster meat weight over a 15 month period. Weights are expressed as percent of the total live weight (shell + meat) of the animal. Samples were taken from a natural population. Each point is the average of 10 individual animals. (mean ± SD).*

DISCUSSION

The seasonal variations in oyster lipids reflected fluctuations in neutral lipid. The polar or phospholipid, which functions chiefly as a structural unit in membranes, remained relatively constant year round. (Gardner and Riley, 1973; Swift, 1977), whereas the neutral lipid is generally accumulated as an energy reserve (Helm et al., 1973; Holland and Spencer, 1973). The large drop in neutral lipid in July coincided with spawning as indicated by the greatly reduced gonad size of oysters sampled. Thompson (1977) found that the bulk of the lipid accumulated in the female scallop gonad was released in the spawned eggs. In the oysters, not only did the content of neutral lipid drastically decline during spawning, but there was a significantly greater loss of 20 and 22 carbon, ω3 and ω6, polyunsaturated fatty acids and 16:lω7 fatty acid than other saturated fatty acids or monoenes. Dawson and Barnes (1966) have reported incorporation of high levels of polyunsaturated fatty acids in eggs of other marine invertebrates.

The high levels of both ω3 and ω6 fatty acids incorporated into the oyster gametes is probably indicative of an essential fatty acid requirement for both series of fatty acids (Carney and Williams, 1968). This type of requirement has been shown for carp (Takeuchi and Watanabe, 1977). Mammals require chiefly 6 fatty acids Alfin-Slater and Aftergood, 1968; Aes-Jorgensen, 1961), while rainbow trout *(Salmo gairdneri)* require mainly ω3 fatty acids (Castell et al., 1972).

The ten fold drop in 16:lω7 in July following spawning may indicate some particular function for this monoene in egg development. This function may occur in sterol metabolism by direct or indirect transformation to 20 and 22 carbon non-methylene interrupted dienes (NMID) whose levels are increased when oysters are fed artificial diets supplemented with cholesterol (Trider and Castell, unpublished data).

Both sterols and phospholipids are part of the membrane structure and the relatively constant level of these lipids is probably related to a constant proportion in tissue membranes. The apparent incorporation of sterols into gametes, indicated by low levels of this lipid in July and August samples, may have some significance in reproduction (Mori et al., 1966; Hayashi, 1971). Thompson (1977) reported a build up of sterol in the scallop, *Placopecten magellanicus,* prior to spawning followed by incorporation into the gametes. It is possible that the oyster, like many arthropods, is unable to synthesize sufficient sterol for its needs (Zandee, 1967). The egg may thus require a relatively high initial level of sterol for tissue synthesis during embryonic development prior to initiation of feeding and a dietary source of sterols. In support of this, studies with ^{14}C labelled acetate (Trider and Castell, unpublished data) showed no incorporation of the label into sterols, suggesting the absence of *de novo* synthesis.

The data show that there is an annual cycle in oyster meat lipid resulting from changes in levels of polyunsaturated fatty acids of the neutral lipid. A similar cycle was observed in the percent meat weights and to a much lesser extent in sterol content. These changes may vary in amplitude from year to year because of environmental factors, but the general pattern should remain the same.

ACKNOWLEDGEMENTS

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LITERATURE CITED

Aaes-Jorgensen, E. 1961. Essential fatty acids. Physiol. Rev. **41**(1):1-51.

Ackman, R.G. 1972. The analysis of fatty acids and related materials by gas-liquid chromatography. In: Progress in the Chemistry of Fats and other Lipids, Ed. R.T. Holman. Vol. **12**:165-284.

Ackman, R.G., J.C. Sipos, and P.M. Jangaard. 1967. A quantitative problem in the open tubular gas chromatography of fatty acid esters from cod liver lipids. Lipids **2**(3):251-257.

Alfin-Slater, R.B. and L. Aftergood. 1968. Essential fatty acids reinvestigated. Physiol. Rev. **48**(4):758-784.

Ansell, A.D. 1972. Distribution, growth and seasonal changes in biochemical composition for the bivalve *Donax vittatus* (DaCosta) from Kames Bay, Millport. J. Exp. Mar. Biol. Ecol. **10**:137-150.

Ansell, A.D. 1974a. Seasonal changes in biochemical composition of the bivalve *Abra alba* from the Clyde Sea area. Mar. Biol. **25**:13-30.

Ansell, A.D. 1974b. Seasonal changes in biochemical composition of the bivalve *Nucula sulcata* from the Clyde Sea area. Mar. Biol. **25**:101-108.

Ansell, A.D. 1974c. Seasonal changes in biochemical composition of the bivalve *Chlamys septermradiata* from the Clyde Sea area. Mar. Biol. **25**:85-99.

Ansell, A.D. and A. Trevallion. 1967. Studies on *Tellina tenuis* DaCosta I. Seasonal growth and biochemical cycle. J. Exp. Mar. Biol. Ecol. **1**:220-235.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **27**:911-917.

Calzolari, C., E. Cerma and B. Stancher. 1971. Gas chromatography applied in determining fatty acids of some gastropods and lamellibranchia from the Adriatic Sea during an annual cycle. Riv. Stal. Sostanze Grasse. **XLVII**:605-616.

Carney, J.A. and D.L. Williams. 1968. Changes in the fatty acids of the reproductive tissues of male rats in essential fatty acids deficiency. Biochem. J. **109**:12

Carroll, K.K. 1963. Acid treated florisil as an absorbent for column chromatography. J. Am. Oil Chem. Soc. **40**(9):413-419.

Castell, J.D., R.D. Sinnhuber, J.H. Wales and D.J. Lee. 1972. Essential fatty acids in the diet of rainbow trout *(Salmo gairdneri)*: growth, fed conversion and some gross deficiency symptoms. J. Nutr. **102**:77-85.

Dare, P.J. and D.B. Edwards. 1975. Seasonal changes in flesh weight and biochemical composition of mussels. *(Mytilus edulis* L.) in the Conway Estuary, North Wales. J. Exp. Mar. Biol. Ecol. **18**:89-97.

Dawson, R.M.C. and H. Barnes. 1966. Studies in the biochemistry of cirripede eggs II. Changes in lipid composition during development of *Balanus balanoides* and *Balanus balanus.* J. Mar. Biol. Ass. U.K. **46**:249-261.

Engle, J.B. 1950. The condition of oysters as measured by the carbohydrate cycle, condition factor and % dry weight. Natl. Shellfish. Ass. Conv. Add.:20-25.

Gardner, D. and J.P. Riley. 1972. The component fatty acids of the lipids for some species of marine and freshwater molluscs. J. Mar. Biol. Ass. U.K. **52**:827-838.

Hayashi, A. 1971. Lipids in shellfish. Oil Chemistry **20**(10):726-736. (Translated from Japanese; FRB Translation Series No. 2113).

Helm, M.M., D.L. Holland and R.R. Stephenson. 1973. The effect of supplementary algal feeding of a hatchery breeding stock of *Ostrea edulis* L. on larval vigour. J. Mar. Biol. Ass. U.K. **53**:287-298.

Holland, D.L. and B.E. Spencer. 1973. Biochemical changes in fed and starved oysters, *Ostrea edulis* L., during larval development, metamorphosis and early spat growth. J. Mar. Biol. Ass. U.K. **53**:287-298.

Loosanoff, V.L. 1942. Seasonal gonadal changes in the adult oysters, *Ostrea virginica,* of Long Island Sound. Biol. Bull. **82**:192-206.

Mehlenbacher, V.C., T.H. Hooper, and E.M. Sallee. 1965. Preparation of methyl esters of long-chain fatty acids. In: Official and tentative methods of the American Oil Chemists Society, Ce 2-66.

Momose, T., Y. Ueda, K. Yamamoto, T. Masumura and K. Chta. 1963. Determination of total cholesterol in blood serum with perchloric acid-phosphoric acid-ferric chloride reagent. Anal. Chem. **35**(1):1751-1753.

Mori, K., H. Tamate and T. Smai. 1966. Histochemical study on the change of 17B-hydroxysteroid dehydrogenase activity in the oyster during the stages of sexual maturation and spawning. Tohoku J. Agricultural Res. **17**(2):179-191.

Stancher, B., E. Cerma and P. Baradel. 1971. Mollusks of the upper Adriatic. Variations of the chemical composition of some gastropods and lamellibranchia during an annual cycle. Rass. Chim. **23**:39-42

Swift, M.L. 1977. Phosphono-lipid content of the oyster, *Crassostrea virginica,* in three physiological conditions. Lipids **12**(5):449.

Takeuchi, T. and T. Watanabe. 1977. Requirement of carp for essential fatty acids. Bull. Jap. Soc. Sci. Fish **43**(5):541-551.

Thompson, R.J. 1977. Blood chemistry, biochemical composition, and the annual reproductive cycle in the giant scallop, *Placopecten magellanicus,* from southeast Newfoundland. J. Fish. Res. Board Can. **34**:2104-2116.

Walne, P.R. 1970. The seasonal variation of meat and glycogen content of seven populations of oysters *Ostrea edulis* L. and a review of the literature. Fishery Investigations Ser. III Vol. XXVI No. **3**:1-35.

Watanabe, T. and R.G. Ackman. 1974. Lipids and fatty acids of the American oyster *(Crassostrea virginica)* and European flat oyster *(Ostrea edulis)* from a common habitat after one feeding with *Dicrateria inornata* or *Isochrysis galbana.* J. Fish. Res. Board Canada **31**(4):403-409.

Watanabe, T. and R.G. Ackman. 1977. Effect of storage on lipids and fatty acids of oysters. Can. Inst. Food Sci. Technol. J. **10**(1):40-42.

Zandee, D.I. 1967. Absence of cholesterol synthesis as contrasted with the presence of fatty acid and synthesis in some arthropods. Comp. Biochem. Physiol. **20**:811-822.

ABSTRACTS OF TECHNICAL PAPERS PRESENTED AT THE 1979 ANNUAL MEETING VANCOUVER, BRITISH COLUMBIA

GROWTH AND METAL ACCUMULATION STUDIES OF OYSTERS *CRASSOSTREA VIRGINICA* AT THE MORGANTOWN STEAM ELECTRIC STATION ON THE POTOMAC RIVER, MARYLAND[1]

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The growth of three size classes of oysters *Crassostrea virginica* Gmelin was observed for a 20-month period beginning in December 1976 at the intake area and discharge canal area of the Potomac Electric Power Company's Morgantown Steam Electric Station (SES), located on the Potomac River in Charles County, Maryland and at a control area in Shade Side, Anne Arundel County, Maryland. Additional oysters were monitored for uptake of copper and nickel.

Growth of oysters was excellent in all three areas during the first season (salinity > 9 ‰) despite canal temperatures in excess of 6°C above intake temperatures. Poor growth occurred in all three areas during the second season due to low salinity (< 6 ‰). Low salinity during 1978 and high canal temperatures eventually resulted in near total mortality among canal oysters.

Metal studies indicated no SES effect on nickel accumulation, but uptake of copper was directly associated with SES operation. Oysters were able to rid themselves of much of this accumulated copper within 2 months of transfer to a control area.

[1]This study was supported by The Potomac Electric Power Company.

DIFFERENTIAL SURVIVAL OF SELECTED STRAINS OF PACIFIC OYSTERS *(CRASSOSTREA GIGAS)* DURING SUMMER MORTALITY

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Throughout the late 1960's and early 1970's summer mortalities of Pacific oysters on the west coast of the United States resulted in repeated losses as great as 65% in certain locations. The severity of the mortalities lessened during the 1970's. In anticipation of recurring severe mortalities a program at the University of Washington's College of Fisheries was developed utilizing selective breeding to obtain oyster stocks resistant to summer mortalities. During 1978 a summer mortality occurred in Rocky Bay, Washington where several experimental selected families had been planted. Differential mortalities between families ranged from less than 10% to greater than 80% compared to a non-selected Japanese stock control mortality of 47.5%. Ninety percent of the families tested had lower percent mortality than the control. Differential mortalities between families were also observed during laboratory elevated temperature challenges.

THE ROLE OF CHEMORECEPTION IN PREDATION BY THE OYSTER DRILL *UROSALPINX CINEREA.* II. BIOASSAY TECHNIQUES

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The purpose of this study was to identify discrete, sometimes subtle, aspects of predation by *Urosalpinx cinerea,* and thereby develop a bioassay in which oyster drills will reliably signal the presence of a feeding attractant released by *Crassostrea virginica.* Such a technique could facilitate rapid screening and isolation of many substances separated from oyster mantle fluid. Initial attempts to develop a bioassay showed that *U. cinerea* responds to oyster mantle fluid by elevating the anterior portion of its shell from the substratum for several minutes or by searching for the source of stimulus. Search for prey consists of five behavioral events which occur in the following sequence: 1) increased locomotion and shell movements, 2) investigation of the source of stimulus with the siphon, 3) investigation with the cephalic tentacles, 4) investigation with the propodium, and 5) crawling upon the surface of the source of the stimulus. Attempts to induce *U. cinerea* to forgo chemomechanical penetration of shell and to perform proboscis extension in response to water passing over injured oyster flesh were successful; whereas water passing over whole live oysters did not evoke proboscis extension. Apparently proboscis extension is stimulated by completion of shell penetration, by a waterborn secondary substance associated with injured oysters, or by contact with extrapallial fluid or surface of the uninjured mantle.

POPULATION STRUCTURE OF THE MANGROVE COCKLE *ANADARA TUBERCULOSA* (SOWERBY, 1833) FROM EIGHT MANGROVE SWAMPS IN MAGDALENA AND ALMEJAS BAYS, BAJA CALIFORNIA SUR, MEXICO

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The population structure of *Anadara tuberculosa* was studied in eight mangrove swamps at Magdalena and Almejas Bays. Some of these populations have been commercially exploited recently, while others have been left undisturbed. In those mangrove swamps being fished a repopulation by strong juvenile classes was noted.

Three population groups were identified in the eight swamps by their height-width regression index. Such an index suggests an anticlockwise circulation within Almejas Bay, and could also explain larval dispersion.

ULTRASTRUCTURE AND MINERAL CHEMISTRY OF MAJOR SHELL REGIONS OF *CRASSOSTREA VIRGINICA*

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We examined a) the ultrastructure of three major regions of the shell of hatchery-reared oysters, *Crassostrea virginica* (Gmelin), grown for about three months in Broadkill Creek, Delaware, and b) and the relationship of this ultrastructure to the distribution and concentration of eight elements in these shell regions. Ultrastructure was studied with the scanning electron microscope, and chemical analyses were made with a graphite furnace attachment on a flame atomic absorption spectrophotometer.

The regions examined included the prismatic calcite of the right valve, the foliated calcite of the right valve, and the foliated calcite of the left valve. Before chemical analyses, shell was cleaned in clorox, rinsed in quartz-distilled water, and broken in a clean room to avoid metallic contamination.

SEM micrographs revealed a significant difference in ultrastructure between the prisms of the prismatic calcite and the laths of the foliated calcite. Also striking was the difference between the compact and the chalky foliated calcite, both composed of laths, but possessing different lath orientations.

The chemical elements studied included Ca, Mg, Sr, Mn, Fe, Zn, Cd, and Cu. Although Ca, Mg, and Fe were relatively uniformly distributed in the three regions of the shell, the concentration of some of the other elements was variable. For ex-

ample, Sr was more concentrated in the foliated calcite of both valves than in the prismatic region of the right valve, and conversely Mn and Zn were more concentrated in the prismatic calcite than in the foliated regions. Distribution of these elements suggests that Sr may be associated with the mineral component of foliated calcite, and Mn and Zn, with the organic matrix of the prismatic stratum, since the latter is characterized by conspicuously more organic matrix than the rest of the shell.

OBSERVATIONS ON THE PREDATION OF OYSTERS BY THE BLACK DRUM *POGONIAS CROMIS* (LINNAEUS) (SCIAENIDAE)

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Experimental feeding studies have confirmed that the black drum, *Pogonias cromis* (Linnaeus), is capable of crushing and consuming oysters. Black drum utilize their chin barbels to help locate oysters; they will ingest and attempt to crush any oyster that fits within their pharyngeal apparatus. Ingested oysters are normally crushed or rejected within 55 seconds; crushing is accomplished by grinding pressure from the drum's upper and lower pharyngeal plates which possess numerous molariform teeth. The tensile strength of the shell apparently controls the size of the oysters that an individual drum can crush. Oysters that are heavily infested with burrowing organisms such as the clam, *Diplothyra smithii* Tyron, and the sponge, *Cliona celata* Grant, appear most susceptible to predation by black drum. Captive drum are capable of separating and crushing clustered oysters, but prefer single oysters if available. Large drum (90+ cm, TL) crushed oysters of up to 11.2 cm (Ln); drum less than 90 cm crushed oysters of up to 7.5 cm and as large as 9.6 cm if they were heavily infested with burrowing organisms.

The rate of oyster predation by captive drum depended more on original drum habitat than on drum size. Average predation rates varied from zero (for drum from habitats without oysters) to 48/day (for drum from oyster reef habitats). During peak feeding periods, moderate to large drum consumed more than two oysters per day for every kilogram of body weight.

Black drum are opportunistic carnivores; those captured from oyster reef habitats preferred oysters over other potential molluscan and crustacean species; those captured from other habitats preferred the dominant bivalve mollusk of their habitat. Captive drum from all habitats, however, readily consumed the common rangia clam, *Rangia cuneata* (Sowerby).

ANATOMY, HISTOLOGY AND ULTRASTRUCTURE OF LARVAL CRASSOSTREID OYSTERS[1, 2]

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Histologic and ultrastructural highlights of the three dimensional relationships of soft tissue organ systems of larval veligers of *Crassostrea virginica* and *C. gigas* are described as follows: (1) organs defining the visceral cavity; (2) the rudimentary vascular system; (3) the digestive system, and (4) the musculature.

The visceral cavity is defined ventrally by the velum, dorsally and laterally by the mantle and posteriorly by the foot and the posterior visceral cavity membrane. The mantle is fused on its anterior aspect dorsal to the anterior adductor muscle. The velum consists of supporting cells, secretory cells and the highly specialized cells which provide for motility of the larva. A ciliary tuft, possibly serving a sensory function, is found in the central part of the velar cup. Parts of the velum also serve as sites of amebocyte extrusion. The mantle consists of cells specialized for secretory functions and forms both shell matrix and periostracum which, in the larva, is continuous between the right and left valves at the umbo. The posterior visceral cavity membrane is an attenuated cellular barrier which surrounds the foot and attaches to the mantle lobes and the foot. The complex foot contains the paired byssus ducts, the pedal ganglion and, depending on the stage of larval development, other recognizable rudiments of adult tissues.

The rudimentary vasculature consists primarily of tissues which will form the heart, lying along the posterior adductor muscle, and the paired gill rudiments. These gill tissues are extensions from the peripheral mantle to the posterior visceral cavity membrane at the base of the foot.

The digestive system is composed of the esophagus, stomach, style sac, paired digestive gland, and intestine. All elements consist of one primary cell layer which is surrounded by an attenuated pericyte layer on the visceral cavity aspect. The epithelium of all digestive organs, except the digestive gland, is, at least in part, ciliated. A spiral groove between the stomach and style sac serves to concentrate the particulate material and form the fecal pellet. The digestive gland consists of large absorbtive cells, secretory cells and undifferentiated cells at the tips of the digestive gland lobes.

The musculature is composed of the two nonstriated adductor muscles and four paired groups of striated retractor muscles. The two principal retractor groups are adjacent and insert ventrally on the foot, mouth and velum. The other two groups are a velar bundle and a pair of fibers inserting ventrally on the posterior visceral cavity membrane.

Functional relationships are discussed in relationship to the new structural findings.

[1] This work was sponsored in part by the New York Sea Grant Institute under grant #344-5047-E (L. Leibovitz) from the Office of Sea Grant, National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce.

[2] This paper was the winner of the Thurlow Nelson Award for the outstanding paper by a junior scientist.

NEW ULTRASTRUCTURAL ASPECTS OF A SERIOUS DISEASE OF HATCHERY REARED LARVAL PACIFIC OYSTERS *CRASSOSTREA GIGAS*[1].

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Ultrastructural examination of tissues, and a possible etiologic agent, in a previously described disease of larval *Crassostrea gigas* (Leibovitz *et al.*, 1978. Proc. World Maricult. Soc., pp. 603-615; Elston, 1979. J. Invertebr. Pathol, 33:71-74) are reported. The tissue changes consisted of the accumulation of irregular electron opaque bodies near the surface of velar epithelial cells. Large accumulations of such materials resulted in detachment of velar epithelial cell fragments. These accumulations also apparently exacerbated tissue damage during movement of the velum by the larvae. Damage of mantle tissue, as described in the original report, was not detected ultrastructurally.

Spherical bodies, previously implicated in severe gastro-intestinal impaction, showed an ultrastructural resemblance to the marine fungus, *Hyalochlorella marina.*

The findings of this and the prior reports are considered together. The prevalent dense inclusions described here correspond to the dense fascicular masses observed histologically as reported in the first paper. These dense bodies also appear to be more important in the disease process than the virus-like particles and associated lesions reported in the second paper. Suboptimal cultural conditions appear to permit the normally present spherical forms to proliferate and consequently interfere with digestive functions.

[1] This work was sponsored in part by the New York Sea Grant Institute under grant #344-5047-E (L. Leibovitz) from the Office of Sea Grant, National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce.

DETECTION OF VIBRIOSIS IN HATCHERY REARED LARVAL OYSTERS: CORRELATION BETWEEN CLINICAL, HISTOLOGICAL AND ULTRASTRUCTURAL OBSERVATIONS IN EXPERIMENTALLY INDUCED DISEASE[1]

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Clinical observations, including tissue changes and behavior are correlated with histological, ultrastructural and fluorescent microscopy findings in experimental vibriosis of larval *Crassostrea virginica. Vibrio* sp. isolates from a Long Island, New York oyster hatchery were inoculated into laboratory cultures of larval oysters at levels of

from 10^3/ml to 10^7/ml. Hatchery culture conditions were simulated in the laboratory. Mortality ranged from 40% to 100% in the five sequential experiments. All experiments were terminated by five days post inoculation.

Behavorial changes were usually evident by two days post inoculation and included decreased swimming acitivity, abnormal patterns and cessation of feeding. Abnormal swimming included "flip-flop", reversed and spinning patterns. Examination of live larvae also indicated that apparently detached retractor muscles resulted in continuously extended vela. Detached cells were observed within the mantle cavity. A general visceral shrinkage was a prevalent response in older larvae. In straight hinge larvae, nearly complete visceral degeneration could occur while relatively intact vela enabled the larvae to continue swimming.

Histological examination indicated that straight hinge larvae with intact but deformed vela often had bacteria filled visceral cavities. Detached cells in the mantle were uniformly associated with selective invasion of the mantle by bacteria. Visceral shrinkage seemed to result from detachment of digestive gland cells and atrophy of other cell types including those of the velum.

Application of the direct immunofluorescent technique to frozen tissue sections confirmed that the bacteria invading mantle tissues and the visceral cavity were *Vibrio* sp. In addition, relatively faint staining of digestive tract surfaces in larvae showing visceral atrophy suggested that this tissue change was associated with attachment of bacterial antigens to the epithelial surfaces of the digestive tract. All immunofluorescent techniques were controlled with specificity tests.

Ultrastructural examination confirmed the detachment of retractor muscles from the vela of straight hinged larvae showing continuously extended vela. In all experiments, some degree of phagocytosis of whole bacteria by larval amebocytes was observed. Attachment of bacteria to larval shell, prior to selective invasion of mantle tissue, apparently contributed to the virulence of the pathogen. The degeneration of the absorptive cells of the digestive gland and their subsequent detachment was confirmed ultra- structurally.

These results indicate that clinical observations can be reliably interpreted to correlate with defined tissue changes and, in some cases, with the early stages of bacterial infection.

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[1]This work was sponsored in part by the New York Sea Grant Institute under grant #344-5047-E (L. Leibovitz) from the Office of Sea Grant, National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce.

DETECTION OF VIBRIOSIS IN HATCHERY LARVAL OYSTER CULTURES: STUDY OF THE INTERRELATIONSHIP OF DIAGNOSIS AND MANAGEMENT VARIABLES IN AN EXPERIMENTAL MODEL[1]

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Previous studies of spontaneous and experimental vibriosis in larval shellfish have suggested that outbreaks of the disease in hatcheries are associated with sudden increases of *Vibrio* populations in larval cultural medium and its ingredients (incoming bay and well water, stock and pooled algal cultures) (Leibovitz , 1979) and the invasion and proliferation of these bacterial organisms in shellfish larvae (Elston and Leibovitz, 1979). In order to influence the course of the disease in individual shellfish hatcheries, more efficient methods of detection, and required management responses needed to modify, prevent and control this economically important disease must be developed. The following study was undertaken to develop an experimental model of larval shellfish vibriosis that could be utilized for the evaluation of experimental variables which may influence the course and detection of the disease. The study was divided into 2 parts. The first part dealt with the influence of selected variables upon the course of the experimental disease that resulted from a single dose of *Vibrio anguillarum* at a concentration of 10^4 per ml of larval culture or culture ingredient (synthetic sea water, or sea water and algal food mixture) given 24 hours after the start of the experiment. The second part dealt with the influence of the same variables upon the course of

the disease that resulted from equal doses of the same organism at the same concentration given daily, after the first 24 hours, and continued for the remaining total experimental period of 5 days.

The experimental model hatchery culture units employed in both parts of the experiment were identical 7 liter capacity clear plastic round bottomed conicals, each filled with 5 liters of one of the following experimental liquid media: (1) a complete larval culture containing synthetic sea water, a standard concentration of algal food and 5-day-old oyster *Crassostrea virginica* larvae at a concentration of 100 larvae per ml of culture at the start of the experiment, (2) synthetic sea water mixed with the standard concentration of algal food, (3) synthetic sea water alone. The conicals containing complete larval cultures were divided into pairs. One of each pair was aerated directly from a small hole at the bottom of the conical, and the other conical of the pair contained a central tubular air lift that moved culture media from the bottom of the conical to an overflow discharge over the top surface of the media in the conical. One pair of the conicals served as uninfected controls; the second and third pairs were infected as above for each part of the experiment, and were changed at 24-hour intervals; the fourth pair were infected as indicated, but were changed at 48-hour intervals.

Other than the frequency of experimental infection, both parts of the experiment were conducted in the same manner: individual 1 ml samples of culture contents were taken from the top and bottom levels of the conical, both before and after conical changes, for enumeration of *Vibrio* organisms and larvae. The liquid contents of each conical was examined at the time of each change for salinity, temperature, pH, ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen levels. In addition, at each change, bottom deposits were collected and cultured on selective *Vibrio* media for quantitative counts.

The results of the experiment indicated parallel increases in *Vibrio* concentrations in both the top and bottom conical samples taken following experimental infection. In single-dose experimentally infected larval conicals, the increase was limited to the first 24 hours following infection, followed by a rapid decline. In multiple daily-dosed experimentally infected larval conicals, however, the increase was sustained or magnified during the remaining portion of the experimental period. In *Vibrio*-infected conicals without larvae, containing synthetic sea water, or synthetic sea water and standard amounts of algal food, *Vibrio* concentrations also increased in top and bottom conical samples during the first 24 hours, and persisted for longer intervals at higher levels than found in similar samples taken from conicals with oyster larvae. This finding suggests that oyster larvae selectively remove *Vibrio* organisms from larval cultures. The concentration of *Vibrio* organisms was much higher than found in either top or bottom samples taken at the same interval. Bottom deposits were the most sensitive indicators of *Vibrio* organisms in the samples taken. In conicals given single infective doses of *Vibrio* organisms, high concentrations of *Vibrio* organisms persisted in bottom deposits, although samples from the conicals were negative for *Vibrio* organisms. In multiple daily *Vibrio* dosed conicals parallel high concentrations of *Vibrio* organisms were found in both top and bottom samples, although these concentrations were lower than found in bottom deposits. The greatest larval mortality was evidenced in those conicals receiving repeated daily *Vibrio* inoculations. In contrast, those which received a single inoculum, experienced limited mortality during the first 24 hours following experimental infection. No significant mortality was observed in this group after this 24-hour period. In both multiple- and single-dosed larval conicals, greater mortality was experienced with direct bottom aeration than with air lifts. In addition, infected larval conicals that were changed at 24-hour intervals suffered greater mortality than those with 48-hour change intervals. All *Vibrio*-inoculated conical cultures evidenced increases in ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen during the experimental infective period. The greatest increases in ammonia-nitrogen, however, coincided with peak larval mortality periods. The practical implications of evaluating management and diagnostic variables in the experimental model are presented.

References

Leibovitz, L. A Study of Vibriosis at a Long Island Shellfish Hatchery. Proceedings of the In-

ternational Council for the Exploration of the Sea. Oct. 2-11, 1978, Copenhagen, Denmark.

Elston, R. and L. Leibovitz. Detection of vibriosis in hatchery-reared larval oysters: Correlation between clinical, histological and ultrastructural observations. Presented at the 1979 Joint Annual Meeting of the National Shellfisheries Association and the Shellfish Institute of North America at Vancouver, British Columbia, June 1979.

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A HISTOPATHOLOGICAL STUDY OF MUSSELS *(MYTILUS EDULIS)* EXPOSED TO PETROLEUM FROM THE *AMOCO CADIZ* SPILL

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Mussels *(Mytilus edulis)* collected from the Quiberon Peninsula in South Brittany, an area uncontaminated by the *Amoco Cadiz* spill, were separated and randomly placed in two cages. One cage was suspended at a depth of one meter in the Bay of Morlaix, an area highly contaminated with oil from the *Amoco Cadiz;* the other cage was similarly suspended at Rade de Brest, a site which was minimally impacted by *Amoco Cadiz* oil. Mussels from both sites were collected 8 and 25 days after suspending the cages, and their digestive glands processed for light and electron microscopy. Digestive diverticula of the mussels from both exposure periods in the Bay of Morlaix had increased amounts of lipid and lysosomes compared to the levels in tissue from the reference mussels from Rade de Brest. Increased numbers of lipid droplets were evident in the primary and secondary ducts, as well as in the blind-ending tubules, but the lysosomal increases were limited to the latter. Within the tubules, the absorptive cells had greater accumulations of both lipid droplets and lysosomes than did the basophilic secretory cells. A morphometric technique was used to quantify these organelle differences, and the resulting data were statistically analyzed. The ratios of areas of lipid:nuclei in cells from two replicate samples of four mussels held at the Bay of Morlaix for 8 and 25 days were significantly higher ($P<0.001$) than lipid:nuclei area ratios for equivalent samples of mussels from Rade de Brest. Similarly, the ratios of areas of lysosomes:nuclei in cells from mussels from the Bay of Morlaix were significantly higher ($P<0.05$) than lysosomes: nuclei area ratios for mussels from Rade de Brest. No significant differences were found, however, in the ratios of areas of digestive residual bodies: nuclei between the two groups of mussels. The elevated amount of lysosomes in mussels from the Bay of Morlaix is indicative of cellular damage, whereas the impact of increased lipid levels on the mussel is not known.

Chemical analyses revealed higher concentrations of aromatic hydrocarbons, dibenzothiophenes, and hydrocarbon metabolites in mussel tissues from the Bay of Morlaix than in tissues from Rade de Brest (Malins, D.C. *et al.*, Analysis for Petroleum Products in the Marine Environments, Proc. 14th European Marine Biology Symposium, Helgoland, 1979), further indicating that the observed cytological changes were related to exposure to oil from the *Amoco Cadiz.*

EVIDENCE FOR SCLEROTIZATION OF THE SHELL MATRIX OF A MARINE BIVALVE

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Scanning electron micrographs of sections of the shell of *Mercenaria mercenaria* display promi-

nent growth lines, especially in the prismatic region of the shell. The lines, however, seem to become indistinct and cannot be readily identified near the growing shell margin. When the shell is decalcified and thin sections of the insoluble shell matrix are treated histochemically, lysine, tyrosine and a phenoloxidase can be identified in regions of the section corresponding to the most recent growth of shell. It is hypothesized that progressive polymerization of the matrix takes place subsequent to shell deposition, the entire process requiring on the order of 2-3 days to go to completion. Cross-linking of the organic molecules is effected by amino-quinone bonds involving epsilon-amino groups and tyrosine. The ultrastructural appearance of growth lines near the edge of calcified sections can be explained by differential solubility of regions of organic matrix exposed to preparatory treatments.

ROLE OF THE ORGANIC MATRIX IN CALCIFICATION OF THE MOLLUSCAN SHELL

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We investigated the phenomena of calcium binding and initiation of calcium carbonate growth by soluble and insoluble organic matrix from the shells of several molluscan species. Repeating the experiments of earlier investigators, we found that the insoluble matrix is unable to catalyze crystal growth from a solution which is saturated with respect to either calcite or aragonite, at pH close to *in vivo* conditions. There was no evidence for uptake of calcium by the insoluble matrix either.

We isolated and purified soluble shell matrix and observed calcium binding in several species. By selectively modifying or blocking specific amino acid residues in the matrix, we were able to test whether individual chemical groups could be attractive sites for calcium in the process of shell formation. An early conclusion was that dicarboxylic acids, which have been implicated as active uptake sites in collagen, are not responsible for the calcium-binding properties of the shell matrix. The effects of other group modifications will be discussed in detail.

SAMPLING PACIFIC OYSTER *CRASSOSTREA GIGAS* THUNBERG LARVAE AND PREDICTING SPATFALL IN PENDRELL SOUND, BRITISH COLUMBIA

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General breeding of Pacific oysters is irregular in southern British Columbia but one area, Pendrell Sound, has provided consistent spatfall since first investigated in 1949. Since then numerous studies have been carried out to sample Pacific oyster larvae, calculate abundance and predict spatfall. The sampling methods were not compared and estimates of larval abundance were frequently erratic. In 1977 and 1978 three larval sampling methods were compared; five minute surface plankton tows, running pipe samples, and standing pump samples. Accurate prediction of the time of spatfall was made from all three methods. However, there was poor agreement in estimates of larval abundance between the three methods and the results did not permit accurate prediction of the extent of spatfall. This may have been due in part to the heavy abundance of larvae in both years. Improvements to sampling methods are suggested.

A MICROCELL DISEASE OF THE BAY SCALLOP *ARGOPECTIN IRRADIANS*

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During a routine survey of parasites in benthic invertebrates around Millstone Point, Connecticut, in June, 1978, one bay scallop from a sample of five was found to contain a microcell parasite. The cell is approximately two to three microns in diameter, with a nucleus of approximately 0.7 to 0.8 microns in diameter. For the most part, the organism is cytozoic being found primarily in large macrophages and leukocytes, but occasionally free in the hemolymph. The heaviest concentration of parasite-containing cells was in the gonads, where the large macrophages appear to

have replaced the testicular and ovarian follicles.

Samples of scallops collected from September through December, 1978 failed to show a similar infection, but occasional cells resembling the microcell organism were found only in the digestive tubule epithelium.

It is not known at this time whether the stages seen in the digestive tubules are life cycle stages of the parasite found in June, 1978 or another parasite, but the former seems most likely.

SHELL STRUCTURE, MINERALOGY AND MICROMORPHOLOGY OF DEEP-SEA THERMAL VENT BIVALVES FROM THE GALAPAGOS RIFT: ECOLOGICAL IMPLICATIONS

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The shell structure and mineralogy of two unnamed bivalve species (Vesicomyidae and Mytilidae) from deep-sea hydrothermal vents on the Galapagos Rift are typical of calcified structures encountered within their respective families. Calcified shell layers of the vesicomyid clam are entirely aragonitic and, from the shell exterior inwards, may be classified as: (1) homogeneous; (2) predominantly fine complex crossed lamellar with patches of irregular complex crossed lamellar; (3) irregular prismatic (pallial myostracum); and (4) cone complex crossed lamellar. From the vacuolated periostracum inwards, the calcified layers of the mytilid shell consist of: (1) fibrous prismatic calcite; (2) nacre; (3) irregular prismatic aragonite (pallial myostracum); and (4) nacre.

The micromorphology and size of the mytilid prodissoconch shell indicates the presence of a planktotrophic larval stage with long-range dispersal capabilities. Recorded regional abyssal currents are probably sufficient to transport larvae of this species hundreds of kilometers. It is suggested that high water temperatures encountered at the submarine thermal vents provide a larval settlement stimulus. Such a behavioral response to elevated temperature, perhaps coupled with a gregarious setting response, would provide a means of concentrating relatively sessile organisms at regions in and around these isolated thermal systems.

DYNAMICS OF OCEAN QUAHOG *ARCTICA ISLANDICA* POPULATIONS OFF THE MIDDLE ATLANTIC COAST OF THE UNITED STATES[1]

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Abundance and size composition estimates of Middle Atlantic ocean quahog, *Arctica islandica*, populations were derived from seven federal (BCF-NMFS) research survey cruises conducted from Cape Cod to Cape Hatteras during 1965-1977. Sampling was performed with an hydraulic shellfish dredge utilizing a transect grid-type survey design, post-stratified to area/depth strata. Standardized tows of four minutes duration using a 121.92 cm (48 in) wide dredge sampled approximately 83 m^2 of bottom per station.

Minimum population size in numbers and biomass (meat weight) was calculated using mean quahog densities (1965-1977) expanded by strata areas. With the surveyed regions, a standing crop of approximately 56.6×10^9 ocean quahogs was estimated, having a biomass of 1.5×10^6 metric tons (mt). The largest proportion of the resource (46%) was located off Long Island, with 44% off New Jersey and 10% off the Delmarva Peninsula. Estimates of equilibrium yields for the Middle Atlantic quahog resource (Long Island through Delmarva) ranged from 3,022 to 45,332 mt, assuming rates of instantaneous natural mortality (M) between 0.01 and 0.10, and mortality of unharvested quahogs due to gear encounters between 40-60% of the amount caught. If $M \leq 0.05$, (implying that $\geq 0.7\%$ of the population survives to age 100), total annual equilibrium yield for the area Long Island-Delmarva is less than 23,000 mt.

Commercial offshore landings in 1977 and 1978 were 7,295 and 9,163 mt, respectively with the bulk of the FCZ landings from off New Jersey and the Delmarva Peninsula. While the accumulated Middle Atlantic ocean quahog population biomass can support substantial increases in annual harvests, present data suggest that the current landings may be near the equilibrium capacity of the resource and the current distribution of fishing effort is disproportionate to the spatial distribution of stock biomass.

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[1]Laboratory Reference No. 79-16, April 1979

PRELIMINARY OBSERVATIONS ON THE EFFECT OF SUBSTRATE COLOR ON LARVAL SETTLEMENT IN THE SEA SCALLOP *PLACOPECTEN MAGELLANICUS*

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Test substrates were used to examine the effect of color on larval settlement in the sea scallop, *Placopecten magellanicus.* Color *per se* did not appear to be critical in influencing numbers of larvae settling on the substrates. The marginally better performance of white substrates over two other colors used is ascribed to the greater efficiency with which spat are retrieved from lighter-colored substrates.

SELECTION FOR FASTER GROWTH IN THE EUROPEAN OYSTER *OSTREA EDULIS:* FIRST GENERATION RESULTS

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A selective breeding program for the European oyster, *Ostrea edulis,* has been established at Dalhousie University. Initially, we are focusing on genetically improving the growth rate of the stock for intensive culture. Results from the first generation of selection for size at 2 years of age are now becoming available. In the first year the top 10% of the 1975 hatchery year class was selected and spawned as separate lines. Compared to contemporaneous offspring from the parents of the 1975 year class the selected lines are 20% heavier after 2 years of growth. Compared to unselected controls drawn from the same year class the selected lines are 9% heavier. With a response to selection on the order of 9 - 20% per generation a selective breeding program can be expected to produce significantly improved strains of oysters in a few generations.

PETROLEUM HYDROCARBONS AND THE OYSTER: THE EFFECTS OF OIL ADSORBED ON PURE KAOLIN CLAY AND NATURALLY POLLUTED SEDIMENTS

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Oysters were chronically exposed to crude and refined oils adsorbed onto fine kaolin clay (oil-clay) and to oil polluted sediments. Nine chronic exposure studies were completed using oil-clay prepared with Iranian light crude oil, Nigerian crude oil, and No. 2 fuel oil. Concentrations ranged from 0.05 ppm to 0.8 ppm total oil adsorbed onto clay. To explore the effects of oil contaminated sediments from the upper Delaware estuary, oysters were treated with natural sediments which contained high concentrations of hydrocarbons.

The three oils varied in toxicity, and except for Iranian light crude, caused increased levels of mortality compared to seawater and clay controls. Iranian light treatment mortalities were only slightly greater than controls; Nigerian crude oil, however, caused about 2 to 6 times greater mortalities than controls; and No. 2 fuel oil resulted in up to 7 times greater mortalities than control treatments. Oysters exposed to oily sediments from polluted areas in the Delaware estuary, at about 0.5 ppm total oil, showed about 4 times the control mortality. Threshold levels which caused

significant adult mortality were determined at about 0.1 ppm for No. 2 fuel oil, and about 0.3 ppm for Nigerian crude oil.

Histological examination showed no pathological conditions in oil-treated oysters which were unique compared to seawater or clay control oysters. No. 2 fuel oil and Nigerian crude oil, however, caused decreased feeding, inhibition of gonad development, and a small but significant increase in gill damage. There was no evidence from these studies to suggest any interaction between the oil treatment and the two parasites, *Minchinia nelsoni*, and *Labyrinthomyxa marina*, in oyster mortality.

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RATIONALE FOR OYSTER HATCHERY DEVELOPMENT IN AN AREA OF HIGH NATURAL PRODUCTION BASED UPON AN EXPERIMENTAL HATCHERY

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Development of an oyster hatchery in an area of high natural production can result in reduced costs due to the lack of a requirement to condition brood stocks to spawn and the lack of a requirement to raise food for the larvae. Based upon an experimental hatchery a facility has been designed that would cost under $4000, could be operated by one person and could be expected to set one million spat per week.

INFLUENCE OF COPPER ON THE GILLS OF THE LITTLENECK CLAM *PROTOTHACA STAMINEA*[1]

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Copper contamination of the marine environment is a subject of increasing concern due to increases in anthropogenic inputs and the high toxicity of copper to marine organisms. In this study, clams were exposed to levels of control = 0.35;7; 18; 39; and 82 μg/l (ppb) total copper for 30 days. Analyses of our exposure sea water by anodic stripping voltammetry indicated that most of the added copper was present as ionic and/or loosely complexed species.

The results indicated that *Protothaca staminea* is very sensitive to copper. Slight but detectable decreases in survival over controls (= 97% survival) were observed after 30 days at 7 and 18 μg/l (∼ 85% survival at both concentrations). At 39 and 82 μg/l, survival after 30 days was only 14 and 3% respectively. Analyses for copper in tissues of clams surviving the 30 day exposure showed that the gills were the primary organ for the concentration of copper. Gills were the only organ to exhibit a correlation between tissue copper concentration and exposure level. Other organs exhibited similar increases in copper when compared to control values, regardless of the exposure concentration.

When gills were analyzed for specific physiological or biochemical effects of copper, the results indicated effects associated with both lethal and sublethal alterations. Disruption of cellular sodium and potassium regulation was observed at the lethal exposure concentration of 39 μg/l but not at the lower concentration (7 and 18 μg/l) in which survival was only slightly decreased compared to controls. (At 82 μg/l, high mortalities did not allow sufficient sample size for analyses.) At the lower exposure concentrations, increases were observed in the activity of the lysosomal marker enzyme acid phosphatase, suggesting a sublethal cytotoxic action of copper and increased lysosomal activity. Additionally, at 7 and 18 μg/l copper, increases in copper were observed on a soluble, low molecular weight, copper-binding protein. A shift in distribution of copper from this low molecular weight protein to larger proteins in copper-exposed animals was consistent with the "spillover" hypothesis proposed by other workers for the toxicity of trace metals. The molecular weight of this copper- binding protein was estimatd at 14,000 daltons.

[1]This study was supported by a research contract with the U.S. Department of Energy.

OBSERVATIONS ON THE PREDATION OF HATCHERY-REARED SPAT AND SEED OYSTERS BY THE STRIPED BURRFISH *CHILOMYCTERUS SCHOEPFI* (WALBAUM) (DIODONTIDAE)

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The predation of hatchery-reared spat and seed oysters [*Crassostrea virginica* (Gmelin)] by the striped burrfish, *Chilomycterus schoepfi* (Walbaum), under laboratory conditions is described and documented. Five burrfish (10-25 cm, TL) crushed and consumed 163 (0.6-3.7 cm, Ln) spat and seed oysters during experiments that determined their size-selectivity and predation rates. Predation rates ranged from 1.0 to 8.2 oysters/fish/day and one fish consumed 23 spat in 5 min. Predation appears to be dependent on both prey and predator sizes; large fish consume larger oysters than small fish consume. During experiments to determine prey-selectivity, one 25 cm burrfish preferred small blue crabs (*Callinectes sapidus* Rathbun) over all other species of prey, but did consume barnacles, mussels, and oysters. In captivity, burrfish appear to be opportunistic carnivores. Striped burrfish appear to be minor oyster predators unless they encounter unattached spat and/or seed oysters on unprotected bottoms in waters of moderate salinity (above 20°/₀₀).

CONTROL OF A FILAMENTOUS BACTERIAL DISEASE OF BRINE SHRIMP

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A strain of a colorless, filamentous bacterium, identified as *Leucothrix mucor*, heavily infests the brine shrimp, *Artemia salina*. The resulting disease is characteristically expressed as a massive cover of the bacterium plus associates and debris over all external body surfaces, giving affected shrimp a moldy appearance. The ultrastructure of the bacterium, unlike that of some other strains, does not reveal an external sheath, distinct middle layer between its inner and outer double membranes, or irrgular blebs extending from cell walls. When undergoing treatment with formalin, potassium permanganate, terramycin, nitrofurazone, cutrine, and low concentration of seawater to control *L. mucor*, the affected shrimp sloughs its entire infestation as a single mat. Primarily because of the toxic response by brine shrimp to most treatments, we consider 100 ppm terramycin the treatment of choice. (Funded in part by the U.S. Department of Commerce, NOAA, National Marine Fisheries Service, PL88-309, Project No. 2-325-R)

OBSERVATIONS ON CONTAINERIZED RELAYING OF POLLUTED OYSTERS IN MISSISSIPPI SOUND

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Four, 15-day purging experiments using naturally contaminated oysters were conducted in approved shellfish-growing waters at Horn Island, Mississippi, to assess the reliability of containerized, off-bottom relaying. The oysters were held in commercial chicken "coops" (86 × 56 × 20 cm) that were fabricated with structural, polyethylene foam. The coops have a solid bottom, a hinged lid, and a capacity of 0.5 barrel (1.5 sacks) of oysters. During the experiments the oysters were relayed in a 3-coop stack and suspended above the bottom and below the mean low water level. Oysters purged fecal coliforms from initial median values of up to 23,000 MPN/100 gm of tissue down to or below the recommended 50 MPN level excluding equipment failure. Mortality rates within the coops ranged from 3 to 12% even though no attempt was made to acclimate the oysters to the higher salinity waters of the study site. The oyster coop system appears to have immediate applicability to the oyster leasing and relaying program in Mississippi if logistic and

legal problems can be solved. The re-usable coops will serve as acceptable purging and transport containers from initial harvesting, through the cleansing period, to the final processing.

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ABSTRACTS OF PAPERS PRESENTED AT A SPECIAL SYMPOSIUM: RECENT ADVANCES IN MOLLUSCAN PHYSIOLOGY

COMPARATIVE PHYSIOLOGY OF INTERTIDAL BIVALVES

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Resistance adaptations in intertidal species of bivalves allow survival at greater extremes of temperature, desiccation, salinity and hypoxia than can be endured by subtidal species. Capacity adaptations permit activities to be maintained at optimal levels during "normal" environmental conditions; their significance should be assessed by determining their contributions to the energy budget of the organism. It is proposed that capacity adaptations to intertidal life be further subdivided into those which are energy-conserving and those which are energy-supplementing.

A number of intertidal animals have been shown to acclimate to slowly changing thermal conditions, and this is presumed to be adaptive. Data presented for *Crassostrea virginica* indicate that this species shows reasonably good thermal compensation of its metabolic rate when acclimated under immersed conditions, but that acclimation regimes involving exposure to air produce different results. It is suggested that aerial exposure be considered in future temperature-acclimation studies on intertidal bivalves.

Growth rate may be regarded as a physiological integration of capacity adaptations, and used to assess the importance of various adaptations observed in or postulated for littoral species. Determining growth rate as a function of aerial exposure level under controlled conditions is a good starting point for studying the comparative physiology of intertidal suspension feeders. A scheme is proposed whereby index values for intertidal compentence based on growth performance are derived and used to classify bivalves into four categories: shore-intolerant; shore-tolerant; shore-competent; and shore-adapted.

The ribbed mussel, *Geukensia demissa*, is a shore-adapted species whose growth rate increases with increasing intertidal height at least to the level of 60% mean aerial exposure.

RECENT ADVANCES IN BIOCHEMICAL CONTROL OF REPRODUCTION, SETTLING, METAMORPHOSIS AND DEVELOPMENT OF ABALONES AND OTHER MOLLUSCS: APPLICABILITY FOR MORE EFFICIENT CULTIVATION AND RESEEDING[1]

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Our research has been aimed at identifying the natural physiological, biochemical and hormonal triggers which are normally required for the efficient control of reproduction, development and growth in the red abalone *(Haliotis rufescens)* and related molluscs. Applications of our findings have resulted in the development of inexpensive, convenient and reliable simple chemical procedures for efficient control of reproduction, larval settling, metamorphosis and early growth, for the improved economic efficiency of cultivation

and reseeding, in a variety of commercially valuable shellfish.

We have found that low concentrations of hydrogen peroxide, when added to alkaline seawater, will reliably induce copious spawning of gravid male and female abalones and certain other species. The peroxide appears to act by stimulating the endogenous enzymatic synthesis of prostaglandins and related compounds in the reproductive tissues of these (and other) animals; the hormone-like prostaglandins physiologically trigger normal release of gametes fully competent for fertilization and early development.

A subsequent critical stage in molluscan life-cycles typically occurs at metamorphosis, with high mortality characteristic of failures at this transition. We recently have identified a family of simple and closely related compounds which are normally required for the efficient induction of settling and metamorphosis of the planktonic *H. rufescens* larvae; these required compounds usually are provided by the specific crustose red algae which normally "recruit" the planktonic larvae, inducing efficient settling and metamorphosis of the larval abalones on these algal surfaces in California coastal waters. Among the most potent and abundant of these normally required algal inducers is the simple amino acid known as γ-amino butyric acid ("GABA"), a potent neurotransmitter in many animal species. The addition of low concentrations of this inexpensive natural inducer is sufficient to trigger rapid and synchronous settling and metamorphosis of *H. rufescens* larvae, with fully efficient development and rapid early growth of the resulting juveniles. We have shown that use of GABA to induce rapid and synchronous metamorphosis and early juvenile development is effective in reducing the serious mortality which otherwise may result from intensive cultivation (and microbial overgrowth of developmentally arrested or retarded larvae) in the absence of such required inducer.

In further analytical studies, these results have been used: to identify and define other principal requirements for optimal cultivation, metamorphosis and early growth of *H. rufescens* and related species; to identify principal sources of early mortality in these species; and to devise a convenient, rapid and reliable new bioassay for the evaluation of water-quality requirements, and the quantitation of interference in metamorphosis and early development from pollutants of industrial, agricultural and urban origin.

Results of these studies, and the resulting techniques developed for convenient biochemical control of reproduction, settling, development, and genetic breeding (with minimization of early mortality and its associated costs) have found wide applicability in research and cultivation with many valuable molluscan species. We know of at least 14 valuable species of gastropod and bivalve molluscs (representing 8 different genera) which can be induced to spawn using the convenient and inexpensive hydrogen peroxide technique; many of these are species which had not previously been induced to spawn by other methods. Similarly, the finding that simple amino acid/neurotransmitter-type compounds are required for normal induction of metamorphosis in red abalone also has wide general applicability. We know of at least 4 species (representing 3 different genera) of molluscs which are induced to settle and begin metamorphosis by GABA; this and related simple compounds are expected to prove effective in the induction of settling and metamorphosis in other species, as well.

---

[1]This research has been supported by the U.S. Department of Commerce (NOAA) — University of California Sea Grant Program, and the University of California.

A PHYSIOLOGICAL APPROACH TO THE SUMMER MORTALITY PROBLEM OF PACIFIC OYSTERS IN WASHINGTON STATE

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The summer mortality phenomenon of the Pacific oyster, *Crassostrea gigas*, has been studied for over two decades by both Japanese and U.S. researchers. Although the characteristics of the mortality are well known by growers and scientists alike, no direct course has been clearly established.

Japanese research on the summer mortality in Matsushima Bay, Japan in the 1960's and recent insights into the reproductive physiology of

Crassostrea gigas support the supposition that during the warm late summer months, oysters in nutrient rich heads of bays are in a state of over-maturation which consequently results in an unusally low level of stored reserves. Without these badly needed reserves, the Pacific oyster conceivably succumbs to stressful conditions characteristic of the high mortality areas such as warm water temperatures and periodic outbreaks of toxic-laden dinoflagellates.

The source of Pacific oysters being used in these studies is F_2 progeny developed in the selective breeding program of the University of Washington. The reproductive physiology of both high and low surviving families is being studied under both field and laboratory-controlled conditions. It is hoped that results from these studies will add to our knowledge of the cause or causes of the summer-mortality phenomenon in Washington state waters.

THE UTILIZATION OF FOOD BY BIVALVE MOLLUSCS

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A knowledge of how bivalves collect food, and what they do with that food is not only central to an understanding of the general biology of bivalves but is also relevant to maximizing the production of cultured species and the evaluation of possible culture sites.

There are several ways in which such knowledge has been acquired, for example:

Feeding cycles, tidal and circadian, have been elucidated and optimal feeding regimes designed accordingly.

Recent advances have been made in digestive enzymology, particularly in the areas of cellulose digestion and protein digestion. Low levels of extra-cellular protein digestion are compatible with the crystalline style, but some styles are particularly resistant to digestion. The carnivorous septibranchs have high levels of extra-cellular protein digestion but retain the style. Proteolytic enzyme activity shows a distinct cycle related to feeding patterns.

Work with labelled food organisms has illustrated rates of uptake and digestion and efficienty.

The significance of the absorption of dissolved organic molecules has been largely ignored but may be important. It is certainly relevant to a new species of gutless *Solemya* which has recently been discovered. This animal has no digestive apparatus and must be presumed to depend upon dissolved organic molecules in its environment. This problem and others relating to the digestibility of particular food species and mixtures of food species remain to be investigated.

Volume 69 — paper by F.M. Serchuk, P.W. Wood, J.A. Posgay, and B.E. Brown: Assessment and Status of Sea Scallop (*Placopecten magellanicus*) Populations off the Northeast Coast of the United States.

Page 163: Left column, second paragraph, line 6 — after "that spatfall" ADD . . . "occurs 35 days after fertilization (Culliney 1974). The distribution of spatfall . . ."

Page 166: Table 3, year 1968, column 5Ze — "994" should be "1025."

Page 169: Table 5, Footnotes 5 and 6 — the "k" values listed in the von Bertalanffy growth equation and the age-weight relationship, ".2297" should be ".2997."

Page 177: Table 10, Columns labeled "≥70 mm per tow" and "<70 mm per tow." These column headings are reversed. They should be: "<70 mm per tow" and "≥70 mm per tow.

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Original papers given at the Annual Association Convention and other papers on shellfish biology or related subjects will be considered for publication. Manuscripts will be judged by the Editorial Committee or by other competent reviewers on the basis or originality, contents, clarity of presentation and interpretations. Each paper should be carefully prepared in the style followed in the 1972 PROCEEDINGS (Volume 63) before submission to the Editorial Committee. Papers published or to be published in other journals are not acceptable.

Manuscripts should be typewritten and double spaced; original and two copies are required to facilitate reviews. Tables, numbered in arabic, should be on separate pages with the title at the top. Scientific names should be underlined. Illustrations preferably should be 8 x 10 inch prints which can be reduced to a size of 6¼ x 8 inches or smaller. Glossy photographs are preferred to originals. Illustrations smaller than a page should be carefully oriented and loosely attached to plain white paper with rubber cement. Legends should be typed on separate sheets and numbered in arabic.

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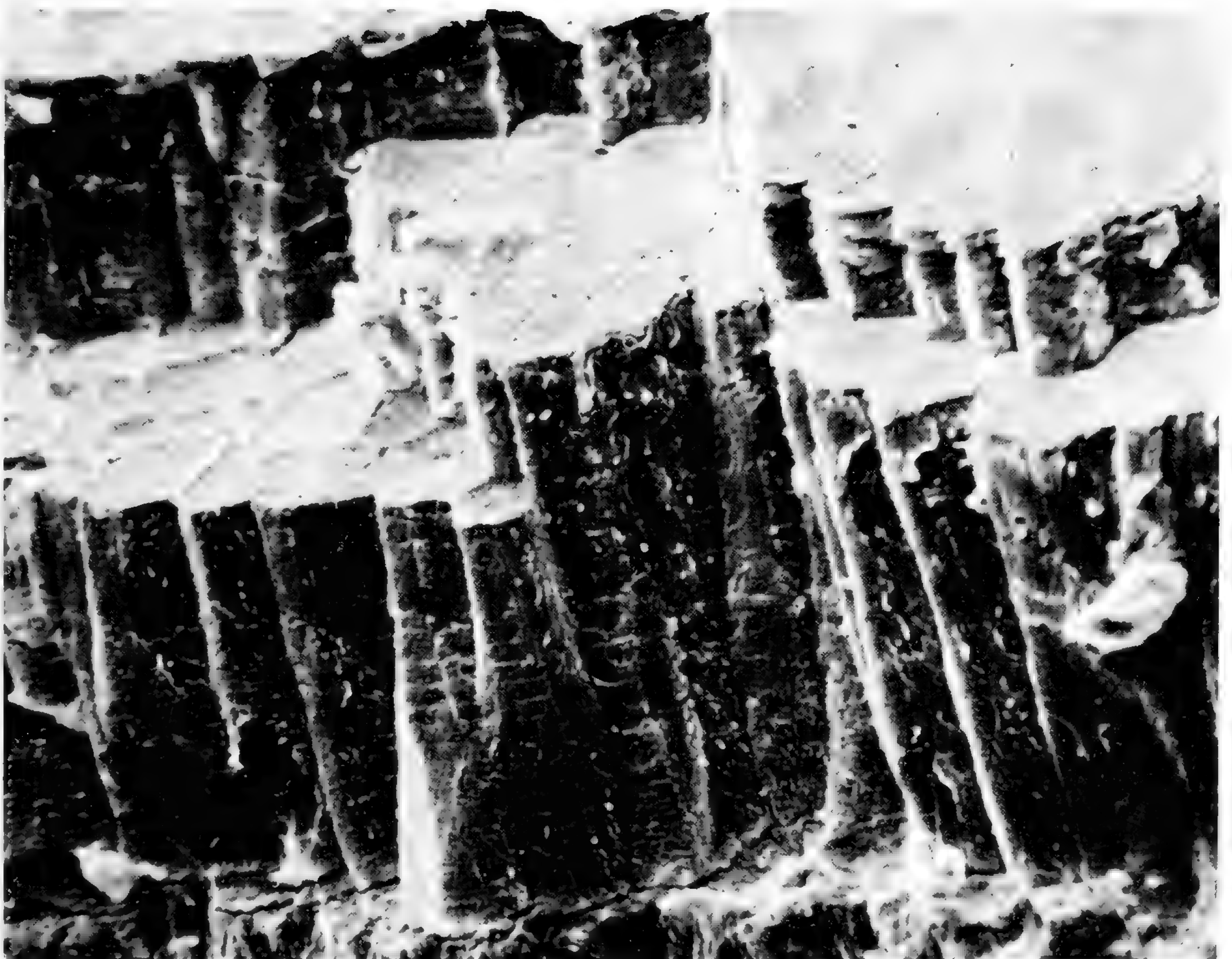

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Scanning electron micrograph of fracture line in oyster *(Crassostrea virginica)* shell.
Courtesy M.R. Carriker, R.E. Palmer and R.S. Prezant. (See page 139).

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FUNCTIONAL ULTRAMORPHOLOGY OF THE DISSOCONCH VALVES OF THE OYSTER *Crassostrea virginica*

Melbourne R. Carriker, Robert E. Palmer, and Robert S. Prezant

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ABSTRACT

We present an illustrated scanning electron microscopical overview of the structure, grouping, and layering of microstructures of the dissoconch valves of the oyster, Crassostrea virginica *(Gmelin). Development and function of microstructures relative to configuration, growth, and function of valves are emphasized. Oysters used in the study were grown either in a local estuary or in a controlled laboratory habitat. The adult oyster shell is composed of four principal kinds of mineralized microstructures (calcitic simple prisms, foliated calcitic laths, chalky calcitic units, and aragonitic myostracal prisms), transitional microstructures, and conchiolin. The periostracum is very thin and nonmineralized. All shell structure contains organic matrix, but that of calcitic prisms is most prominent. Calcitic prismatic structure is present on both right and left valves; that of the left valve has been overlooked in previous studies. Prisms increase in size away from the margin of the valves due to fusion of older prisms. Multilayering of prismatic strata occurs primarily in the right valve. The bulk of both valves consists of foliated and chalky structure. Laths in the region of valves between the adductor muscle scar and ventral shell edge generally point ventrally; those between adductor muscle scar and hinge are variably oriented. Motility of the mantle on the ventral side may partly explain this orientation. Chalky shell, composed of blades and leaflets, bounds a system of pores. The adductor muscle-facing surface of the myostracum is smooth while its ventral edge is a narrow transitional zone laid down in advance of muscle attachment. Conchiolin patches commence as thin granular layers on laths. A band of ligostracal prisms is deposited in advance of deposition of ligamental resilium and tensilia as the shell grows. A rugose, pitted, foliated structure is laid down ventral to this and probably holds the mantle isthmus close to the shell. The resilium is reinforced by aragonitic fibers; tensilia lack these. Transitional zones of granular crystallites join adjacent prismatic, foliated, chalky, and myostracal layers. Umbonal plicae are present in young dissoconchs and strengthen attachment of the left valve to the substratum. Microscopic shell annuli are present in the outer prismatic layer, resilium, chondrophoral and nymphal ligostraca, and adductor myostraca. The study provides new insights on shell structure and suggests profitable avenues for research on shell formation.*

INTRODUCTION

The shell of the oyster, *Crassostrea virginica* (Gmelin), is composed of four principal kinds of mineralized microstructures. These include calcitic simple prisms, foliated calcitic laths; chalky calcitic units, aragonitic myostracal prisms (Galtsoff, 1964; Carter, 1979, 1980). From these basic structural units, their transitional forms, and the supporting periostracal and conchiolinal organic matrix, the oyster mantle fashions the ultrastructurally complex shell that encloses and supports its soft tissues. Development of the valves reflects changes accompanying increase in size from prodissoconch I to the mature individual (Carriker and Palmer, 1979a) and mechanical stress resulting from antagonistic functioning of ligament and adductor muscle (Wainwright, 1969). The wonder is that so apparently simple a biological structure as mantle epithelium can produce the complexity and diversity of organic-mineralized forms realized in shell ontogeny.

In view of the economic importance of *Crassostrea virginica*, its usefulness as an experimental animal (Galtsoff, 1964), the substantial research that has been conducted on it during the last several decades (Baughman, 1947; Joyce, 1972), and the recent flood of literature on ultrastructure of the skeletons of many other biological species (Scanning Electron Microscopy, 1968-1979), it is surprising that more research has not been conducted on the fine structure of the valves of the oyster. Principal publications on the ultrastructure of shell microstructures of *C. virginica* include those by Watabe et al. (1958), Tsujii et al. (1958), and Watabe and Wilbur (1961) using transmission electron microscopy to study replicas of surfaces; and those by Watabe (1965) and Travis and Gonsalves (1969) on the transmission electron microscopy of ultrathin sections of shell. The ultrastructure of the shell of other species of oysters (primarily the pearl "oyster" *Pinctada radiata*), examined by replication and ultrathin sections, was treated by Wada (1961, 1972, and earlier papers) and Nakahara and Bevelander (1971). With the exception of the research by Margolis and Carver (1974) on chalky shell, and Carriker and Palmer (1979a) on newly set dissoconchs, no investigations on the scanning electron microscopy of the dissoconch of *C. virginica* have been reported. Wise (1969, 1970), Wise et al. (1971), and Watabe (1974) investigated the shell of other species of oysters (principally pearl "oysters") with scanning electron microscopy. Reviews of the literature on oyster shell microstructures are included in the publications of Taylor et al. (1969), Grégoire (1972), and Wilbur (1972).

Scanning electron microscopy makes possible the study of the structure, grouping, and layering of molluscan shell units with respect to shell form and size. An architectural study of this kind has not been attempted before on the oyster. We present here such an ultramorphological overview of the dissoconch valves of *Crassostrea virginica*. Emphasis is placed on the development and function of shell microstructures relative to the configuration, growth, and function of the valves.

Our observations are integrated with those of earlier workers using transmission electron microscopy on the shell of this and related species of oysters in the genera *Crassostrea* and *Ostrea*. Individuals in these genera possess a substantial inner layer of foliated calcite (Taylor et al., 1969) in contrast to the nacreous aragonitic inner layer of pearl "oysters" (see Wada, 1972, for example). Ultrastructural morphogenesis of prodissoconch and early dissoconch valves of *C. virginica* was described by Carriker and Palmer (1979a) and provides the setting on early ontogeny for the present investigation.

MATERIALS AND METHODS

Oysters

The valves of 62 oysters *(Crassostrea virginica)* were studied. Height of valves (dorsoventral dimensions) averaged 2 to 5 cm; occasional specimens as short as 1.7 mm, and as tall as 8 cm, were also examined. Oysters for the study were taken from four different habitats: a) wild oysters setting and growing indigenously in Broadkill River, Lewes, Delaware, a natural estuary where oysters grow rapidly, b) oysters spawned from brood stock originally collected in Broadkill River, set on mylar sheets, raised to small spat size in the maricultural facility of the College of Marine Studies, University of Delaware, and grown suspended from a floating dock in Broadkill River, c) oysters spawned and grown on

mylar sheets and on glass slides in the maricultural facility in a flow-through system in which seawater was changed every second day, and d) oysters spawned and grown on mylar sheets in the maricultural facility in a system in which seawater was largely recycled (see Palmer and Carriker, 1979b, for descriptions of the systems). During the growing period the exterior of valves of oysters was brushed carefully with a soft toothbrush every one to two weeks to prevent fouling organisms from developing on the surface.

Preparation of specimens for electron microscopy

Oysters were opened (under running tap water) by cutting off a part of the valves with a thin, high speed, diamond-coated wheel (22 mm in diameter) or by prying the valves apart at the hinge with a sharply pointed oyster knife. The adductor muscle was cut from the valves without scratching the interior of the valves, and muscle attached to the adductor muscle scar was removed by gentle rubbing with the dull edge of a wet human fingernail. Muscle remaining on the scar was removed by application of Clorox.

For study of the surface of the ligament facing the mantle isthmus, valves of whole oysters were sawed under running water in an anterior-posterior line just dorsal to the adductor muscle with the diamond wheel. For examination of chondrophores and nymphae of the hinge area, valves, still held together by the ligament, were immersed in 10% Clorox for 48 hr (Clorox = 5.25% sodium hypochlorite); valves were then separated and residual ligamental material was removed gently with a camel's hair brush.

Radial sections (dorsoventral axis from hinge to ventrum) of valves were sawed under running water with a Gillings-Hamco thin-sectioning machine (Gillings and Buonocore, 1959) after dried valves had been embedded in an epoxy resin (Epon 815). Radial surfaces were polished with alumina or boron carbide (Jones and Gadomski, 1978). Diamond paste was tried, but was unsatisfactory as it left plastic grit on the polished surface.

Different samples of valves were treated as follows in preparation for examination with the scanning electron microscope: a) exterior and interior surfaces were brushed gently with a soft toothbrush under running tap water to remove loose tissue fragments and organic films, b) organic matter in shell surfaces was dissolved in Clorox in a range of concentrations of full strength, 20%, and 5%, for intervals ranging from 5 sec to 1 min, depending on the solubility of the organic material and the degree of removal of organic material desired, and c) mineral components of shell were etched in 0.1N HC1 for 30 sec. In addition, unanticipated etching of minerals (more delicate than that achieved with solvents listed in b and c) was found in parts of the prismatic shell of medium sized, Broadkill River oysters held on a windowsill in the laboratory for 10 days in October. These oysters were maintained in a glass bowl of still seawater changed every second day from a supply of Broadkill River seawater that was held without changing in a pail beside the bowl.

For study with the scanning electron microscope, pieces of shell, usually less than 1 cm in longest dimension, were cut from oyster valves with the 22 mm diamond-coated wheel under running water. Shell sawdust released by the saw was removed by flowing water and by light brushing under water.

Controlled fracturing of a specific area of a valve was done by sawing with the 22 mm wheel from opposite sides in a straight line to approximately 5 mm of the desired fracture area. A block approximately 1 cm square was sawed around the part to be fractured. Then, by hand, or with pliers whose tines were covered with soft felt, the block was fractured apart at a line joining the original saw marks. Fragments fractured by shattering of whole valves with a hammer were also examined. No fluids were allowed to touch the fractured surfaces, unless etching was intended.

Desiccation of shell specimens, necessary to minimize or eliminate charging in the scanning electon microscope, was achieved in one of four ways: a) drying in an oven at 70°C, b) immersion in several changes of absolute ethyl alcohol or acetone, c) freeze drying, or d) critical point drying from absolute ethyl alcohol into carbon dioxide. Procedures a and b worked well for small pieces of shell and when allowed to dry for long periods of time; for shell with large proportions of organic matter, such as prismatic layers, or shell

with adductor muscle or ligament attached to it, procedures c and d worked best.

Shell specimens were mounted with silver paint on scanning electron microscope aluminum pin stubs that had been cleaned in acetone. Different types of double adhesive tape were tried for mounting, but resulting images on the display screen of the cathode ray tube were generally best after mounting with silver paint. Mounted specimens were held in an oven at 70°C for several days prior to coating with metal in vacuum.

Mounted specimens were coated in vacuum with two or more layers of carbon and gold (400-600 Å) and examined with the scanning electron microscope within a day. Clearer images were obtained after heavy metal coating than with the generally prescribed thinner coats. When surfaces of specimens were highly irregular, additional gold was evaporated on them with a sputter coater. In preparation for transmission electron microscopy, small pieces of the resilium of the ligament were fixed in 2% glutaraldehyde and postfixed in 1% osmium tetroxide in a phosphate buffer at pH 7.4, embedded in Epon 812, ultrathin sectioned, and stained with uranyl acetate and Sato lead.

Micrographs and Analysis

A total of 1350 scanning electron micrographs was taken of the fine structure of the valves of *Crassostrea virginica* during the period February, 1977-January, 1980. The rationale in scanning was to thoroughly explore the basic ultrastructure, range of variation, and transitional zones of shell surfaces, layers, and microstructures. Each ultrastructure was analyzed in at least three different oyster shells. Unetched natural surfaces, etched surfaces, and fractures proved the most informative. Polished surfaces, whether etched or not, tended to mask ultrastructure. The tendency of shell to fracture naturally along the boundaries of organic matrix was invaluable in the study of ultramorphology of microstructures. Scanning electron micrographs were taken at effective magnifications ranging from about 20 to 20,000X at accelerating voltages of 15 to 30 Kv.

To facilitate analysis of microstructures and regions of the shell, micrographs were classified in the following categories and are treated in this sequence in the sections that follow: periostracum, prismatic structure, foliated structure, chalky structure, myostracum, conchiolin patches, chondrophores, nymphae, ligament, umbonal plicae, annuli, worn exterior surfaces, and solubilized interior surfaces. After analysis, the minimum number of micrographs that clearly illustrates the ultramorphology of each microstructure, layer, and region was chosen for inclusion.

Insofar as we have been able to determine, we include only normal structures and microstructures. Normality was inferred if macroscopic growth and appearance of valves conformed to those of what we have come to recognize along the East Coast of the United States as "healthy" oysters.

Dimensions of microstructures and layers in the shell of oysters vary widely in different regions of the same individual and among different individuals. For ease in comparing dimensions, the actual horizontal field width of each micrograph is given in its accompanying caption.

TERMINOLOGY

Several writers have recently clarified the descriptive nomenclature for molluscan shell structures (Taylor et al., 1969; Kobayashi, 1971; Grégoire, 1972; Carriker and Palmer, 1979a; Carter, 1979, 1980). To avoid further ambiguity, we list briefly the principal terms employed here, terms basically those proposed by Taylor et al. (1969) and Carter (1979, 1980), and some used by Carriker and Palmer (1979a) for ultrastructures of newly set *C. virginica* (Fig. 1).

The exterior of recently deposited shell of dissoconch valves is covered by a thin organic *periostracum*. This overlies the thin stratum of *prismatic structure* that constitutes the outer region of both valves and is most prominent on the right valve. Prismatic structure consists of compact, closely joined layers of calcitic, regular, simple *prisms*. On the external surface of the valves, primarily of the right valve, the ventral rim of individual layers of prisms tends to flare outward forming overlapping, imbricated *scales* (the "spurs" of Nakahara and Bevelander, 1971). On older parts of valves, prismatic structure is generally eroded, and these scales are thus absent, exposing the underlying *foliated structure* (the

calcitostracum, subnacreous, or nacreous layer of several authors), which with chalky shell forms the inner bulk of the valves. *Foliated structure* consists of fine sheets, or *folia* (or *laminae*), grouped into larger *lenticular folia.* Individual folia are composed of small elongate *laths* (*tablets* or *lamellae* of other authors), joined together by organic matrix. On the interior surface of valves, especially near the ventral margin, laths are arranged regularly like tiles on a roof. Between the adductor muscle scar and the umbones, lath directions are irregular and more variable. Carter (1980, and personal communication) refers to the irregularly oriented laths as complex crossed foliated structure. Lenses of *chalky structure,* consisting of microstructures arranged irregularly in a spongy pattern, occur commonly in foliated structure. Carter (personal communication) suggested that chalky shell represents a structural modification of foliated structure (see also Carter, 1980); our observations lend support to this suggestion. The adductor muscle scar is the external surface of the *myostracum,* which is composed of aragonitic, *irregular simple prisms.* Occasionally oysters deposit *conchiolin patches* over the interior surface of the foliated structure. The *ligament* in the umbonal hinge consists of a prominent central part, the *resilium,* and anterior and posterior extensions, *tensilia.* The resilium is reinforced by *aragonitic fibers,* while tensilia lack them. The resilium is attached to umbones at *chondrophores,* and tensilia at *nymphae* (Galtsoff, 1964). Surfaces of chondrophores and nymphae support a prismatic layer, the *ligostracum,* which bonds the ligament to the umbonal shell. On the right valve ligostracal prisms are aragonitic; on the left valve, a mixture of calcite and aragonite (Carriker and Palmer, 1979b). Prominent *umbonal plicae,* or folds, extend from the left umbo ventrally on either side of the valve, especially in young dissoconchs. *Annuli,* rings of interrupted growth or changes in pattern of structure, occur in the prismatic structure of both valves and in the ligostraca of chondrophores and nymphae. Boundaries between different kinds of microstructures are marked by sharp to gradual *transitional zones* (Watabe et al., 1961).

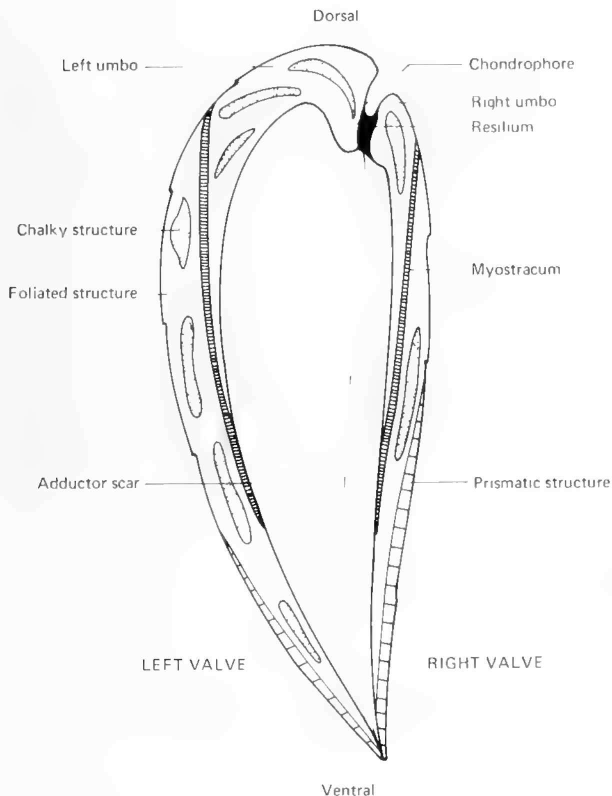

FIGURE 1. Crassostrea virginica, *diagrammatic drawing of anterior half of valves, sectioned dorsoventrally through middle of hinge, thickness of valves exaggerated to illustrate major shell regions. Oyster 5 cm high.*

ULTRAMORPHOLOGY

In this section we describe the ultrastructure, arrangement, and variation of dissoconch microstructures relative to shell layers and shell form. Observations of earlier investigators are integrated with our findings. The major regions of the valves of *Crassostrea virginica* are outlined in Figure 1.

Periostracum, Right Valve

The periostracum, present as an external cover only on the unworn prismatic structure of the valve, is so inconspicuous (Taylor et al., 1969) that in many places the outer layer of the organic envelope (or sheath) of prisms and the overlying periostracum are difficult to differentiate (Fig. 3, 4).

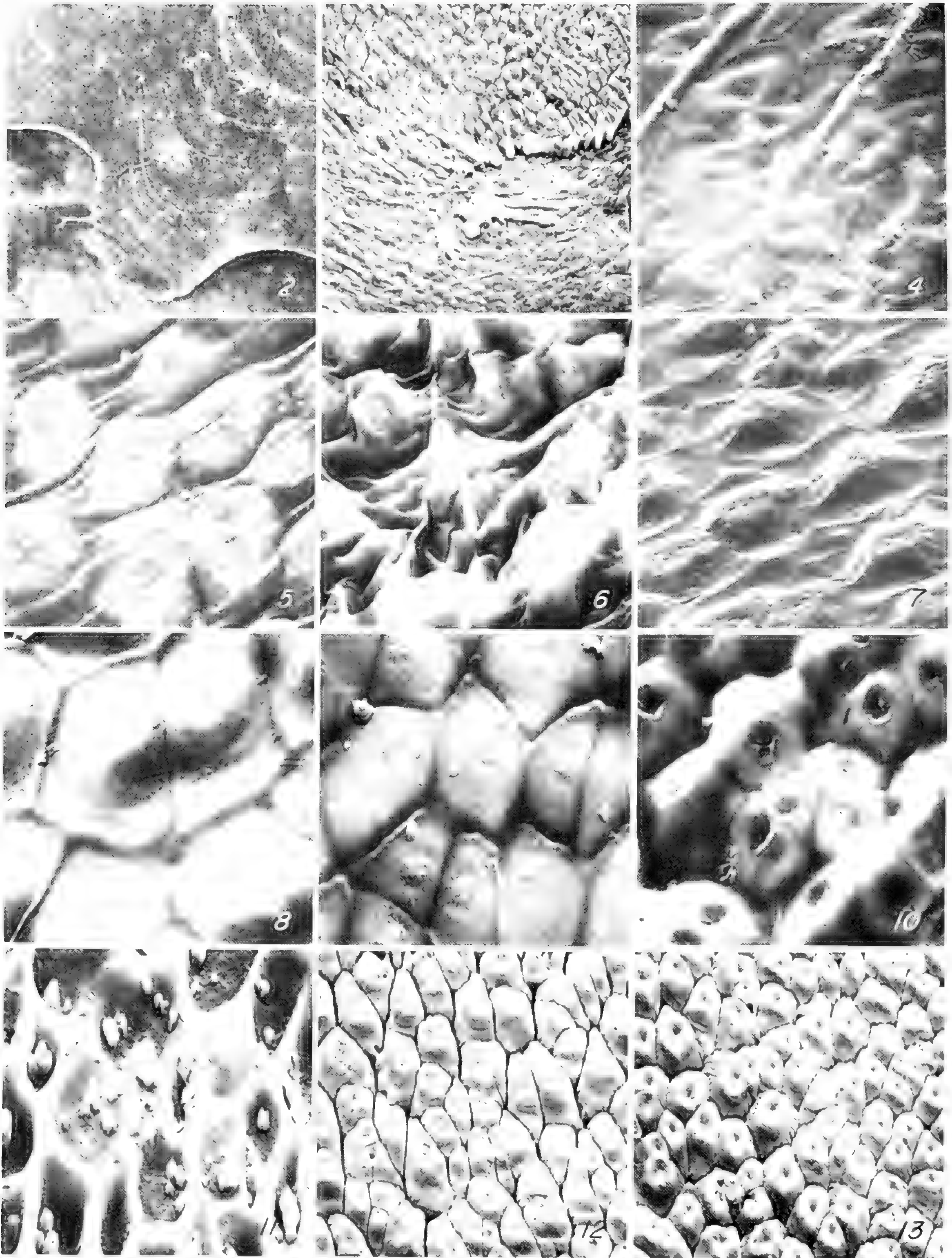
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FIGURE 2. *Prismatic scale, right valve. 100% Clorox 15 sec. Horizontal field width = 2.5 mm.*
FIGURE 3. *Periostracum, exterior surface, right valve. Periostracal folds overlying prisms at spot where prismatic growth was interrupted and then resumed. 100% Clorox 1 min. Horizontal field width = 240 µm.*
FIGURE 4. *Periostracum, exterior surface, right valve. Periostracal folds overlying prisms. 5% Clorox 1 min. Horizontal field width = 30 µm.*
FIGURE 5. *Periostracum, exterior surface, right valve. Periostracal ridges overlying prisms. 20% Clorox 10 sec. Horizontal field width = 30 µm.*
FIGURE 6. *Periostracum, exterior surface, right valve. Dense periostracal folding. 15%* H_2O_2 *1 min. Horizontal field width = 60 µm.*
FIGURE 7. *Prisms, exterior surface, with shallow boss, right valve. 100% Clorox 5 sec. Horizontal field width = 32 µm.*
FIGURE 8. *Prisms, exterior surface, smooth domed, right valve. 15%* H_2O_2 *1 min. Horizontal field width = 30 µm.*
FIGURE 9. *Prisms, exterior surface, with punctate central disk, right valve. Brushed clean. Horizontal field width = 30 µm.*
FIGURE 10. *Prisms, exterior surface, central disk depressed, right valve. 15%* H_2O_2 *1 min. Horizontal field width = 60 µm.*
FIGURE 11. *Prisms, exterior surface, depressed, right valve. Brushed clean. Horizontal field width = 60 µm.*
FIGURE 12. *Prisms, exterior surface, central disk with concentric circles, right valve. 100% Clorox 1 min. Horizontal field width = 80 µm.*
FIGURE 13. *Prisms, exterior surface keeled, sides grooved, right valve. 100% Clorox 1 min. Horizontal field width = 120 µm.*

Elevation of the periostracum into folds and wrinkles is common. Ridges are more numerous where prismatic growth has been interrupted than where normal development occurs (Fig. 3). Periostracal ornamentation varies in the same valve, and in valves of different individuals, from one or two ridges per prism (Fig. 5), to crowded folding and crumpling (Fig. 6). Thickness of the periostracum is variable, and ranges from one to several micrometers.

The periostracum is entirely organic and nonmineralized (Taylor et al., 1969). Hirata (1951) possibly mistook the prismatic layer for the periostracum when he noted that the periostracum in his specimens contained crystalline materials in a cellular structure.

Prismatic Structure, Right Valve

The ornamentation of prismatic scales ranges from flutings and ruffles to relatively smooth, gracefully undulating, overlapping terraces. Growth annuli in scales are clear, especially near the edge of each scale (Fig. 2).

Prismatic structure of the right valve consists of generally discrete, parallel, columnar, closely packed calcitic units (prisms) of variable size, polygonal in cross section, and delineated from each other by relatively thick, nonmineralized, generally simple, conchiolin walls.

Exterior surface. The central part of the exterior surface of most prisms is elevated in the form of a shallow boss (Fig. 7). The shape of the boss varies from that of a relatively smooth dome (Fig. 8) to an elevation with a conspicuous disk in the center (Fig. 8-14). The disk can be raised (Fig. 9), or depressed (Fig. 10-14, 16). The dome in some valves can be sharply keeled (Fig. 13, 14). Occasionally, the surface of prisms is deeply depressed below the surface of the periostracum, giving the impression of exaggerated boundaries between them (Fig. 11). Some prisms assume an overlapping, tongue-like form (Fig. 15), with shallow striae running parallel to the long axis of each prism. The central disk of each prism can be relatively smooth (Fig. 8, 13, 14), conspicuously punctate (Fig. 9), or characterized by a series of concentric circles (Fig. 12, 16). At a higher magnification, the pattern of circles within the central disk ap-

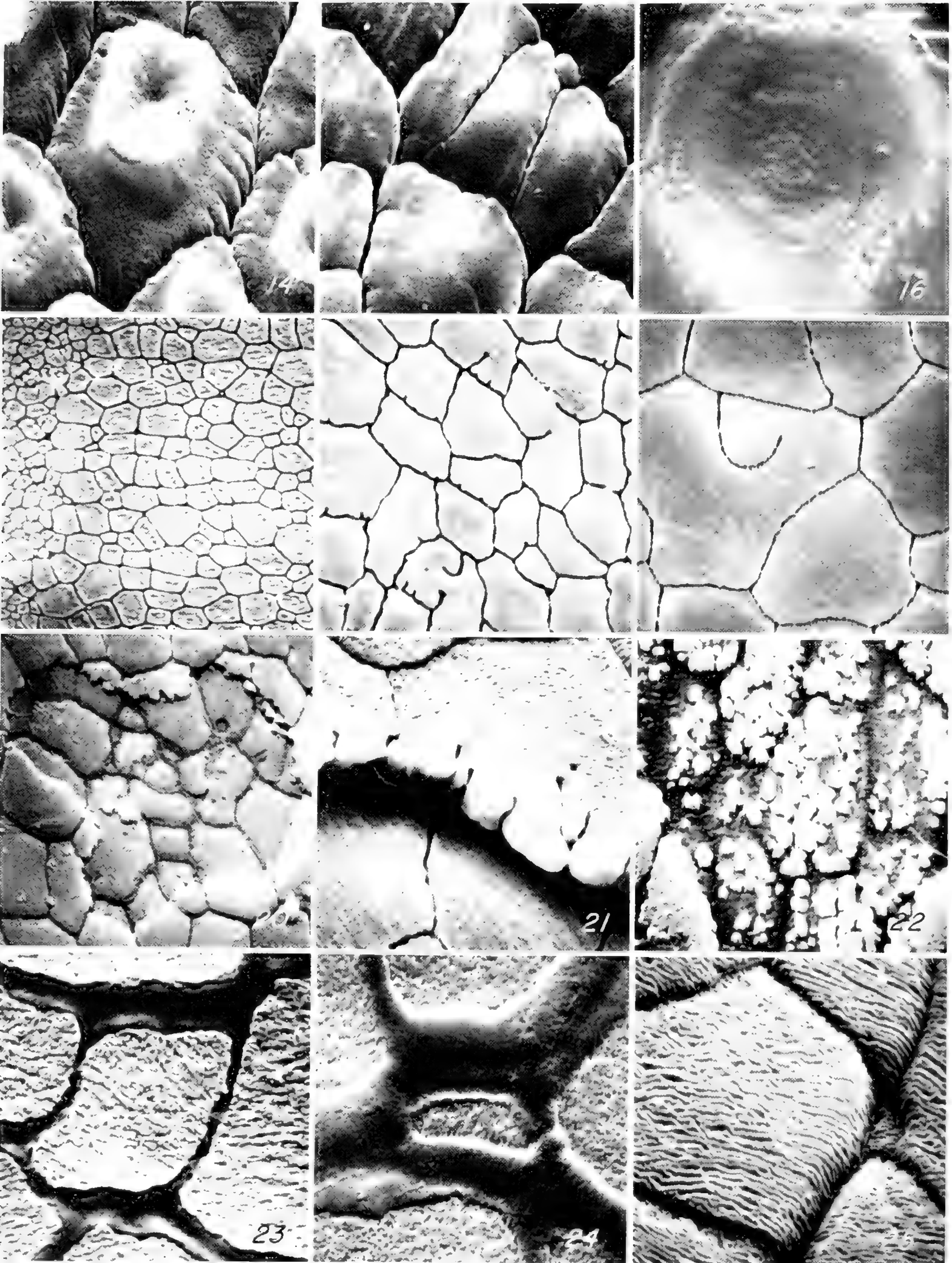
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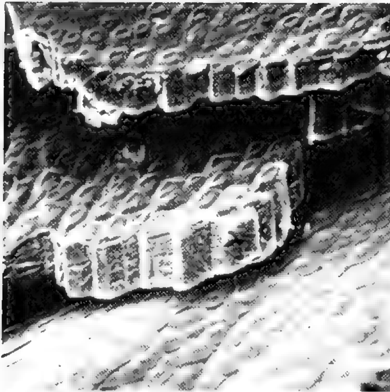
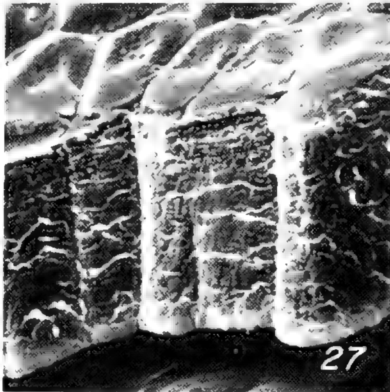
FIGURE 14. *Single prism, exterior surface. High magnification of Fig. 13. Horizontal field width = 30 μm.*
FIGURE 15. *Prisms, exterior surface, overlapping, tongue-shaped, right valve. 100% Clorox 1 min. Horizontal field width = 30 μm.*
FIGURE 16. *Single prism, exterior surface, concentric circles in central disk, right valve. 20% Clorox 10 sec. Horizontal field width = 10 μm.*
FIGURE 17. *Prisms, interior surface, shell margin to left, right valve. 5% Clorox 1 min. Horizontal field width = 120 μm.*
FIGURE 18. *Prisms, interior surface, indentations, right valve. 100% Clorox 1 min. Horizontal field width = 60 μm.*
FIGURE 19. *Prisms, interior surface, curved indentation, right valve. 100% Clorox 1 min. Horizontal field width = 30 μm.*
FIGURE 20. *Prisms, interior surface, incompletely formed layer, right valve; most of organic matrix still present. 20% Clorox 10 sec. Horizontal field width = 60 μm.*
FIGURE 21. *Prisms, interior surface, edge of newly forming layer, right valve; organic matrix removed. 100% Clorox 1 min. Horizontal field width = 30 μm.*
FIGURE 22. *Prisms, interior surface, coalescence of crystallites to form prisms, right valve. Brushed clean. Horizontal field width = 30 μm.*
FIGURE 23. *Prisms, interior surface, approaching full size, right valve. Brushed clean. Horizontal field width = 15 μm.*
FIGURE 24. *Prisms, interior surface, organic matrix spreading out of interprismatic junctures over surface, right valve. Brushed clean. Horizontal field width = 15 μm.*
FIGURE 25. *Prisms, finely rugose interior surface, right valve. 20% Clorox 10 sec. Horizontal field width = 15 μm.*

pears distinctly granular (Fig. 16). It is probable that some of the punctate marks in the disk are a part of the periostracum (Fig. 9), and that treatment with Clorox partially removes them with the periostracum (Fig. 12). Organic walls separating prisms are often raised above the general surface of the valve as crests that divide most, but not all, of the prisms (Fig. 7-10). When the surface of the valve is treated with Clorox to dissolve the organic matter, deep, distinct grooves are exposed in place of the conchiolinal prismatic envelopes (Fig. 12-15). The outer part of interprismatic surfaces of mineral prisms often display vertical furrows, some so pronounced as to give the appearance of crenulations (Fig. 12-14).

Interior surface. Mineral crystals appear to develop in the sheet of organic material deposited by the mantle margin along the edge of the valve from minute, randomly distributed, crystalline bodies. These crystallites coalesce, increase in size, and make contact with other crystals forming characteristic polygonal outlines in the new layer of prisms. The surface area of prisms increases away from the margin of the shell (Fig. 17). Indentations occur in the sides of some prisms, some penetrating deeply in graceful curves into the prisms (Fig. 18, 19).

New layers of prisms on the interior surface of the valve are not always formed uniformly, and incomplete areas sometimes occur (Fig. 20, 21) in which lateral growth of prisms appears to take place by sideways extension of existing prisms (Fig. 20, 21). Deposition of additional layers of prisms on the internal side of the valve can start with minute crystallites that coalesce and form polygonal figures (Fig. 22, 23), much as takes place at the mantle margin. The proportion of conchiolin to mineral core among prisms decreases as crystallites increase in size (Fig. 17, 22, 23). Interprismatic walls are generally less conspicuous on the interior than the exterior surface of prismatic structure (Fig. 20, 25). The surface of prisms adjacent to the mantle is finely rugose (Fig. 23), a feature that is emphasized by treatment with Clorox (Fig. 21, 25).

Fracture surfaces. The free margin of prismatic scales consists of a single layer of short prisms (Fig. 26). The interprismatic conchiolin fractures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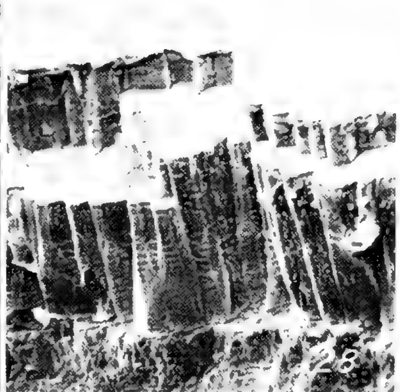
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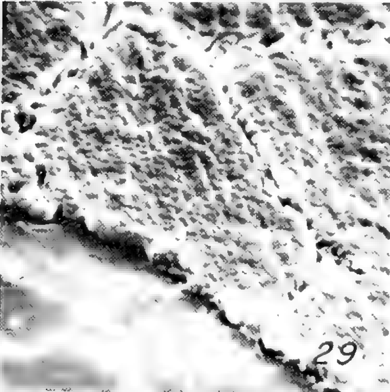
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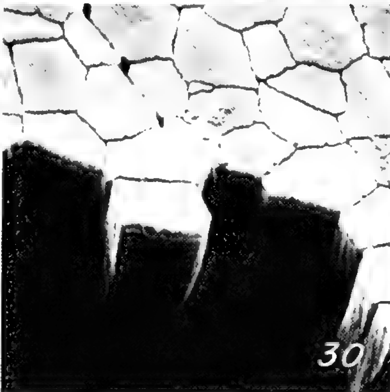
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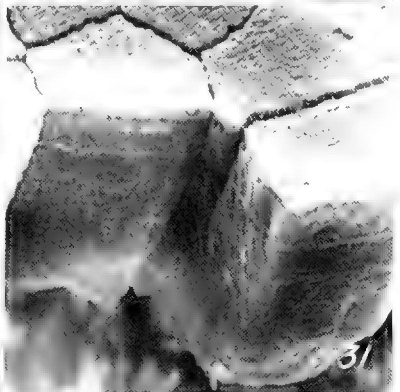
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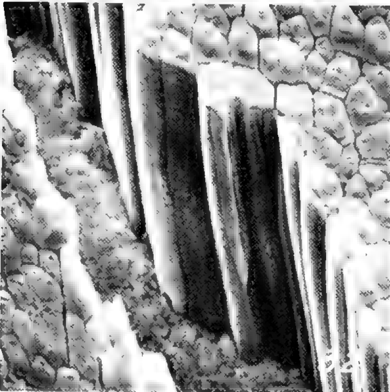

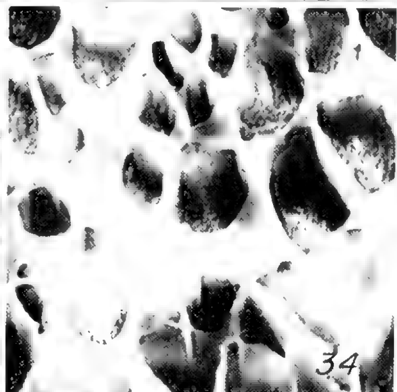
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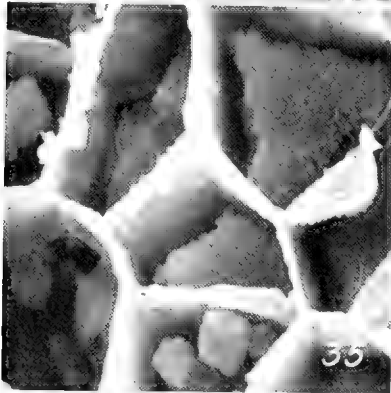
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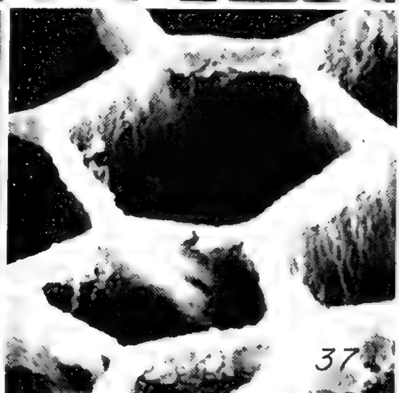
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FIGURE 26. *Prisms, fracture of two scales, right valve. Brushed clean. Horizontal field width = 160 μm.*
FIGURE 27. *Prisms, fracture of single scale showing interprismatic scale-like conchiolin, right valve. Brushed clean. Horizontal field width = 40 μm.*
FIGURE 28. *Prisms, multilayered fracture, over foliated structure, right valve. Horizontal field width = 160 μm.*
FIGURE 29. *Conchiolin sheet between layers of prisms (lower left), and thin layer of mineral prisms (upper right), fracture, right valve. Horizontal field width = 15 μm.*
FIGURE 30. *Prismatic scale, interior surface, fractured after treatment with 100% Clorox 1 min, right valve. Horizontal field width = 60 μm.*
FIGURE 31. *Higher magnification of Figure 30 to show stratification of internal structure of mineral core. Horizontal field width = 30 μm.*
FIGURE 32. *Long prisms, fracture, external view, right valve. 100% Clorox 1 min. Horizontal field width = 120 μm.*
FIGURE 33. *Prismatic scale fracture, exterior at top, margin of valve to the right, right valve. No cleaning treatment. Horizontal field width = 320 μm.*
FIGURE 34. *Prismatic organic sheaths, exterior surface, with partially dissolved mineral inside, etched in laboratory seawater, right valve. Brushed clean. Horizontal field width = 60 μm.*
FIGURE 35. *Prismatic organic sheaths, exterior surface, with partially dissolved mineral cores, etched in laboratory seawater, left valve. Brushed clean. Horizontal field width = 30 μm.*
FIGURE 36. *Organic sheaths of prisms from which mineral cores dissolved in laboratory seawater, fracture of exterior prismatic scale, left valve. Brushed clean. Horizontal field width = 30 μm.*
FIGURE 37. *Organic sheaths of prisms from which mineral cores dissolved by immersion in 0.1N HC1 for 30 sec, sawed polished surface, right valve. Horizontal field width = 15 μm.*

in a scale-like design (Fig. 27). Away from the ventral margin beneath prismatic scales, prismatic structure becomes tightly multilayered and complex, and prisms in most layers are considerably longer than those at the margin (Fig. 28). Adjacent horizontal layers of prisms are joined by a sheet of nonmineralized conchiolin (Fig. 28, 29). Prismatic structure usually fractures cleanly at conchiolin interfaces (Fig. 26-32).

Fractures of prismatic scales viewed from the interior surface of the valve emphasize the varied polygonal shape of prisms (Fig. 30, 31). Individual prisms consist of transverse bands that are clearly visible after the organic sheath has been chemically removed and probably represent growth increments (Fig. 31). Fine strands between prisms represent fragments of conchiolin left undissolved by Clorox.

Thick prismatic layers are characterized by long columnar-shaped prisms (Fig. 28, 32). In scales, the long axis of prisms tends to bend toward the margin of the shell in a direction from exterior to interior of the valves (Fig. 33).

Organic sheaths. The polygonal shape of organic sheaths mirrors that of mineral prisms (Fig. 34-37). Remnants of partially dissolved exterior parts of sheaths are illustrated in Figure 34. An organic layer bounds both exterior and interior ends of mineral prisms (Fig. 36). Dissolution of prismatic scales by Clorox (Fig. 37), rather than more gently in seawater (Fig. 34-36), results in prismatic envelopes that are rough and pitted, probably as a result of the action of the corrosive solvent.

Previous studies. The general shape and size of prisms of *Crassostrea virginica* illustrated here correspond with those given by previous investigators for the same species. At the developing margin, crystallites range in diameter from 0.01 to 8 μm, farther in they average 9.1 μm, and near foliated structure, 44.6 μm (Tsujii et al., 1958). Thickness of organic sheaths ranges from 0.16 to 0.75 μm (Tsujii et al., 1958), and averages 0.5 μm (Travis and Gonsalves, 1969). In our rapidly growing oysters in Broadkill River, maximal surface dimension of prisms in a transect from near the shell edge to the foliated structure of the right valve varied from about 14 to 23 μm (Palmer and Carriker, 1979b). The fracture section in Figure 28 shows prisms approximately 60 μm long. In other

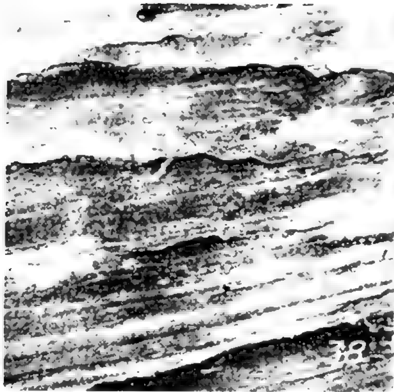
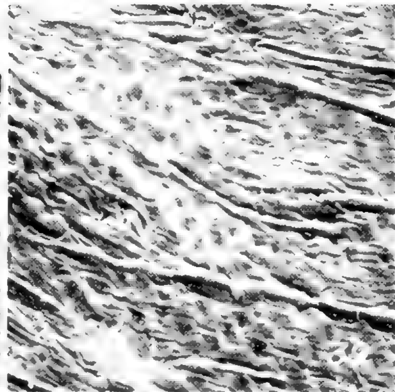
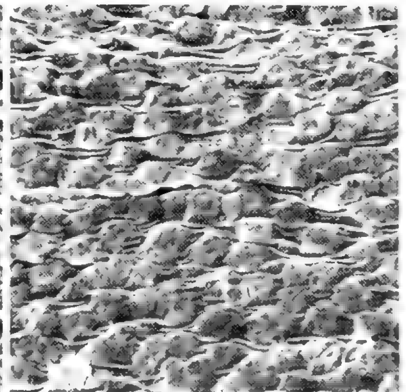
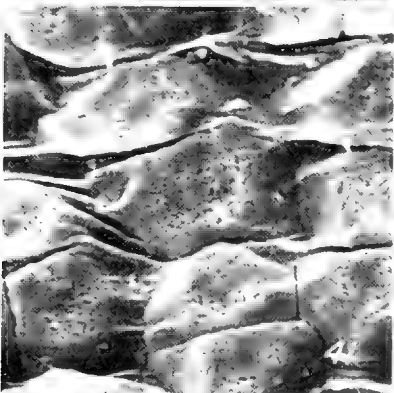
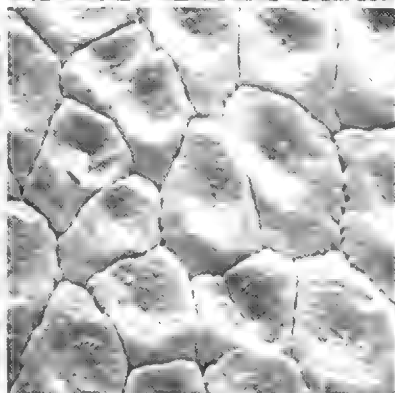
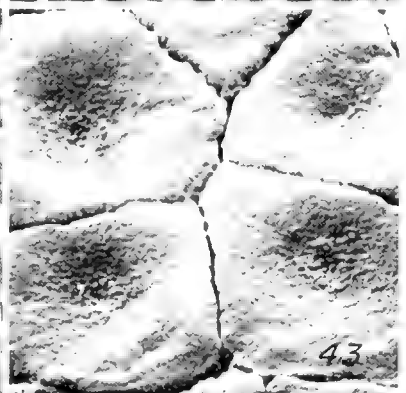
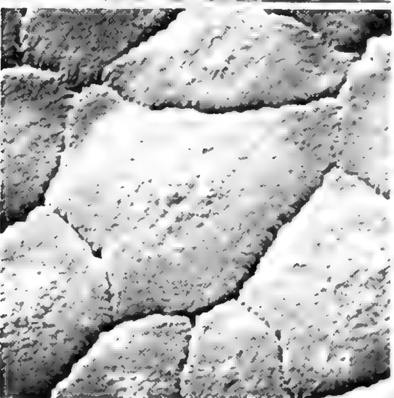
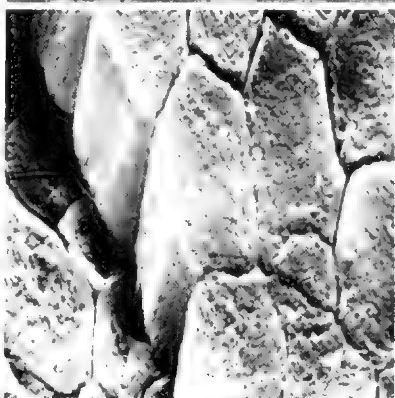
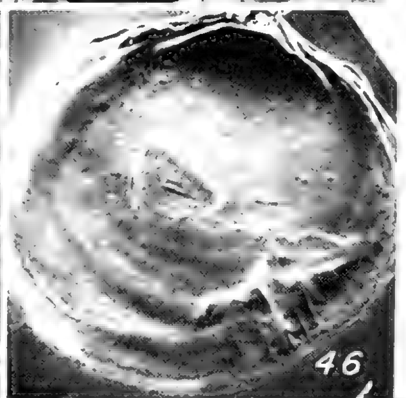
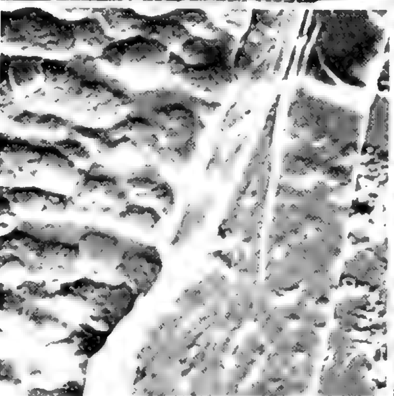
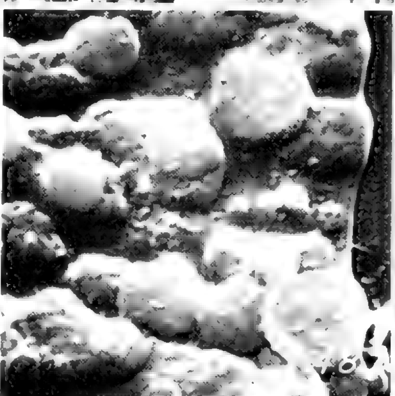
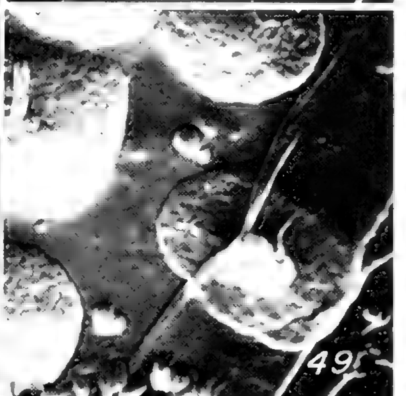













FIGURE 38. *Exterior prismatic surface, left valve. 100% Clorox 5 sec. Horizontal field width = 3.2 mm.*
FIGURE 39. *Periostracum, exterior surface, left valve. Periostracal folds overlying prisms. 100% Clorox 1 min. Horizontal field width = 120 μm.*
FIGURE 40. *Periostracal folds, exterior surface, left valve. 100% Clorox 5 sec. Horizontal field width = 140* μm.
FIGURE 41. *Periostracal folds around prisms, high magnification of Figure 40. Horizontal field width = 35 μm.*
FIGURE 42. *Prisms, exterior concave surfaces, left valve. 100% Clorox 1 min. Horizontal field width = 30 μm.*
FIGURE 43. *Prisms, exterior surface, concave granular structure, left valve. 100% Clorox 1 min. Horizontal field width = 15 μm.*
FIGURE 44. *Prisms, exterior surface, elevated granular structure, left valve. 100% Clorox 1 min. Horizontal field width = 15 μm.*
FIGURE 45. *Prisms, exterior surface, fracture, left valve. 100% Clorox 1 min. Horizontal field width = 30 μm.*
FIGURE 46. *Left valve of spat attached to glass, interior surface. No cleaning treatment. Horizontal field width = 2.0 mm.*
FIGURE 47. *Margin of shell, spat in Figure 46. Partially wrinkled organic sheet to right and developing crystallites in sheet to left. Horizontal field width = 15 μm.*
FIGURE 48. *Margin of shell, spat in Figure 46. Crystallites forming in organic sheet atop a previous sheet with crystallites. Horizontal field width = 7.5 μm.*
FIGURE 49. *Margin of shell, spat in Figure 46. Edge of organic sheet folded to left over a large crystallite, with newly forming crystallite atop it. Horizontal field width = 7.5 μm.*

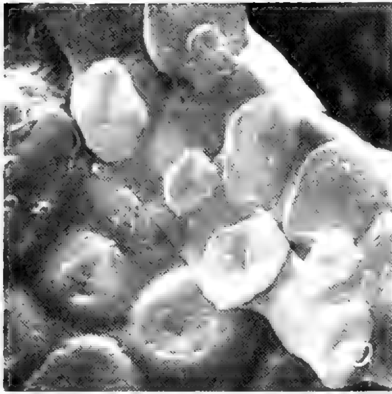
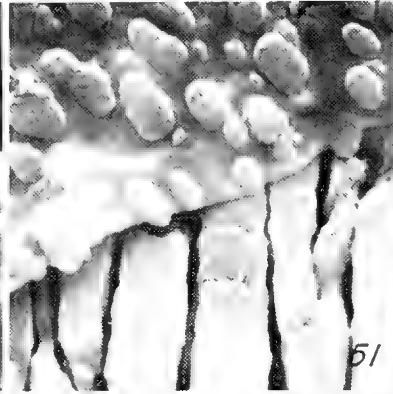
oysters, length of prisms ranged from 32 to 84 μm (Travis and Gonsalves, 1969).

We confirm the observations of Tsujii et al. (1958), Galtsoff (1964), Travis and Gonsalves (1969), and Taylor et al. (1969) that prisms of *Crassostrea virginica* are highly variable in size and are characterized by a large proportion of organic matrix. Taylor et al. (1969) reported, in other species of bivalves, that the conchiolinal wall between adjacent prisms, which can appear as either a groove or a low ridge, is sometimes discontinuous and projects into the mineral prism as a flange.

Taylor et al. (1969) noted that calcitic prisms of bivalves possess transverse striations. Grégoire (1972) referring to these as growth lines, reported that they consist of transverse conchiolin shreds disposed in ring-shaped strands anchored at irregular intervals on the inner surface of prism sheaths. Transverse striations are also conspicuous on the lateral walls of mineral prisms of *Crassostrea virginica* (Fig. 31). We confirm as well the observations of Tsujii et al. (1958) that the inner surface (against the mantle) is rugose, characterized by roughly parallel minute grooves (Fig. 25).

Within each prism of the shell of *Crassostrea virginica* according to the transmission electron microscopical study of Travis and Gonsalves (1969), there is present a conspicuous intraprismatic organic matrix organized into small elongated compartments with walls about 650 Å thick. These walls are clearly evident in ultrathin sections demineralized on grids. Each compartment encloses a mineral crystallite. The length of each compartment is about 6000 A and parallels the long axis of the prism, and the width of each compartment is about 1000 Å. Mutvei (1979) also found a complex organic matrix in demineralized lamellae of *Mytilus edulis*. The surface of the partially dissolved mineral prism in Figures 34 and 35 suggests the protrusion of crystallites from a surface in which the intraprismatic matrix has been partially dissolved.

In *C. gigas* Wada and Suga (1976), using an electron microprobe, found that sulphur and chlorine were present in high concentrations, and calcium in low concentrations, in the organic matrix of the prismatic structure.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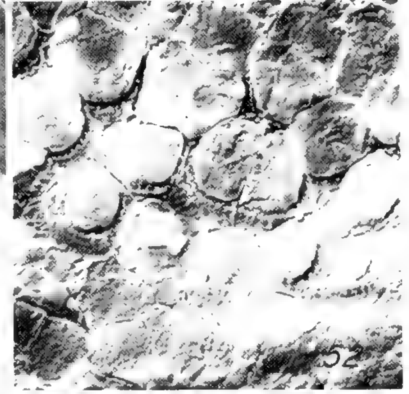
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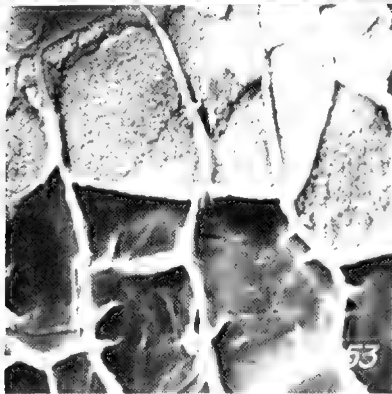
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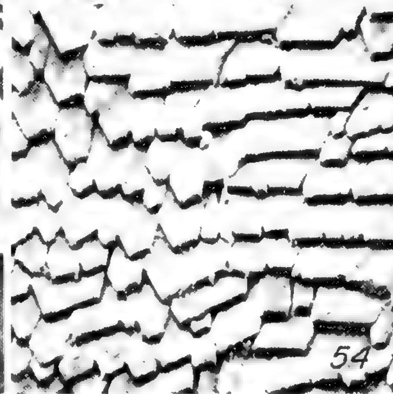
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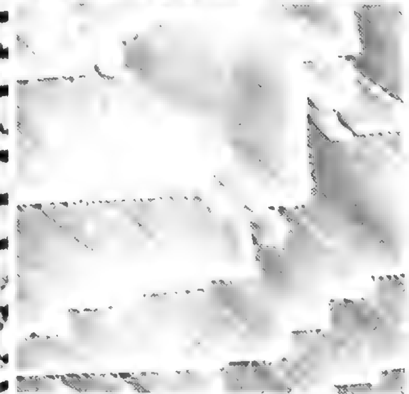
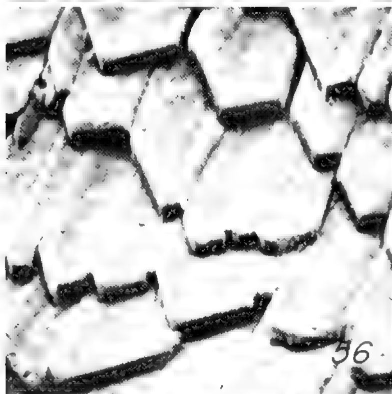
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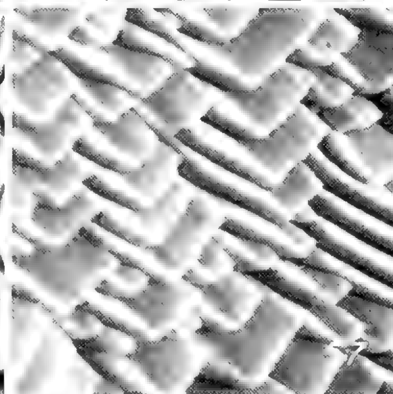
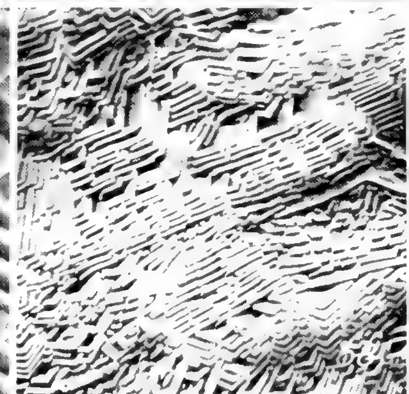
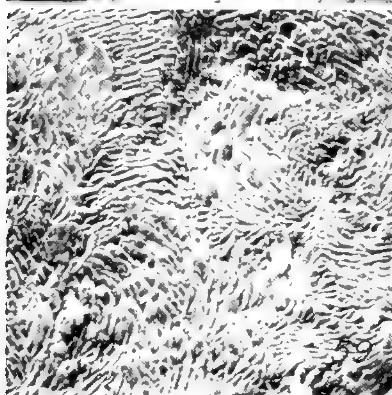
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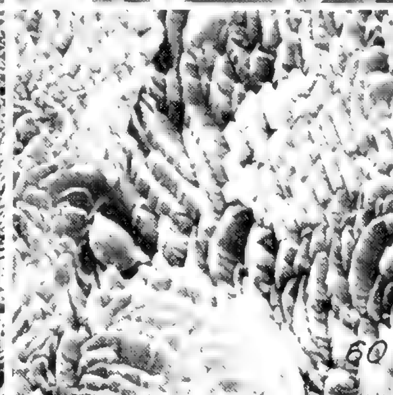
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FIGURE 50. *Margin of shell, spat 1.2 cm high; edge folded to left over exterior of valve, exterior surface of prisms exposed at left and interior at right. No cleaning treatment. Horizontal field width = 30 μm.*
FIGURE 51. *Margin of shell, interior surface, left valve; newly forming crystallites in organic sheet (upper half) underlying older layer of elongate prisms (lower half). 20% Clorox 10 sec. Horizontal field width = 15 μm.*
FIGURE 52. *Margin of shell, exterior surface, left valve; thin prisms in incomplete prismatic layer. 100% Clorox 1 min. Horizontal field width = 60 μm.*
FIGURE 53. *Prism sheaths in thin prismatic layer, left valve, mineral cores dissolved in laboratory sea-water. Brushed clean. Horizontal field width = 30 μm.*
FIGURE 54. *Laths, parallel to each other, interior surface, between adductor muscle and ventrum. 5% Clorox 15 sec. Horizontal field width = 15 μm.*
FIGURE 55. *Laths, very large, parallel to each other, interior surface, covered by thin coating of conchiolin, between adductor muscle and ventrum. 5% Clorox 1 min. Horizontal field width = 15 μm.*
FIGURE 56. *Laths with ridges, interior surface, between adductor muscle and ventrum. 5% Clorox 15 sec. Horizontal field width = 7.5 μm.*
FIGURE 57. *Laths, sharply angular, interior surface, between adductor muscle and ventrum. Brushed clean. Horizontal field width = 7.5 μm.*
FIGURE 58. *Laths, sharply angular pattern, interior surface, between adductor muscle and umbones. Brushed clean. Horizontal field width = 30 μm.*
FIGURE 59. *Laths, rosette pattern, interior surface, between adductor muscle and umbones. 100% Clorox 1 min. Horizontal field width = 30 μm.*
FIGURE 60. *Laths, rosette pattern, interior surface, between adductor muscle and umbones. 100% Clorox 1 min. Horizontal field width = 15 μm.*
FIGURE 61. *Laths, ends sharply truncated, interior surface, between adductor muscle and umbones. 100% Clorox 1 min. Horizontal field width = 7.5 μm.*

Periostracum, Left Valve

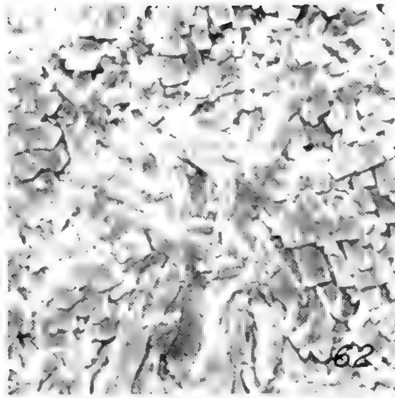
In contrast to the thick, flexible, pigmented, prismatic layers overlaid by prismatic scales in the right valve, the prismatic stratum of the left valve is thin, relatively smooth (Fig. 38), and creamy white, reflecting the color of the thick underlying foliated structure. The unworn surface of prismatic structure of the left valve is coated with a thin, organic, periostracal sheet, which is even thinner than that of the right valve. Prisms show clearly through the periostracum. Its surface varies from smooth, to minutely ridged, to prominently folded and creased (Fig. 39). Frequently the periostracum forms microscopically conspicuous ridges and grooves between rows of prisms, giving the impression of tiny scales (Fig. 40, 41).

All oysters attach to the substratum by their left valve. In no case has it been possible to prove attachment by the right valve (Galtsoff, 1964; Stenzel, 1971). The adhesive cement of settling oysters (*Ostrea edulis*, Cranfield, 1975) is applied directly onto the periostracum of prodissoconch II; presumably the cement is extended under the dissoconch spat onto the periostracum as well, but this matter has not been investigated.

Prismatic Structure, Left Valve

Prisms of the outer calcified layer of the left valve possess the same well-known honeycomb-like structure that characterizes those of the right valve. However, interprismatic conchiolinal walls and prismatic layers are thinner, and prisms are shorter in the left than in the right valve, and scales are small or absent.

Exterior and fracture surfaces. In contrast to the generally bossed exterior of prisms of the right valve, the exterior of prisms of the left valve tends to be concave (Fig. 39, 42, 43), flat (Fig. 52), or slightly domed (Fig. 44). Ornamentation in the center of each prism varies from the typical disk with concentric, punctate marks characteristic of prisms of the right valve, to slightly depressed (Fig. 43) or elevated (Fig. 44) granular areas. Corrugations are present along the exterior edges of mineral prisms (Fig. 42-45), but are not as deep as analogous furrows in prisms of the right valve.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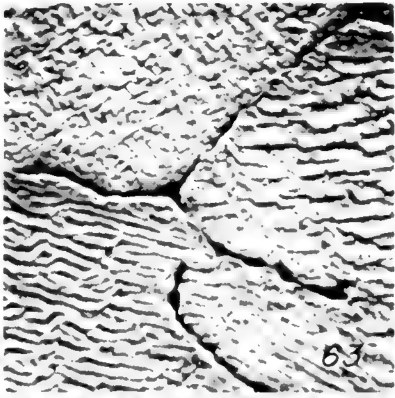
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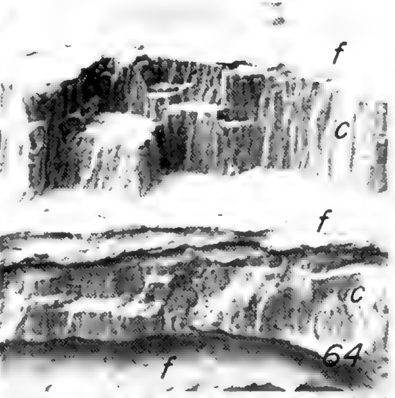
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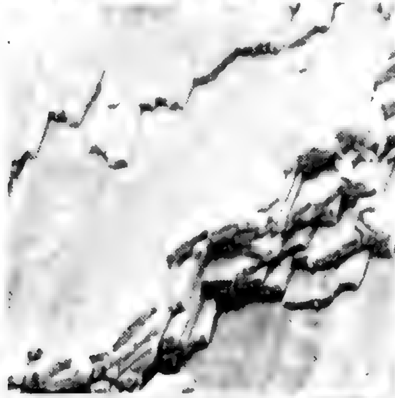
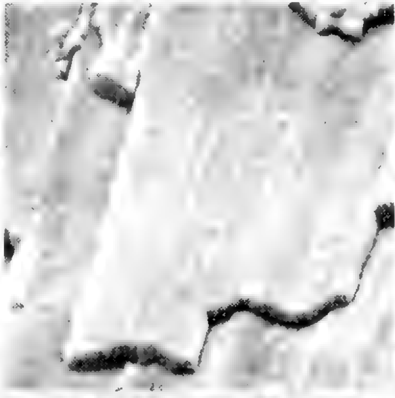
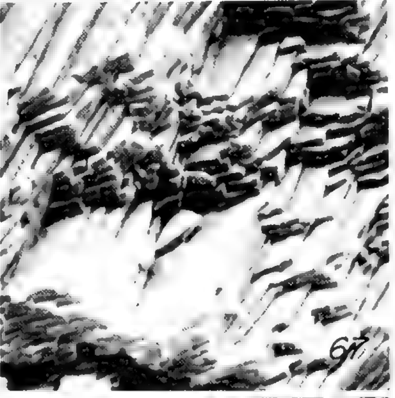
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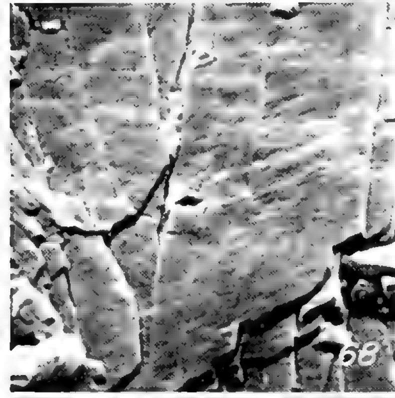
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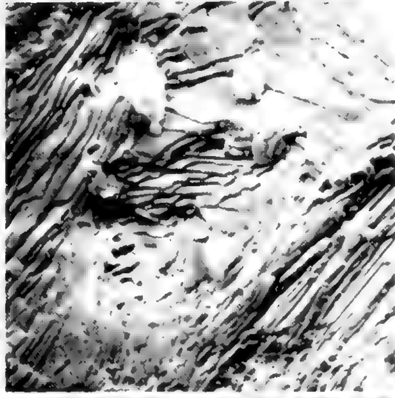
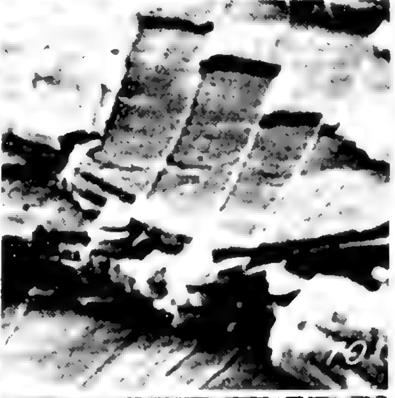
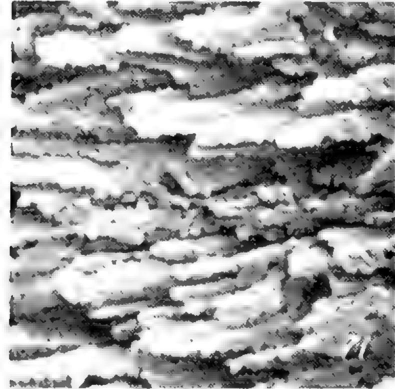
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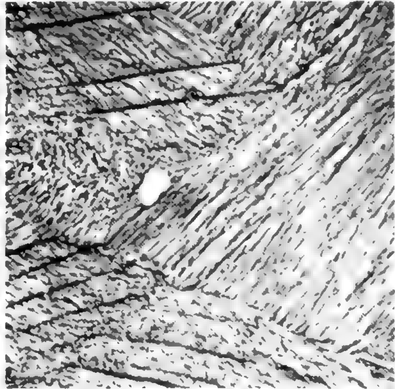
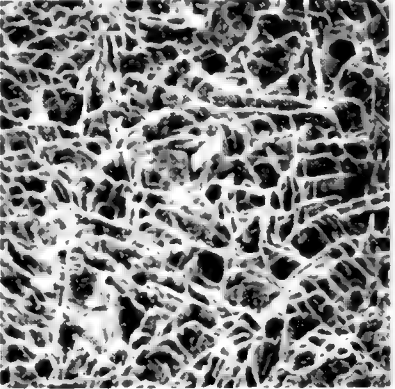

FIGURE 62. *Laths, branching, bending, interior surface, between adductor muscle and umbones. 100% Clorox 5 sec. Horizontal field width = 40 μm.*
FIGURE 63. *Prisms overlaid by initial layer of laths, interior surface, near valve margin; boundaries of prisms outlined by dark conchiolin. Brushed clean. Horizontal field width = 15 μm.*
FIGURE 64. *Stratification of foliated and chalky structure, fracture; interior surface of valve at top. No cleaning treatment. F, regularly foliated structure; c, chalky structure. Horizontal field width = 3 mm.*
FIGURE 65. *Laths, chevron marked, fracture. No cleaning treatment. Horizontal field width = 15 μm.*
FIGURE 66. *Laths, dimpled surface, fracture. No cleaning treatment. Horizontal field width = 7.5 μm.*
FIGURE 67. *Laths, dimpled surface, end view, fracture. No cleaning treatment. Horizontal field width = 15 μm.*
FIGURE 68. *Laths, parallel lined, fracture. No cleaning treatment. Horizontal field width = 15 μm.*
FIGURE 69. *Laths, heterogeneously directed, fracture. No cleaning treatment. Horizontal field width = 30 μm.*
FIGURE 70. *Laths, intercrystalline organic matrix shows where crystals removed by fracturing. No cleaning treatment. Horizontal field width = 10 μm.*
FIGURE 71. *Laths, polished surface treated with 100% Clorox 30 sec. Horizontal field width = 7.5 μm.*
FIGURE 72. *Laths, polished surface treated same as that in Figure 71. Lines mark grooves of organic matter. Horizontal field width = 60 μm.*
FIGURE 73. *Chalky structure, angular pattern, surface against mantle. 5% Clorox 1 min. Horizontal field width = 60 μm.*

Marginal surface. Ultrastructure of the prismatic margin of the left valve was studied in young oysters growing on glass surfaces (Fig. 46). An organic membrane is deposited by the mantle edge over (that is, inside) and beyond a previous organic layer and developing prisms (Fig. 47). Supported by the glass surface, the new membrane retains its identity after preparation for scanning electron microscopy. Minute crystallites (calcospherites, or mineralized granules, Galtsoff, 1964; spherulites, Taylor et al., 1969) take form in the new membrane, in the zone extending beyond the previously mineralized layer (Fig. 47, 49), as well as in the mineralizing layer (Fig. 48, 51). Shape of crystallites varies from roughly round to oval. As crystals grow, they assume their definitive form that ranges from elongated (Fig. 51) to polygonal with the typical central disk (Fig. 50). The thinness of prismatic strata in the left valve is illustrated in Figure 52, where an external layer of prisms in the process of formation was left incomplete as the next inner layer was formed. Partial dissolution in seawater of the mineral content of prisms in a shallow layer illustrates the internal configuration of the conchiolin sheaths (Fig. 53) and occasional projections of each sheath into the mineral core.

Previous studies. Taylor et al. (1969) observed that in three of the species of oysters examined by them, and probably in all species of oysters, the outermost layer of only the right valve consists of simple calcitic prisms. Other investigators have also overlooked the prismatic layer in the left valve of oysters. In view of the inconspicuousness of this layer, it is not surprising that it has been missed. Galtsoff (1964) in *Crassostrea virginica* and Taylor et al. (1969) in *Anodonta cygnea* illustrated the formation of prisms at the margin of the shell with light micrographs. These, at that magnification, compare favorably with our scanning electron micrographs.

Foliated Structure, Both Valves

The inner and most massive layer of each valve is composed of calcitic foliated structures (pseudonacre, subnacre, calcitostracum of many authors). On the inner surface of valves foliated structure consists, for the most part, of long, tabular laths (tablets, blades, lamellae of some authors) joined laterally to form thin sheets or folia. Folia in turn are grouped into larger lenticular folia. In many areas single folia are inclined slightly to the inner surface of the valves, and laths successively and regularly overlap in parallel

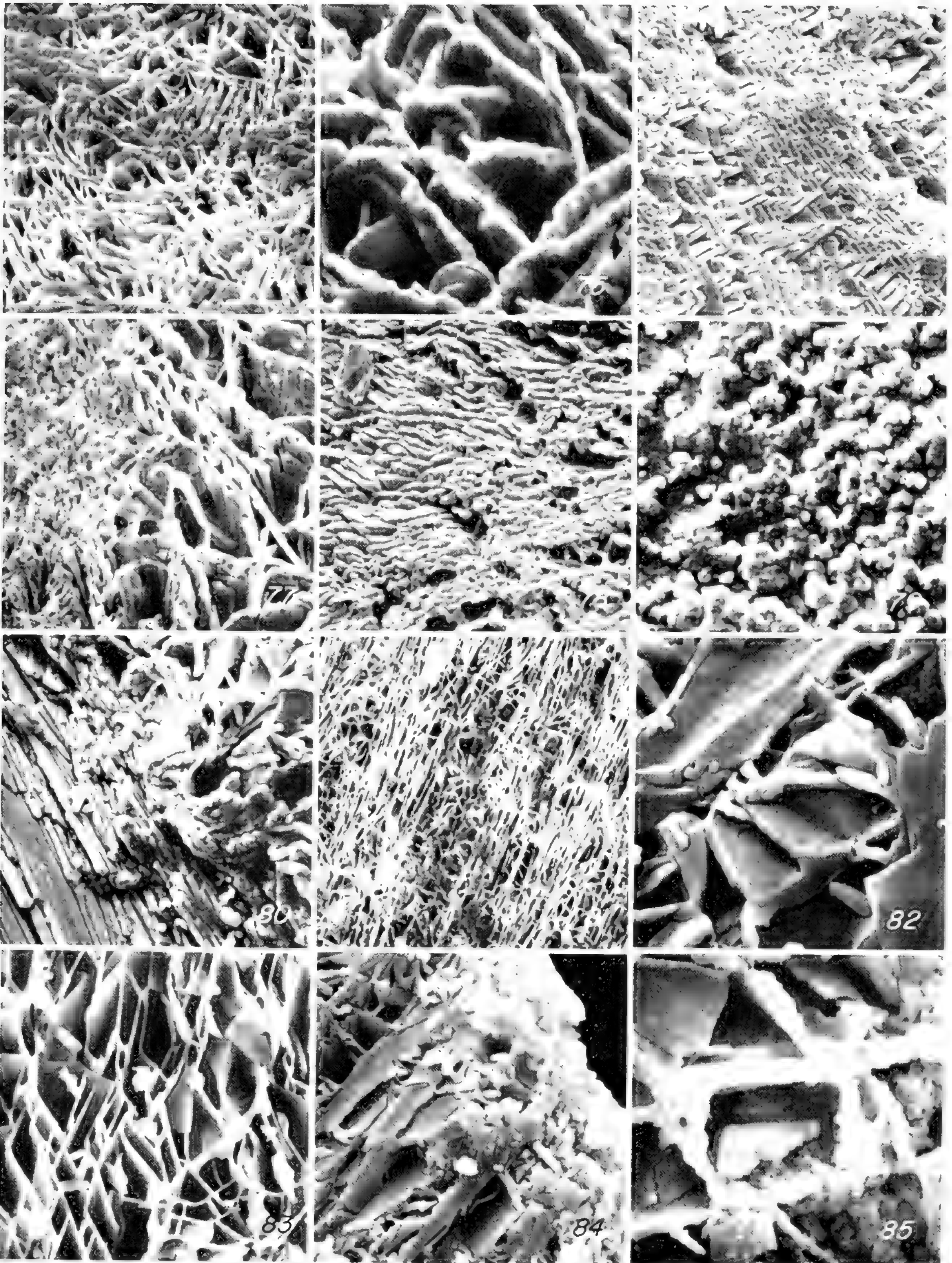
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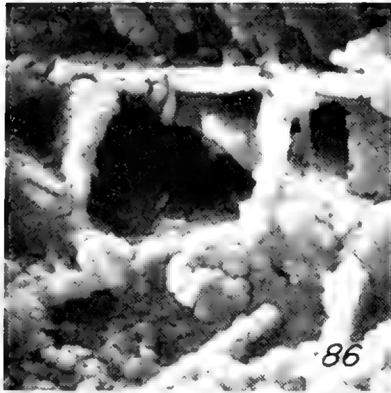
FIGURE 74. *Chalky structure, floral pattern, surface against mantle. 5% Clorox 1 min. Horizontal field width = 60 μm.*
FIGURE 75. *Chalky structure, deep pore spaces, surface against mantle. 5% Clorox 1 min. Horizontal field width = 15 μm.*
FIGURE 76. *Transition between chalky and foliated structure, surface against mantle. No cleaning treatment. Horizontal field width = 35 μm.*
FIGURE 77. *Boundary between typical chalky structure (at right) and initiation of granular material (left) transitional to foliated structure, surface against mantle. 5% Clorox 1 min. Horizontal field width = 30 μm.*
FIGURE. 78. *Thin layer of foliated laths over chalky structure, surface against mantle. 5% Clorox 1 min. Horizontal field width = 30 μm.*
FIGURE. 79. *Initiation of chalky structure (granular material) on conchiolin patch, surface against mantle. No cleaning treatment. Horizontal field width = 20 μm.*
FIGURE. 80. *Transition from foliated laths (left) through granular material (center) to chalky structure (right), fracture. No cleaning treatment. Horizontal field width = 30 μm.*
FIGURE 81. *Chalky structure, fractured parallel to long axes of blades. No cleaning treatment. Horizontal field width = 120 μm.*
FIGURE 82. *Chalky structure, blades and leaflets, fracture. No cleaning treatment. Horizontal field width = 15 μm.*
FIGURE 83. *Chalky structure, irregular honey-comb pattern, fracture. No cleaning treatment. Horizontal field width = 280 μm.*
FIGURE 84. *Chalky structure, fracture at right angle to mantle surface; chalky structure (left), transitional granular material at mantle surface (right). No cleaning treatment. Horizontal field width = 30 μm.*
FIGURE 85. *Chalky structure, fracture. 100% Clorox 30 sec. Horizontal field width = 7.5 μm.*

in a design often described as similar to that of "tiles on a roof." Thin walls of intercrystalline conchiolin delineate the laths.

Interior (or mantle-facing) surface. Especially in the region of the valves between the ventral margin and the adductor muscle, laths for the most part remain parallel to each other, and the growing front of each faces the ventral margin of the valves (Fig. 54-57). In the area between the adductor muscle and the umbones, different clusters of laths are generally oriented in various directions relative to each other, in some spots, for example, in sharply angular designs (Fig. 58), in others in exquisite rosette patterns (Fig. 59, 60), and in still others in a disorderly array of branching, bending laths (Fig. 62). Both the width and exposed length of laths vary greatly between adjacent laths, between folia, between different areas of shell, and between different individuals (Fig. 54-62). In some places laths are closely joined to their neighbors (Fig. 55), whereas in others, gaps of varying width can occur between them (Fig. 54, 56). The surface of some laths appears smooth (Fig. 55, 57); in others, gently dimpled (Fig. 54, 56). Some laths bear slight ridges that run parallel to the long axis (Fig. 54); and in others, the former positions of crystal fronts (probably growth halts) are visible (Fig. 56).

Exposed rows of folia near the ventrum of the valves are generally straight (Fig. 54-56), whereas toward the umbones, adjacent laths are increasingly angled to each other (Fig. 60). The growing front of folia can be sharply truncated (Fig. 56, 61), or bevelled (Fig. 57). In some cases the growing front of folia can be in close contact with underlying folia (Fig. 56, 61), while in others it can be elevated to varying degrees, leaving a space between folia (Fig. 55, 58, 60). This space is generally clean (Fig. 58), but occasionally short fibers extend from the underside of the growing front of one folium to the exterior surface of the underlying folium (Fig. 55). These fibers could be remnants of organic matrix, perhaps consolidated by drying.

Intercrystalline conchiolin is not conspicuous in micrographs of most folia, but can be inferred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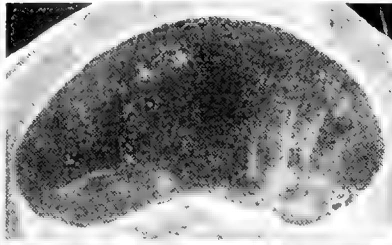

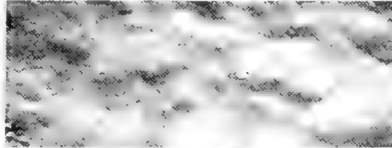

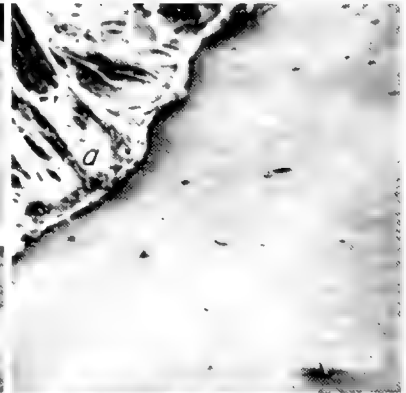
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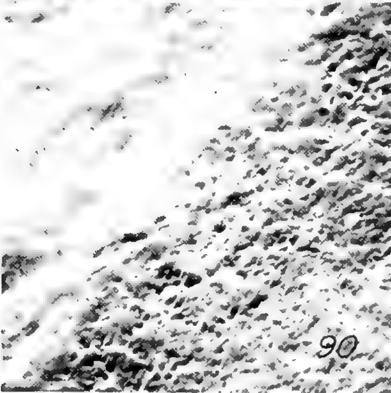
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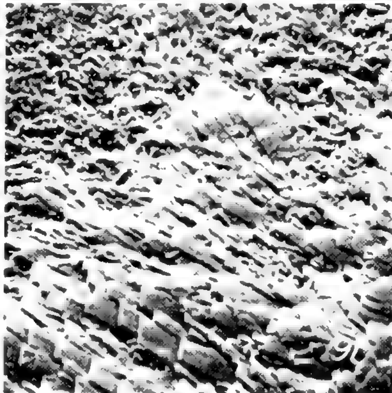

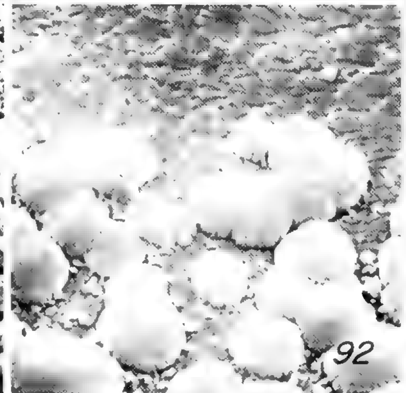
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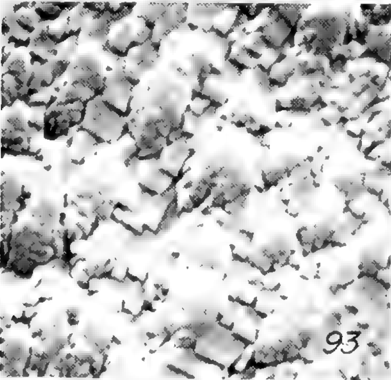
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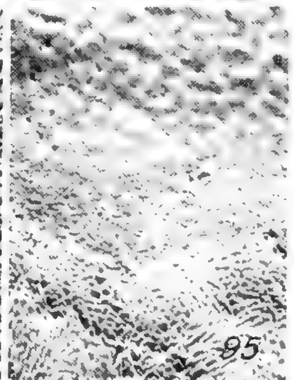
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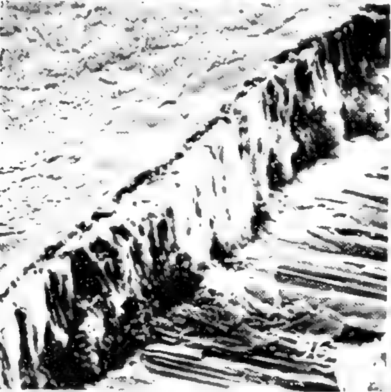

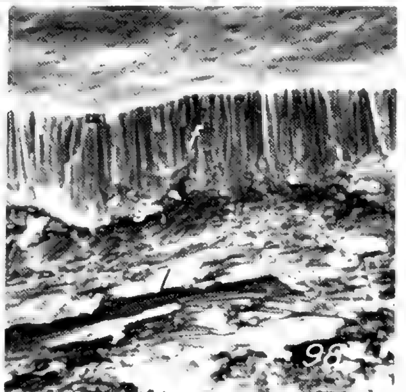
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FIGURE 86. *Chalky structure, fracture. 0.1N HC1 30 sec. Horizontal field width = 7.5 µm.*
FIGURE 87. *Adductor muscle scar of young oyster, right valve. Anterior, right; posterior, left; ventral, top; dorsal, bottom. 20% Clorox 10 sec. Horizontal field width = 7.5 mm.*
FIGURE 88. *Enlargement of area in center of adductor muscle scar in Figure 87. Horizontal field width = 30 µm.*
FIGURE 89. *Transitional myostracum (m) between adductor muscle (a) and foliated structure (f) on ventral side of adductor muscle scar, left valve. Brushed lightly, 5% Clorox 15 sec, critical- point dried. Horizontal field width = 1 mm.*
FIGURE 90. *Transition between myostracum (upper left) and granular structure (lower right) of Figure 89. Horizontal field width = 30 µm.*
FIGURE 91. *Transition between granular (top) and foliated lath structure (bottom) of Figure 89. Horizontal field width = 30 µm.*
FIGURE 92. *Transition between muffin-like microstructures of ventral edge of adductor myostracum (bottom) and foliated lath structure (top). 20% Clorox 10 sec. Horizontal field width = 30 µm.*
FIGURE 93. *Transition between mulberry-like microstructures of ventral edge of adductor myostracum and foliated lath structure. 20% Clorox 10 sec. Horizontal field width = 15 µm.*
FIGURE 94. *Narrow transitional zone between smooth adductor myostracum (left), granular zone (middle), and foliated structure (right) on mid anterior edge of adductor muscle scar. 20% Clorox 10 sec. Horizontal field width = 80 µm.*
FIGURE 95. *Transition between smooth adductor myostracum (top), granular structure (middle), and foliated lath structure (bottom) on dorsal side of adductor muscle scar. 20% Clorox 10 sec. Horizontal field width = 45 µm.*
FIGURE 96. *Dorso-ventral fracture through transitional myostracum on ventral side of adductor muscle; organic sheet (upper left), fracture of myostracal prisms (center diagonal), fracture of foliated structure (lower right). 5% Clorox 15 sec. Horizontal field width = 30 µm.*
FIGURE 97. *Myostracum, fracture through center of adductor scar (left); foliated structure (right). 20% Clorox 10 sec. Horizontal field width = 30 µm.*
FIGURE 98. *Myostracum, vertical fracture through center of adductor scar; surface of myostracum (s), prisms exposed by fracture (f), transitional granular structure (g), foliated structure (l) laths oriented in various directions. Surface treated with 100% Clorox 5 sec prior to fracturing. Horizontal field width = 35 µm.*

(from fractures, Fig. 70) to be present in boundaries between laths (Fig. 55, 56).

Fracture surfaces. Foliated structure does not fracture as cleanly at intercrystalline conchiolinal boundaries as prismatic structure, but nonetheless foliated fractures provide valuable information on the form of laths. Thick parts of the shell frequently consist of several layers of foliated structure alternating with chalky structure (Fig. 64).

The types of foliated microstructures facing the mantle described in the previous section are illustrated in fracture surfaces in Figures 65-69. Laths and folia are generally tightly joined to each other. Surface texture ranges from smooth (Fig. 68), to slightly dimpled (Fig. 66, 67), to parallel lined (Fig. 66-68), to chevron marked (Fig. 65). Chevron marks represent the growth halts of crystal fronts; since these laths are uniformly parallel, a characteristic of foliated structure near the ventral margin of the valves, it is likely that the chevrons point in the direction of the margin. The thickness of most laths is relatively uniform (Fig. 67). Extreme disorganization of laths is not uncommon (Fig. 69), nor is the meeting of laths at diverging angles (Fig. 68) (complex crossed foliated structure of Carter, 1980).

Only rarely will a fracture occur in which the intercrystalline organic matrix is clearly exposed, outlining boundaries of adjacent laths by distinct conchiolinal ridges (Fig. 70).

In fracture sections treated with Clorox to remove organic matrix, individual laths stand out clearly (Fig. 71). What appear to be consecutive series of thin chevron-shaped growth increments

along the long axis of each lath are also evident. Each increment is composed of granular subunits. Fracture surfaces, the organic matter dissolved by Clorox (Fig. 72), also occasionally expose deep, often straight, grooves at roughly right angles to the long axes of the laths.

Previous studies. Of all the regions of oyster shell, foliated structure has received the most attention from ultramorphologists. The most detailed description was given by Taylor et al. (1969).

In shadowed replicas, Tsujii et al. (1958), Watabe et al. (1958), Watabe and Wilbur (1961), and Watabe (1965) observed the general configuration of laths and folia described in this paper. Earlier work was reviewed by Taylor et al. (1969) and Gregoire (1972). Tsujii et al. (1958) and Watabe et al. (1958) reported that the angle of the apex of the growing front of laths ranged from 81° to 125°, with higher frequencies of angles between 96° and 100°. Watabe et al. (1958) suggested that the "true" angle is probably 90°; they also reported dendritic growth of laths.

Watabe and Wilbur (1961) described the transition zone between foliated and prismatic structure. This zone begins as a thin sheet of aggregates of granular blocks about 0.8 μm in width that are formed by the fusion of minute crystallites. These granules develop along the edges and corners of laths and prisms and on conchiolin patches, coalesce, and pave the way for formation of folia or chalky shell, as the case may be. The relationship, if any, between organic membranes and crystallites in transition zones has not been elucidated.

On the average, the thickness of each lath is about 0.2 μm. The length is difficult to determine because of indefinite orientation of long axes of laths relative to fracture surfaces; the length of the longest lath observed free is 9 μm (Watabe and Wilbur, 1961). A thin layer of conchiolin is present about every 30 crystalline layers (Watabe and Wilbur, 1961), an observation reminiscent of the sheets of organic matter dissolved in Figure 72.

According to Watabe (1965), each lath is composed of subunits (lamellae: 100-400 Å wide and 150-200 Å long) arranged in parallel across the width of the lath. This substructure appears to be reflected in the pattern of dissolution of laths treated by us with Clorox (Fig. 71). Watabe also reported that an interlamellar matrix is absent in oyster foliated structure, but an intercrystalline matrix, about 120-200 Å thick, separates individual laths. An intracrystalline matrix surrounds each lamellar subunit.

The most striking differences between prismatic and foliated structure are the conspicuously greater quantity of organic matrix in prismatic shell, and the larger size of prisms than of laths.

Chalky Structure, Both Valves

Islands of relatively soft, porous, chalky white, calcitic structure occur randomly on the interior surface of the valves. These lenses of chalky shell are normal components of the valves, become buried in foliated structure as valves thicken, are more common in the left valve than in the right valve, and increase in number and size in older oysters.

Chalky deposits consists of smooth, blade-shaped microstructures (blades) of various sizes, oriented perpendicular to the inner surface of the valves. Leaf-like microstructures (leaflets) branch at several angles from the central blades, enclosing pores among the blades and leaflets, and giving chalky shell its characteristic spongy appearance.

Interior (mantle-facing) surface. The surface of well developed chalky structure lying against the mantle epithelium is typically spongy in appearance, the free ends of blades being the most prominent features (Fig. 73). Blades occur in parallel rows, in floral designs, or at various angles to each other (Fig. 74). Pore spaces among the blades and leaflets are distinct and extend deep among the microstructures (Fig. 75).

Transitional zones between foliated structure and chalky structure are morphologically variable. Where chalky shell develops over the ends of laths, size of the space among the laths increases as new shell is deposited (Fig. 76) until the spongy chalky structure is fully developed. As foliated structure forms over chalky structure, the ends of blades become covered with a granular material (Fig. 77, 84), which in turn forms the base for foliated laths (Fig. 78). Formation of chalky shell on the sides of foliated laths (Fig. 80) or over conchiolin patches (Fig. 79) likewise is preceded by deposition of finely granular material.

Fracture surfaces. The often parallel arrangement of blades of chalky shell is most evident in

large lenses fractured parallel to the long axes of blades (Fig. 81). Some leaflets contact opposing blades, in section forming an irregular honeycomb type of pattern (Fig. 83); while others project only part way between blades (Fig. 82). The angle of leaflets to blades is variable (Fig. 81-83). The distance between rows of blades is also variable; in some strata, blades are close together with little pore space among them, whereas in others, blades are longer, and pore space is larger (Fig. 81). Granular material deposited over ends of blades in transition to foliated structure adheres closely to the ends of blades and soon fills pores among the blades (Fig. 84). Fracture surfaces of chalky shell etched lightly with Clorox (Fig. 85) or with acid (Fig. 86) are similar, except that after acid a granular substructure was more conspicuous after Clorox. Etching did not reveal the relationship of conchiolin to the blade-leaf structure.

Previous studies. The optical microscopy of chalky shell was reviewed by Korringa (1951), Galtsoff (1964), and Stenzel (1971). The only investigators to study the ultrastructure of chalky shell of *Crassostrea virginica* (Margolis and Carver, 1974) published a brief description illustrated with two scanning electron micrographs of fracture surfaces treated with Clorox and acid. They identified blade-shaped crystals oriented with long axes perpendicular to the inner surface of the valves, branching intermediate crystals, the interface between chalky and foliated shell, and the porous nature of chalky structure. Taylor et al. (1969) included two scanning electron micrographs of fractures of chalky shell of *Ostrea edulis.* The chalky shell of the two species appears similar.

Organic matter is present in the chalky shell of *Crassostrea virginica* (Weiner and Hood, 1975). These researchers reported 0.91% soluble and insoluble nondialyzable organic matter in chalky layers, and a lesser quantity, 0.58%, in foliated structure, figures somewhat in agreement with those of Korringa (1951) for two analyses of *Ostrea edulis:* 1.1 and 0.8% in chalky structure and 0.5 and 0.6% in foliated calcite. In *C. gigas* magnesium, sulfur, sodium, and chlorine are present in chalky structure in higher concentrations, and calcium in lower concentration, than in the surrounding foliated structure (Wada and Suga, 1976). The ultrastructural relationship of conchiolin to mineral microstructures of chalky shell has not been determined and is not evident in our micrographs.

Myostracum, Both Valves

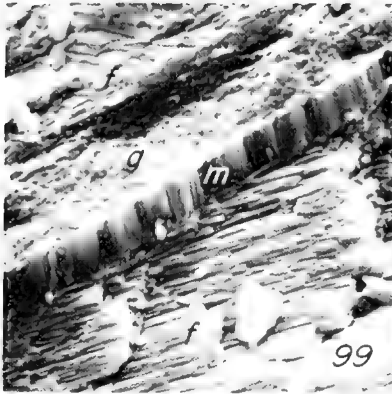
Myostracum (hypostracum of other authors), shell deposits secreted in the attachment areas of muscles (Oberling, 1964), is limited in *Crassostrea virginica* to sites of the large single adductor muscle and the small, single, vestigial Quenstedt's muscle just ventral to the hinge. The oyster lacks pallial and pedal muscles, and thus pallial and pedal myostraca are absent. The mantle is attached securely to the shell only at the periphery of the adductor muscle-shell juncture. Thus, when the adductor muscle is cut from both valves, the oyster slides easily out of the shell.

Adductor myostraca are pigmented, the color ranging in different individuals from light lavender-white to dark purple. Adductor myostracum is a thin, but sturdy, well-developed, aragonitic, irregular, simple prismatic layer. As the oyster grows the adductor muscle increases in size and migrates ventrally. Myostracal layers left behind the adductor muscle become buried under foliated structure, and can be traced in dorsoventral sections as a thin line leading to the mid-umbonal region in each valve (Fig. 1).

Inner (adductor muscle-facing) surface. In young oysters the adductor muscle scar tends to be broadly ovate-crescent shaped (Fig. 87). With increasing growth, the shape and dimensions of the scar area become more variable (Galtsoff, 1964; Stenzel, 1971). The anterior-posterior dimension of each scar occupies roughly one-third of the length of the valve in the area of the scar.

The surface of the scar from which adductor muscle has been removed with Clorox is extremely smooth and shallowly wavy (Fig. 88). Although the adductor muscle is comprised of two distinct parts (approximately a two-thirds, dorsal, translucent area, and a ventral, one-third, milky-white area) no ultrastructural difference was evident in the myostracum of the two areas.

In front of the ventral advancing edge of the adductor muscle there is a narrow transitional zone of newly deposited myostracum about 0.5 to 1.0 mm wide, which precedes the muscle (Fig. 89). On the surface this zone consists of a smooth, typically myostracal inner part adjacent to the base of the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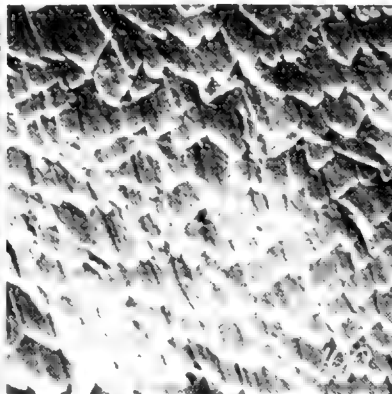

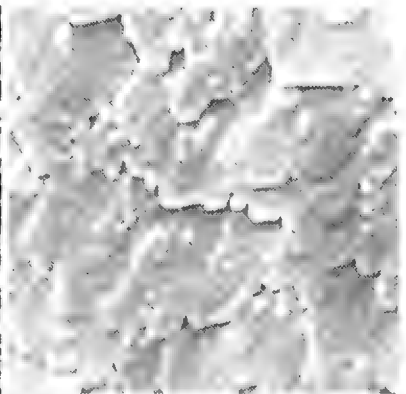

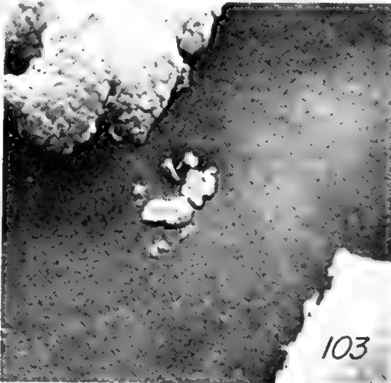
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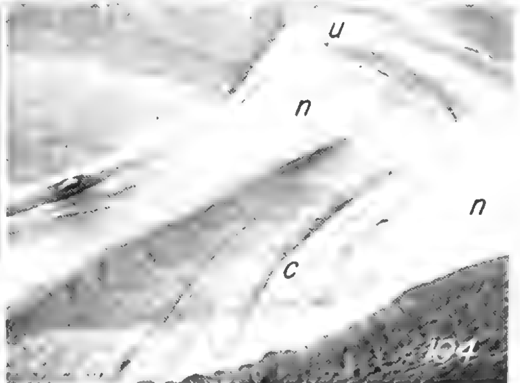
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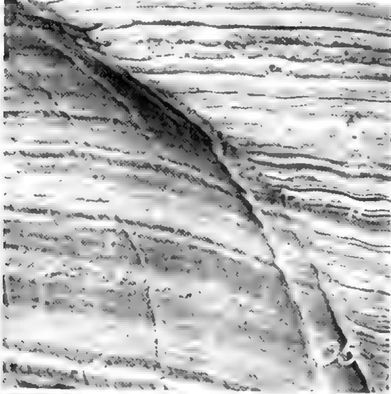

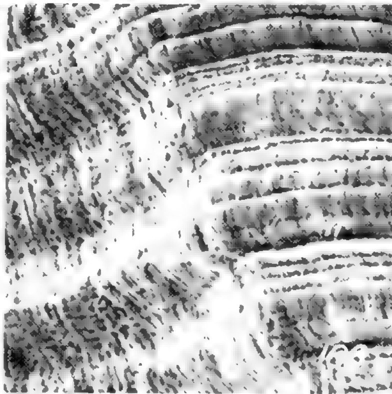

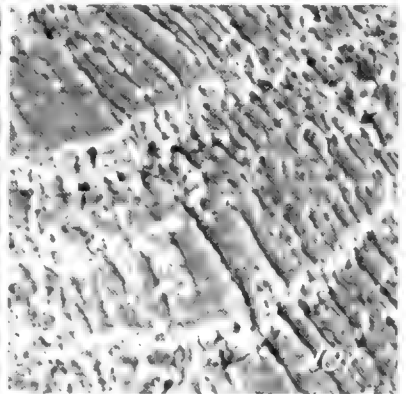

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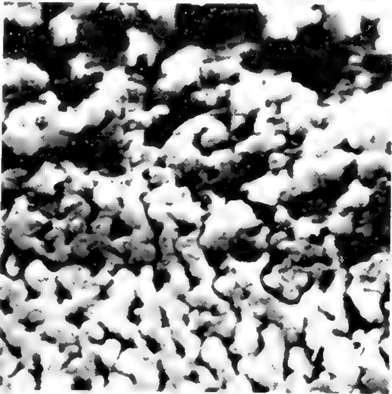

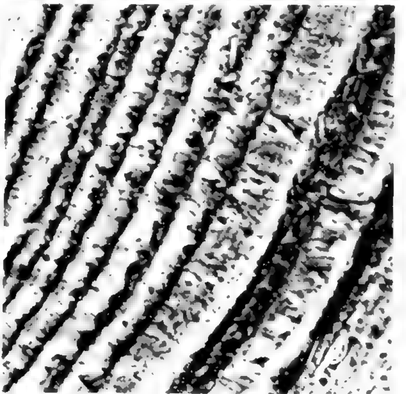

FIGURE 99. *Stratum of adductor myostracum (m) buried in foliated structure (f) in region between adductor scar and umbone, granular structure (g); fracture. 20% Clorox 10 sec. Horizontal field width = 60 μm.*
FIGURE 100. *Thin layer of conchiolin over foliar laths. 5% Clorox 1 min. Horizontal field width = 15 μm.*
FIGURE 101. *Thin granular layer of conchiolin over foliar laths. No cleaning treatment. Horizontal field width = 10 μm.*
FIGURE 102. *Thick granular layer of conchiolin over foliated structure. No cleaning treatment. Horizontal field width = 10 μm.*
FIGURE 103. *Conchiolin patch (smooth dark surface) and initial deposition of foliated structure (upper left, middle, and lower right). 5% Clorox 1 min. Horizontal field width= 15 μm.*
FIGURE 104. *Hinge surface of left valve of young oyster with anterior to right; u, umbo; n, nympha; c, chondrophore; m, contact with mantle isthmus. Ligament dissolved in 100% Clorox, freeze dried. Horizontal field width = 5 mm.*
FIGURE 105. *Chondrophore (c) and nympha (n) of hinge of left valve. Ligament dissolved in Clorox. Horizontal field width = 1 mm.*
FIGURE 106. *Higher magnification of chondrophore and nympha in Figure 105. Horizontal field width = 125 μm.*
FIGURE 107. *Ligostracal prisms of chondrophore of left valve. Ligament dissolved in Clorox. Horizontal field width = 60 μm.*
FIGURE 108. *Chondrophoral-mantle isthmus juncture; c, chondrophore; s, smooth surface of juncture; r, rugose foliated structure. Ligament dissolved in Clorox. Horizontal field width = 475 μm.*
FIGURE 109. *Ligostracal prisms forming in advance of the ligament (lower half) in smooth zone (s, Figure 108). Upper half, ligostracal prisms under ligament. Ligament dissolved in Clorox. Horizontal field width = 15 μm.*
FIGURE 110. *Ligostracal prisms of nympha of left valve. Ligament dissolved in Clorox. Horizontal field width = 60 μm.*

adductor muscle and an outer zone that ranges from smoothly to coarsely granular where it overlaps foliated structure (Fig. 90, 91). In some individuals, on the ventral side of the scar the structure of encroaching myostracum is finely granular and crystallites intermingle with, fill around, and eventually cover foliated laths (Fig. 91). In other individuals the transitional area is a gradient of granular, discrete, muffin-like microstructures that are deposited over folia and finally merge into a solid sheet of smooth myostracum (Fig. 92). In a third type, the transitional zone consists of mulberry-like microstructures ranging in complexity from single to multiple granular mounds (Fig. 93). These coalesce to form myostracum during the migration. The distinctly granular nature of all transitional forms is characteristic.

The transitional zone between myostracum and foliated structure on the anterior and posterior sides of the adductor scar is usually much narrower than that on the ventral side (Fig. 94). Transitional myostracum in this area is generally finely granular.

On the dorsal side of the adductor scar, where foliated structure grows over and buries abandoned myostracum, there is also a granular transitional zone (Fig. 95). This zone is narrow in some individuals (Fig. 95), and in others can be much wider and can consist of several terraces. Growth of foliated calcite over the myostracum extends ventrally on anterior and posterior sides of the scar to about the midline where a reversal occurs and myostracum overgrows the foliated calcite.

Fracture surface. Proof that fully formed myostracum precedes the advance of the adductor muscle is clear in a dorsoventral fracture of a valve through the ventral side of the scar (Fig. 96). Myostracal prisms are present in the entire zone of smooth myostracum, and a granular stratum, homologous with the outer granular zone of transitional myostracum, lies between myostracal and foliated layers. What appears like a thin organic sheet lies over the myostracum just ventral to the edge of the adductor muscle (Fig. 96).

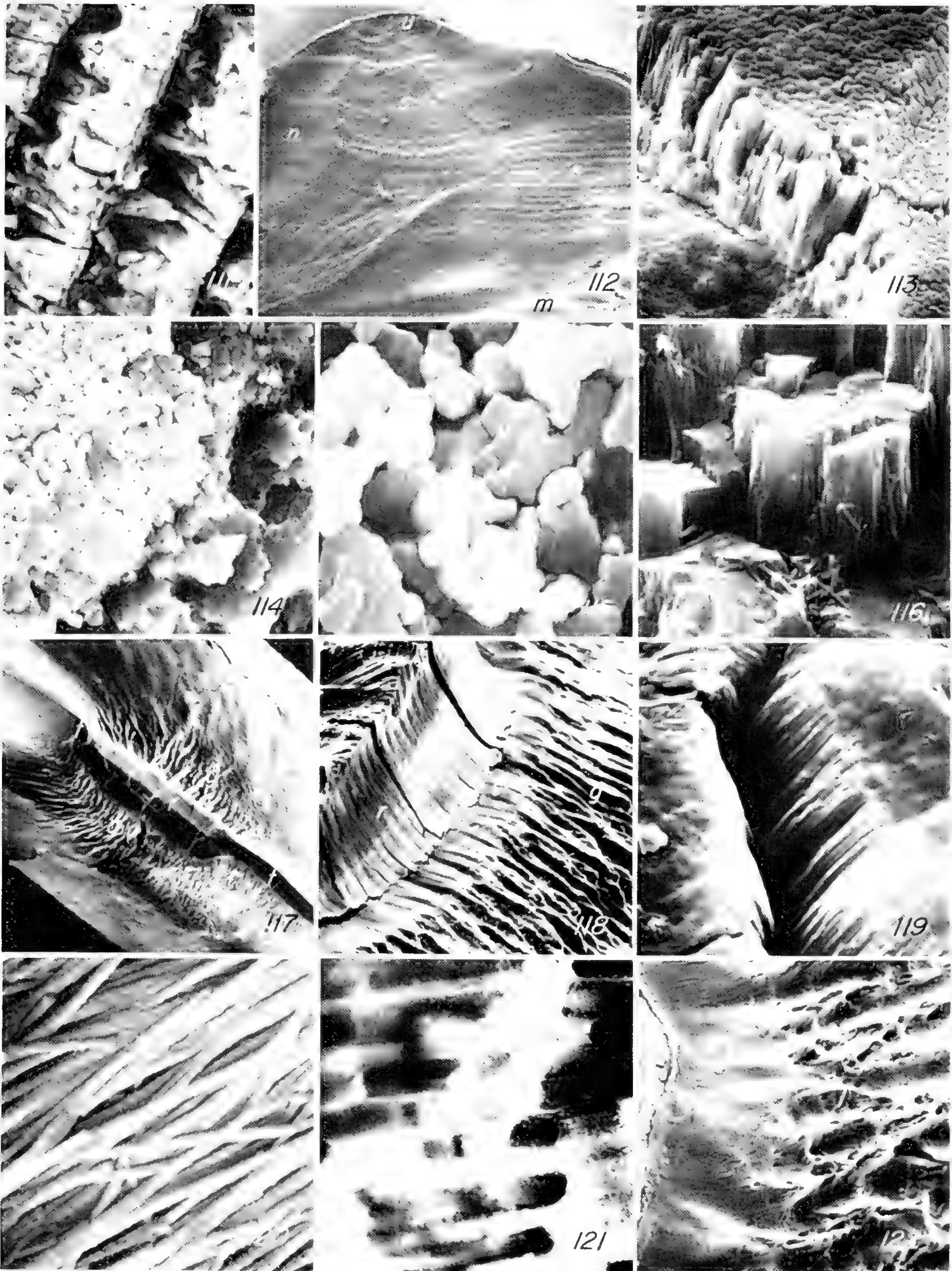
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FIGURE 111. *Higher magnification of nymphal prisms in Figure 110. Ligament dissolved in Clorox. Horizontal field width = 12 μm.*
FIGURE 112. *Hinge surface of right valve of young oyster, anterior to left; u, umbo; c, chondrophore; n, nympha; m, contact with mantle isthmus. Ligament dissolved in Clorox. Horizontal field width = 0.6 mm.*
FIGURE 113. *Ligostracal prisms of nympha of right valve, fracture; prisms, upper right; underlying foliated structure, lower left. Ligament dissolved in Clorox. Horizontal field width = 55 μm.*
FIGURE 114. *Nymphal prisms, vertical view, right valve; prisms at lower right fractured at various terraced levels, traces of aragonitic fibers. Ligament dissolved in Clorox. Horizontal field width = 30 μm.*
FIGURE 115. *Higher magnification of cross fractured nymphal prisms in Figure 114. Horizontal field width = 7.5 μm.*
FIGURE 116. *Side view of cross fractured nymphal prisms in Figure 114. Horizontal field width = 9 μm.*
FIGURE 117. *Ligament in place in hinge between left valve (lower left) and right valve (upper right), interior view. r, resilium; t, tensilia; g, rugose zone of foliated structure. 20% Clorox 10 sec. Horizontal field width = 3.8 mm.*
FIGURE 118. *Fracture of resilium on valve; f, fracture surface of resilium exposing growth bands; r, resilium; s, ligostracal prisms deposited in advance of formation of resilium; g, rugose zone of foliated structure. 20% Clorox 10 sec. Horizontal field width = 1 mm.*
FIGURE 119. *Crack in resilium exposing aragonitic fibers. Surface of resilium treated with 20% Clorox for 10 sec prior to cracking. Horizontal field width = 7.5 μm.*
FIGURE 120. *Aragonitic fibers of resilium, organic part of resilium dissolved in 100% Clorox. Horizontal field width = 3.8 μm.*
FIGURE 121. *Aragonitic fibers in place in resilium, ultrathin section, transmission electron micrograph. Glutaraldehyde and osmium tetroxide fixation. Horizontal field width = 1.4 μm.*
FIGURE 122. *Mantle-facing surface of smooth zone of ligostracum (s) that precedes formation of resilium (r); g, pitted rugose area of foliated structure. 20% Clorox 10 sec. Horizontal field width = 475 μm.*

The thinness of the myostracal stratum is evident in fracture sections from which adductor muscle has been dissolved with Clorox (Fig. 97, 98). The irregular simple prisms are oriented normal to the surface of the layer, and differ strikingly from the regular simple prisms in the external prismatic structure of both valves. Myostracal prism outlines are highly irregular, with re-entrant angles. Although individual prisms are bounded by thin, often discontinuous bands of organic matrix in bivalves (Taylor et al., 1969), these walls were not visible in scanning electron micrographs of *Crassostrea virginica* even after treatment with Clorox (Fig. 97, 98).

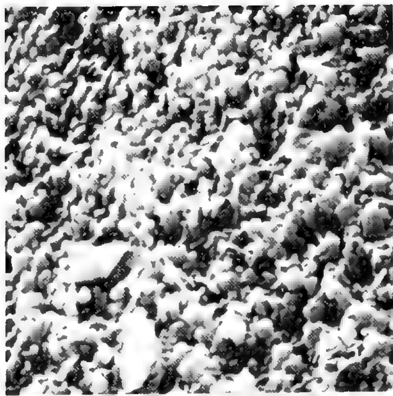
The stratum of myostracum that becomes buried in foliated structure between the scar and the umbonal region is clearly visible in fractures (Fig. 99). Transitional granular shell material, demonstrated in surface views of the scar is also evident on both sides of the myostracal layer sandwiched between strata of foliated structure (Fig. 99).

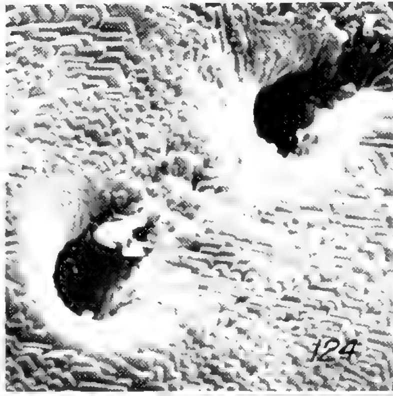
Previous studies. The myostracum of *Crassostrea virginica* has not been examined ultrastructurally. From optical microscopy, Stenzel (1971) described the shift of the adductor muscle and adductor myostracum ventrally in order to retain their relative location in the shell cavity. Taylor et al. (1969), in a transmission electron microscopical study of the myostracum of several species of bivalves, examined briefly the myostracum of *Ostrea irridescens* and *O. hyotis* with replicas.

Conchiolin Patches

Inner (mantle-facing) surface. From time to time greenish-brownish conchiolin patches, or sheets, are deposited randomly by the mantle on the inner surface of foliated structure. In fracture surfaces these organic patches appear as thin lines interleaved among strata of foliated structure.

Patch conchiolin is deposited first as an extremely thin layer that completely covers foliar laths, blurring their outlines (Fig. 100). As the con-

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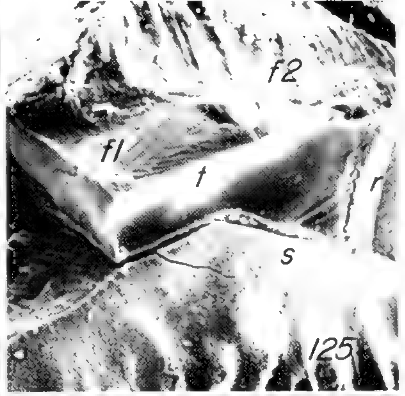
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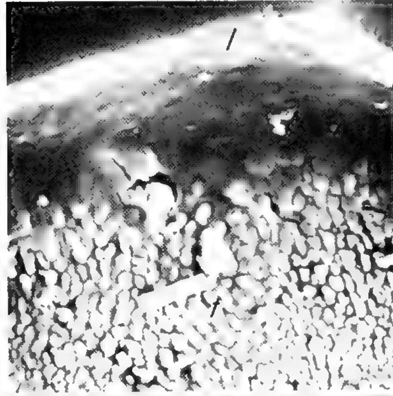
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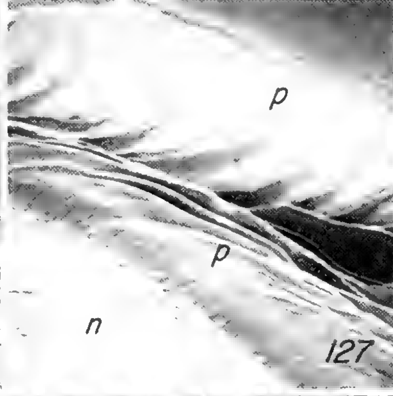
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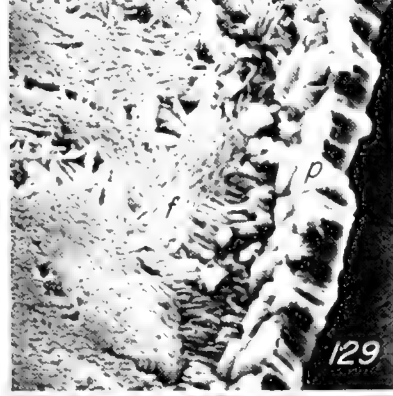
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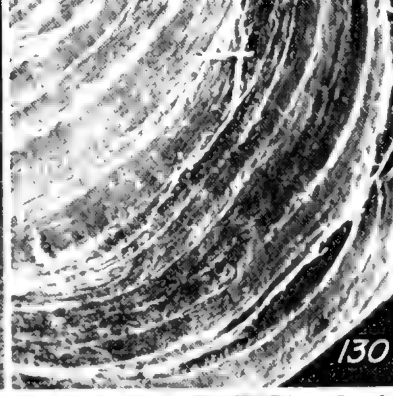
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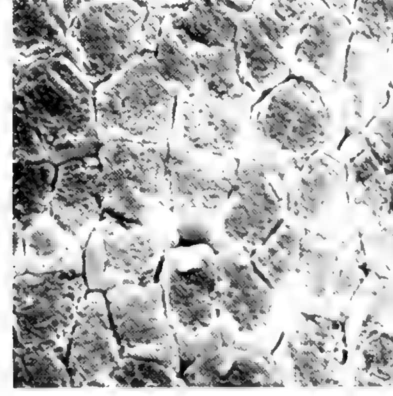

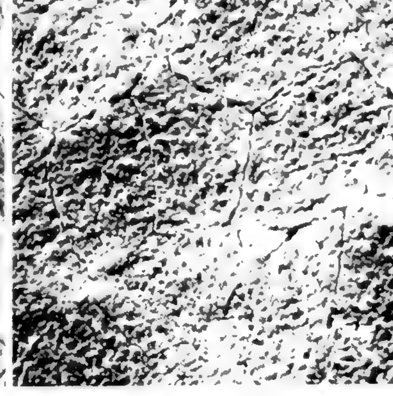

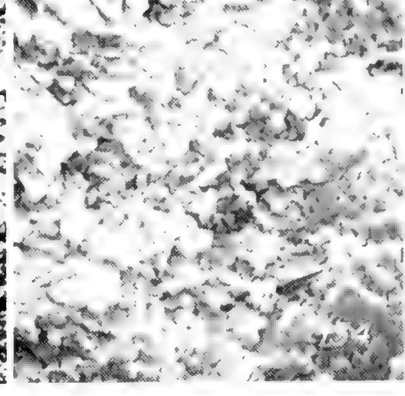

FIGURE 123. *Higher magnification of surface of ligostracum (s) in Figure 122. Horizontal field width = 15 μm.*
FIGURE 124. *Higher magnification of surface of pitted rugose area (r) of foliated structure in Figure 122. Horizontal field width = 30 μm.*
FIGURE 125. *Interior surface (facing subligamental epithelial ridge of mantle) of tensilium (t); f_1, fracture of tensilium; f_2, fracture of resilium; r, interior surface of resilium; s, ligostracum; c, foliated structure. 20% Clorox 10 sec prior to fracturing. Horizontal field width = 475 μm.*
FIGURE 126. *Mantle facing surface of tensilium (t) and ligostracum (l). 20% Clorox 10 sec. Horizontal field width = 30 μm.*
FIGURE 127. *Umbonal plicae (p) of young oyster; n, nympha. Ligament dissolved in Clorox. Horizontal field width = 1.9 mm.*
FIGURE 128. *Umbonal plicae, outer row; higher magnification of Figure 127. Horizontal field width = 1 mm.*
FIGURE 129. *Upper surface of single plica; f, thin layer of foliated structure; p, prismatic scale. Higher magnification of Figure 127. Horizontal field width = 120 μm.*
FIGURE 130. *Closely spaced annuli on surface of right valve formed during late fall. No cleaning treatment. Horizontal field width = 1.9 mm.*
FIGURE 131. *Accumulation of conchiolin at an annulus in valve in Figure 130. Horizontal field width = 60 μm.*
FIGURE 132. *Surface of naturally worn prismatic structure of right valve, erosion of organic matrix exceeded that of mineral prisms. 100% Clorox 1 min. Horizontal field width = 60 μm.*
FIGURE 133. *Surface of naturally worn prismatic structure of left valve, wear of organic and mineral components about equal. 100% Clorox 1 min. Horizontal field width = 30 μm.*
FIGURE 134. *Surface of naturally worn foliated structure, left valve. Brushed clean. Horizontal field width = 30 μm.*

chiolin thickens, granules (presumably also conchiolinal in nature) are added, and outlines of laths become increasingly indistinct (Fig. 101). Upon further deposition, laths are totally obscured and a solid pavement of granular material forms (Fig. 102). The surface of the patch often becomes exceedingly smooth (Fig. 103). The next layer of foliated structure forms directly on the patch, in most cases, apparently, with no or little intervention of transitional granular shell material (Fig. 103).

Previous studies. Korringa (1951) observed conchiolin sheets in *Ostrea edulis* and hypothesized that they were secreted by the oyster either as a defense against the intrusion of burrowing worms, or "for no obvious reason." Taylor et al. (1969), from observations of conchiolin patches in *Ostrea cuccullata,* suggested that deposition of these layers seems to be a reaction by the animal against excessive corrosion of the older parts of its shell. The matter needs further study.

Hinge Chondrophores and Nymphae

Interior surfaces of the hinge that interface with the ligament are morphologically complex. In young dissoconch oysters the hinge base in each valve consists of a central gully, the chondrophore (resilifer of other authors), flanked anteriorly and posteriorly by elevated shelves, the anterior and posterior nymphae (anterior and posterior bourrelets of other authors). The chondrophore on each valve first forms in the late pediveliger stage, and increases in size as valves grow, forming an often serpentine, widening depression leading from the umbo to the mantle isthmus between widening nymphal plateaus (Fig. 104, 112) (Carriker and Palmer, 1979a,b). In older oysters the chondrophoral valley in the left valve becomes deeper with age, and the corresponding one in the right valve becomes elevated onto a supporting buttress with the associated nymphae depressed below it on anterior and posterior sides. The left valve bears a pronounced open umbonal cavity deep beneath the platform that carries the ligament (Galtsoff, 1964; Stenzel, 1971). As the oldest part of the ligament deteriorates and crumbles, chondrophoral and nymphal surfaces become exposed, and these, too, erode and lose their characteristic microstructures.

Ligostracal layers. A thin, distinct, mineralized

layer, the ligostracum, binds the foliated calcite of the shell in the umbonal region to the organic ligament (Carriker and Palmer, 1979b). This layer is ultrastructurally, and generally mineralogically different from the underlying foliated structure. The ligostracum of the hinge of the right valve is aragonitic; that of the left valve is calcitic with traces of aragonite. We could not determine whether or not the aragonitic part is ultrastructurally different from the calcitic part. On the left valve the ligostracal surface of both the chondrophore and nymphae is conspicuously annulated (Fig. 105), growth lines being more obvious on nymphae (Fig. 106) (Palmer and Carriker, 1979b). The ligostracum of the right valve is also annulated, but less prominently.

Ligostracal prisms of the chondrophore of the left valve tend to lie obliquely to the surface of the layer, range in diameter from a fraction of a micrometer to about 6 μm, and are interrupted periodically by growth lines (Fig. 107). Thickness of the layer averages 8 to 16 μm. Prisms are spaced apart and bear deep pits.

Ligostracal prisms of the chondrophore of the right valve and of nymphae of left and right valves are generally oriented at nearly right angles to the surface of the underlying foliated structure, and form a distinct layer that can be removed by fracturing (Fig. 110, 113). At the exterior surface, nymphal prisms are spaced slightly apart and tend to merge toward the base of the layer (Fig. 114, 115, 116). Nymphal prisms of the left valve are considerably more pitted and irregular (Fig. 111) than those of the right valve (Fig. 114). Diameter of distal ends of ligostracal prisms of the chondrophore of the right valve and of nymphae of both valves ranges from a fraction of a micrometer to about 4 μm. In fracture sections the nymphal layers average 15 μm in thickness and can taper to half this thickness at the edges. The central part of the right chondrophoral ligostracum increases to 30 μm.

Chondrophoral and nymphal ligostraca form a sharp boundary at the ligament-mantle isthmus interface (Fig. 104, 108). The chondrophoral surface frequently is thrown into wave-like folds that parallel the dorsoventral axis of the chondrophore (Fig. 108, upper right). Below the ventral boundary of the chondrophore the shell surface is relatively smooth for a short distance (Fig. 108). This narrow zone is ligostracal structure that forms in advance of the ligament (Fig. 109). Ventral to this ligostracal band lies a wider area, the rugose zone, in which the shell surface is thrown into conspicuously pitted, deep valleys and sharply pointed crests (Fig. 104, 108).

Previous studies. Other than that reported by Carriker and Palmer (1979b) and Palmer and Carriker (1979), no ultrastructural research has been conducted on the layers and microstructures of the mineralized parts of the hinge of *Crassostrea virginica.*

Hinge Ligament

The ligament consists of a relatively thin band of dark, elastic, organic material hidden from outside view within the opening-closing pivotal axis of the valves in the umbonal region. The ligament is a nonliving, secreted material that forces the valves apart when tension of the adductor muscle is released. Although prodissoconch shells of the oyster possess articulating teeth (Carriker and Palmer, 1979a), these are lacking in the dissoconch. The youngest part of the ligament lies in contact with the subligamental epithelial ridge of the mantle isthmus on the inside of the valves; oldest strata face the outside between the umbones, and as exposed to seawater, microbial action, and elastic fatigue failure, crack and crumble and become nonfunctional (Trueman, 1951, 1964; Galtsoff, 1964; Stenzel, 1971).

The ligament is composed of three distinct parts: a) the light brown central resilium (cartilage, fibrous ligament, inner layer of other authors) attached to the chondrophore of each valve; and b) anterior and c) posterior dark green tensilia (lamellar ligament, outer layer of other authors) located anteriorly and posteriorly of the resilium (Fig. 117) and attached to the nymphae of the hinge (Fig. 104). The periostracum, which may cover the outside of the ligament in bivalves (Trueman, 1964), is visible only in young dissoconch oysters before erosion of the umbones commences. When valves are closed, the resilium is compressed because of its considerable thickness, while the thinner tensilia are stretched slightly; both thus serve to open the valves when the adductor muscle relaxes (Trueman, 1951; Galtsoff, 1964).

Ultrastructure of ligament. The sturdy resilium

is securely attached to the ligostracal layers of the hinge, and is composed of transverse strata (representing growth bands) and longitudinal ridges that parallel similarly oriented waves in the chondrophores (Fig. 118, 125).

The conchiolin of the resilium is reinforced with white, aragonitic fibers (Stenzel, 1963) that are placed dorsoventrally in the ligament and about normal to its ventral growing border. These mineralized fibers are clearly visible in fracture sections in the surface of the ligament facing the subligamental ridge (Fig. 119). When the organic matter of the ligament is dissolved with Clorox, the fibers remain as long slender needles with a slightly nodular surface and the suggestion of growth marks on the surface of each fiber (Fig. 120). The diameter of the fibers is relatively constant, approximately 0.15 μm. The relationship of the organic matrix to the aragonitic fibers is disclosed in the transmission electron micrograph of a thin section of undecalcified ligament (Fig. 121). The thickness of each fiber is approximately twice that of the matrix binding fibers to each other in fixed specimens.

Adjacent to the interior ventral border of the resilium lies a smooth band of ligostracum (Fig. 122, 123). This precedes the formation and advance of the growing resilium. Ventral to the exposed ligostracal zone is the conspicuous rugose region (Fig. 104, 108, 117, 118, 122, 125), characterized by numerous, randomly distributed holes. The foliated nature of this part of the valves is accented by laths that surround and outline the walls of the holes (Fig. 124).

Tensilia, in contrast to the resilium, are smooth and homogeneous in appearance (Fig. 125, 126). Fracture sections show lines that could represent either growth bands or fracture lines (Fig. 125). The boundary between tensilia and the resilium is sharp (Fig. 125). A ligostracal zone precedes the growing margin of tensilia (Fig. 126), as it does before the resilium. Organic matter of the ligament is deeply enmeshed among the prisms of the ligostracum (Fig. 122, 126), affording a strong hold for the ligament in the shell.

Previous studies. The ligament of *Crassostrea virginica* was examined ultrastructurally only by Galtsoff (1964). He studied sections of the resilium with the transmission electron microscope, and observed, but did not identify, the fibers. These were investigated ultrastructurally in *Pinctada radiata* and *Mytilus edulis* by Bevelander and Nakahara (1969) and mineralogically in *C. virginica* by Stenzel (1963) who determined that the resilium consists of both conchiolin and aragonitic fibers.

Umbonal Plicae

Umbonal folds of shell that extend ventrolaterally from the nymphae along both sides of the left valve are especially noticeable in young oysters, up to about 15 mm in height, growing attached to a smooth substratum (Fig. 127). The inner row of these plicae, adjacent to the nymphae, are close-set, tend to follow the contour of the edge of the valve, and appear like extensions of nymphal growth bands. Plicae of the outer row are higher and more widely separated, and flare outward, forming keels over the basement sheet of shell deposited upon the substratum (Fig. 128). In older oysters these plicae tend to disappear, or a few continue, sometimes as exaggerated folds, along the sides of the valve.

Ultrastructurally, the central layer of each plica is composed of prisms that are extensions of prismatic scales on the exterior of the valve (Fig. 129). Plical prisms are exposed on the under side (that facing the substratum) of plicae. On the upper side (exterior), each plical prismatic layer is covered by a thin deposit of foliated structure (Fig. 129).

The outer row of umbonal plicae was illustrated by Galtsoff (1964, his Fig. 32) in young *Crassostrea virginica* 8.5 to 10 mm high growing attached to tar paper. The row is especially prominent on the anterior side of the valve and is similar to what we have observed in our specimens. The presence of plicae only on the left valve suggests that they support the umbonal region and facilitate adhesion to the substratum.

Wear of Exterior Shell Surfaces

Just as the exterior part of the ligament deteriorates with age, so the exterior surface of the valves becomes worn upon exposure (Galtsoff, 1964; Stenzel, 1971). The imbricated prismatic layers (scales), located on the exterior surface, are the first to be eroded, exposing the foliated structure to view in older parts of the valves.

Wear may occur as a result of dissolution by organic and inorganic acids and chelators in relatively quiet water over highly reducing organic sediments, abrasion by suspended pelting sediments (especially sands) in flowing seawater, dissolution by bacteria and fungi, and burrowing by a wide range of species of shell-penetrating algae, fungi, and small invertebrates (Carriker and Smith, 1969). In cases where erosion of organic matrix by bacteria and fungi exceeds that of mineral prisms by acids and chelators, conchiolin envelopes of microstructures tend to be exaggerated (Fig. 132). Where wear of both organic and mineral components progresses at about the same rate, prism boundaries are less conspicuous (Fig. 133). In situations where mineral cores of prisms are dissolved first, the configuration of organic sheaths is clearly exposed (Fig. 34-36, 53).

Structural outlines of laths of foliated structure tend to be erased soon after the layer is exposed and wear begins (Fig. 134). Obliteration of intercrystalline organic boundaries occurs quickly, probably because the proportion of organic matrix to mineral microstructures is considerably lower in foliated than in prismatic structure. Pits of various sizes and depths are common in the mineral part of eroding microstructures, probably as a consequence of differential solubilization of various mineral constituents of crystals (Carriker, 1978).

Dissolution of Interior Shell Surfaces

Normally during the growing season mantle-facing surfaces of both prismatic and foliated strata of valves are clean and microstructures are clear-cut and sharply outlined (Fig. 23, 60, 74). If, however, oysters are forced to remain closed, dissolution occurs. In several oysters (approximately 2-3 cm high) held shut in air at room temperature for three days, we observed mild to severe dissolution of microstructures at magnifications ranging from 4,000 to 10,000. In heavily affected areas, edges and surfaces of microstructures were dissolved, and occasionally pits of various sizes and depths were etched, resulting in indistinct, or even obliterated, microstructural form. Solubilization was spotty, being prominent in some surfaces, and scarcely noticeable in others. Elevated regions of the shell surface were least affected.

In contrast to our findings, Watabe et al. (1958) noted that etching of the growing surface appears to be a common, rather than a rare, event. It is possible that some of the dissolution that they observed could have resulted from the treatment of their experimental bivalves. Oysters were collected in March on the North Carolina coast from bay water at 8°C, and maintained for 15 hours to 9 days in aerated seawater at about 20°C in the laboratory.

Degree of dissolution of interior shell surfaces varies roughly with the duration of anaerobiosis; during shorter periods of closure, solubilization is less, and during longer periods it may be so extreme as to completely obliterate the surface form of microstructures. During anaerobic metabolism, neutral substances can be converted into organic acids, lowering the pH of the extrapallial fluid (de Zwaan and Wijsman, 1976), resulting in dissolution. Succinic acid has been implicated as one possible solvent (see Wilbur, 1972, for review). The fundamental cause of inter- and intracrystalline dissolutional patterns in shell lamina is still an open question. Quite likely, differential solubility of the different constituents of microstructures (soluble and insoluble organic matrix, minor and trace minerals) is part of the answer (Carriker, 1978).

FUNCTIONAL ULTRAMORPHOLOGY

Knowledge of the structure and formation of molluscan shell has expanded rapidly during the last two decades (Wilbur, 1964, 1972, 1976; Taylor et al., 1969; Kobayashi, 1971; Grégoire, 1972). Although little is known about the control of form and orientation of microstructures or of thickness and configuration of shell layers (lamina), our understanding of the manner in which shell is formed from mineral crystals and organic material has advanced significantly (Wilbur, 1976). Formation of multilayered shells in bivalves is a continuous process that can be summarized in five steps, conceptualized here (adapted from Wilbur, 1976) to provide a basis for discussion of some of these events in the ultramorphogenesis of the shell of *Crassostrea virginica:*

1) Shell microstructures, composed primarily of $CaCO_3$, develop in the extrapallial space, from mineral and organic ingredients passed through the mantle epithelium;

2) The mantle edge secretes a sheet of periostracum that becomes tanned and provides the substratum on which the outer shell layer forms;
3) On the interior surface of the valves, microstructures form in the organic matrix or on the surface of microstructures previously formed;
4) Mineral crystal seeds grow, orient, and coalesce in the organic matrix, sandwiching the matrix between them as they enlarge laterally to form a single lamina one microstructure thick; and
5) New laminae, sometimes several at a time, form atop previous laminae producing terrace-like configurations.

Prism Formation

In *Crassostrea virginica* an extremely thin organic sheet is laid down by the mantle edge (Fig. 47). Crystallites, beginning as very small, roughly rounded bodies, possibly at electrically active point defects (Distler, 1979), increase in diameter away from the edge of the membrane (Fig. 48-50). They soon make contact laterally, apparently squeezing the organic matrix between them to make conchiolin sheaths (Fig. 50). Crystallization (inorganic), according to Distler (1979), should be considered replication in an electrically active crystal; this theory is reminiscent of Digby's semiconductor hypothesis of calcification in organisms (Simkiss, 1976). However, growth stages of shell prisms in *C. virginica* lack the orderly form of inorganic crystals, and it is not until their edges crowd upon the interprismatic matrix that they assume the characteristic prism morphology.

Our micrographs suggest that crystallites are embedded within the marginal organic sheet (Fig. 47-51), much as Nakahara and Bevelander (1971) found in *Pinctada radiata* in prisms developing in scales (spurs). Whether the marginal organic sheet in *Crassostrea virginica* is periostracum or conchiolin is not clear in the micrographs; it is likely a combination of both.

Increase in size of prisms away from the growing margin could reflect geometric selection (Taylor et al., 1969), a case in which prisms grow at irregular rates, and slow growing ones are crowded out for lack of space (Fig. 17). It is more likely, however, that as the prismatic layer thickens, fusion occurs between prisms. Coalescence of adjacent prisms is suggested where a conchiolin spur extends part way across a prism (Fig. 18, 19) and by the studies of Palmer and Carriker (1979) and Tsujii et al. (1958). How current concepts explaining mantle growth relate to prism formation in the oyster is not clear (see Waller, 1979, for discussion of these concepts).

The matrix separating prisms is generally high in the center with grooves at its edge (Fig. 8); Tsujii et al. (1958) explained that this is the form to be expected if approaching crystal edges squeeze the matrix. Yet this is not always the case on exterior (Fig. 10, 11, 50) or interior surfaces (Fig. 23). Taylor et al. (1969) observed that the central boss characterized by punctate marks or concentric growth rings appears to represent the original spherulite that developed on the periostracal substratum (Fig. 9, 12, 44). This observation is partly supported by the form of developing crystals at the margin of the valves (Fig. 50).

Crenulated edges of prisms on the exterior surface of the valves (Fig. 14) could form as a result of the lateral growth (possibly from squeezing) against the organic matrix. The grooving probably serves as an anchorage for the matrix wall, and thus contributes to the strength of the structure.

The periostracum on both valves occasionally becomes folded and wrinkled (Fig. 3, 39). As Figure 47 shows, the newly secreted organic sheet at the valve margin is easily convoluted, and this can explain the formation of ridges in the periostracum.

Successive layering of prismatic strata is present primarily on the right valve. Prismatic scales seem to form in the manner described by Nakahara and Bevelander (1971) for *Pinctada radiata;* that is, after establishment of a single layer of short prisms (Fig. 26), the mantle retracts, then re-extends beyond the first scale to form a new scale separated from the first. Whether scales in *Crassostrea virginica* are enveloped on both exterior and inner surfaces by periostracum, as stated by Nakahara and Bevelander (1971) for *P. radiata,* is difficult to determine, but our micrographs (Fig. 27) suggest the presence of periostracum only on the outside. The reason for formation of single

surface scales, in contrast to thick prismatic shell composed of several layers of prisms (Fig. 28) deeper in the valves, is uncertain. Individual layers of prisms in multilayered formations are also separated from each other by a sheet of organic matrix.

The shell forming process occurs in two distinct phases Galtsoff (1964): a) movements of the mantle to provide an organic matrix upon which the shell is formed, and b) deposition of the mineral shell materials. According to Galtsoff conchiolin is secreted as a clear, viscous, sometimes stringy substance from the periostracal groove and inner mantle surface. During release of the secretion, the mantle actively expands and retracts, spreading fluid conchiolin over the shell surface. Because of this action, the proximal part of the newly formed valve receives a larger amount of conchiolin than the distal zone. Soon after conchiolin is secreted, mineralization occurs within it.

The mantle lobes are muscularly active and highly contractile. They may stretch a considerable distance beyond the edge of the valves, withdraw some distance inside the shell (since the ventral parts of the mantle are attached only at the adductor muscle), roll into a tube, or form ridges that serve as temporary channels for discarding mucus and foreign particles. These movements can involve the entire surface of the mantle, or only a small part of it, depending upon the intensity of stimulation received by tentacles of the middle and inner lobes. In a closed oyster, the mantle edges are withdrawn to about midway between the distal margin of the gills and the edges of the valves (see also Galtsoff, 1964).

Muscular activity of the mantle complicates any explanation of prism formation. If the mantle surface remained fixed in position relative to the valves, close association between epithelial cell groups of the mantle and growing microstructures of the valves could be hypothesized. Since, however, the mantle is mobile, alternative postulates are necessary. These could include: a) the mantle returns to a precise position relative to growing microstructures of each valve after each excursion or change in form, and accretion of microstructures is under close cellular control, or b) there is no close association between cell groupings of the mantle epithelium and microstructures of the growing shell surface. In the latter case, after random nucleation of mineral crystallites and formation of the first layer of prisms on the periostracum, shell growth would take place, from chemical substances in the extrapallial fluid, by addition to existing shell microstructures, much as inorganic crystals form. A further possibility is that the mantle, stimulated by the inner surface of the shell could continue to produce the same type of microstructures (H. Haskin, personal communication). Unfortunately, there is little to support either postulate. Because of the mobility of its mantle, the oyster may be an excellent bivalve for experimentation on the perplexing relationship, if any, between cell groups of the mantle epithelium and formation of shell microstructures.

In an optical micrograph Stenzel (1971) showed, as we have, that the long axis of prisms of relatively thick scales of the right valve of oysters is inclined to the outer surface of the prismatic layer of the valve (Fig. 33). Angling probably contributes to the structural strength of the scale, and reduces breakage of the valve edge. It is difficult to explain, though, how angling takes place. If the assumption is made that the mantle, during deposition of successive intraprismatic microlayers, returns to the same position relative to the valves, then angling could be explained by a further assumption, that proliferation of mantle epithelial cells occurs not only at the mantle margins, but continues inside the edge (Stasek and McWilliams, 1973). Accordingly, with increase in size (area) of the mantle, each cluster of cells responsible for depositing a specific shell microstructure would be advancing radially, resulting in a bending in the long axes of prisms. This provocative problem calls for more attention.

The ultrastructural appearance of the mantle surface of prismatic strata indicates that some mechanism exists for maintaining the height of the matrix and crystal surface at approximately the same level. Tsujii et al. (1958) suggested that movements of the mantle probably accomplish this by distributing secreted shell material in the extrapallial space. They admitted, however, that this suggestion does not explain the presence of the delicate sculpture on prism surfaces.

Individual prismatic layers tend to be approximately uniform in thickness (Fig. 26), though thickness of different strata, and thus length of prisms, can be highly variable. Control of thick-

ness of laminae of nacreous shell, according to Wilbur (1974), must operate on individual lamellae as well as on an entire lamina; the same is probably true of prismatic shell. The compartment hypothesis of Bevelander and Nakahara (1971), which was postulated to explain the uniform thickness of laminae, was found untenable by Erben and Watabe (1974), and in its place Wilbur (1976) proposed two other hypotheses: 1) secretion of organic material by the mantle in amounts that would result in a matrix layer of uniform thickness in which crystals grow, and b) periodic sclerotization of a layer of matrix secreted by the mantle that would inhibit crystal growth upon contact with the hardened organic layer. Degens (1976) suggested a variant of hypothesis b) in which secretion of an intercrystalline matrix stops biocrystal growth, and release by the mantle of a carrier protein that combines with the mineralization matrix reactivate biocrystal formation. Both hypotheses a) and b) are based on a controlled sequence of secretion of given amounts; in the case of a), of organic matrix, and of b), of phenoloxidase. However, as J. Gordon (personal communication) points out, controlled release is not necessary if a finite time is allowed for polymerization to occur. Our observations on *Crassostrea virginica* suggest that in some respects both may apply. For example, at one phase of development matrix sheaths can be flush with the crystal surface (Fig. 23), supportive of hypothesis a); and in a second one, the intercrystalline matrix seems to grow out over the crystal surface (Fig. 24), suggestive of the beginning of sclerotization. Hypothesis b) is also strongly suggested by the striking box-like configuration of prism sheaths from which mineral cores have been dissolved (Fig. 36). In other respects the hypotheses are less helpful: not all areas of a stratum form simultaneously (Fig. 22), at the margin two or more layers of prisms develop at the same time (Fig. 47, 48), and the substructure of prisms can be rather regularly stratified (Fig. 25, 31). The irregularity of boundaries of prisms (Fig. 18-20) supports the concept that crystallites grow and coalesce randomly until their boundaries crowd upon compressed matrix sheaths to form layers. Without doubt, much remains to be explained about the processes that control prism formation and layer thickness.

In some bivalves, at least, prismatic structure could be necessary for initiation of growth of foliated structure. This suggestion is based on the observation that development of the former always precedes that of the latter in *Crassostrea virginica*. What determines initiation of growth of foliated calcite upon prismatic structure is still an unsolved question. The change from one type of structure to the other is not abrupt, as demonstrated by the transition zone between the two (Fig. 63).

A curious observation reported by Galtsoff (1964) deserves mention. On several occasions he found "fairly large quantities" of a white powdered material (analyzed as calcite and a small proportion of gypsum) in front of the discharge areas of healthy oysters maintained in glass trays in running seawater. He suggested that the particles were mineral crystals formed by the mantle and not incorporated in the shell. Ultrastructurally, it is difficult to imagine how mantle epithelial cells can excise and discharge shell particles roughly 100 to 200 μm in size into the mantle cavity. Examination of the particles in Galtsoff's photograph (his Figure 101) disclosed that they are angular, possess several flat, concave, or convex facets that meet to form ridges and points — and closely resemble in form and size the chips that are excavated from shell by burrowing sponges (Cobb, 1969; Rützler and Rieger, 1973). Consequently, what Galtsoff saw were probably shell fragments excavated by sponges inhabiting the shells of his oysters.

Lath Formation

Pearl "oysters" (family Pteriidae, for example) and edible oysters (family Ostreidae, for example) are not closely related systematically, and the inner layer of their valves differs markedly. That of pearl "oysters" (for example, *Pinctada martensii;* Watabe, 1965) is aragonitic (nacreous) and microstructures (lamellae) are hexagonally shaped disks arranged in layers separated from each other by relatively thick interlamellar organic matrix and oriented parallel to the surface of the mantle. The inner layer of ostreid shell, on the other hand, is calcitic and composed of a structure that ranges from regularly to highly irregularly foliated layers, and microstructures are long, thin laths surrounded by relatively thin matrix walls.

Watabe's (1965) hypothesis, suggestive of Wilbur's hypothesis b), proposed that the interlamellar matrix over lamellae in pearl "oysters" inhibits lamellar growth and accounts for the generally uniform thickness of laminae. In contrast, Watabe noted, foliated structure of ostreids, with its reduced amount of matrix, is less well regulated, with the result that multilayers of microstructures can be formed simultaneously. The relatively low proportion of matrix may also explain, at least in part, the wide range of orientation of laths in the broad morphological spectrum from regularly to highly irregularly foliated structure (Fig. 54, 60, 74).

All shell laid down initially on the outside of the valves of *Crassostrea virginica* by the mantle is prismatic. Foliated structure, deposited next, underlies the prismatic and therefore originates on the organic matrix of prismatic structure. As shown by Watabe and Wilbur (1961) and by us, this occurs through a thin transitional zone of minute aggregating crystallities that develop into foliar laths (Fig. 63). This sequence raises the question whether a layer of prismatic shell is a prerequisite for development of foliated shell in this species. That this is not the case in all bivalves (Pectinacea, for example), is indicated by the fact that prismatic structure has been lost in some of them (Waller, 1975).

Laths probably originate on organic matrix. Whether nucleating crystallites form within the matrix, coalesce as they grow, and push the surrounding matrix against neighboring growing crystals to form intercrystalline matrix and incipient laths, is questionable. Probably another model is called for as random growth of laths would more likely yield round or polygonal, rather than rectangular, shapes.

Once formed, individual laths continue to grow at the free ends facing the mantle. Direction of growth can take place at a slight angle to the mantle surface and tightly over underlying laths, or can range to nearly right angles to the surface with increasing space between lath ends as the structure of chalky shell is approached. Because growth of microstructures is primarily by incorporation of mineral material with only small amounts of intra- and intercrystalline matrix, Watabe (1965) considers foliated shell organically economical. This is important in light of the observation of Price, Thayer and Montgomery (1975) that over one half of the organic matter in *Crassostrea virginica* is in its shell. The precise relationship of intercrystalline matrix and mineral crystal at the growing end of each lath has not been explored. The chevron-shaped increments visible on the ends of some laths (Fig. 65) suggest, as do fractures of laths that have been etched (Fig. 71), that development is incremental, the mineral packaged between thin sheets of intracrystalline matrix.

Various hypotheses have been offered to explain the direction of growth of laths relative to the valves: direction of growth of the mantle, fibrillar arrangement of the matrix, direction of currents of extrapallial fluids (Grégoire, 1972), local concentration differences of $CaCO_3$, adsorption of impurities, and presence of neighboring laths (Watabe et al., 1958; Watabe and Wilbur, 1961). Galtsoff's (1964) observations on the movement of mantle lobes suggest another explanation. Microscopy shows that the prevailing direction of long axes of laths in the area of the valves between the adductor muscle and the ventral edge of the valves is ventral (Fig. 54). Galtsoff (1964) demonstrated that the mantle is very active in this area, moving back and forth in a dorsoventral direction. In contrast, orientation of laths in the area between the adductor muscle and the hinge is erratic and variable. In this area, the mantle is relatively inactive because lobes are joined to the adductor muscle and held firmly at the hinge. Circumstantial evidence thus points to back and forth movements of the mantle as determining, at least in part, the direction of long axes of laths, and absence, or relative absence, of movement as allowing other factors (perhaps some of the ones listed by Watabe et al., 1958; Watabe and Wilbur, 1961; Grégoire, 1972) free play, resulting in heterogeneous orientation. Wainwright's (1969) report that bivalve shell develops tensile stresses in its outer layers and compressive stresses in the inner layers between the adductor muscle and the hinge, could also explain the varying orientation of laths in oyster shell.

Chalky Shell

Several suggestions have been offered to explain the cause of chalky shell formation. All, however, were refuted by Galtsoff (1964). He noted, for ex-

ample, that chalky structure does not result from secondary solution of calcium salts of the shell since dissolution resulting from closed valves is not localized and occurs over the entire inner surface of the valves. The same logic would apply to the comment of Margolis and Carver (1974) that chalky shell could be deposited during periods of maximum ventilation and reduction of $CaCO_3$ saturation. Galtsoff also observed that detachment of the mantle from the inner surface of the shell does not result in deposition of chalky material. He lifted the mantle from the shell surface by insertion of thin, shallow, plastic cups. Foliated calcite was deposited on the surface of the cups facing the mantle, but not on the surface of the valves, while conspicuous chalky areas were formed in places where the mantle was in close contact with the shell. In short, other than the possible effect of rate of deposition (Carter, 1980), there is yet no explanation for the cause of formation of this curious type of foliated structure. It can be said, though, as Stenzel (1971) pointed out, that chalky deposits do not require as much shell material per unit volume of valves as the more solid regularly foliated structure.

In terms of current theories on shell formation it is difficult to visualize development of chalky microstructures primarily because of the presence of interstitial spaces among blades and leaflets. Chalky shell microstructures originate in a transitional zone of small crystallites (Fig. 80) bathed in extrapallial fluid. From these crystallites blades emerge, and from the sides of blades come leaflets. Scanning micrographs of blades (Fig. 82) show what appear to be layers of deposition, suggesting that strata of new mineral are laid down following the form established during initial stages of growth of blades. According to Korringa (1951) and Weiner and Hood (1975), there is approximately a third more conchiolin in chalky shell than in regularly foliated calcite; scanning electron microscopy does not indicate whether this matrix is primarily on the exterior of chalky microstructures or within them. Nucleation of leaflets, presumably on the surface of matrix of blades, follows theory, but what determines the site of nucleation, the direction of growth of leaflets, and why some leaflets contact opposing microstructures while others terminate in space is still enigmatic. Even if the mantle surface were in close apposition to forming microstructures, it is problematical whether individual clusters of cells could control the spatially restricted deposition of shell material that results in chalky microstructures.

Inasmuch as chalky shell is deposited in the aqueous environment of the extrapallial space, it is logical to assume that composition of fluid trapped in interstitial pores of chalky shell reflects that of the extrapallial fluid. Korringa's (1951) data suggest that this may not always be the case. He had pure samples of chalky shell of *Ostrea edulis* "extracted in water" and the resulting solution titrated for chloride. The quantity of "sea-salts" obtained (6.5% as compared to 0.1% each in prismatic and regularly foliated structure) is surprising. Pore fluid in chalky deposits (Fig. 73, 74) in the region of the valves between the adductor muscle and the ventral edge of the valves could exchange with seawater when mantle lobes retract or curl, or otherwise move, exposing the inner shell surface to seawater. As new layers of shell are deposited, salts could become trapped in chalky pores. Still, this is much less likely to be the case in regions of the valves between the adductor muscle and the hinge. Korringa did not indicate from what part of the valves his samples were taken.

In any case, it is important to know whether density and viscosity of the pore fluid exceed that of seawater and what happens to extrapallial fluid when the mantle lobes move. Extrapallial fluid includes proteins, mucopolysaccharides, glycoproteins, organic acids, and several inorganic ions (Wilbur, 1972; Wada and Fujinuki, 1976). In *Mercenaria mercenaria* fluid, mucopolysaccharides account for about one-fifth and protein about four-fifths of the nondialyzable material (Crenshaw, in Wilbur, 1972). Concentration of this material is greatest in the blood, intermediate in extrapallial fluid, and lowest in mantle cavity seawater (Crenshaw, 1966). Furthermore, biomineralization in bivalves probably occurs under conditions of saturation or low supersaturation of shell minerals (Crenshaw, 1972; Wada and Fujinuki, 1976). These reports indicate, therefore, that extrapallial fluid is denser than seawater and thus exchange of chalky pore fluid with mantle cavity seawater in *Crassostrea virginica* could be minimal. The viscosity of extrapallial fluid has not

been reported, though, and this would obviously affect the extent and rate of mixing of the two fluids.

Another factor to be considered in exchange of fluids is the stability of the layer of extrapallial fluid on the surface of the valves when the mantle is elevated and seawater is admitted. If extrapallial fluid is dense and viscous enough, much of it could cling to the shell surface and await the repositioning of the mantle. It is more likely that a new supply of fluid is secreted when the mantle returns. This is an interesting problem, and its solution may well contribute to the knowledge of shell formation.

Fracture sections of chalky shell taken at right angles to the internal surface of the valves (Fig. 81) demonstrate that transversely placed leaflets soon close off underlying pores from the extrapallial space. Therefore exchange of mantle cavity seawater and pore fluid, if it occurs at all, does so primarily near the surface of the shell. Korringa (1951) did not describe the treatment his samples of chalky shell received prior to analysis, so that the possibility of salt contamination as an explanation for the high concentration of sea salts in his chalky shell cannot be ruled out.

Myostracum

The union of the adductor muscle and myostracal surface in *Crassostrea virginica* is a powerful one. The muscle can withstand a pulling force as great as 10 kg; beyond this, the muscle breaks in the middle rather tearing from the muscle scar when valves are forced apart (Galtsoff, 1964).

The surface of the adductor muscle scar from which adductor muscle has been dissolved with Clorox appears extremely smooth at relatively low magnifications of the scanning electron microscope (Fig. 88, 89, 97). At higher magnifications with the transmission electron microscope, Nakahara and Bevelander (1970) found minute pores measuring about 30 Å in the myostracal prismatic surface of *Pinctada radiata*. Tompa and Watabe (1976) noted that the columellar muscle attachment in gastropods is similar to that reported by Nakahara and Bevelander (1970) for *P. radiata*. A tendon sheath secreted by tendon cells of the adhesive epithelium (see also Waller, 1979) sends fibers into the myostracum. During mineralization these are embedded in prisms. The myostracal pores therefore represent sites of organic fiber penetration. A search for possible pores in the myostracum of *Crassostrea virginica* has not been undertaken.

On the basis of their discovery, Tompa and Watabe (1976) suggested that as the oyster increases in size, the adhesive epithelium proliferates in the direction of growth (ventrally in *Crassostrea virginica)*, forming an additional zone for attachment for the migrating muscle. Our observations on *C. virginica* confirm this.

The thin "organic sheet" covering the new myostracal zone ventral to the adductor muscle (Fig. 96) in *Crassostrea virginica* appears similar to the adhesive epithelium described by Nakahara and Bevelander (1970) and Tompa and Watabe (1976), and the "organic film," 2 μm thick, discovered by Galtsoff (1964) between the adductor muscle and the myostracal surface in decalcified histological sections. Our observations tend to support the conclusion of Tompa and Watabe (1976) that the adhesive epithelium is generally more extensive in area than the cross sectional area of the adductor muscle affixed to it.

According to Galtsoff (1964), the attachment "organic film" of the adductor muscle contains collagen, an important supportive constituent of connective tissues. When he immersed adductor muscles affixed to myostracum in a solution of collagenase, they became detached in 36 hours, whereas controls did not.

When the shell of *Mercenaria mercenaria* is decalcified, the adductor muscle retains its attachment to the conchiolin of the myostracum (Crenshaw and Watabe, 1969). This connection probably reinforces the attachment of the muscle to the shell. Whether tendon sheath fibrils in *Crassostrea virginica* are extended primarily into the thin interprismatic conchiolin, or into the mineral crystals, or both, has yet to be determined.

Hinge and Ligament

The hinge-ligamental complex of bivalves is admirably structured, not only to support ontogenetic growth of the dissoconch, but also to facilitate its purely mechanical function of opening the valves (Galtsoff, 1964). The resilium is composed principally of a calcified protein (Trueman, 1951); in *Crassostrea virginica* the

percentage by weight of $CaCO_3$ in dried, powdered resilium is approximately 92% (Kahler et al., 1976). This high concentration of mineralized material as aragonitic fibers contributes to the incompressibility of this part of the ligament. The principal amino acid in the resilium, glycine, is present in amounts almost thrice those of the next most abundant amino acids, aspartic acid and serine. Glycine concentration is directly related to resilience (Kahler et al., 1976), which corresponds with the strength of the resilium under compression (Stenzel, 1963: Galtsoff, 1964; Wainwright, 1969). Tensilia consist principally of a quinone-tanned protein, which contributes to their tensile quality.

In young oysters the chondrophore in both valves is depressed, whereas in older oysters the chondrophoral valley of the left valve becomes deeper, and that in the right valve becomes correspondingly elevated. The tongue and grooving of chondrophores and nymphae in the two valves minimizes sheer, in much the same way that articulating teeth in the hinge of prodissoconch II of the larvae do (Carriker and Palmer, 1979a). With enlargement of the valves with age, prevention of sheer continues to be important.

Oysters have evolved to a sedentary existence, attaching to the substratum invariably by the left valve, which is generally larger, deeper, thicker, and heavier than the right one, that acts as a lid (Galtsoff, 1964; Stenzel, 1971). Variations in the organization of the chondrophoral and nymphal ligostracum may reflect these morphological differences in the valves and consequently the opening-closing function of the dissimilar valves. For example, in contrast to the shallow annulation of the ligostracum of the hinge surface of the right valve, that in the left valve, especially in the chondrophore, is marked. Also, whereas ligostracal prisms of the right valve and those of nymphae of both right and left hinge surfaces are oriented more or less at right angles to the surface of the ligostracum, ligostracal prisms of the chondrophore of the left valve are placed obliquely to the surface of the ligostracum.

It is likely that prominent annuli in the overall ligostracum of the left valve, as well as obliquely placed prisms in the chondrophoral ligostracum of the left valve, are associated in some way, not only with opening and closing of the valves, but also with adhesion of the ligament to the "bottom" component of the lidded shell container.

The principal function of the ligostracum is to bind the ligament to the umbonal foliated shell. The finely tuberculous, pitted, spaced arrangement of the distal ends of the prisms provides a functional surface for the bonding. The ligostracal stratum in the right valve is aragonitic, while that in the left is calcitic with traces of aragonite. Since muscle attachment sites (myostracal prisms) in molluscs are invariably aragonitic, it appears that aragonitic, or partially aragonitic, prisms possess qualities which enhance attachment, not only of muscle fibers, but also of ligament (Carriker and Palmer, 1979b).

The elastic property of the ligament of oysters from different geographic regions (expressed as the pulling force of the adductor muscle), varies from 252 g to 79 g per cm^2 of muscle area (Galtsoff, 1964). There is no information on whether this range of values is associated with ecological conditions. Variation in the size of the adductor muscle of *Mytilus edulis* from different regions was reported by Hancock (1965), who suggested that variability of this organ could be influenced by environment. This may well be the case, but variation of both ligament and adductor muscle as a result of genetic influence is also a possibility, and awaiting study.

Shell Annuli

Interruption of the normal structural configuration of molluscan shell at the growing margin, resulting from environmental and physiological change, is marked subsequently in the shell by macroscopic and microscopic "growth lines" or annuli. Careful search by Lutz (1976) and by us (Palmer and Carriker, 1979) failed to reveal discernible annuli preserved in the foliated structure of *Crassostrea virginica*.

Macroscopic annuli, however, are frequently present in the prismatic structure of the right valve. Most conspicuous are gross surficial annuli that coincide with annual winter periods when little or no growth takes place. Annuli include a few to many prismatic scales between them. The age in years can often be approximated in rapidly growing oysters on the basis of these annual bands.

Annuli in the prismatic layer of the right valve of *Crassostrea virginica* become closely and irregularly spaced during the middle to late fall in temperate zones as the temperature of seawater drops, and prior to the cessation of shell growth during the winter (Fig. 130). Ultrastructurally each annulus is characterized by an accumulation of conchiolin in the form of a folded, wrinkled sheet (Fig. 131). The folded structure is reminiscent of the organic sheet secreted in advance of mineralization by the mantle edge (Fig. 47). The structure of the annulus suggests that perhaps a sudden drop in temperature inhibited crystallite nucleation, but not organic secretion, and with a subsequent rise in temperature, a new, fully formed, prismatic layer developed under it.

During the warm growing season when rapid shell growth is taking place, coarse to fine annuli are visible on the surface of newly formed prismatic layers (Fig. 2, 38). Their temporal meaning has yet to be determined.

Although not conspicuous in young dissoconchs, the adductor myostracum in older oysters is characterized by annuli that range from very fine to coarse and broad. These growth lines are subparallel to the ventral margin of the myostracal scar, and were also noted by Stenzel (1971). The association between myostracal annuli and growth rate has not been determined.

Conspicuous ultrastructural annuli are present in the resilium (Fig. 118, 125), and in the ligostracum of chondrophores and nymphae (Fig. 104, 105) of the shell (see also, Palmer and Carriker, 1979). In oysters raised in the Broadkill River, approximately one annulus was present on the surface of nymphae for each tidal cycle. In oysters cultured simultaneously in controlled systems in the laboratory, an average of 4 to 5 nymphal annuli were formed per day. More bands were present on nymphae than on the corresponding adjacent chondrophore (Palmer and Carriker, 1979). The chronological significance of annuli in the hinge structure of oysters is unknown.

Galtsoff (1964) and Stenzel (1971) from optical observations, described briefly and illustrated the laminated nature of the ligament and the banding of the surface of chondrophores and nymphae in *Crassostrea virginica*. Growth of the ligament is incremental in three dimensions: ventrally, between surfaces of chondrophores and nymphae, and anteroposteriorly (Fig. 125). The subligamental epithelium of the mantle isthmus, which produces the ligament and associated shell hinge structures, thus not only increases the size of the ligament in three directions as the animal grows, but also spreads the tensilia apart anteropsteriorly to accommodate the growing resilium. Ligamental layers in bivalves represent local modification of shell layers, and thus growth bands of the ligament and corresponding bands in valves are generally homologously comparable (Owen et al., 1953; Trueman, 1964; Kahler et al., 1976). In *Crassostrea virginica*, however, because of the absence of annuli in foliated structure, and rapid wear of the thin prismatic layers in both valves, correspondence of banding in shell and ligament is not easily determined.

Evolution of Mechanical Properties

Relatively few species of bivalves secrete principally calcitic shells (Waller, 1975, 1978; Carter, 1979). Those that do are confined to an epifaunal existence and include species that cement themselves to the substratum (Taylor and Layman, 1972). Early dissoconchs of *Crassostrea virginica* are relatively thin-shelled, but with age, individuals produce increasingly thick valves characterized in extreme cases by massive quantities of foliated and chalky calcite.

It is likely that the phylogenetically first molluscan shells were entirely aragonitic, that simple prismatic calcite evolved from aragonitic simple prisms and that foliated microstructures evolved from simple calcitic prisms (Waller, 1975, 1979; Carter, 1980). Because of the insular position of most chalky lenses, it is possible that chalky calcite evolved from foliated calcite, but there is no evidence to support this conjecture. In view of the fact that foliated calcite of oysters is the weakest of all shell structures tested (compression, bending, impact, microhardness, density) in a wide range of species of bivalves (Taylor and Layman, 1972), it is worth noting that oysters have evolved into such successful biological species.

Thus, foliated calcite, although a poor replacement for nacre because of its inferior mechanical properties (Waller, 1975), should possess some

redeeming features. What these might be have not yet come to light, although various hypotheses have been proposed. Taylor and Layman (1972) postulated that original shell materials could have been selected for reasons other than mechanical strength, such as lower expenditure of energy for secretion, or rapidity of deposition. Degens (1976) concluded that calcified tissues were not developed for carbonate deposition, but were secreted as excretory byproducts of metabolic processes. Carter (1980) pointed out that foliated structure could possess such ecological advantages as localization of fractures resulting from impacts, low density, and volumetric economy of secretion (i.e., calcite fills a larger volume per mole than aragonite, Stenzel, 1964), but the importance of these advantages in evolution will require further evaluation as microstructural history becomes better understood.

There is no obvious correlation between the content of organic matrix in molluscan valves and the strength of the shell, yet shell is harder than inorganic calcite or aragonite (Taylor and Layman, 1972). The composite nature of shell (Wainwright, 1969) thus contributes qualities of hardness and pliability not present in nonbiogenic polymorphs of calcium carbonate. A good example of pliability is that contributed by a high concentration of organic matrix in the prismatic margin of the right valve of *Crassostrea virginica,* which provides a flexible shell closure (Carter, 1980) against the more densely mineralized thick left valve margin. The oyster is often surrounded by dense concentrations of suspended particles, including fine sand, and actively discharges these from the mantle cavity by forcefully snapping its valves together (Galtsoff, 1964). The flexible right margin yields to hard particles when it contacts the less yielding left valve margin, and reduces the possibility of fracture especially of the valve edges.

Environmental Effects

Although environmental factors can have a marked influence on the gross morphology of the valves of *Crassostrea virginica* (Medcof and Kerswill, 1965; Galtsoff, 1964; Wilbur, 1964, 1972; Stenzel, 1971; Ruddy et al., 1975; Palmer and Carriker, 1979), little is known about the influence on shell microstructure of environmentally related modifications of secretory mechanisms. Palmer and Carriker (1979) discovered that prisms of the right valve were significantly larger in oysters maintained in a natural estuary during the summer where growing conditions were good than those of oysters held under laboratory cultural conditions. Furthermore, oysters in the estuary deposited approximately two microgrowth annuli in the ligostracum of the hinge per day, while cultured oysters formed 4 to 5 per day. Wada (1961), studying nacre formation in the pearl "oyster," observed seasonal variations in the size of aragonitic lamellae, Kennish and Olsson (1974) studied the effect of temperature on growth banding in *Mercenaria mercenaria,* and Lutz (1976), Lutz and Rhoads (1977), and Carter (1980) examined microstructural changes in other species of bivalves. A seasonal study has not been conducted on the microstructures of the shell of *C. virginica.*

Environmentally associated microstructural studies of bivalves have emphasized primarily the effects of a composite environment (Galtsoff, 1964) or seasonal influences (Wada, 1961; Lutz, 1976). The effect of isolated single factors (while other factors in the environment are held more or less constant) on the size, shape, proportion of organic matrix to mineral crystal, substructure, and chemistry of microstructures has not been attempted. Prisms of the right valve of *Crassostrea virginica,* because of their relatively large size and thick organic envelope, would be good subjects for study. Among physical factors, the influence of temperature, salinity, and light suggest themselves as initial parameters for examination. Obvious chemical factors would be a range of concentrations of calcium, magnesium, strontium, and dissolved organic matter. Oysters have the capacity to concentrate several elements in their shell as density of these elements increases in ambient seawater (Carriker et al., 1980). Moreover, several elements occur heterogeneously distributed in different major regions of the valves, suggesting that ultrastructural effects might differ from one type of microstructure to another.

RECAPITULATION

Our study provides an atlas for the comparison of normal oyster shell ultramorphology with ana-

tomical, functional, and disease-caused anomalies that can occur in valves of oysters cultured in either field or controlled environmental systems (Galtsoff, 1969). Our study also lays the foundation for investigations correlating the distribution and concentration of chemical elements in shell with ultrastructural layers and microstructures of the valves, in somewhat the way Wada and Suga (1976) correlated the distribution of several elements with the major regions of valves of Japanese bivalves (including *Crassostrea gigas*) using light microscopy and an electron microprobe. The uncommon capacity of oysters to concentrate certain chemical elements in their mineralized tissues is well documented (Carriker et al., 1980). Moreover, although various theories have been proposed to describe the remarkable process of extracellular formation of shell in molluscs (Wilbur, 1972; Erben and Watabe, 1974; Degens, 1976; Simkiss, 1976; Wilbur, 1976; and others), the mechanism(s) still remains largely unexplained. Simkiss (1976) suggested that a new relevance in the investigation of shell formation is needed. Our analysis of the functional ultramorphology of oyster valves provides some new insights on shell structure and suggests profitable avenues for future research on the intractable problem of shell formation.

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LITERATURE CITED

Baughman, J.L. 1947. An Annotated Bibliography of Oysters, with Pertinent Material on Mussels and other Shellfish and an Appendix on Pollution. Texas A & M Res. Fd., Agr. Mech. Coll. Tex., College Station. 794 p.

Bevelander, G. and H. Nakahara. 1969. An electron microscope study of the formation of the ligament of *Mytilus edulis* and *Pinctada radiata*. Calc. Tiss. Res. **4:** 101-112.

Carriker, M.R. 1978. Ultrastructural analysis of dissolution of shell of the bivalve *Mytilus edulis* by the accessory boring organ of the gastropod *Urosalpinx cinerea*. Mar. Biol. **48:** 105-134.

Carriker, M.R. and R.E. Palmer. 1979a. Ultrastructural morphogenesis of prodissoconch and early dissoconch valves of the oyster *Crassostrea virginica*. Proc. Nat. Shellfish. Assoc. **69:** 103-128.

Carriker, M.R. and R.E. Palmer. 1979b. A new mineralized layer in the hinge of the oyster. Science **206:** 691-693.

Carriker, M.R. and E.H. Smith. 1969. Comparative calcibiocavitology: summary and conclusions. Amer. Zool. **9:** 1011-1020.

Carriker, M.R., R.E. Palmer, L.V. Sick and C.C. Johnson. 1980. Interaction of mineral elements in seawater and shell of oysters *(Crassostrea virginica)* cultured in controlled and natural systems. J. Exp. Mar. Biol. Ecol. **46:** 279-296.

Carter, J.G. 1979. Comparative shell microstructure of the Mollusca, Brachiopoda and Bryozoa. Scanning Electron Microscopy/1979/II: 439-456.

Carter, J.G. 1980. Environmental and biological controls on bivalve shell mineralogy, microstructure, and architecture. *In:* D.C. Rhoads and R.A. Lutz, ed., Skeletal Growth of Aquatic Organisms: Biological Records of Environmen-

tal Change, Vol. 1, Geobiol. Ser., Plenum Publ. Co., p. 69-113.

Cobb, W.R. 1969. Penetration of calcium carbonate substrates by the boring sponge, *Cliona*. Amer. Zool. **9:** 783-790.

Cranfield, H.J. 1975. The ultrastructure and histochemistry of the larval cement of *Ostrea edulis* L. J. Mar. Biol. Assoc. U.K. **55:** 497-503.

Crenshaw, M.A. 1966. The compostion of the extrapallial fluid of *Mercenaria mercenaria*. Physiologist **9,** No. 3 (abstract).

Crenshaw, M.A. 1972. The inorganic composition of molluscan extrapallial fluid. Biol. Bull. **143:** 506-512.

Crenshaw, M.A. and N. Watabe. 1969. The muscle attachment of the shell of *Mercenaria mercenaria*. Amer. Zool. **9,** No. 4 (abstract No. 510).

Degens, E.T. 1976. Molecular mechanisms on carbonate, phosphate, and silica deposition in the living cell. Topics in Current Chemistry, Inorganic Chemistry **64:** 1-112.

de Zwaan, A. and T. Wijsman. 1976. Anaerobic metabolism in Bivalvia (Mollusca). Characteristics of anaerobic metabolism. Comp. Biochem. Physiol. **54B:** 313-324.

Distler, G.I. 1979. Crystallization as a matrix replication process, *In:* A.A. Chernov, ed., Growth of Crystals. Vol. 11. Trans. from Russian by J.E.S. Bradly. Consultants Bureau (Plenum), N.Y., p. 44-56.

Erben, H.K. and N. Watabe. 1974. Crystal formation and growth in bivalve nacre. Nature **248:** 128-130.

Galtsoff, P.S. 1964. The American oyster *Crassostrea virginica* Gmelin. Fishery Bull. Fish & Wildl. Serv. **64:**1-480.

Galtsoff, P.S. 1969. Anomalies and malformations in the shells of *Crassostrea virginica*. Nat. Cancer Inst. Monogr. **No. 31:** 575-580.

Gillings, B. and M. Buonocore. 1959. An apparatus for the preparation of thin serial sections of undecalcified tissues. J. Dent. Res. **38:** 1156-1165.

Grégoire, C. 1972. Structure of the molluscan shell. In: M. Florkin and B.T. Scheer, ed., Chemical Zoology, Vol. 7, Mollusca, Academic Press, New York, p. 45-102.

Hancock, D.A. 1965. Adductor muscle size in Danish and British mussels and its relation to starfish predation. Ophelia **2:** 253-267.

Hirata, A.A. 1951. The composition of the internal surface layer of the shell of the oyster as studied by histochemical and radioautographic methods. Thesis, Master of Arts, Duke University, Durham, N.C., 68 p.

Jones, R.L. and S.T. Gadomski. 1978. Boron carbide metallurgical polishing to avoid interference in energy dispersive X-ray analysis. Scanning **1:** 198.

Joyce, E.A., Jr. 1972. A Partial Bibliography of Oysters, with Annotations. Spec. Sci. Rept. No. 34, Mar. Res. Lab., Fla. Dept. Nat. Res., St. Petersburg, Fla., 846 p.

Kahler, G.A., F.M. Fisher, Jr. and R.L. Sass. 1976. The chemical composition and mechanical properties of the hinge ligament in bivalve molluscs. Biol. Bull. **151:** 161-181.

Kennish, M.J. and R.K. Olsson. 1974. Effects of thermal discharges on the microstructural growth of *Mercenaria mercenaria*. Environmental Geol. **11:** 41-64.

Kobayashi, I. 1971. Internal shell microstructure of recent bivalvian molluscs. Sci. Rept. Niigata Univ., Ser. E, Geol. & Mineral., **No. 2:** 27-50.

Korringa, P. 1951. On the nature and function of "chalky" deposits in the shell of *Ostrea edulis* Linnaeus. Proc. Calif. Acad. Sci. **27:** 133-158.

Lutz, R.A. 1976. Annual growth patterns in the inner shell layer of *Mytilus edulis* L. J. Mar. Biol. Assoc. U.K. **56:** 723-731.

Lutz, R.A. and D.C. Rhoads. 1977. Anaerobiosis and a theory of growth line formation. Science **198:** 1222-1227.

Margolis, S.V. and R.E. Carver. 1974. Microstructure of chalky deposits found in shells of the oyster *Crassostrea virginica*. Nautilus **88:** 62-65.

Medcof, J.C. and C.J. Kerswill. 1965. Effects of light on growth of oysters, mussels, and quahaugs. J. Fish. Res. Bd. Canada **22:** 281-288.

Mutvei, H. 1979. On the internal structures of the nacreous tablets in molluscan shells. Scanning Electron Micros. **1979/2:** 457-462.

Nakahara, H. and G. Bevelander. 1971. The formation and growth of the prismatic layer of *Pinctada radiata*. Calc. Tiss. Res. **7:** 31-45.

Oberling, J.J. 1964. Observations on some struc-

tural features of the pelecypod shell. Mitt. Naturforsch. Ges. Bern **20:** 1-63.

Owen, G., E.R. Trueman and C.M. Yonge. 1953. The ligament in the Lamellibranchia. Nature **171:** 73-75.

Palmer, R.E. and M.R. Carriker. 1979. Effects of cultural conditions on the morphology of the shell of the oyster *Crassostrea virginica*. Proc. Nat. Shellfish. Assoc. **69:** 58-72.

Price, T.J., G.W. Thayer, M.W. LaCroix and G.P. Montgomery. 1975. The organic content of shells and soft tissues of selected estuarine gastropods and pelecypods. Proc. Nat. Shellfish. Assoc. **65:** 26-31.

Ruddy, G.M., S.Y. Feng and G.S. Campbell. 1975. The effect of prolonged exposure to elevated temperatures on the biochemical constituents, gonad development and shell deposition of the American oyster, *Crassostrea virginica*. Comp. Biochem. Physiol. **51B:** 157-164.

Rützler, K. and G. Rieger. 1973. Sponge burrowing: fine structure of *Cliona lampa* penetrating calcareous substrata. Marine Biology **21:** 144-162.

Scanning Electron Microscopy, Vol. 1968-1979.

Simkiss, K. 1976. Cellular aspects of calcifications, *In:* N. Watabe and K.M. Wilbur, ed., The Mechanisms of Mineralization in the Invertebrates and Plants. Univ. S. Ca. Press, Columbia, p. 1-31.

Stasek, C.R. and W.R. McWilliams. 1973. The comparative morphology and evolution of the molluscan mantle edge. Veliger **16:** 1-17.

Stenzel, H.B. 1963. Aragonite and calcite as constituents of adult oyster shells. Science **142:** 232-233.

Stenzel, H.B. 1964. Oysters, composition of the larval shell. Science **145:** 155-156.

Stenzel, H.B. 1971. Oysters. Part N, Vol. 3, Mollusca 6, Bivalvia. Treatise on Invertebrate Paleontology, Geol. Soc. Amer. & Univ. Kansas, p. N953-N1224.

Taylor, J.D., W.J. Kennedy and A. Hall. 1969. The shell structure and mineralogy of the Bivalvia, Introduction, Nuculacea-Trigonacea. Bull. Brit. Mus. (Nat. Hist.) Zool. Supplement **3:** 1-125.

Taylor, J.D. and M. Layman. 1972. The mechanical properties of bivalve (Mollusca) shell structure. Palaeontology **15:** 73-87.

Tompa, A.S. and N. Watabe. 1976. Ultrastructural investigation of the mechanism of muscle attachment to the gastropod shell. J. Morph. **149:** 339-352.

Travis, D.F. and M. Gonsalves. 1969. Comparative ultrastructure and organization of the prismatic region of two bivalves and its possible relation to the chemical mechanism of boring. Amer. Zool. **9:** 635-661.

Trueman, E.R. 1951. The structure, development, and operation of the hinge ligament of *Ostrea edulis*. Quart. J. Micros. Sci. **92:** 129-140.

Trueman, E.R. 1964. Adaptive morphology in paleoecological interpretation, *In:* J. Imbrie and M. Newell, ed., Approaches to Paleoecology, John Wiley, N. Y., p. 45-75.

Tsujii, T., D.G. Sharp and K.M. Wilbur. 1958. Studies on shell formation. VII. The submicroscopic structure of the shell of the oyster *Crassostrea Virginica*. J. Biophys. Biochem. Cytol. **4:** 275-280.

Wada, K. 1961. Crystal growth of molluscan shells. Bull. Nat. Pearl Res. Lab. **7:** 703-828.

Wada, K. 1972. Nucleation and growth of aragonite crystals in the nacre of some bivalve molluscs. Biomineralization **6:** 141-159.

Wada, K. and T. Fujinuki. 1974. Physiological regulation of shell formation in molluscs. I. Chemical composition of extrapallial fluid. Bull. Nat. Pearl Res. Lab. **18:** 2085-2110.

Wada, K. and T. Fujinuki. 1976. Biomineralization in bivalve molluscs with emphasis on the chemical composition of the extrapallial fluid, *In:* N. Watabe and K.M. Wilbur, ed., The Mechanisms of Mineralization in the Invertebrates and Plants. Univ. S. Ca. Press, Columbia, p. 175-190.

Wada, K. and S. Suga. 1976. The distribution of some elements in the shell of freshwater and marine bivalves by electron microprobe analysis. Nat. Pearl Res. Lab. **20:** 2219-2240.

Wainwright, S.A. 1969. Stress and design in bivalved mollusc shell. Nature **224:** 777-779.

Waller, T.R. 1975. The origin of foliated-calcite shell microstructure in the subclass Pteriomorpha (Mollusca: Bivalvia). Bull. Amer. Malacological Union **1975:** 57-58.

Waller, T.R. 1978. Morphology, morphoclines, and a new classification of the Pteriomorphia

(Mollusca: Bivalvia). Philos. Trans. Roy. Soc. London, Ser. **B 284:** 345-365.

Waller, T.R. 1979. Scanning electron microscopy of shell and mantle in the Order Arcoida (Mollusca: Bivalvia): Functional anatomy, calcification, and evolution. Smithsonian Contr. Zool. No. 313, 58 p.

Watabe, N. 1965. Studies on shell formation. XI. Crystal-matrix relationship in the inner layers of mollusk shells. J. Ultrastructure Res. **12:** 351-370.

Watabe, N. 1974. Crystal growth of calcium carbonate in biological systems. J. Crystal Growth **24/25:** 116-122.

Watabe, N. and K.M. Wilbur. 1961. Studies on shell formation. IX. An electron microscope study of crystal layer formation in the oyster. J. Biophys. Biochem. Cytol. **9:** 761-772.

Watabe, N., D.G. Sharp and K.M. Wilbur. 1958. Studies on shell formation. VIII. Electron microscopy of crystal growth of the nacreous layer of the oyster *Crassostrea virginica*. J. Biophys. Biochem. Cytol. **4:** 281-286.

Weiner, S. and L. Hood. 1975. Soluble protein of the organic matrix of mollusk shells: a potential template for shell formation. Science **190:** 987-989.

Wilbur, K.M. 1964. Shell formation and regeneration. *In:* K.M. Wilbur and C.M. Yonge, ed., Physiology of Mollusca, Vol. 1, Academic Press, N. Y., p. 243-282.

Wilbur, K.M. 1972. Shell formation in mollusks. *In:* M. Florkin and B.T. Scheer, ed., Chemical Zoology, Vol. 7, Mollusca. Academic Press, N. Y., p. 103-145.

Wilbur, K.M. 1976. Recent studies of invertebrate mineralization. *In:* N. Watabe and K.M. Wilbur, ed., The Mechanisms of Mineralization in the Invertebrates and Plants, Univ. S. Ca. Press, Columbia, p. 79-108.

Wise, S.W. 1969. Study of molluscan shell ultrastructures. Scanning Electron Microscopy **1969:** 205-216.

Wise, S.W. 1970. Microarchitecture and mode of formation of nacre (mother-of-pearl) in pelecypods, gastropods, and cephalopods. Eclogae Geol. Helvetiae **63:** 775-797.

Wise, S.W. and J. deVilliers. 1971. Scanning electron microscopy of molluscan shell ultrastructures: screw dislocations in pelecypod nacre. Trans. Amer. Micros. Soc. **90:** 376-380.

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DIFFERENTIAL SURVIVAL OF SELECTED STRAINS OF PACIFIC OYSTERS *(Crassostrea gigas)* DURING SUMMER MORTALITY[1/2]

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ABSTRACT

Throughout the late 1960's and early 1970's summer mortalities of Pacific oysters on the west coast of the United States resulted in repeated losses as great as 65% in certain locations. The severity of the mortalities lessened during the 1970's. In anticipation of recurring severe mortalities a program at the University of Washington's College of Fisheries was developed utilizing selective breeding to obtain oyster stocks resistant to summer mortalities. During 1978 a summer mortality occurred in Rocky Bay, Washington where several experimental selected families had been planted. Differential mortalities between families ranged from less than 10% to greater than 80% compared to non-selected Japanese stock mortality of 47.5%. Ninety percent of the families tested had lower percent mortality than the control. Differential mortalities between families were also observed during laboratory elevated temperature challenges.

INTRODUCTION

Mass mortalities of the Pacific oyster *(Crassostrea gigas)* have been the subject of investigations in the United States and Japan for the past 20 years. During the 1960's when mortalities reached 50% in areas of California and Washington, the National Marine Fisheries Service (NMFS) coordinated a study of the problem. At the termination of that study in 1972, many characteristics of the epizoology had been defined; however, no causative agent had been isolated (Glude, 1975; Koganazawa, 1975). In both Japan and the United States, mortalities were always associated with areas of high productivity, high nutrient levels, and water temperatures exceeding 20°C and were coincident with the period of maximum gonad condition for spawning.

Further studies sponsored by Sea Grant at the University of Washington College of Fisheries demonstrated that mass mortalities could be induced in the laboratory by holding oysters in water warmer than 18°C and increasing the nutrients (Lipovsky and Chew, 1972). Examination of dead and dying oysters revealed the presence of large numbers of bacteria *(Vibrio* spp.) in the normally sterile pericardial fluid (Grischkowsky and Liston, 1974). Although it could not be determined that these were causative pathogens, it was believed that they played a significant role in the laboratory mortality. During these bacteriological investigations of the early 1970's no significant field mortalities occurred; thus

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bacterial levels in moribund oysters from the field could not be monitored.

Anticipating that mass field mortalities could conceivably recur, a breeding program was begun in order to develop strains of the Pacific oyster resistant to summer mortality. It was assumed for the purposes of selection, that summer mortality and laboratory mortality were of similar etiology.

Massive mortalities did occur during the summer of 1978 in Rocky Bay, Washington, a test site for the experimental oysters. This paper describes the performance of these oysters in the field and during laboratory testing.

METHODS AND MATERIALS

The experimental design consisted of: 1) selection by elevated temperature (21°C) challenge; 2) spawning of survivors, larval rearing, and growing the juveniles at selected test sites; 3) evaluation of each family group by field and laboratory performance; and 4) selection by elevated temperature challenge prior to production of the next generation (Figure 1).

Parent challenges were performed in 1976 in the Environmental Protection Agency Saltwater Laboratory, Manchester, Washington. Progeny were challenged in the University of Washington, College of Fisheries portable shellfish laboratory, Manchester, Washington. Spawning and larval rearing were conducted in the N.M.F.S. Aquaculture facility, Manchester, Washington. Experimental and control seed oysters were planted in Rocky Bay, Washington for grow out and monitoring (Figure 2).

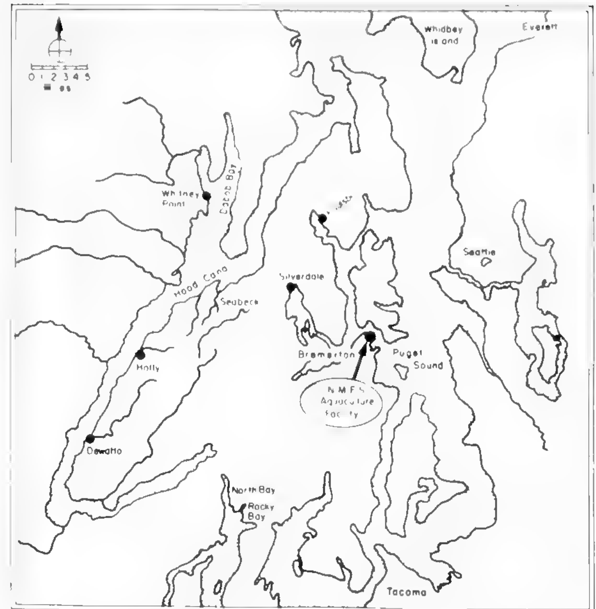

FIGURE 2. *Map showing the location of the study areas.*

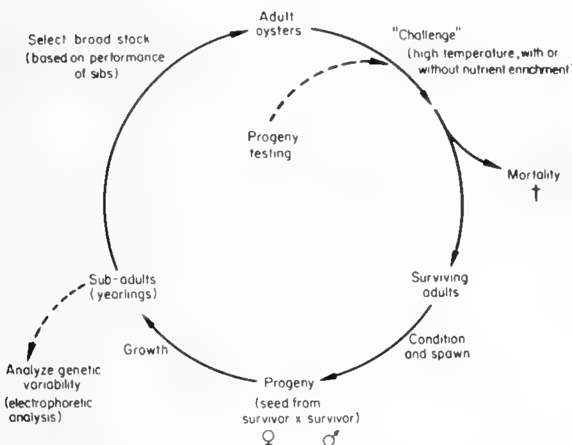

FIGURE 1. *Diagram of the selection design and genetic analysis used in breeding oysters for resistance to mortality during simulated summertime stress.*

Parent Challenges. Two year old Japanese stock adult oysters were thermally challenged in 160 liter circular fiberglass containers and in 160 liter fiberglass and acrylic aquaria. Temperature was maintained by aquarium immersion heaters at 21°C. Renewal of sea water was done at two day intervals using unfiltered, heated sea water. Dead oysters were removed daily. At 60% mortality the surviving oysters were placed in a system of running unfiltered unheated water (9 to 12°C).

Progeny Rearing. Oyster families were produced by mixing the eggs of a selected female with sperm of a selected male. Larvae from each family were reared separately and set on a string of oyster shell cultch on which the shells were spaced at 25 cm intervals. The shell strings were then staked to the sediment in Rocky Bay during Spring of 1977, in order to test the families under commercial growing conditions and still maintain their identity. Seed size at planting ranged from 2 to 15 mm. Two plots were utilized, one at approximately the +1 tidal level (lower plot) and one at about +1½

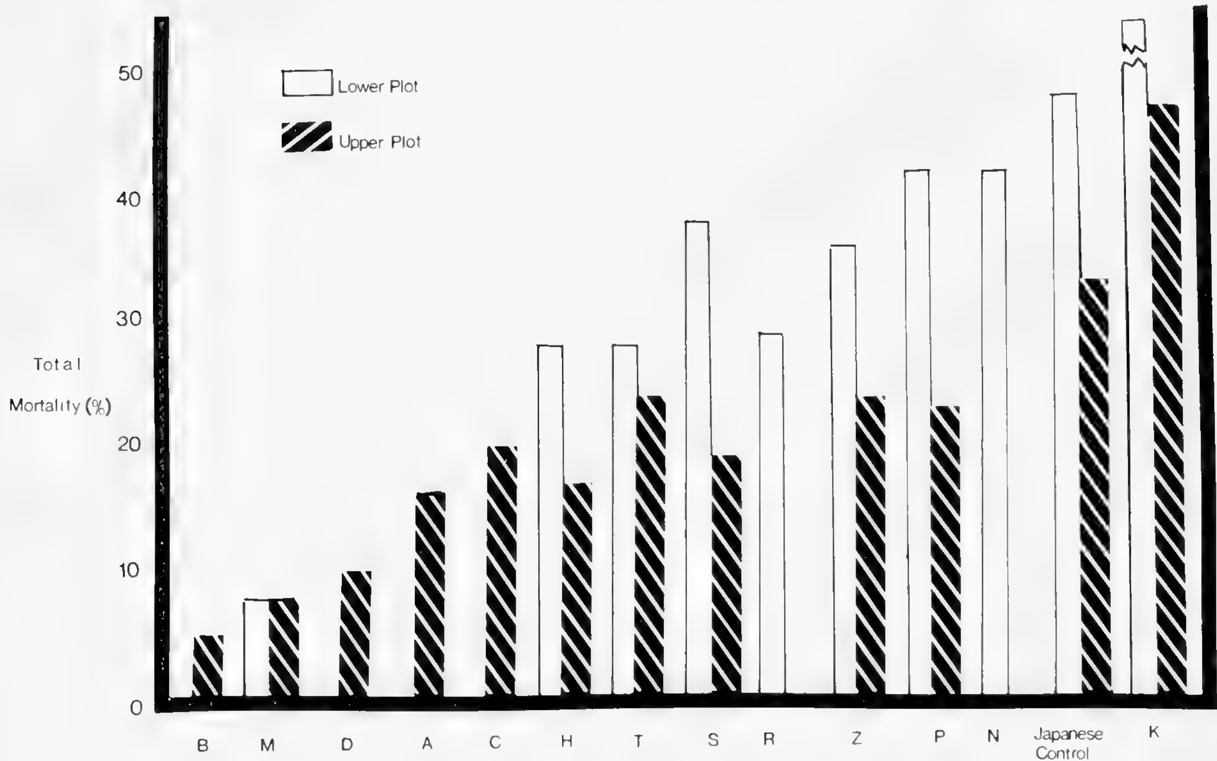

FIGURE 3. *Field mortality levels for the upper and lower plots in Rocky Bay.*

(upper plot). The oysters in these plots were observed monthly during the first summer, every two months during the following fall, winter and spring and every fortnight from mid-June through August 1978.

Progeny Challenges. Three separate tests were conducted on F_1 progeny retrieved from the field. Vessels for these 3 challenges were fiberglass and acrylic aquaria (160 1) and fiberglass troughs (85 1). Challenge temperature was 21°C. The sea water was unfiltered, and renewed every other day. Dead oysters were removed daily; shell size (height and length) was recorded for all oysters.

Bacterial Investigation. Samples of moribund oysters were collected several times through the summer of 1978 for histological preparations and pericardial fluid analysis.

Data Analysis. Mortality data from field and laboratory were compiled and compared graphically. Shell size data from the laboratory challenges were used to determine statistical size differences between surviving and dead oysters at LT_{50} using Students T-test for independent means.

RESULTS AND DISCUSSION

During the summer of 1978, massive mortalities approaching 60% occurred among the oysters in Rocky Bay. Differential mortality was apparent between the selected family groups. By August 31, total percent mortality of the families ranged from 5.1% to 47.3% in the upper plot and from 7.4% to 85.5% in the lower plot in Rocky Bay (Figure 3). Total mortality of the controls on August 31 was 33% and 48% in the upper and lower plots respectively. The similarity in performance between families present at both test areas was very high. A linear regression comparing the survival of the families present in both plots resulted in a slope of 0.50 and a correlation coefficient of 0.954.

Differential mortality between families was also apparent in the results from the laboratory testing. The combined results of these tests were compared at both the time of 25% mortality of the Japanese stocks ($LT25_J$) and 50% mortality ($LT50_J$) (Figure 4). Mortalities at $LT25_J$ ranged from 6.3% to 53.8%. For ease of comparison, the field mortality

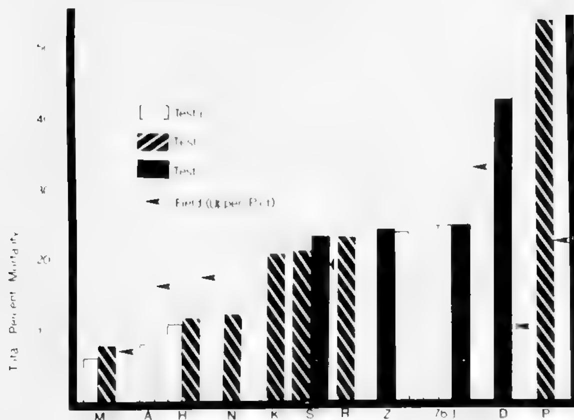

FIGURE 4. *Laboratory mortality levels for three different tests at the LT_{25J}. Field mortality for Rocky Bay upper plot is indicated for comparison.*

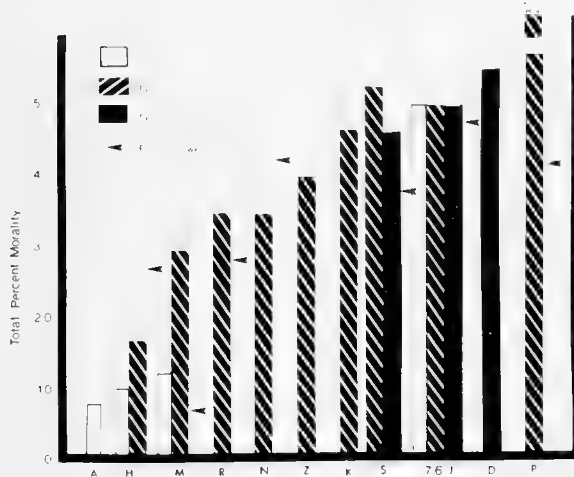

FIGURE 5. *Laboratory mortality levels for three different tests at LT_{50J}. Field mortality for Rocky Bay lower plot is indicated.*

levels from the upper plot are indicated. Although some discrepancies exist between the results of laboratory vs. field testing (e.g., families P and D) there is general agreement between laboratory and field results. Those families which performed well in the field (M and H) also performed well in the laboratory. Similarly, at LT50$_J$, differential mortality was noted (Figure 5). Again, the laboratory challenge appears in general to reflect the mortality potential of families in the field. Those families exhibiting good survival in the field (H, M, and R) performed satisfactorily in the laboratory.

Although the parent stock had been chosen by thermal challenge in the laboratory and variation in response to selection would be expected because of the phenotypic basis, the field mortality of each family was compared to the mortality level (total mortality during challenge) of the parents (Table 2). Survival of the offspring did not appear to be directly related to the level of parent survival. Some of the families were related by a common parent. Those with a common dam were families P and N, C and Z. Those with a common sire were A and K, R and P. It is interesting to note that the families related by a common sire represent a relatively wide divergence of field survival (A, 16.3 of K, 47.3, and R, 28.6 of P, 41.9) compared to families related by a common dam (P, 41.9 of N, 42.2 and C, 20.0 of Z, 24.1) (Table 2).

Height and length measurements were used to calculate cross-sectional areas and these were compared to mortality figures to determine if there was a relationship between size and survival. Sizes of those oysters which died through LT50 from a particular family were compared to sizes of all other oysters of that family in that test (Table 1). In 9 of the 16 cases analyzed the mean size of mortalities was larger than survivors at LT50. The difference was significant in only 3 cases ($P < 0.05$) as determined by Student's T-test.

The overall mean size (H $\times$ L) between families varied from 34.1 cm^2 (76$_J$ #1) to 52.1 cm^2 (R #2). There was no direct correlation between this mean size and level of mortality experienced either in the laboratory or in the field, as indicated by very low linear regression slopes and correlation coefficients. In the laboratory for all tests, at LT25$_J$ a regression of mortality on size resulted in a slope (b) of 0.13 and correlation coefficient (r) of 0.31; at LT50$_J$ $b = 0.03$, $r = 0.10$. In the field upper plot results were $b = 0.03$ and $r = 0.07$, and lower plots results were $b = 0.04$ and $r = 0.21$.

During the field mortalities of 1978, samples of moribund oysters were taken for bacteriological examination. No bacteria were found either in the pericardial fluid or in stained histological sections. It thus appears that bacteria noted in the studies of the early 1970's (Grischkowsky and Liston, 1974) may have been an artifact of the laboratory.

SUMMARY AND CONCLUSIONS

In order to determine whether selective breeding

TABLE 1. *Student T-test for shell dimensions (Height × Length) comparing mortality and survivor sizes from laboratory challenges at each LT_{50} for all families tested and for non-selected Japanese stock (76_J).*

Test	Family	Mean Area Mortality	Mean Area Survival	T Test	Degrees of Freedom	‘0.05
I	H	40.4	45.1	0.36	63	1.94
	A	47.4	26.6	3.03	13	1.77
	M	36.8	41.7	0.82	7	1.90
	76_J	34.2	34.0	0.05	21	1.72
II	H	52.4	40.8	1.47	14	1.76
	K	45.4	47.2	0.39	43	1.65
	S	46.8	41.8	1.06	28	1.77
	M	38.8	34.7	1.18	47	1.65
	P	51.4	36.8	2.54	7	1.89
	N	45.6	48.6	−0.63	22	1.72
	R	58.1	45.2	1.56	13	1.77
	76_J	44.1	32.1	2.32	37	1.65
III	D	49.3	51.2	−9.59	60	1.65
	S	44.1	41.0	0.71	36	1.65
	Z	49.1	53.4	−1.17	57	1.65
	76_J	37.0	38.8	0.19	21	1.72

TABLE 2. *Families, their respective field mortalities, the source bays and challenge mortality for their respective parents. LB-Liberty Bay, RB-Rocky Bay, SB-South Bay, HC-Hood Canal.*

Family	Percent Mortality Upper	Percent Mortality Lower	Parent Source Male × Female	Parent Mortality
A	16.3		LB × SB	59.6 × 75.0
B	5.1		RB × RB	58.0
C	20.0		LB × SB	59.6 × 75.0
D	10.3		RB × RB	58.0
H	17.3	27.5	SB × SB	75.0
K	47.3	85.5	LB × SB	59.6 × 75.0
M	7.5	7.4	LB × LB	59.6
N		42.2	RB × RB	74.8
P	22.6	41.9	RB × RB	74.8
R		28.6	RB × RB	74.8
S	18.7	38.0	RB × RB	58.0
T	24.0	27.5	RB × HC	58.0 × n.c.
Z	24.1		LB × SB	59.6 × 75.0

could be utilized as an effective method to combat summer mortality of Pacific oysters, a breeding program was developed utilizing survivors of elevated temperature challenges as brood stock. Two-year-old progeny survival was compared to non-selected two-year oysters grown from imported Japanese seed. Thirteen full sib families were tested in the field and/or laboratory. A wide differential in field mortality between the families was noted ranging from 5.1% to 85.5% in the field compared to Japanese stock control mortality of 47.15%. Approximately 90% of the families exhibited higher survival than the control. In general those families that showed high survival in the field were also among those of high survival in the laboratory. Previous investigations of Pacific oyster mortalities implicated a size selection against larger oysters (Glude, 1975). Size does not appear to be a factor in survival potential during laboratory challenge. Though previous work implied that bacteria could be a factor during oyster field mortality (Grischkowsky and Liston, 1974), histological preparations of moribund oysters from the 1978 Rocky Bay mortality did not support that implication.

During 1974-1975, seven F_1 families were tested by laboratory challenge. Two of the seven families consistently showed significantly improved survival compared to Japanese controls. At that time it was conditionally stated that the value of this selective breeding program would be substantiated when selected hatchery crosses performed as well in the field as they had in the laboratory. The performance of the experimental families during the summer of 1978 in Rocky Bay indicates the value of selective breeding programs for increased survival during summer mortality.

LITERATURE CITED

Glude, J.B. 1975. A summary report of the Pacific Coast oyster mortality investigations 1965-1972. Proc. Third U.S.-Japan Meeting on Aquaculture at Tokyo, Japan, October 15-16, 1974; 1-28.

Grischkowsky, R.S. and J. Liston. 1974. Bacterial pathogenicity in laboratory-induced mortality of the Pacific oyster *(Crassostrea gigas,* Thunberg) Proc. Nat. Shellfish Assoc. 64:82-91.

Køganazawa, A. 1975. Present status of studies on the mass mortality of cultural oysters in Japan and its prevention. Proc. Third U.S.- Japan Meeting on Aquaculture at Tokyo, Japan, October 15-16, 1974; 29-34.

Lipovsky, V.P. and K.K. Chew. 1972. Mortality of Pacific oysters *(Crassostrea gigas):* the influence of temperature and enriched sea water on survival. Proc. Nat. Shellfish. Assoc. 62:72-82.

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SOME OBSERVATIONS ON SPAT SETTLEMENT, GROWTH RATE, GONAD DEVELOPMENT AND SPAWNING OF A LARGE BRAZILIAN OYSTER

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ABSTRACT

Field observations and laboratory experiments indicate that rapid growth of oyster spat takes place during the first three months after settlement, and this is confirmed by the results obtained independently from two widely separated localities of the Paraiba River estuary. A maximum of 16 spat per valve settled on clean empty shells. Settlement of spat and their growth rates are very much dependent on physico-chemical, biological, especially circulation of food, and topographical characteristics of the culture sites. Spawning of this large oyster usually coincides with the heavy rainfall during winter, June-July, and the combined effect of salinity reduction and changes in temperature acts as strong stimulus to spawning. The sexes are separate, but the males slightly outnumber the females. Eggs are small and liberated with mucus in large numbers. Increased mortality was found to accompany in prolonged decreasing salinity, predation, pests, and fouling organisms. Though natural causes of mortality appeared to be less, if spawning coincided with torrential tropical rainfall for prolonged periods exceeding one week, natural calamity would be imminent.

INTRODUCTION

Oyster cultivation is perhaps the second established fish farming enterprise in Brazil. Despite her large sources of natural oyster beds and the vast stretches of estuaries, Brazil produces only about 0.054% of the world's oyster production (FAO, 1970). Therefore, the long term culture prospects are very exciting. During the last three successive years, field and laboratory experiments were carried out at different estuarine localities to determine the settlement of spat and their growth rates so that the cultivation of these large Brazilian oysters could be made into a profitable industry which would contribute significantly to a steady supply of protein rich food.

MATERIALS AND METHODS

Spat were collected fortnightly, particularly shortly after peak spawning, during the winter, from the main segments of the Paraiba River estuary by suspending "shell strings."

Spat collected on these collectors were carefully counted, the substrate shells spaced out, and transferred to two different but remotely separated culture sites (Figure 1). They were then resuspended in specially selected rearing areas where the water depth varied between 2.0 and 3.0 m under tidal flows. These waters were generally rich in food and detritus year-round (Singarajah, 1978a).

A complete account of the methods employed in

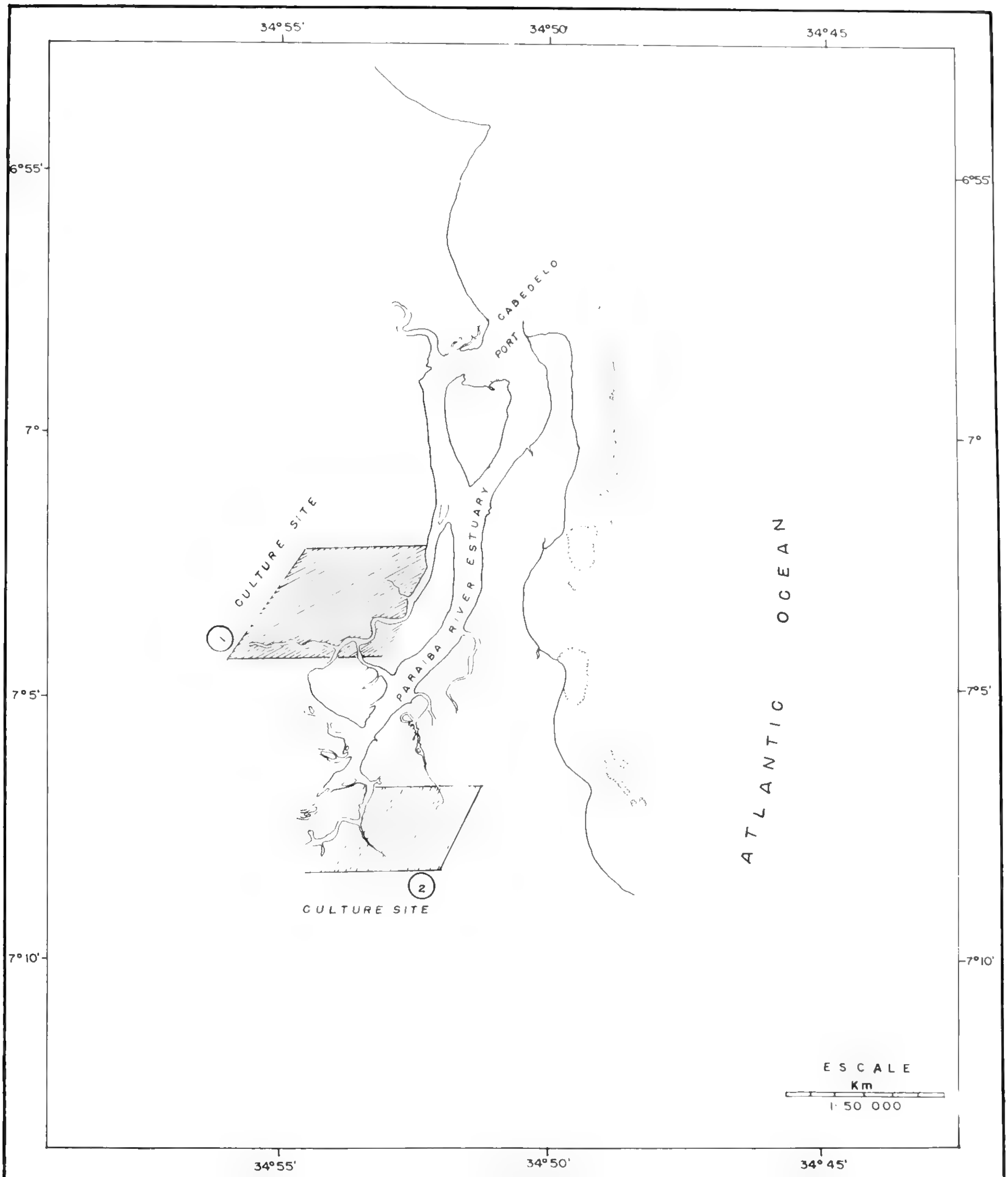

FIGURE 1. *Paraiba River estuary, showing the oyster culture sites.*

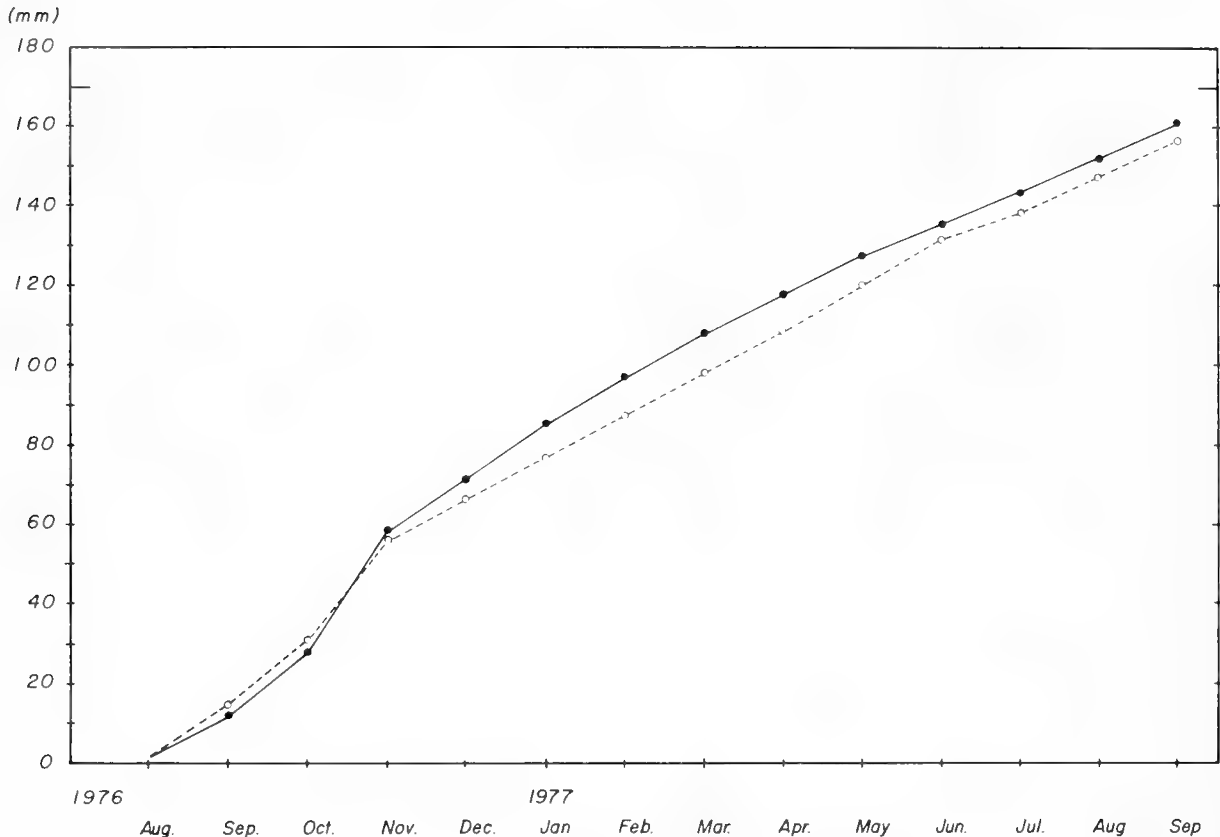

FIGURE 2. *Growth curve in height in* Crassostrea paraibanensis *"Salinas" solid line and "Livramento" broken line.*

field research and a description of the estuarine regions has been presented elsewhere (Singarajah, 1978b). Briefly, it should be mentioned that this estuary covers an area of about 36.9 km² and the intertidal zone of both banks of the estuary is largely dominated by mangroves. The river mouth is about 11 m deep and has direct access to the North Atlantic Ocean.

To measure growth rates, carefully isolated valves, on which the spat had settled, were placed in steel wire trays and suspended about 10 cm above the evenly formed muddy bottom, at a depth of about 2.5 m below low tide level, in the path of the main water ways.

Body dimensions, wet and dry weights were determined to the nearest millimeter and milligram respectively. Fresh gonad samples were examined under a stereoscopic microscope immediately after the oysters were shucked, and appropriate tissues were fixed in Bouin's fluid for histological sectioning. Sections were cut between 12 and 15 μ, using a sliding microtome.

Egg size was determined by means of an ocular micrometer fixed in one of the eye pieces of a binocular microscope.

In order to estimate the number of eggs, fully mature female oysters, after being carefully shucked, were kept individually in Petri dishes containing filtered estuarine water collected from the same areas where the oysters were taken. When the eggs were liberated, they were then carefully transferred by pipet to small test tubes containing Gilson's (1898) fixative. After fixation they were washed and, after suitable dilutions, known portions were counted in a specially designed perspex counting chamber.

The height-weight relationship was derived by examining 1000 specimens at random from each culture field and all field and analysis data were transferred to an IBM computer for processing.

Abiotic and biotic factors such as salinity, temperature, pH, transparency, current velocities and tidal fluctuations were determined, together with qualitative and quantitative analyses of plankton samplings. Bottom samples of water in depths exceeding 2.6 m were checked for any salinity stratification between surface and bottom layers. Prodissoconch larvae were isolated from fresh collections of plankton samples obtained at the oyster sites.

RESULTS

Spat settlement

Clean empty shells collected the greatest number of spat, ranging from 12 to 16 per valve. Median sized valves (130 × 60 mm) dropped, individually, close to the main water inlets collected an average of 16 spat per valve. The right shallow valves often favoured more spat settlement than those of the deeply cupped left valves. Larvae settled quite easily on both surfaces but the outer darker surfaces attracted more larvae than the inner smooth surface. The greatest numbers of spat usually settled at both oyster sites in the month of August, corresponding with the spawning period. Some of these results confirm the observations made by Padilla (1967) who, working with larvae of the Chilean oysters, also found that settlement occurred to the maximum extent on the empty shells which he suspended below the neap tide. First settlement was noticed 18-21 days after the collectors had been suspended.

Growth rate of spat

The steel wire trays, which contained carefully isolated spat, facilitated weekly measurements of growth rate more precisely. The results obtained at both oyster beds were comparable. Growth was quite rapid during the first three months, followed by slow but gradual growth (Figure 5). The most critical period was the third month when maximum growth took place, and the spat reached an average size of 57 × 32 × 20 mm by the end of this period. The average growth rate for the three-month period was 0.62 mm / day. Subsequent growth continued at an average rate of 0.37 mm / day until fourteen months and then growth was considerably decreased, and weekly measurements were of little value. Although the vast majority of the oysters reached marketable size of 157 to 161 mm in fourteen months, fattening did not take place until later.

Well-defined eccentric rings were evident only in three to four year old oysters, and these were readily discernable on the anterior-lateral sides of the valves where the rings were laid down in extremely thin layers (Figure 3). These rings are inconspicuous in oysters less than two years old. In the fully mature three to four year old oysters, the number of rings vary, an average being 84 that could be seen by the naked eye on each valve. The data collected on the formation of these rings suggest that the individual variations might be due to environmental conditions and metabolic state of the animals.

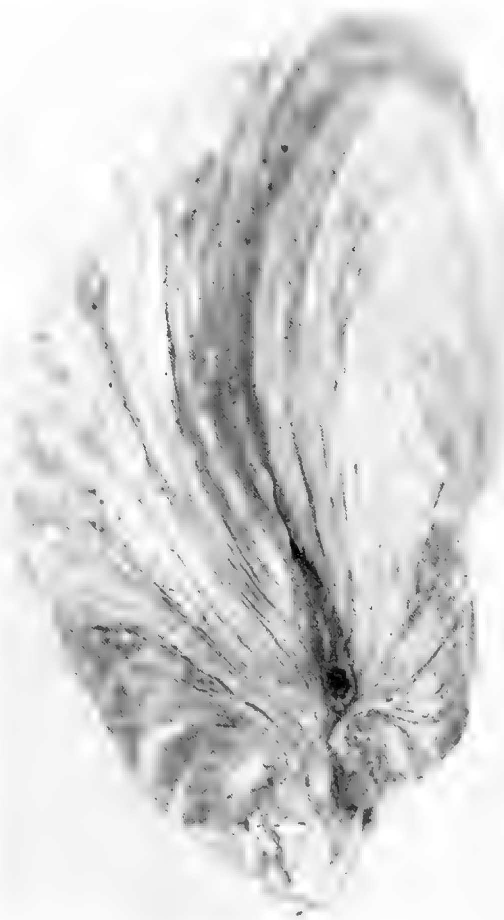

FIGURE 3. *The large Brazilian oyster,* Crassostrea paraibanensis, *showing several eccentric rings. Lateral view; life size: 206 × 96 × 72 mm.*

TABLE 1. *Number in samples and sex ratios of oysters studied for Salinas and Livramento estuaries.*

	Salinas				Livramento			
		Percentage				Percentage		
	No. in Sample	Male	Female	Indeterminate	No. in Sample	Male	Female	Indeterminate
1976								
September	98		7.1	92.8	88	2.3	9.1	88.6
October	56	14.3	10.7	75.0	50	40.0	6.0	54.0
November	52	11.5	7.7	80.8	46	23.9	13.6	56.5
December	72	50.0	27.8	22.2	62	16.1	19.4	64.5
1977								
January	96	52.1	41.6	6.3	55	38.2	49.1	12.7
February	80	56.2	25.0	18.8	90	50.0	33.3	16.7
March	94	22.3	57.5	20.2	102	36.3	39.2	24.5
April*	241	49.7	45.6	4.7	250	48.8	50.0	1.2
May	44	40.9	43.1	17.5	53	43.4	37.7	18.9
June	75	50.6	49.4		81	34.6	50.6	14.8
July	38	42.1	44.7	13.2	46	30.4	63.1	6.5
August	54	33.3	29.6	37.1	79	50.6	8.9	40.5

*Oysters are harvested during early part of April, usually biannually.

Gonad development and spawning

The sexes are separate and generally the males slightly outnumbered the females (Table 1). Gonad development in this species is gradual and, before the onset of spawning time, the gonads become enlarged into a retort-shaped creamy or yellowish structure, with the narrow end lying next to the adductor muscle. The fully matured paired ovarian follicles or spermatic ducts are generally packed with gametes which are at different stages of gametogenesis but of the same generation. The appearance of the eggs becomes more conspicuous in May, ripe by the early part of June, with a peak spawning during the end of June and the first week of July, and the process continues till the end of October.

The spawning period coincides with the torrential tropical rainfall, under which the environmental conditions such as salinity, temperature and pH also fluctuate quite considerably. Similar observations were recorded by Hornell (1910) for Madras backwater oysters and he suggested that the low salinity might have some effect on the spawning of such oysters.

Young oysters reach maturity as early as twelve to fourteen months and the smallest mature females measured an average 60 × 50 × 30 mm and weighed 32 g. When such oysters were shucked and kept in Petri dishes containing filtered estuarine water, large numbers of eggs were liberated, enmeshed in small squirts of mucus which the oysters released intermittently and the whole process appeared to be completed within an hour. It was noticed that the eggs were initially smaller in size, round and opaque and floated with the mucus with which they were entangled; when gradually freed from the mucus, they settled to the bottom due to their own density and swelled up into sperical or slightly conical form, probably by absorption of water, and became translucent.

The size and number of eggs estimated for different oysters by different authors (Cole, 1941; Cleland, 1950; Galtsoff, 1930; Quayle, 1969; Ranson, 1940; Mattox, 1949) differ quite markedly (Table 2), but these figures were for different genera and species. Unfortunately, these authors gave no details of the estimations. Despite great care being taken in this study the slight reduction in the size of the eggs treated with fixative might be due to shrinkage caused during interaction of the fixative.

Height - weight relationship

The height-weight relationship was derived by

TABLE 2. *Details of size and number of eggs estimated in various oysters by different authorities.*

Authority	Year	Genus	Species	Diameter of egg in microns	No. of eggs/ spawning	Comments
1. Cole	1941	*Ostrea*	*edulis*		91,000	01 Y. Old
					218,100	02 Y. Old
					462,000	03 Y. Old
					902,000	04 Y. Old
2. Clelands	1950	*Gryphaea*	*commercialis*	50	15,000,000	Per CC
3. Galtsoff	1930	*Crassostrea*	*virginica*	50	115,000,000	Oyster of Normal Dimension
4. Quayle	1967	*Crassostrea*	*gigas*	50	50 to 100,000,000	Marketable Size
5. Mattox	1949	*Crassostrea*	*rhizophorae*		170,000,000	61.5 MM In shell Length
6. Ranson	1940	*Gryphaea*	*angulata*		1 to 2,000,000	Unspecified
7. Singarajah	1980	*Crassostrea*	*paraibanensis*	43*	748,800	12-14 M. Old
				44*	1,050,000	2 Y. Old
				47+	15,400,000	3 Y. Old
				45=	29,680,000	4 Y. Old

Measured while the eggs were: *Entangled in mucus
+Soon after freeing from the mucus
=After fixing in Gilson's fixative

least square estimates; the general formula:

$$W = a H^{b}$$

was fitted, where W = weight, H = height, a is a constant and b is an exponent. The values for a and b were obtained from the data. The logarithmic equation expressed by Log W = −7.519746 + 2.698430 Log H and the equation in exponential form is $W = 0.000542\ H^{2.698430}$, where W is in grams and H in millimetres, sexes combined (Figure 5).

Of the six hundred adult and four hundred juvenile specimens examined at random from each oyster bed, the vast majority were mature and showed individual variations in height between 60 to 206 mm, and in weight, with shells intact, 55 to 1050 g. The tallest ones were by no means the heaviest ones but greatest weights were mostly due to lateral growth along the anteroposterior axis of the shell. The net weight of the wet flesh, after shucking and drying by blotting on filter paper, varied between 18 and 92 g; and their dry weights were 1.2 to 2.9 g. However, the unshucked oysters usually retained a sufficient amount, 10 to 20 ml, of a fluid mixture of water and mucus.

Environmental conditions

Hydrological conditions at the two oyster sites varied somewhat during the winter's torrential tropical rainfall and remained constant during the fairly dry summer. Temperature, salinity (Figure 4), pH, abundance of plankton as food, turbidity and lunar periodicity have often been attributed as environmental variables which influence estuarine life. Among these, temperature was considered as the single most important influencing factor (Galtsoff, 1938; Loosanoff et al., 1950; Sato, 1936; Bargeton, 1943; Quayle, 1969) and a critical temperature for mass spawning of oysters was

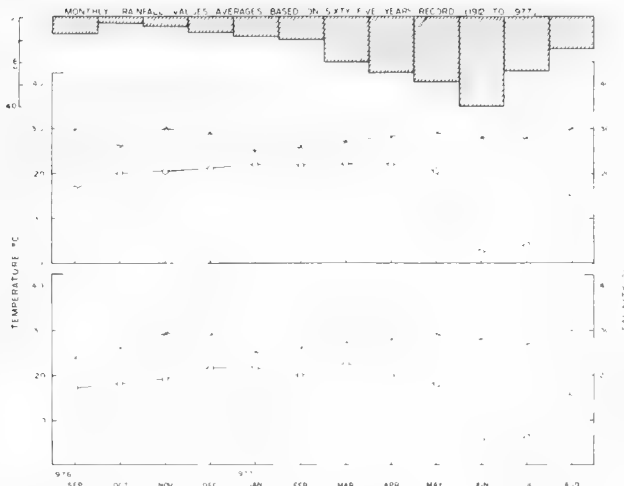

FIGURE 4. *Monthly temperature (x) and salinity (O) values are averages based on four stations at "Salinas" (bottom) and "Livramento" (top), estuaries respectively.*

also described (Coe, 1931; Ingle, 1951). However, despite the emphasis on temperature, it was found during this study that the temperature remained fairly constant throughout the year, particularly so during May to July when the peak spawning appeared, though salinity became considerably reduced to as low as 2.5‰ (Figure 4). In view of the present observations, temperature normally seems to have little effect on the spawning of *Crassostrea paraibanensis.*

Salinity is an equally important factor influencing oyster growth. The northeastern part of Brazil has two distinct seasons: winter, with torrential rain from April to the end of August and summer, with occasional showers from September to March. During the summer the salinity is usually high reaching a maximum of 22.60‰ . However, under the torrential rain, particularly during June-July, salinity is considerably reduced. Furthermore, the slight differences in salinity between the two oyster sites might be due to their proximity to the sea and also the amount of fresh water runoff. Despite wide fluctuations seasonally with depth and under tidal flows, these oysters showed a tolerance to wide variations of salinity. Heavy rain and tidal flows also stirred up the muddy bottom detritus, greatly increasing suspended materials nutrients; turbidity correspondingly reduced light penetration. A full account of the composition and density of plankton of the culture sites and the trophic relationship have been reported earlier (Singarajah, 1978a, c).

Mortality

Observations on the natural causes of mortality have shown that the mortality rate of oysters in Paraiba River estuary has been fortunately low. However, because of spawning, which usually takes place during the winter when rainfall is maximum in this area, a natural calamity could be imminent. For example, if the rainfall were to be consistently heavy for prolonged periods, as occurred in June-July of 1977 with an average rainfall of 10.6 mm / day, extreme reduction of salinity could persist for more than a week thus resulting in considerable damage to oyster production. Field studies and laboratory experiments indicated that during prolonged salinity reduction the oysters kept their shells tightly closed and didn't feed. Eventually, in excess of one week, some of them died and the shells began gaping. The impact of such extreme changes in environmental conditions are likely to have some long term effect which is not noticeable immediately.

Other main causes of mortality are predation and fouling. The commonest predators appeared to be gastropods, puffer fish, and crabs, especially the blue crab, *Callinectes sapidus* which appeared to be a voracious selective feeder on oyster larvae. Mortality of the spat might also be due to competition for space on setting substratum and for food, particularly with barnacles, hydroid colonies, especially *Sertularia* and sponges. A potential pest, the gammarid amphipod, *Ampelisca brevisimulata,* has also been frequently found on these oysters.

Culture of oysters and present status

Of the two commercially important and well recognized oysters, *Crassostrea rhizophorae* and *Crassostrea paraibanensis,* only the latter is popularly cultured at present because of its enormous size, pleasant taste, and economic potential. Many experimental and pilot schemes currently are under way to increase production.

The new methods, including raft culture, were recently introduced to replace some of the old and

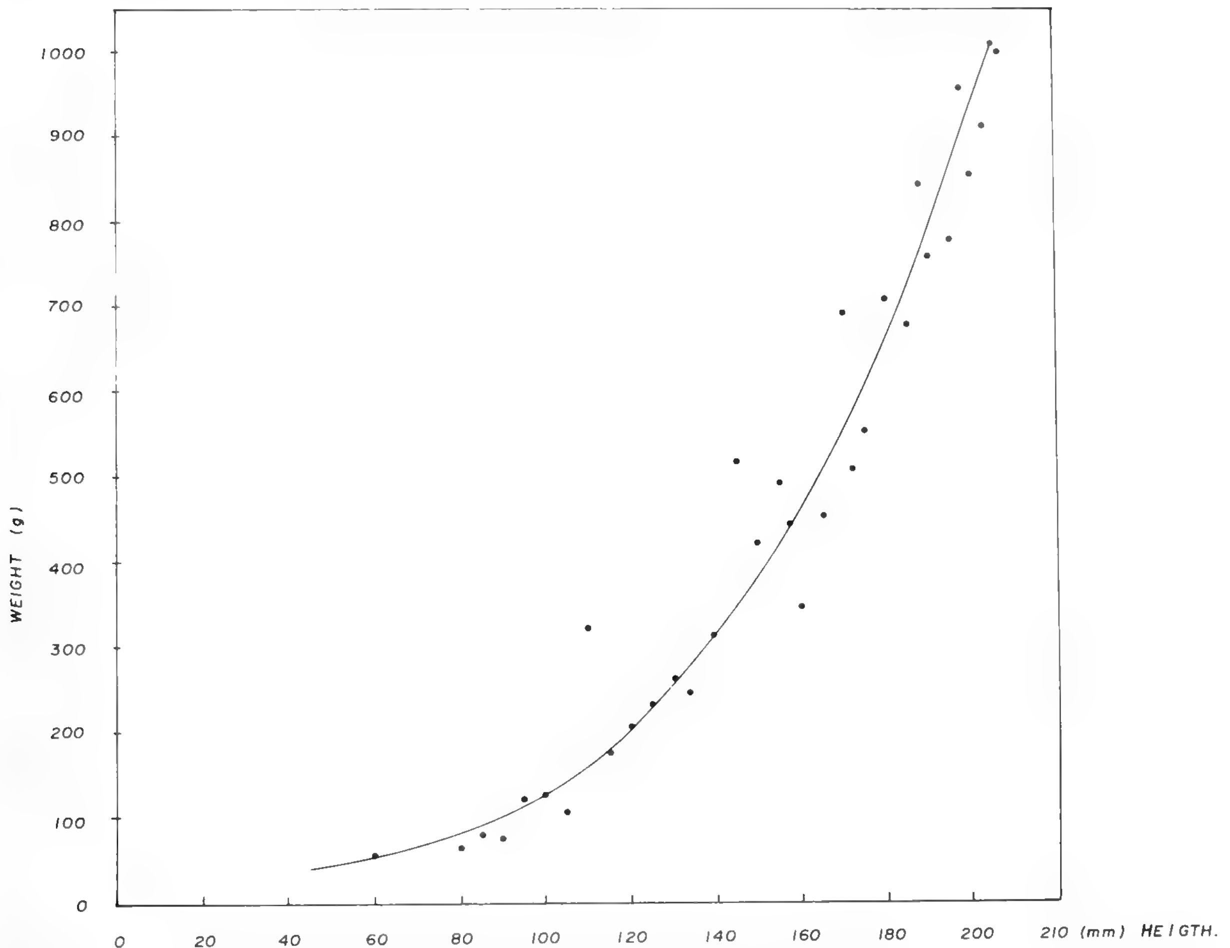

FIGURE 5. *Height-weight relationship in* Crassostrea paraibanensis.

obsolete techniques. Spat are collected and transplanted to growing and fattening grounds which are always at depths more than 2 m, under low tide level. The growing and fattening areas are divided by dams into plots, one hectare each, with constant circulation of food materials along the tidal current which varies diurnally.

The current yield of oysters is far too low, amounting to only five to eight tons of meat per hectare for two years. It is estimated that not more than 50 to 200 metric tons are sold in the whole of northeastern part of Brazil. With the new, improved and effective techniques, some of the privately owned oyster beds are expected to double production in 1982 and increase it four times in subsequent years.

The oysters are hand shucked and the meat sold at the local markets. The oyster is consumed in different ways, the commonest being the steamed lemon preparation.

There are many new oyster projects, supported by the Government agencies, in the northeastern part of Brazil and culture details warrant separate publication elsewhere.

DISCUSSION

Oyster spat settlement and growth rates are very much dependent on physico-chemical and biological conditions, and topographical characteristics of the estuary. The greatest numbers of larvae usually settled in the month of August in both culture sites. This suggests the possibility of collecting spat for future large scale cultivation of this species.

Korringa (1952) noticed that Ostrea edulis ". . . larvae prefer to settle down with the umbo of the shell pointing in a very definite direction, irrespective of the angle at which the collector is placed, provided the latter is placed not precisely horizontal. The pull of gravity is no doubt involved in this phenomenon." In view of the present observations, it is doubtful whether gravity alone or any other adhesive factor is also involved in this phenomenon and, no doubt, more work is required, although the direction of the current had some influence and swift currents tended to induce settlement and increase the number of potentially transplantable larvae. Other abiotic and biotic factors greatly contribute to the distribution of the larvae and selection of suitable substrate for completion of their life cycle.

Loosanoff (1949) correlated the increased growth in oysters with increased temperature, but this alone could not have influenced the exaggerated growth rate in this species, particularly during the third month as can be seen from Figure 5. Perhaps some neurosecretory factor might have been responsible for the greatly increased growth. Ingle (1967) also showed extremely rapid growth rate in "coon reef" oyster larvae during the first forty-two days.

It is difficult to assess the importance of the small variability in the growth rate of spat between the two sites, which were essentially under similar experimental conditions, but the small difference might be associated with nutritional factors. Plankton samples from both oyster sites showed significant quantitative rather than qualitative differences (Singarajah, 1978a). Ryther (1968) showed that a high yield of oysters resulted from high concentration of food in the water and the rate at which it is brought to the shellfish by tides or currents. Davis et al. (1964) has shown that the rate of growth of larvae of American oysters at different temperatures was critically affected by the type of food organisms available and that they preferred naked algae such as chrysophytes - *Monochrysis lutheri* and *Isochrysis galbana* than chlorophytes - *Chlorella sp.* These authors further suggested that the preference might be due to enzyme systems which act on the algal cellular components. Lackey et al. (1953) also suggested that the preferred food in adult shellfish were diatoms and dinoflagellates.

Galtsoff's (1930) estimation of eggs for a single spawning of *Crassostrea virginica* appears to be too high even though it is a prolific species since it is relatively smaller than *Crassostrea paraibanensis.* Galtsoff appeared to have relied heavily on the dimensions of the gonads during spawning, and Burkenroad (1947) suggested that "there might be an error in the calculation by the misplacing of a decimal point." Other differences might be due to species variations and environmental conditions. However, the main advantage in the present estimation of egg numbers has been that continued release of eggs from the gonads could be demonstrated during spawning. Perhaps, the curious coincidence of spawning with the heavy torrential tropical rainfall, under which the environmental conditions such as salinity, temperature, and pH fluctuate, might be an adaptive value allowing the eggs to be more easily disseminated, even though the large number of eggs produced by this species is far in excess of what an estuarine habitat can support. Nevertheless it is important in the maintenance of an endemic adult population.

Butler (1949) showed that gametogenesis of oysters living in areas of low salinity was inhibited. Others (Seno et al., 1926), working with Japanese oysters, thought that low salinity might adversely affect the development of eggs, and Chanley (1957) showed that the optimum salinity for growth of recently set *Crassostrea virginica* was 15.00 to 22.50 parts per thousand.

Loosanoff (1953) observed that a sharp reduction in salinity decreased the pumping rate of the American oysters while Fingerman (1959) showed that both temperature and salinity profoundly affected ciliary activity of *Crassostrea virginica,* and Shinkawa (1961) also showed similar effects in *Crassostrea gigas.* In *Crassostrea paraibanensis,* the reduction of salinity seemed to have had relatively little effect, except acting as a stimulus to spawning. On the other hand, most of the oysters taken during the heavy rainy period had their valves more tightly closed. When they were brought to the laboratory and examined, general physiological activities, including the tentacular movements, were continued but generally at a reduced rate. Their digestive tracts were almost empty and the meat volume also reduced which might be due to exhaustion resulting from spawning and starvation under reduced salinity.

Prytherch (1928) attached far too much importance to pH; he suggested that the spawning of American oysters was influenced by higher alkalinity. Calabrese et al. (1966) found that the pH range for normal embryonic development of oysters was between 6.75 to 8.75 and that the lower pH limit for survival of oyster larvae was 6.00. They further observed that the maximum and minimum pH levels at which the American oyster would spawn were 10.00 and 6.00 respectively.

Accurate mortality estimates are difficult to obtain in benthic species such as oysters. The evidence seems to suggest that a major source of mortality seems endemic through predation, fouling and competition, and the greatest mortality in benthic oysters is in the planktonic stage. From the evidence already considered, it would appear that extreme changes in salinity over a prolonged period have some effect on mortality, and certain populations may be reduced, but the species is not entirely eliminated. These oysters have evolved an adaptive strategy to cope with natural environmental instability and calamity.

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LITERATURE CITED

Bargeton, M. 1943. Modifications histologiques de la zone des gonades apres la ponte chez *Gryphaea angulata* Lamk. Bull. Biol. Fr. Belg. **77**:97-101.

Burkenroad, M.D. 1947. Egg number in *Ostrea virginica* Science **106**:342.

Butler, P.A. 1949. Gametogenesis in the oyster under conditions of depressed salinity. Biol. Bull., **96** (3):263-269.

Calabrese, A. and H.C. Davis. 1966. Spawning of American oyster, *Crassostrea virginica*, at extreme pH levels. Vegliger. **11**(3):235-236.

Chanley, P.E. 1957. Survival of some juvenile bivalves in water of low salinity. Proc. Nat. Shellf. Assoc. **84**:52-65.

Cleland, K.W. 1950. Intermediary metabolism of unfertilized oyster eggs. Proc. Linn. Soc. N.S. Wales. **75**:296-319.

Coe, W.R. 1931. Sexual rhythm in the California oyster *(Ostrea lurida)*. Science **74**:247-249.

Cole, H.A. 1941. The fecundity of *Ostrea edulis*. J. Mar. Biol. Assoc. U.K. **25**:296-319.

Davis, H.C. and A. Calabrese. 1964. Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *Crassostrea virginica*. U.S. Fish Bull. **63**(3):643-655.

F.A.O. 1970. Year book of Fishery Statistics. 30 (1969).

Fingerman, N. 1959. Technical Notes No. 50: Effects of temperature and salinity in the oyster, *Crassostrea virginica*. Comm. Fish Rev., **21**(2):10-11.

Galtsoff, P.S. 1930. The fecundity of the oyster. Science **72**:97-98.

Galtsoff, P.S. 1938. Physiology of reproduction of *Ostrea virginica*. Biol. Bull. **14**(1,3):461-486.

Gilson, G. 1898. Cellules musculo-glandularies la Celule. **14**:89-105.

Hornell, J. 1910. Notes on an attempt to ascertain the principal determining factor in the oyster spawning in Madras backwaters. Madras Fish Bull. **4**:25-31.

Ingle, R.M. 1951. Spawning and setting of oysters in relation to seasonal environmental changes. Bull. Mar. Sci. Gulf. Carib. **1**(2):111-135.

Ingle, R.M. 1947. Artificial food for oysters. Sea Frontiers **13**(5):296-303.

Korringa, P. 1952. Recent advances in oyster biology. Quart. Rev. Biol. **27**(4):266-308 and 339-365.

Lackey, J.B., G.K. Grough, Jr. and G.B. Glancy. 1953. General character of plankton organisms in waters overlying shell-fish producing grounds. Proc. Nat. Shellf. Assoc. **52**:18-22.

Loosanoff, V.L. and C.A. Nomejko. 1949. Growth of oysters, *O. virginica* during different months. Biol. Bull. **97**(1):82-94.

Loosanoff, V.L. and H.C. Davis. 1950. Spawning of oysters at low temperatures. Science **3**:521-522.

Loosanoff, V.L. 1953. Temperature for maturation of gonads of Northern oysters. Biol. Bull. **103**(1):80-96.

Mattox, N.T. 1949. Studies on the biology of the edible oyster, *Ostrea rhizophorae* Guilding, in Puerto Rico. Ecol. Monogr. **19**(4):341-356.

Padilla, M. and J. Orrego. 1937. La fajacion larval de ostras sobre collectores experimentales em Quetamahue, 1966-1967. Boletim Ceietifico. Inst. Fom. Pesq., **5**:1-14.

Prytherch, H.F. 1928. Investigations of the physical conditions controlling spawning of oysters and occurrence, distribution, and setting of oyster larvae in Milford harbour, Connecticut. Fish Bull. U.S. **44**:429-503.

Quayle, D.B. 1969. Pacific oyster culture in British Columbia. Bull. Fish. Res. Bd. Can. **169**:1-192.

Ranson, G. 1940. La biologia de L'huitre. Rev. Path. Comp. **40**:122-131.

Ryther, J.H. 1968. The potential of the estuary for shellfish production (oysters-clams). Proc. Nat. Shellf. Assoc. **59**:18-22.

Sato, S. 1936. Ciliary movement of the gills of an oyster, *Ostrea gigas*, in relation to room temperature. Bull. Jap. Soc. Sci. Fish, Tokyo. **4**(6):409-410.

Seno, H., J.K. Hori, and D. Husakabei. 1926. Effects of temperature and salinity on the development of the eggs of the common Japanese oyster, *Ostrea gigas*. Thumberg. J. Imp. Fish Inst. Tokyo. **22**:41-47.

Shinkawa, H. 1961. Studies on the vertical distribution of Japanese oysters. Sci. Reports. Tohku. Univ. Fourth Ser. Biol. **27**(1):19-38.

Singarajah, K.V., 1978a. Hydrographic conditions, composition and distribution of plankton in relation to potential resources of Paraiba River estuary. Rev. Nordest. Biol. (Univ. Federal da Paraiba). **1**(1):125-144.

Singarajah, K.V. 1978b. Culture of a giant oyster, a new species *Crassostrea paraibanensis*. S. Internat. Simposio Brasileiro de Aquicultura, Recife, July, pp. 245-253.

Singarajah, K.V. 1978c. Culture of estuarine benthic and nectonic first trophic level feeders. V. Simposio Latinamericano, sobre Oceanografia, November, São Paulo. pp. 13-14.

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POPULATION STRUCTURE OF THE MANGROVE COCKLE *Anadara tuberculosa* (Sowerby, 1833) FROM EIGHT MANGROVE SWAMPS IN MAGDALENA AND ALMEJAS BAYS, BAJA CALIFORNIA SUR, MEXICO

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ABSTRACT

The population structure of Anadara tuberculosa *was studied in eight mangrove swamps at Magdalena and Almejas Bays, on the west coast of Baja California Sur, México. Some of these swamps have had recent commercial exploitation, while others were undisturbed. In those mangrove swamps being fished, a repopulation by strong juvenile classes was noted.*

Three population groups were identified in the eight swamps by their height-width regression index. Such an index suggests a counterclockwise circulation within Almejas Bay, which could also explain larval dispersion.

INTRODUCTION

The mangrove cockle, *Anadara tuberculosa*, is one of the most intensively exploited species in the mangrove areas of the western coast of México (Baqueiro et al., 1978).

The increasing demand and over-exploitation of the mainland mangrove ecosystems has forced commercial fishermen to the coast of Baja California Sur, where mangrove communities, including *A. tuberculosa* populations, had been undisturbed except for occasional fishermen. Therefore, it became important to make an estimate of the population of *A. tuberculosa* to establish a management policy to prevent further over- exploitation of the species.

There are few references on the biology of this species. Flores (1971) refers to the anatomy and reproductive cycle for a population from the coast of Sinaloa, México. Squires, et al. (1978) describe the population structure of a commercially exploited population on the coast of Colombia.

MATERIALS AND METHODS

Study area

The study was conducted in Magdalena and Almejas Bays which are part of a coastal lagoon system located on the west coast of Baja California Sur, México, between 25°N, 113°37'W and 25°20'N, 112°30'W. The lagoons are separated from the Pacific Ocean by several islands of which Magdalena, Margarita and San Lorenzo are the most prominent (Figure 1).

Along the coast there are numerous indentations where mangrove swamps are formed. The most conspicuous swamps are San Buto with an area of 32.9 km², El Curry with 3.5 km², La Herradura with 26.4 km², Salinas with 24.1 km², El Alacrán with 4.1 km², El Cayuco bordered by

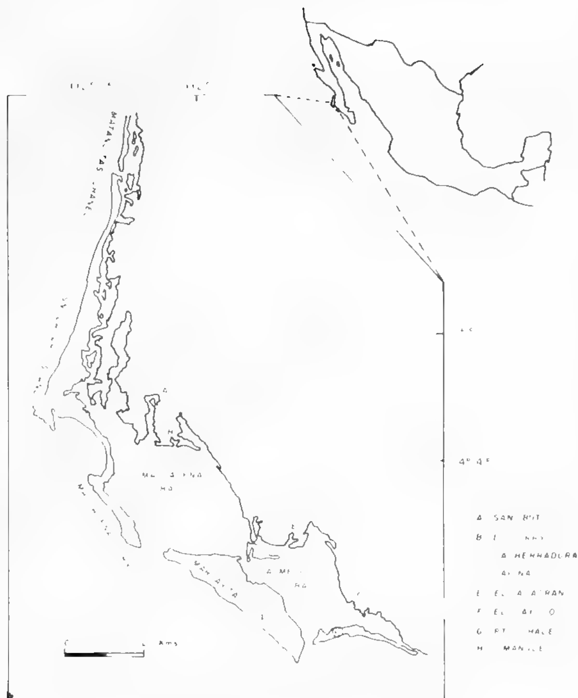


FIGURE 1. *Study Area and Sampling Sites*

mangroves that make an area of 18.2 km², Puerto Chale with 30.5 km² and Isla Mangle a sand barrier with an area of 29.4 km² of mangroves. Within these swamps numerous canals and banks are formed which make a suitable habitat for the settlement of *A. tuberculosa.*

The hydrology of the lagoons is quite stable (Alvarez et al., 1970). Sea temperatures fluctuate between 17°C in March and 27°C in August and salinities between 34.2 ‰ in October and 35‰ in June. Within the mangrove swamps the water circulation seems to be limited to the inward and outward flow of tides, as changes in temperature from 35°C to 29.4°C and salinity from 30 ‰ to 40 ‰ were noted during the sampling period.

Tides have an average range of 2.86 m (Instituto de Geofísica, 1978), ranging from −0.21 m to +1.98 m during the sampling period.

Sampling Methods

Sampling took place from 15 to 25 October, 1978. The number of stations established at each swamp depended upon the size of the swamps. At each station from 4 to 6 m² of bottom was sampled during low tide. The cockles were collected by digging with a fork 40 cm. deep into the mud. A square meter was marked with a rope which was passed in between the mangrove roots.

The length, height and width of every animal was measured to tenths of mm with vernier calipers, the data tabulated, and frequency histograms of 2 mm classes and cumulative percent curves were drawn. Skewness was used to determine the predominate length, where a positive values of Sk meant dominance of animals longer than the mean and negative values meant dominance of animals shorter than the mean.

Differences within the population groups was calculated by means of height-width regression indexes and Student's "t" tests.

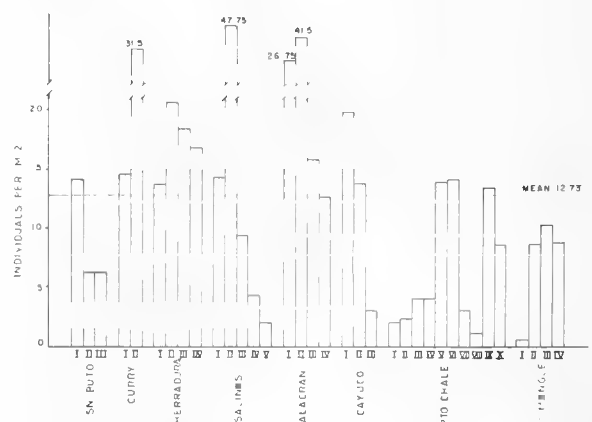


FIGURE 2. *Abundance per m² of* Anadara tuberculosa *at eight swamps in Magdalena and Almejas Bays.*

RESULTS

Distribution and Abundance

The distribution of *Anadara tuberculosa* was not homogeneous throughout the mangrove swamps. It reached its highest density between the roots of the red mangrove, *Rhizophora mangle,* in muddy bottoms rich in organic matter. Low density populations were found between the pneumatophores of the black mangrove, *Avicenia germinans* where the sediments were more compact and with higher amounts of fiber. *A. tuberculosa* was never found in sandy or shelly bottoms even in the presence of *R. mangle.* It occupied the littoral zone from high to low tide levels, along the edges of canals and in all intertidal banks.

TABLE 1. *Abundance and meristic characteristics of* A. tuberculosa *populations in Magdalena and Almejas Bays, Mexico.*

Swamp	D	Length (mm)					Height (mm)		Width (mm)	
		Max.	Min.	Mean.	S	SK	Mean	S	Mean	S
SAN BUTO	8.8	118	16	65.07	0.49	4.2	42.04	8.7	34.05	9.52
EL CURRY	23	84	42	64.12	.22	−.23	48.21	6.74	41.35	7.40
HERRADURA	17.22	88	36	60.88	.51	.55	44.71	6.65	37.53	7.90
SALINAS	15.49	80	32	60.73	.54	−.49	48.05	6.3	34.88	7.19
EL ALACRÁN	24.12	80	24	56.18	.26	−.69	42.6	7.3	35.24	8.49
CAYUCO	12.16	78	36	62.35	.46	−1.41	48.65	7.06	42.62	7.83
PTO. CHALE	6.59	94	40	71.56	.22	−2	52.97	7.34	45.44	8.48
I. MANGLE	7.0	96	18	65.01	0.23	.04	47.49	8.89	39.59	8.3

D = Density per m^2.
S = Standard deviation.
SK = Skewness.

The variations in density are presented in Figure 2. The highest density was found in the Salinas swamp which had an average of 47.75 individuals per m^2 at station II and an average of 15.49 per m^2 for the whole swamp. However the highest average density was found at El Alacrán with 24.12 individuals per m^2 (Table 1, Figure 2). Cockle density varied from 0.6 to 10.25 animals per m^2 at Isla Mangle, and from 2 to 47.75 at Salinas; it was more uniform at La Herradura with 15.6 to 20.5 individuals per m^2 and at El Alacrán with 12.5 to 41.5 per m^2.

Population Structure

Figure 3 shows the length frequency histograms of the different population groups.

Both the smallest and the largest cockles were found at San Buto. In this location the widest range of classes was also encountered (Figure 3a). It was possible to identify three class groups at San Buto: Group 1, juveniles with a wide range of sizes from 16.1 to 58 mm and a dominant class between 52.1 - 54 and 54.1 - 56 mm; Group 2, sizes from 58.1 to 80 mm with a dominant class of 64.1-68 mm; and Group 3, which was formed of organisms larger than 80 mm. The presence of group 3 explains the large Sk value (4.2) which signifies a dominance of organisms larger than the mean. Although the largest range of sizes was found in this swamp, the density per square meter was very low, with an average of 8.8 individuals per m^2. The cockle population found at El Curry (Figure 3b) showed a smaller range of sizes with a slight dominance of smaller animals (Sk=-0.23). Here two size groups were detected: one of 42.1 to 64 mm with a dominant class at 62.1 - 64 mm and another from 64.1 to 84 mm with dominant classes between 68.1 and 74 mm. The population density at El Curry with an average of 23 individuals per m^2, was higher than at San Buto.

The swamp of La Herradura (Figure 3c) showed a population with a slight dominance of adults (Sk=0.55). Group differentiations were not clear, but when considering dominant classes two groups could be identified: one of juveniles from 36.1 to 60 mm with a dominant class of 56.1 - 58 mm, and a group of adults from 60 to 68 mm with dominant classes at 62.1 - 68 mm and 68.1 - 70 mm. The density at this swamp was slightly greater than average with 17.27 individuals per m^2.

The population of Salinas was very uniform with a slight dominance of juveniles (Sk=-0.49). There was a distinctive class at 62.1-64 mm for the main population group (54.1 to 68 mm) preceded by juveniles of 32.1 to 54 mm and followed by larger adults from 68.1 to 80 mm (Figure 3d). Although the population density was high at one station (47.75 per m^2), the average was only slightly above the mean.

At El Alacrán there was a wide range of sizes with a minimum of 24.1 mm and maximum of 80 mm. It was difficult to divide the population into

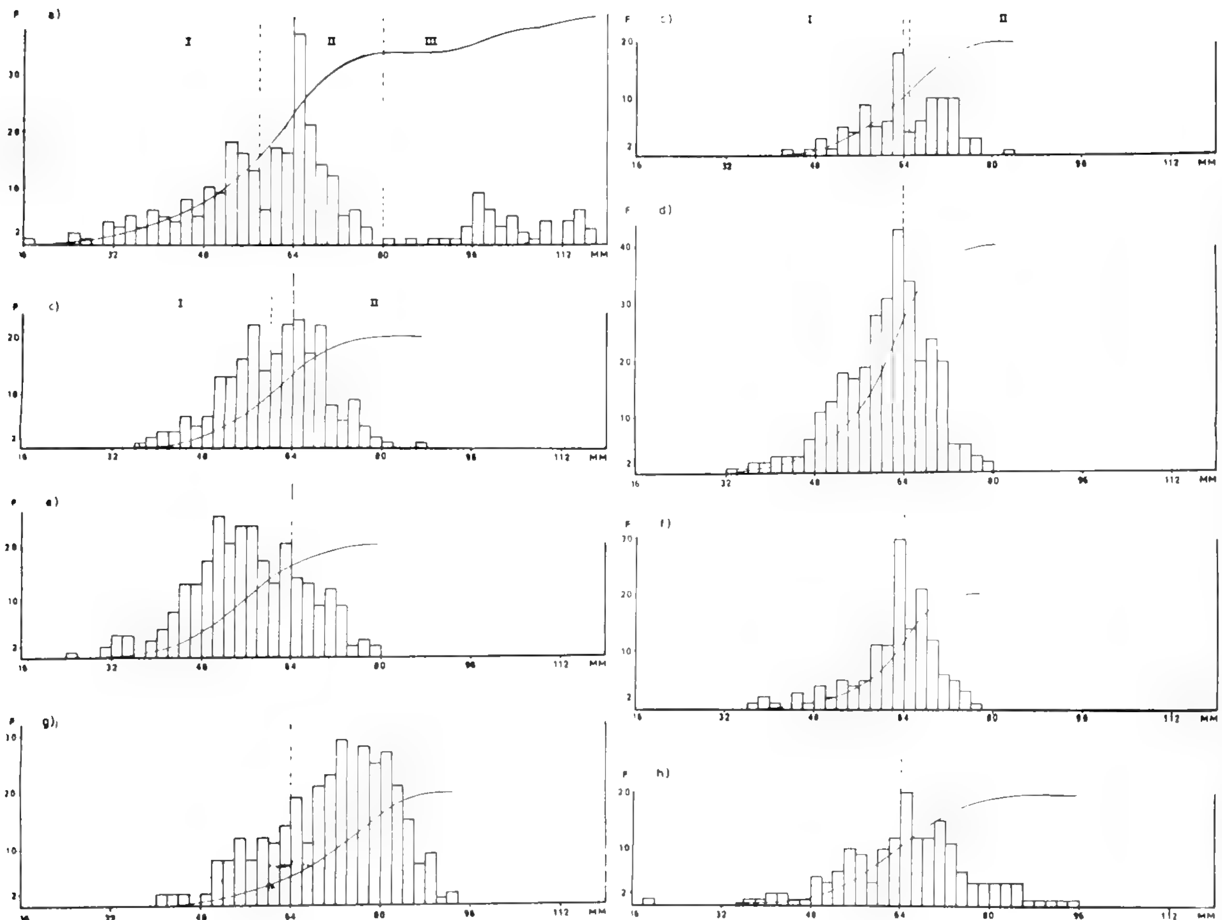


FIGURE 3. *Size frequency histograms of the different population groups at each swamp.*

three size groups. Juveniles ranged from 24.1 to 54 mm, which gave the population a Sk of -0.69. One group of adults measured from 54.1 to 64 mm and another group of adults was larger than 64.1 mm. Dominant classes of 50.1-52 mm, 54.1-58 mm and 62.1-64 mm respectively were found for each group (Figure 3e). The highest average density was observed in this swamp, however the mean size was smallest (56.18 mm).

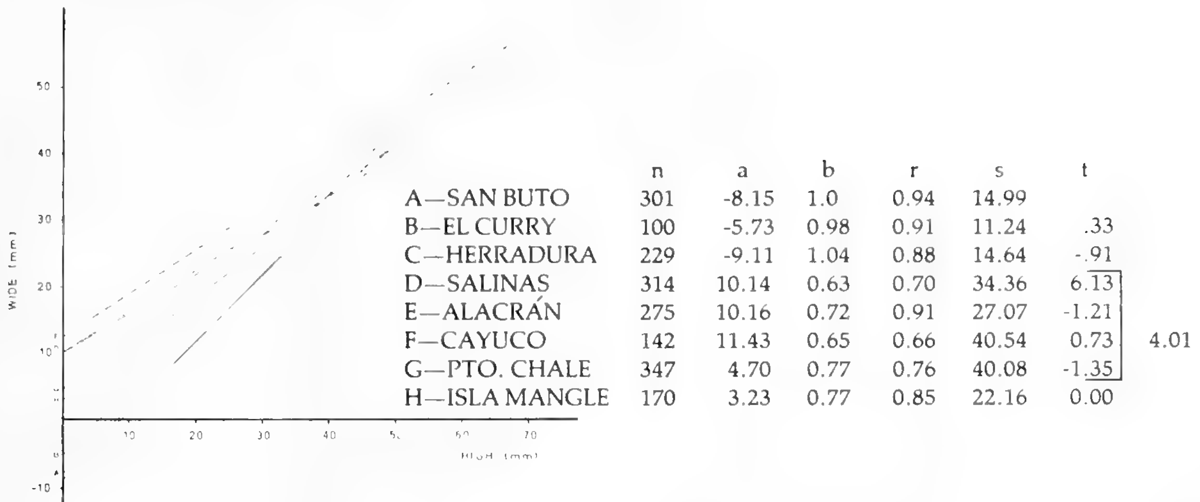
The population of El Cayuco (Figure 3f) showed a narrow range of classes with a minimum length of 36.1 mm and a maximum of 78 mm. This population could be divided into two groups: one of juveniles which gave a Sk of -1.41 with its dominant class between 62.1-64 mm, and a group of adults with a dominant class of 66.1-68 mm.

Puerto Chale presented a wide range of sizes and the largest overall mean size (71.56 mm). Two groups were noted: juveniles and small adults of 40 to 64 mm with no distinctive class, and a second group of larger adults with two dominant classes of 72.1-74 mm and 76.1-84 mm respectively (Figure 3g). In this swamp the lowest density was noted for any locality although some stations showed an average greater than the mean.

At Isla Mangle the population showed an ample range of sizes with three distinct groups: juveniles of 18 to 60 mm, adults with a dominant class of 64.1-66 mm, and a third group of larger adults of 70 to 96 mm. This population showed no Sk and its density was very low. There were many places with no *A. tuberculosa* even though the biotope seemed adequate.

Regression Analysis

Differences in the shell shape of *A. tuberculosa*



	n	a	b	r	s	t	
A—SAN BUTO	301	-8.15	1.0	0.94	14.99		
B—EL CURRY	100	-5.73	0.98	0.91	11.24	.33	
C—HERRADURA	229	-9.11	1.04	0.88	14.64	-.91	
D—SALINAS	314	10.14	0.63	0.70	34.36	6.13	
E—ALACRÁN	275	10.16	0.72	0.91	27.07	-1.21	
F—CAYUCO	142	11.43	0.65	0.66	40.54	0.73	4.01
G—PTO. CHALE	347	4.70	0.77	0.76	40.08	-1.35	
H—ISLA MANGLE	170	3.23	0.77	0.85	22.16	0.00	

FIGURE 4. *Regression lines of the different population groups of* Anadara tuberculosa *at Magdalena Bay, Mexico.*

were observed in some localities. Individuals from San Buto, El Curry and La Herradura were somewhat cylindrical with a similar height-width ratio, while farther south the shells were more flattened. To test whether this was a characteristic that could be used to determine the relationship amongst individuals from different localities, the regression coefficient of each group was calculated. After have analyzed length-height, length-width and height-width ratios the latter was chosen.

This ratio showed a higher correlation and its regression lines could be used to separate the groups better. A rectilineal correlation was chosen rather than the exponencial proposed by Hickman (1979) as it presented values with higher significance (Figure 4).

From Figure 4 it can be seen that the individuals form three different groups. Lines A, B and C indicate nearly cylindrical shells from the swamps of San Buto, El Curry and La Herradura, all within Magdalena Bay. The regression analyses detected no difference between these three swamps. A very significant difference was detected between the last swamp of the first group and the first of the second (4.01). One group was indicated by lines G and H from Puerto Chale and Isla Mangle with intermediate shell flattening of its cockle populations. Lastly, lines D, E and F indicate a more flattened shell from Salinas, El Alacrán and El Cayuco. This group presents some difference from the second group and a very large difference from the first one (T=1.35 and 6.13 respectively).

DISCUSSION

There are no reports on the currents of Magdalena and Almejas lagoons. However from the work of Alvarez et al. (1970) on salinity and temperature in the area, it can be concluded that the main flow of water from the Pacific ocean is restricted to tidal currents through Magdalena Channel and Margarita or Almejas Channels. These currents indicate that there is some exchange of water from Magdalena to Almejas Bay. The intermediate position of Isla Mangle and Puerto Chale cockle populations could be explained by a counterclockwise circulation within Almejas Lagoon, which moves larvae northward to El Cayuco, El Alacrán and Salinas. Although this has yet to be proved the shape and position of sand bars and barriers support the hypothesis.

Populations of *A. tuberculosa* within Magdalena Bay appear to have considerable potential for recovering from commercial fishing operations, as evidenced from the high density of small cockles in those swamps where commercial fishing was taking place.

The shell shape of *A. tuberculosa* was different

throughout the sampling area, thereby allowing identification of individuals from certain localities within short distances from one another. Distribution of larvae in Magdalena and Almejas Lagoons is not as important for sustaining the populations as the population in the neighboring swamps.

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REFERENCES

Alvarez, B.S., L.A. Galindo and A.C. Barragan 1970. Características hidrológicas de Bahía Magdalena, B.C.S. *Cienc. Mar. Univ. Auton.* Baja Calif. **2**(2):94-110.

Baqueiro, C.E., L.R. Alvarez y C.B. Kensler 1978. Análisis de la producción nacional de mariscos para el período de 1970-1976. *Congr. Nal. Oceanogr.* 1978. Ensenada, México. 18 pp.

Flores, M.A. 1971. Contribución al conocimiento biológico de la pata de mula, *Anadara (Anadara) tuberculosa* (Sow., 1833). *Tesis profesional Esc. Nal. Cienc. Biol. Inst. Pol. Nal.* México. 130 pp.

Hickman, R.W. 1979. Allometry and growth of the green-lipped mussel *Perna canaliculus* in New Zealand. *Marine Biology* **51**:311-327.

Instituto de Geofísica 1978. Tabla de pronóstico de mareas para 1978. Puertos del Oceano Pacífico. *Anales del Inst. Geof. Univ. Nal. Autón.* México. **23**(1,B):35-47.

Squires, H.J., M. Estevez, O. Barona and O. Mora 1978. Mangrove cockles, *Anadara* spp. of the Pacific Coast of Colombia. *Veliger* **18**(1):57-68.

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DISTANCE FROM SHORE AND GROWTH RATE OF THE SUSPENSION FEEDING BIVALVE, *Spisula solidissima*

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ABSTRACT

Annual growth lines in the shell of the suspension feeding bivalve Spisula solidissima *were used to determine growth rates and test the hypothesis that growth rates should decrease with increasing distance offshore. Despite an assumed reduction in food abundance for suspension feeders with increased distance offshore,* S. solidissima *grows faster at distances greater than 5 km from shore than near shore. Intercorrelations between other variables tested as predictors of growth rates prevented determining what variables were causing greater growth rates offshore. Water temperature, depth, and population density of* S. solidissima *are discussed as possible important influences on growth.*

INTRODUCTION

For marine invertebrate suspension feeders several factors have been shown to influence growth rate: temperature (Orton, 1928; Pratt and Campbell, 1956; Loosanoff, 1958); salinity (Wilbur and Owen, 1964); sediment type (Swan, 1952; Pratt, 1953; Greene, 1975; Peddicord, 1977); population density (Ohba, 1956; Beukema, et al, 1977; Peterson, 1978); and food availability (Coe, 1948; Pratt and Campbell, 1956). Our current knowledge of coastal marine environments suggests that food availability should vary inversely as a function of distance from shore.

The predicted gradient of decreased availability of food for suspension feeders with increased distance offshore is a consequence of several processes. First, phytoplankton productivity is higher nearer shore (Ryther and Yentsch, 1958; Smayda, 1973). Second, benthic microalgae production decreases with increasing water depth (Grontved, 1960). Third, because not all marsh primary productivity is consumed *in situ* (Teal, 1962; Odum and de la Cruz, 1967; Nixon and Oviatt, 1973) the concentration of marsh-derived detritus is high near shore. Finally, shallower depths near shore result in more food reaching benthic organisms as well as greater resuspension of bottom material

(for example benthic diatoms) by wave activity.

The object of this study was to test the hypothesis that growth rates of a suspension feeding organism decreased with increased distance offshore. To do this we determined the growth rate of the commercially important Atlantic surf clam, *Spisula solidissima* (Dillwyn), at different locations in the Middle Atlantic Bight. This species is heavily fished in the U.S. (Jones et al., 1978) and also important in certain Canadian waters (Caddy and Billard, 1976). Annual growth lines in the shell of *S. solidissima* permit an accurate determination of growth rate (Jones et al., 1978). We also gathered available data on a variety of other factors in an attempt to better explain the observed variations in growth.

MATERIALS AND METHODS

Source of Samples

Samples of *Spisula solidissima* were obtained from 21 stations in the Middle Atlantic Bight (Figure 1, Table 1). Samples from stations 1-10, 12 and 21 were collected during the National Marine Fisheries Service shellfish assessment cruises of 1974 and 1977. Dr. Harold Haskin of the Rutgers University Oyster Research Laboratory collected samples from stations 11 and 13 in 1975. Samples from stations 14-20 were collected by Snow Food, Inc. of Point Pleasant, New Jersey in 1977. Most samples were collected using a hydraulic dredge.

Preparation and Age Determination

Cross sections of the shells of *Spisula solidissima* were prepared and analyzed for growth lines according to the methods of Jones et al. (1978). A single cut was made through one valve along the line of maximum dorso-ventral height. Shell heights were measured as the straight line distance from the umbo to the exit of each growth line at the shell's external surface,

TABLE 1. Spisula solidissima. *Environmental data. Terms defined in text.*

Station	Sample Size	Distance	Latitude	Depth	Sediment	Mintemp	Max-temp	SDtemp
1	8	51km	36°20′	29.0m	3.12	11.0°C	18.5°C	2.5
2	12	40	36°50′	24.4	3.34	7.5	17.5	3.7
3	10	18	36° 50′	17.4	2.70	6.0	21.0	5.5
4	8	50	36°55′	31.0	3.34	9.0	17.0	2.8
5	6	32	37°15′	16.4	3.12	6.0	20.0	6.0
6	10	40	37°25′	33.8	3.34	7.5	16.0	2.9
7	5	20	38°20′	21.9	3.34	5.0	17.5	3.4
8	11	12	38°25′	26.0	3.34	4.0	16.0	3.6
9	9	22	38°25′	23.7	3.34	4.0	17.5	4.0
10	9	30	38°35′	32.9	3.00	5.0	16.0	3.3
11	10	4	38°55′	11.0	3.00	4.0	22.0	4.7
12	5	14	39°01′	14.6	3.94	4.0	16.0	4.7
13	12	3	39°24′	5.2	3.14	2.5	20.0	4.6
14	20	<1	39°48′	7.3	3.00	2.5	20.0	5.8
15	20	2	39°45′	9.7	3.34	2.5	20.0	5.9
16	14	<1	40°05′	7.0	3.00	2.5	20.0	6.0
17	19	<1	40°05′	7.0	3.00	2.5	20.0	6.0
18	20	2	39°48′	10.5	3.27	2.5	20.0	5.9
19	10	2	40°05′	16.9	3.00	2.5	20.0	5.9
20	10	18	40°05′	28.0	3.00	2.5	17.0	4.5
21	9	2	40°55′	21.0	3.75	2.0	18.5	5.7

measured to the nearest 0.5 mm with a hand-held ruler. We measured narrowly separated growth lines under a dissecting microscope at 25X.

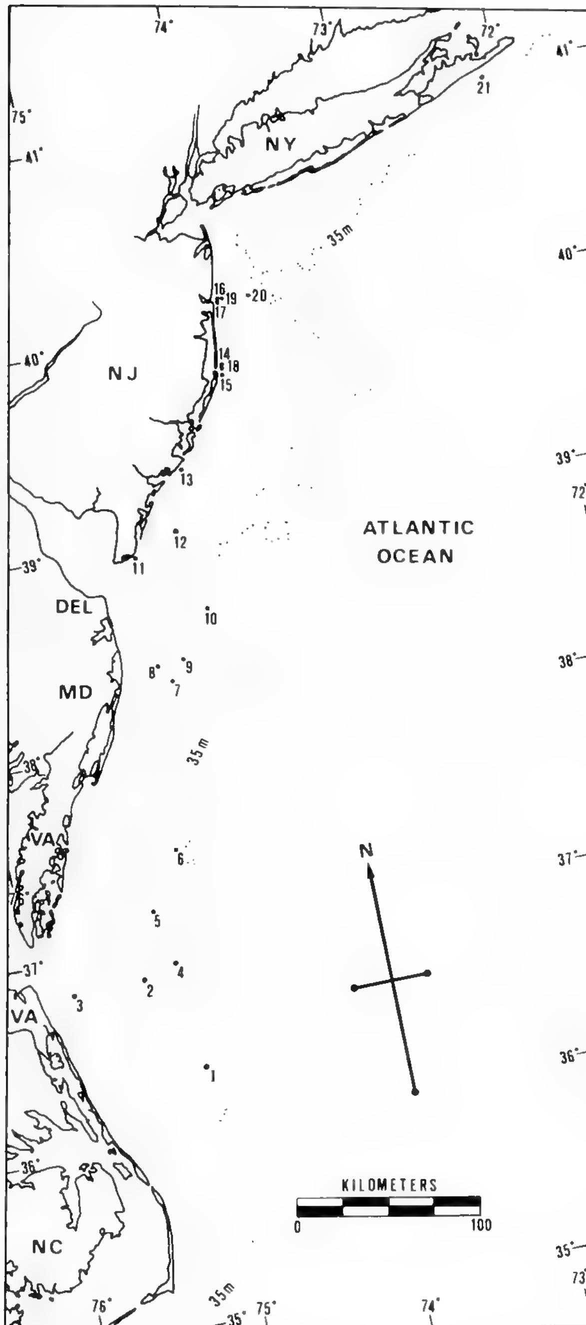

FIGURE 1. Spisula solidissima. *Locations of 21 stations from which specimens of* S. solidissima *were collected.*

Environmental Data

The following data were assembled for each station: 1) distance from shore, 2) average minimum temperature, 3) average maximum temperature, 4) standard deviation in average monthly temperature throughout the year, 5) average sediment grain size, and 6) water depth. The latitude and longitude of each station were determined at the time of sampling. Distance from shore is the shortest straight line distance between the station and the shore measured to the nearest 0.1 km on United States Coast Guard maps.

Water temperatures at all stations were taken from the monthly mean temperature profile maps of Walford and Wicklund (1968) which are based on 50 years of data. Minimum temperature (mintemp, Table 1) and maximum temperature (maxtemp, Table 1) were read from maps with the lowest mean bottom temperature (usually January) and highest monthly mean bottom temperature (usually September). Minimum and maximum temperatures were used because growth in bivalves is inhibited at temperature extremes (Coe, 1945; Loosanoff, 1958; Orton, 1928; Pratt and Campbell, 1956) and these temperatures are most likely to influence growth rates. The SDtemp column of table 1 gives standard deviation of monthly mean temperature at each station.

Sediment grain size was derived from maps of sediment distribution compiled by Milliman (1972) for all stations except 11, 13 and 21. Because the Milliman (1972) maps are based on one sample every 16 km the data are approximate; however, they are the most detailed sediment maps available. The percentages, by composition, of silt and clay (particles smaller than 62 μm), very fine sand (62-125 μm), medium fine sand (125-500 μm), coarse sand (500-2000 μm) and gravel (particles greater than 2000 μm) at each station were determined by taking the midpoint of the percent ranges reported for each sediment class. When necessary these percentages were corrected to equal 100% by dividing the percent in each class by the total percent. These percentages were then multiplied by a factor assigned to each sediment class rank order (1- silt and clay, 2- very fine sand, 3- medium fine sand, 4- course sand and 5- gravel) and the results summed and divided by 100 to provide a sediment index for each station.

Grain size for station 21 was obtained from Sanders and Kumar (1975). For stations 11 and 13 sediment samples were taken with a Peterson grab sampler within 1.6 km of the clam specimens. Percentages of particle sizes were determined by Dr. H.H. Haskin (Rutgers University, Oyster Research Laboratory) and the sediment index computed as above.

Depths for stations 1-13 and 21 were recorded with a fathometer when the samples were taken. Depths for stations 14-20 were read from United States Coast Guard maps. The depth on the map nearest the sample station was used.

Data Analysis

The clams analyzed grew over different years and encompass a wide range of sizes. As a result, before growth rates relative to sample sites could be determined, the raw measurements of height relative to age had to be corrected for two biases: 1) differences in growth rates that were a function of the age of the individual, since growth rates usually decrease with increasing age in bivalves (Levinton and Bambach, 1969), and 2) differences in growth rates that were a function of growth over different periods of time, because not all years are equally favorable for bivalve growth (e.g. Fairbridge, 1953; Green, 1957; Seed, 1969).

To correct for the first factor, differences in growth rate as a function of age, we examined a variety of models which express the relation between shell height and the amount an individual grew the preceding year to attain that shell height. The amount an individual grows each year can be thought of as a percentage of total growth and, as expected, decreases with age. A linear equation gave the best fit. The linear equation, derived from pooling growth for individuals, from all stations, was then used to calculate the deviation of an individual's growth in a given year from the predicted value. The resultant set of residuals represent age-corrected growth and allow comparison among individuals of different ages.

The age-corrected growth residuals constitute a new data set and were used to correct for the second factor, growth differences due to yearly environmental fluctuations. Pooling data from all stations, the mean age-corrected residual for each year back to 1953 was determined. These residuals provide a measurement of the growing quality of a year. A comparison of these mean residuals shows large differences in the favorability for growth among years. The mean age-corrected residuals were subtracted from the respective yearly residuals of each individual. This removes the effect of yearly environmental variability from the growth of each clam but makes the assumption that all individuals were affected equally in any given year. The mean of all the new residuals at a station represents the age-corrected and year- corrected growth rate for individuals at that station.

The age-corrected and year-corrected growth rates were correlated with the environmental data. Pearson product-moment correlation coefficients were computed for all relationships between independent variables and between independent and dependent variables. Partial correlation coefficients, controlling for each of the independent variables, were also computed. Multiple step-wise regressions were used to determine the amount of variance explained by each of the variables. Regressions were run 1) in which no restrictions were placed on the entrance order of the variables, and 2) in which each of the independent variables in turn was programmed to enter on the first step. The entire analysis described above was done for all stations together, and was repeated for inshore (distance < 5 km) and offshore (> 5 km) groups separately in order to determine whether yearly environmental fluctuations affected inshore and offshore populations differently.

Growth curves were constructed for offshore and inshore clams using the average shell height attained by individual clams at a given age. Since relationships of age versus size are usually reported in terms of shell lengths, height data were converted to lengths using the average height/length ratio for all our clams of 0.76 (Jones, et al., 1978). There were no significant differences in the height/length ratio between stations or ages.

RESULTS

Inshore and offshore growth curves are shown in Figure 2. The curves clearly indicate that clams in the offshore group (> 5 km) grow faster than those in the inshore group (< 5 km). The shape of the two curves is similar with rapid growth for the first 4 or 5 years followed by slower growth after-

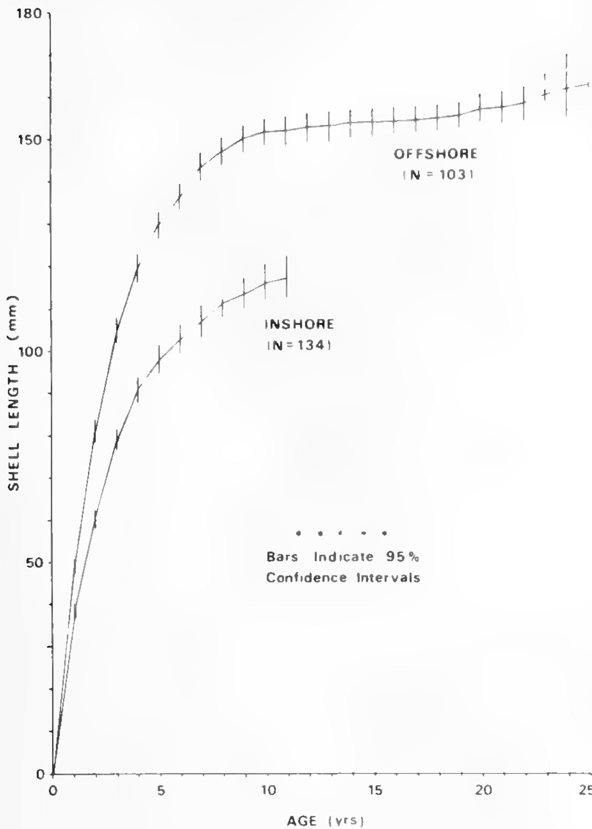

FIGURE 2. *Growth curves for* Spisula solidissima *determined on the basis of annual internal growth banding. The offshore curve is based on 103 individuals while the inshore curve is based on 134. Progressively larger bars with increased age result from declining sample sizes since all clams measured were not 25 years (offshore) or 11 years (inshore) old at time of collection.*

wards; but, by age 10, offshore clams average 152 mm in length while inshore clams average 116 mm. Inshore clams older than 11 years were rare and none were older than 16 years. In contrast clams 25 years and older occurred commonly offshore.

As Figure 1 indicates, the majority of the nearshore stations are in the northern part of the study area off New Jersey, while most of the offshore stations are farther out, off Maryland and Virginia. This does not appear to affect the results, however. In local areas where inshore and offshore stations both exist the pattern of faster growth offshore still holds (see Jones et al. 1978, for curves).

The age- and year-corrected growth residuals, standard error, and number of measurements made for each station are presented in Table 2.

Pearson correlation coefficients (Table 3) support the trend indicated in the growth curves; growth is strongly and positively correlated with distance offshore. Growth is also positively correlated with depth and minimum temperature and negatively correlated with latitude and standard deviation in temperature. The strong inter-correlations between independent variables make it impossible to isolate which variable(s) is controlling the trend of greater growth with increasing distance offshore.

The strong inter-correlations between independent variables also render the results of the step-

TABLE 2. Spisula solidissima. *Age- and year- corrected growth residuals and standard error for each station. Number of measurements at a station represents the sum of the ages of all clams analyzed from that station.*

Station	Corrected Growth	Standard Error	No. of Measurements
1	16.430	1.467	33
2	5.017	0.568	113
3	−1.373	0.902	64
4	7.829	0.854	76
5	11.141	0.951	55
6	6.969	0.663	104
7	−2.313	0.594	98
8	4.344	0.676	121
9	−1.534	0.434	189
10	0.462	0.507	161
11	5.125	0.469	117
12	5.255	0.843	70
13	−5.077	0.469	87
14	−2.241	0.345	171
15	0.676	0.727	133
16	−4.832	0.342	146
17	−5.157	0.343	206
18	−5.905	0.359	206
19	−4.410	0.414	107
20	−1.161	0.306	220
21	3.882	0.606	114

TABLE 3. Spisula solidissima. *Pearson correlation matrix showing correlation coefficients and significance levels (s) of correlation between (1) independent variables and growth in* Spisula *and (2) independent variables themselves. Variables defined in text.*

	Sediment	Depth	Mintemp	Maxtemp	Latitude	Distance	SDtemp
Depth	0.1587 s=0.246						
Mintemp	−0.0029 s=0.495	0.6432 s=0.001					
Maxtemp	−0.5297 s=0.007	−0.7625 s=0.001	−0.3216 s=0.078				
Latitude	0.0949 s=0.341	−0.5475 s=0.005	−0.9273 s=0.001	0.2495 s=0.138			
Distance	0.0731 s=0.376	0.7915 s=0.001	0.9379 s=0.001	−0.5283 s=0.007	−0.8679 s=0.001		
SDtemp	−0.1846 s=0.212	−0.7994 s=0.001	−0.7329 s=0.001	0.6964 s=0.001	0.6467 s=0.001	−0.7849 s=0.001	
Growth	0.2855 s=0.105	0.5370 s=0.006	0.7748 s=0.001	−0.2990 s=0.094	−0.6672 s=0.001	0.7323 s=0.001	−0.5241 s=0.007

wise regressions and partial correlations inconclusive. Minimum temperature enters first and explains 60% of the variance when step-wise regression was run without restrictions on the entrance order of the variables (Table 4). Distance and latitude, when entered first, explain 54% and 45% of the variance, respectively. In the partial correlations (not shown) when one independent variable is held constant the correlations between the other independent variables and growth are severely reduced or become insignificant. The exception is sediment which attains correlation coefficients of 0.80, 0.74 and 0.52 (all significant at 0.01 level of confidence) when minimum temperature, distance, and depth are controlled for respectively.

When the statistical tests are run separately for offshore and inshore stations, no significant correlations between independent variables and growth for inshore stations are present. For offshore stations distance and sediment have correlation coefficients of 0.53 and 0.52 respectively with growth (significant at 0.05 level of confidence). The inshore stations do not span a wide enough range of environmental conditions to result in significant correlations between the independent variables and growth. The results are of interest, however, because distance is still a reasonable predictor of growth when stations beyond 5 km from shore are considered alone. Separating the samples into inshore and offshore also reduces the effect of latitude because, as mentioned above, most of the inshore stations are from the northern section.

TABLE 4. Spisula solidissima. *Multiple regression analysis of variance in growth rate of* Spisula. *Variables enter in successive steps commensurate with their ability to explain proportions of the variance. Increment R Square indicates the percent of the variance explained by each variable. Total R Square sequentially sums the percent of variance explained. Variables defined in text.*

Step	Variable	Increment R Square	Total R Square
1	Min temp	0.600	0.600
2	Sediment	0.083	0.683
3	SD temp	0.022	0.705
4	Depth	0.011	0.716
5	Max temp	0.019	0.735
6	Latitude	0.002	0.737
	Distance	Insufficient significance level to enter	

All steps significant at 0.05 level

DISCUSSION

The results do not support the hypothesis that growth rates of a suspension feeder decrease with increasing distance offshore. Our conception of the concentration of food types utilized by *Spisula solidissima* may be wrong. Alternatively, other variables besides food abundance may be more significant in influencing growth rates. Temperature, water depth, and density might be more limiting to the growth of *S. solidissima* than food abundance.

Coe (1948) and Jorgenson (1966) report that phytoplankton, benthic microalgae, and detritus are all food sources for suspension feeding bivalves. The exact diet of *Spisula solidissima* is not known but it is unlikely to be different from other suspension feeders. During certain seasons of the year food abundance may have a dominating influence on growth rates and inshore individuals may grow faster. Averaged over the entire year, however, food concentration is not an adequate predictor of growth rate.

The intercorrelations between the independent environmental variables obscure the importance of each to the growth of *Spisula solidissima*. Of the variables considered, minimum temperature and water depth are capable of accounting for the faster growth rates offshore. Although not quantified in this study, decreased density of *S. solidissima* farther offshore could also maintain the pattern of faster growth offshore.

A correlation between growth and temperature has been found for the bivalves *Ostrea edulis* (Orton, 1928), *Mercenaria mercenaria* (Pratt and Campbell, 1956), *Mytilus edulis* (Coe, 1945) and *Crassostrea virginica* (Loosanoff and Nomejko, 1949; Loosanoff, 1958). *In situ* studies in shallow water show that the growth of *Spisula solidissima* stops during January, February and March on Cape Cod, Massachusetts (Belding, 1910). Although lack of growth during winter months might be only an indirect effect of temperature, Saila and Pratt (1973) report that below 4°C and above 26-28°C surf clams are inactive in the laboratory.

High temperature could inhibit growth in *Spisula solidissima* as it does in other bivalves. However, none of the stations sampled had a monthly mean temperature higher than 22°C. Very shallow, inshore stations may temporarily experience temperatures high enough to inhibit growth.

One facet of environmental stability was measured by the standard deviation in temperature (Table 3). Greater water depth offshore would also increase environmental stability by reducing wave action on the bottom. At shallow stations wave action might periodically remove clams from the sediment as well as subject them to high levels of turbidity and continually shifting sand, reducing growth.

Commercial clammers from Point Pleasant, New Jersey claim that densities of *Spisula solidissima* are greater nearer shore. Haskin (1978) found that surf clams from southern New Jersey decrease in density with distance offshore (within 4.8 km from shore). While quantitative data on the densities of surf clams over the entire Middle Atlantic Bight are not detailed enough to detect trends in densities, this pattern would be predicted based on Rowe et al.'s (1974) observations of decreasing biomass with increasing distance offshore. Density of bivalves has been shown to have an effect on their growth rates.

Competition at higher densities reduces growth (Ohba, 1956; Beukema, et al., 1977; Peterson, 1978). If lower densities of *S. solidissima* occur offshore this could in part account for the faster growth rates of offshore populations. This hypothesis remains to be tested.

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LITERATURE CITED

Belding, D.L. 1910. The growth and habits of the sea clam *(Mactra solidissima)* Rep. Comm. Fish. Game 1909, Commonwealth Massachusetts Public Doc. **25**:26-41.

Beukema, J.J., G.C. Cadee and J.J.M. Jansen. 1977. Variability of growth of *Macoma balthica* in the Wadden Sea in relation to availability of food. *In:* Biology of Benthic Organisms, pp. 69-77. Ed. by B.F. Keegan, P.O. Ceidigh and P.J.S. Boaden. New York: Pergamon Press.

Caddy, J.F. and A.R. Billard. 1976. A first estimate of production from an unexploited population of the bar clam *Spisula solidissima.* Fish. Res. Board Can. Tech. Rep. **648:** 13 p.

Coe, W.R. 1945. Nutrition and growth of the California sea mussel *(Mytilus californianus).* J. Exp. Zool. **99**:1-30.

Coe, W.R. 1948. Nutrition, environmental conditions and growth of marine bivalve mollusks. J. Mar. Res. **7**:586-601.

Fairbridge, W.S. 1953. A population study of the Tasmanian commercial scallop, *Notovola meridionalis* (Tate). Aust. Mar. Freshwater Res. **4**, 1-41.

Green, J. 1957. The growth of *Scrobicularia plana* in the Gwendraeth estuary. J. Mar. Biol. Assoc. U.K. **36**:41-47 (1957).

Greene, G.T. 1975. Incremental shell growth patterns as affected by environment in *Mercenaria mercenaria.* 67 pp. A.B. thesis, Department of Geology, Princeton University.

Grontved, J. 1960. On the productivity of microbenthos and phytoplankton in some Danish fiords. Meddr. Danmarks Fisk. - og Havunders. N.S. **3**(3):55-92.

Haskin, H.H. 1978. The onshore surf clam resource along the southern New Jersey coast. Proc. Natl. Shellfish Assoc. **68**:79-80.

Jones, D.S., I. Thompson and W.G. Ambrose. 1978. Age and growth rate determinations for the Atlantic surf clam *Spisula solidissima* (Bivalvia: Mactracea), based on internal growth lines in shell cross-sections. Mar. Biol. **47**:63-70.

Jorgensen, C.B. 1966. Biology of Suspension Feeding. London: Pergamon Press, 357 p.

Levinton, J.S. and R.K. Bambach. 1969. Some ecological aspects of bivalve mortality patterns. Am. J. Sci. **268**:97-112.

Loosanoff, V.G. 1958. Some aspects of behavior of oysters at different temperatures. Biol. Bull. **114**:57-70.

Loosanoff, V.G., and C.A. Nomejko. 1949. Growth of oysters, *Crassostrea virginica* during different months. Biol. Bull **97**:82-94.

Milliman, J.D. 1972. Atlantic continental shelf and slope of the United States — Petrology of the sand fraction of sediments, northern New Jersey to southern Florida. U.S. Geol. Surv. Prof. Pap., **529-J.**

Nixon, S.W. and C.A. Oviatt. 1973. Ecology of a New England salt marsh. Ecol. Monogr. **43**:463-498.

Odum, E.P. and R.A. de la Cruz. 1967. Particulate organic detritus in a Georgia salt marsh — estuarine ecosystem. *In:* Estuaries, pp. 383-388. Ed. by G.H. Lauff. Washington: Am. Assoc. Adv. Sci. Publ. 83.

Ohba, S. 1956. Effects of population density on mortality and growth in an experimental culture of a bivalve *Venerupis semidecussata.* Biol. J. Okayama Univ. **3**:169-1973.

Orton, J.H. 1928. On rhythmic periods in shell growth in *Ostrea edulis* with a note on fattening. J. Mar. Biol. Assoc. U.K. **15**:365-427.

Peddicord, R.K. 1977. Salinity and substratum effects on condition index of the bivalve *Rangia cuneata.* Mar. Biol. **39**:351-360.

Peterson, C.H. 1978. The effects of varying density on growth, reproduction, and survivorship of two infaunal, suspension feeding bivalves. Bull. Ecol. Soc. Am. **59**:(in press).

Pratt, D.M. 1953, Abundance and growth of *Venus mercenaria* and *Callocardia morrhuana* in relation to the character of bottom sediments. J. Mar. Res. **12**:60-74.

Pratt, D.M., and D.C. Campbell. 1956. Environmental factors affecting growth in *Venus mercenaria.* Limnol. Oceanogr. **1**:2-17.

Rowe, G.T., P.T. Polloni and S.G. Horner. 1974. Benthic biomass estimates from the Northwestern Atlantic ocean and the northern Gulf of Mexico. Deep-Sea Res. **21**:641-650.

Ryther, J.H. and C.S. Yentsch. 1958. Primary production of continental shelf waters off New York. Limnol. Oceanogr. **3**: 327-335.

Saila, S. and D. Pratt. 1973. Mid-Atlantic Bight Fisheries. *In:* Coastal and Offshore Env-

ironmental Inventory — Cape Hatteras to Nantucket Shoals, Ch. 6:1-125. Univ. R.I. Mar. Publ. Ser. 2.

Sanders, J.E. and N. Kumar. 1975. Holocene shoe string sand on inner continental shelf off Long Island, New York. Bull. Am. Assoc. Petrol. Geol. **59**:997-1009.

Seed, R. 1969. The ecology of *Mytilus edulis* on exposed rocky shores. Oecologia **3**:317-350.

Smayda, T.H. 1973. A survey of phytoplankton dynamics in the coastal waters from Cape Hatteras to Nantucket. *In:* Coastal and Offshore Environmental Inventory — Cape Hatteras to Nantucket Shoals, Ch. 3: 1-100. Univ. R.I. Mar. Publ. Ser. 2.

Swan, E.F. 1952. The growth of the clam *Mya arenaria* as affected by the substratum. Ecology **33**:530-534.

Teal, J. 1962. Energy flow in the salt marsh ecosystem of Georgia. Ecology **43**:614-624.

Walford, L.A. and R.I. Wickland. 1968. Serial atlas of the marine environment. Folio 15. Monthly sea temperature structure from the Florida Keys to Cape Cod, 2 pp., 16 plates, New York: American Geographical Society of New York.

Wilbur, K.M. and G. Owen. 1964. Growth. *In:* Physiology of mollusca. Vol. 1, pp. 211-242. Ed. by K.M. Wilbur and C.M. Yonge. New York: Academic Press, 473 pp.

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ASPECTS OF REPRODUCTION OF HARD CLAMS *(MERCENARIA MERCENARIA)* IN GREAT SOUTH BAY, NEW YORK[1]

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ABSTRACT

Hard clams (Mercenaria mercenaria) *were repeatedly induced to spawn in the laboratory. Unfertilized spawned ova ranged in size from 50 to 97 μm and were characterized by a bimodal size-frequency distribution. In spite of the high variability in egg production among individuals, correlation between size (length) and egg production of clams from Great South Bay, N.Y. was significant; 15 to 25% of the variation in fecundity was attributable to the difference in size of clams. Maximum egg production recorded for a single female over the spawning season was 16.8 million eggs. No significant differences in fecundity, size of eggs or larval survival were detected between clams from two diverse Bay habitats. Quantitative comparison between gonads of clams from the Bay, and those spawned for this study suggested that laboratory spawning tends to underestimate natural fecundities. The proportion of sexes was approximately equal. The smallest clam to spawn was a sublegal female 33.1 mm in length. Seed clams were capable of producing viable spawn, but had extremely low fecundities. The significance of the results was examined in the context of local management practices.*

INTRODUCTION

The hard-shell venerid clam *Mercenaria mercenaria* (L.) supports the most important and lucrative commercial fishery in New York State. In 1975 reported commercial landings alone were nearly 8.7 million pounds of meats, for which clammers received 14.3 million dollars, representing 23.4% by weight and 50.8% by value of all marine fish and shellfish species landed in the State that year (McHugh and MacMillan, 1976). Production is centered in Great South Bay, Long Island.

This study was undertaken to determine 1) sex-ratios, 2) size at first reproduction, 3) size-specific fecundity of hard clam populations in Great South Bay, and 4) to identify possible differences in fecundity and quality of spawn between two diverse Bay habitats.

The earliest information on egg production by hard clams is an unsubstantiated statement (Belding, 1931) indicating that a 2.5 in. (63.5 mm) clam annually produces an average of about two million eggs. Fecundity, expressed as the total number of eggs spawned per female during the reproductive season, has been reported for hard clams from Long Island Sound (Davis and Chanley, 1956) and Southampton Water, England

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(Ansell, 1967). In these earlier studies egg production was correlated with shell cavity volume, but results were somewhat conflicting, and small clams were not adequately represented. No fecundity estimates have been published for Great South Bay clams.

The influence of environmental factors, primarily temperature and food availability, on the reproductive ability of bivalves has been well documented (Ansell and Loosmore, 1963; Galtsoff, 1964; Loosanoff, 1965; Bayne, 1975). In the present study egg size and larval viability were used as indicators of the quality of spawn. A positive correlation between egg size and larval survival has been reported in various fish species (Bagenal, 1973), and recently in *M. mercenaria* (Michael Castagna, personal communication).

Several management practices employed in Great South Bay have proceeded without a critical evaluation of their adequacy, and require information about the reproductive capacity of hard clams. For example, mature spawner clams are annually transplanted from colder Long Island Sound waters into Great South Bay, in the belief that this will extend the local spawning season. Hard clams are locally marketed in three size categories, based on their shell width: littlenecks [25.4 mm (1 inch) to 36.5 mm (1 7/16 inch)], cherrystones [36.5 mm to 41.3 mm (1 5/8 inch)], and chowders (greater than 41.3 mm). Spawner transplants usually involve chowders, because they have a low market value. However, unsupported statements in the literature have indicated that old, "blunt" clams are less efficient spawners (Belding, 1931) or do not reproduce, although they contain spawn (Heppell, 1961). Therefore, size-specific fecundity is more relevant to the management of the fishery than the average number of eggs produced annually during the clam's reproductive lifespan. Such information should also aid in deciding the fate of Bay areas with high densities of chowders. This will depend on whether such grounds contribute a valuable source of seed for reproduction of other areas, or inhibit setting and survival of young clams, as has been suggested (Greene, 1978).

Clams less than 1 in. (25.4 mm) in thickness (width) cannot be legally harvested or marketed in New York State [N.Y. Conservation Law, Art. IV, Div. of Fish and Game, sec. 314(1)]. The restriction was enacted in response to wholesalers' concern that clam beds would be exhausted as a result of the high demand for small clams. A 1 inch size limit was selected in consideration for all sectors of the industry, since a larger size (1 1/8 inch) would not allow marketing of clams small enough for consumption on the half-shell (State of N.Y. Twenty-Second Annual Report of the Commissioners of Fisheries, Jan. 1894). However, the significance of the size limit from a biological standpoint was not assessed. The relative contribution to reproduction by the smaller clams is of interest in evaluating whether the current size limitation is adequately protecting the spawning stocks.

MATERIALS AND METHODS

Clams for the study were sampled from natural populations at two locations in Great South Bay: the mouth of Carmans River in Brookhaven Town waters, and a second site near Fire Island Inlet and Sexton Island in Islip Town waters (Figure 1). Random samples were collected at depths of approximately 1.1 to 1.5 m using conventional hand tongs operated from a small boat. The two sampling areas were selected because they represent very diverse Bay environments, and because they could provide the wide range of sizes of clams required for the study. In addition, a sample of 44 seed clams (i.e. sublegal clams) was collected from the Mud Creek area (Patchogue Bay), to determine size at first spawning.

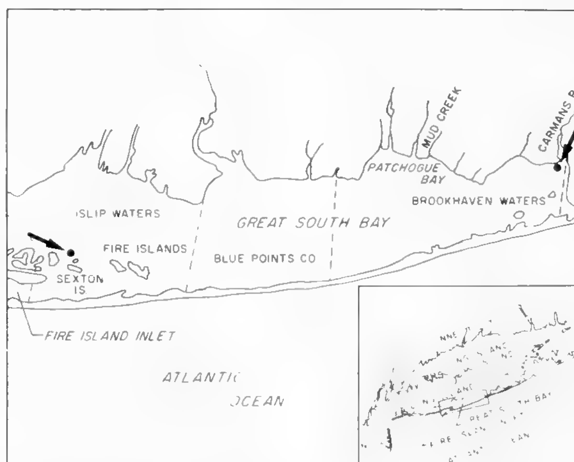

FIGURE 1. *Sampling locations in Great South Bay, New York.*

Conditions at the mouth of Carmans River are relatively unfavorable for clam growth; clams evidence slow growth, blunting and severe summer and winter breaks in shell growth (Greene, 1978). Setting or set survival in the area has been unsuccessful since 1973. The populations are composed of approximately 50% littlenecks and 50% cherrystones. The area was polluted by major additions of duck farm wastes until 1977 and has consequently been closed to shellfishing for many years. The Carmans River estuary has been dominated in the past by the presence of small phytoplankton forms (smaller than 10 μm) from early spring, through the summer and into the autumn as a result of nutrient input from duck farms (Ryther, 1954). Remnant sludge deposits still cover much of the bottom, and high densities of *Nannochloris*, a small chlorophyte, were still found in the area in the autumn of 1977, although nutrient additions from duck farms have abated (Carpenter, 1979). These small algal forms may constitute an inadequate food source for oysters and clams (Glancy, 1956). Sediments have a high silt and organic content (Greene et al., 1978), and salinities are low and extremely variable as a result of direct river input.

In contrast, the Sexton Island site, which is only lightly harvested, offers favorable conditions for clam growth. Clams reach higher maximum lengths than in Carmans River (Greene, 1978). Populations are dominated by larger size classes (cherrystones and chowders). The substrate has a relatively low organic content (Jones, unpublished). Salinities are relatively high, ranging from 26.5 to 30.9 ppt*, and strong tidal currents resulting from its vicinity to Fire Island Inlet ensure renewal of food and oxygen.

Clams were sampled in early May 1978, before the onset of spawning in the Bay. On sampling days ambient water temperatures were 9.7°C at the Sexton Island site and 16.1°C at the mouth of Carmans River. Spawning may start in early June in tributaries of Long Island Sound, and in July in the Sound proper (Loosanoff, 1937c). Carriker (1961) established that spawning in Little Egg Harbor, New Jersey, occurred over a median daily range of water temperatures of 22°C to 30°C. Histological examination of animals sampled from Great South Bay concurrently with this study by J. Kassner (unpublished) reveals that the bulk of the clams are ripe by the end of May, and appear to be ready to spawn as soon as the temperature reaches an appropriate triggering level.

*Suffolk County Department of Health Serv., Marine Monitoring Annual Data Reports for 1977 and 1978 (unpublished).

Conditioning

Clams were conditioned at the Marine Sciences Research Center's Flax Pond Laboratories on Long Island's north shore. They were numbered and held in a conditioning tank supplied with unfiltered water from the laboratory running seawater system at a rate of approximately 6 l/min., and were provided with adequate aeration. A thermoregulated chiller maintained water temperature at 17°C ± 1°C. Clams relied on the natural food supply provided by incoming pond waters; supplementary feeding was not attempted. Salinity ranged from 23 to 31 ppt, with a mean of 25 ppt.

Spawning

After approximately 40 days of conditioning, the clams were subjected to standard thermal and chemical spawning stimuli at intervals of 20-30 days, until they no longer spawned. Davis and Chanley (1956) found no significant difference in egg production whether clams were spawned at 3, 7 or 14 day intervals.

The spawning procedure was similar to that used by Davis and Chanley (1956) and Ansell (1967). Clams were placed individually in shallow spawning dishes containing seawater sequentially filtered through 1 μm, 0.45 μm, and 0.2 μm cartridge filters (Pall Tinity, Micro Corp.). The dishes were arranged in a water bath supplied with running seawater, the temperature of which could be closely controlled. Clams were subjected to periodic heating and cooling cycles and stimulated with non-viable, frozen sperm. Identified males were isolated from females for subsequent use.

As the season advanced, it became increasingly difficult to induce spawning. Therefore, clams were placed directly in running seawater, and stimulated with fresh sperm. At the first indication of spawning, the clam involved was drained, and removed to a dish with filtered seawater to complete spawning.

Water containing the eggs was successively drained through Nitex® screens of 183 μm and 25 μm bar mesh. The larger mesh removed any debris or faeces; the smaller mesh retained the eggs, which were concentrated and resuspended in a measured volume of filtered seawater. After the eggs were uniformly suspended with a Plexiglass® plunger, a subsample was transferred with a pipette to a volumetric flask, fixed with formalin to make up a final 1% solution, and the flask filled to a fixed volume with filtered seawater. Samples were refrigerated until counting.

The number and size of eggs in the range 43.5 μm to 99.7 μm was determined using a Particle Data Electrozone Celloscope®, equipped with a Digital PDP8/H computer programmed to function as a multichannel analyzer. A calibrated 480 μm orifice was used. Samples were usually counted within 24 to 48 hours of spawning, and invariably within five days of spawning, since it was determined that no significant changes in size or concentration occurred within this period. As eggs tend to rapidly settle out of suspension, they were resuspended using the particle counter's automatic mixer, until just before counting. At least three replicate counts were made on each sample. Samples were sufficiently diluted so that all counting occurred below the threshold concentration (approximately 1500 eggs/ml) determined experimentally to result in negligible coincident events. A Hewlett Packard 9830® computer was used to plot the size-frequency distributions.

Larval Culture Technique

Seawater used for culturing of embryos was collected in a settling tank and sequentially filtered through 1 μm, 0.45 μm and 0.2 μm filters. It was then pumped into a second settling tank and filtered immediately before use through a final 0.2 μm filter. Short-term larval rearing was conducted without aeration at 25°C in 250 ml graduated polypropylene beakers, fitted with paraffined paper covers.

The concentrations of the original egg and sperm suspensions were estimated using a Spectronic 20® Bausch & Lomb spectrophotometer (wave-length = 610 nm). Filtered seawater was used as a blank. Sperm concentrations were calibrated against counts made using a hemacytometer (Bricelj, 1979).

An average density of 120 eggs/ml in 230 ml of total incubation volume was prepared. *M. mercenaria* larvae are relatively tolerant of overcrowding in cultures. Loosanoff et al. (1963) showed that most eggs reared at a density as high as 250 eggs/ml develop into straight-hinged larvae after 48 hours. Before being brought to a fixed volume, eggs were inseminated with an aliquot of sperm suspension, calculated so that the final sperm/egg ratio was approximately 1.8×10^5 sperm cells/100 eggs (Range: 1.3×10^5 to 2.5×10^5 sperm cells/100 eggs). This selected ratio lies within a range that tends to maximize production of normal larvae with a minimum wastage of gametes (Bricelj, 1979). Freshly spawned sperm (not more than one hour old) was always used. Although sperm can be more readily obtained by stripping, this requires sacrificing the animal, and does not allow a reliable estimate of the concentration of active, viable gametes present in the extract (Rose and Heath, 1978). To reduce the chances of gametic cross incompatibility, sperm was usually pooled from two or three males from the same site as the female parent.

Percent fertilization was determined from fresh subsamples two to four hours after insemination. The total number of larvae, early straight-hinged and trocophores, was obtained from Sedwick-Rafter counts of samples fixed with Lugol's solution 24 hours after combination of gametes. Percent larval survival was calculated relative to the total number of fertilized ova.

Histological analysis

A subsample of clams was examined histologically at the beginning of the experimental period to assess the condition of the gonads and to ensure that they had not spawned prior to the fecundity experiments. Gonadal tissue was preserved in Bouin's solution, dehydrated, cleared and embedded in paraffin using standard histological methods. After sectioning at 10 μm, specimens were stained in Delafield's hematoxylin and counterstained in an aqueous solution of eosin.

Although no quantitative estimate has been given, both experimentally and naturally spawned clams are known to retain ripe unspawned eggs in the gonad at the end of the spawning season (Loosanoff, 1937a; Davis and Chanley, 1956;

Porter, 1964, and Keck et al., 1975). Therefore, gonad sections of "spent" clams from this study were compared with those of clams sampled from Brookhaven waters in October, presumably the end of the spawning season. The mean number of ripe residual oocytes, including ova free in the lumen and those attached to the follicle walls, per unit area of gonadal tissue, was determined for each individual.

Clams were sexed from microscopic examination of smear preparations of the gonad. Sex of the smaller clams was determined from histological examination.

Sizing of clams

Shell length (antero-posterior dimension) and width (greatest lateral dimension) of clams used in this study were determined with vernier calipers, to the nearest 0.1 mm. Total body volume and shell cavity volume were also determined, by measuring water displacement, for purposes of comparison with earlier studies. The relationships of length to width, total volume and shell cavity volume, defined by linear regression equations using a double logarithmic transformation, are shown in Table 1. These equations can be used to convert and re-interpret earlier results in terms of the commercial size categories of clams, which are widely used by baymen, hatchery operators and resource managers.

RESULTS AND DISCUSSION

Oocyte size-frequency distributions

Ripe spawned oocytes of *M. mercenaria* are generally described as being approximately 70-73 μm in diameter (Loosanoff and Davis, 1963). When first discharged they are surrounded by a gelatinous membrane about 25 μm thick, which soon swells to a thickness of 95 μm, so that the overall diameter of the ovum is approximately 270 μm (Carriker, 1961). Results of this study indicate that mature oocytes, excluding their gelatinous envelope, can vary widely in size from about 50 to 97 μm.

Oocyte size-frequency distributions of clams from all sampling sites, obtained by plotting the diameter of unfertilized spawned eggs as a func-

TABLE 1. *Relationship between* M. mercenaria *shell length (L) in mm, and total volume (V_T), volume of the shell cavity (V_{sc}) in ml, and shell width (W) in mm, described by linear regression equations.*

Sexton Island	$\log V_{sc} = 2.8124 \log L - 3.6148$	r=0.9907	n=61
Carmans River	$\log V_{sc} = 2.8455 \log L - 3.6704$	r=0.9816	n=38
Southampton Water England (Ansell, 1964)	$\log V_{sc} = 3.1653 \log L - 4.2652$		
Sexton Island	$\log V_T = 2.9566 \log L - 3.6798$	r=0.9940	n=61
Carmans River	$\log V_T = 2.9018 \log L - 3.5643$	r=0.9863	n=38
Great South Bay[a]	$\log V_T = 2.9532 \log L - 3.6657$	r=0.9910	n=99
New England[b]	$\log V_T = 2.9527 \log L - 3.6771$	r=0.9998	n=78
Southampton Water (Ansell, 1964)	$\log V_T = 3.0497 \log L - 3.7867$		
Sexton Island	$L = 1.0769 + 1.8953 W$	r=0.9746	n=61
Carmans River	$L = 1.2108 + 1.8296 W$	r=0.9651	n=40

[a] Combined data from both stations
[b] Calculated from Belding's (1931) data for all clams ≥ mm in length

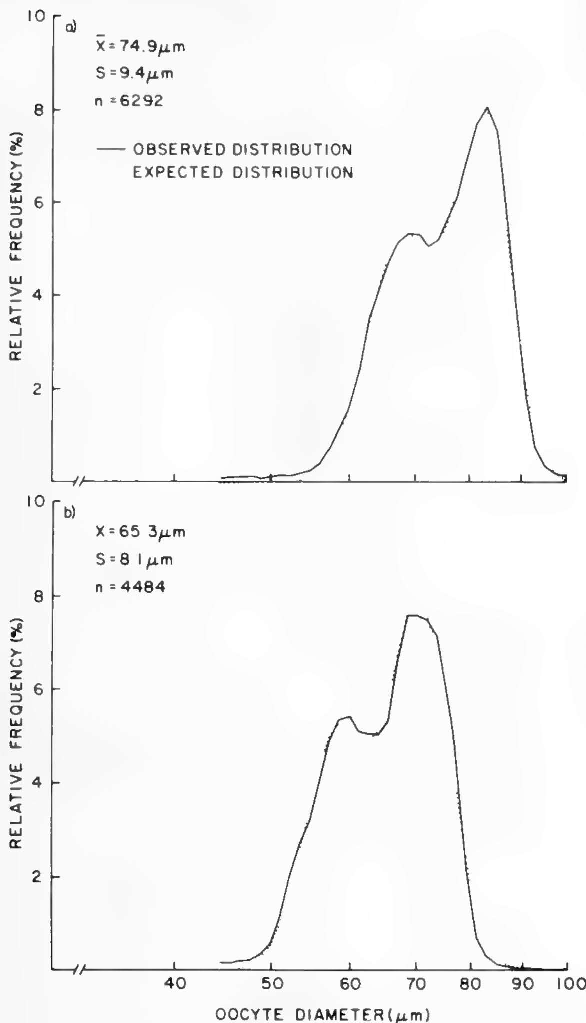

FIGURE 2. *Size (diameter)-percent relative frequency distributions of unfertilized oocytes spawned by* M. mercenaria *(semi-logarithmic scale). a) Clam from Sexton Island, Great South Bay; b) transplant clam from Long Island Sound.*

tion of percent relative frequency, were found to be consistently bimodal. A representative example is given in Figure 2.

A computer program (Beech, 1975, sec. 4.3.2.) was adapted and used to resolve the observed heterogeneous distribution into a sum of two Gaussian components. Given an estimate of the maximum height of each peak, oocyte diameter corresponding to the maximum height, and half-width at half-height of each peak as input parameters, the program performs a least squares fit to experimental data, and produces refined values of the parameters of each normal component (mean and standard deviation). These final values result from successive iterations, repeated until the change in parameters is acceptably small. Agreement between observed and calculated distributions was extremely good, as shown in Figure 2. The difference between them was not significant ($P \geq 0.05$), as tested using the Kolmogorov- Smirnov two-sample test for large sample sizes (Sokal, unpublished).

The means and 95% confidence intervals for both components, obtained for first spawnings of a sample of eleven clams are given in Table 2. An average of 56% (Range = 42.8 - 68.9%) of the oocytes released at one time correspond to one normal distribution $\bar{x} \pm s_{\bar{x}} = 67.0 \pm 0.68\ \mu m$), and an average of 44% (Range = 31.1-57.2%) correspond to the second component ($\bar{x} \pm s_{\bar{x}} = 80.7 \pm 0.74\ \mu m$). These percentages were calculated by integration of each normal component using Simpson's approximation (Orr et al., 1973, program 48).

No significant difference ($P \geq 0.75$) in the mean size of oocytes spawned by clams belonging to the three marketable size classes was detected using analysis of variance (Sokal and Rohlf, 1969, sec. 9.2). Size-frequency distributions of eggs spawned by seed clams did not differ in either size or appearance, from those spawned by older clams.

As the spawning season progressed, clams tended to release some immature oocytes as part of their spawn. This was indicated by the appearance of a third, small peak in some size-frequency distributions, below about 40 μm, and corresponding to between 1 and 7% of small, immature oocytes identified in microscope counts. This discharge of developing ova may have resulted from the strong spawning stimulation.

Size-frequency distributions of oocytes released late in the spawning season were otherwise similar in shape to those of oocytes from first spawnings. Size spectra of "early" and "late" spawn ($n = 16$) were compared using the Kolmogorov-Smirnov two-sample test. The time elapsed between the spawnings compared ranged from about four to fifteen weeks. This comparison showed that for eleven clams (69% of the total), there was a significant difference in the two distributions ($P <$

TABLE 2. *Mean diameter and 95% confidence interval (in μm) of oocytes from first spawnings of* M. mercenaria. *Values are given for both normal components of the bimodal size-frequency distribution, for eleven clams from the Sexton Island sampling site in Great South Bay, and two transplant clams from Long Island Sound. Parameters obtained by least squares fit to experimental data (see text).*

	GREAT SOUTH BAY CLAMS			
	Component I $\bar{x}1$(μm) 95% CI	Component II $\bar{x}2$(μm) 95% CI	($\bar{x}2$-$\bar{x}1$)	No. of Oocytes
	65.7 (51.3-84.0)	78.6 (72.0-85.8)	13	5294
	68.5 (56.0-83.7)	82.8 (73.2-93.6)	14	3863
	65.1 (54.3-78.1)	79.4 (68.5-92.1)	14	5223
	68.2 (51.3-90.9)	80.6 (74.2-87.7)	12	5708
	67.8 (52.6-87.5)	80.7 (73.2-89.0)	13	5572
	72.5 (56.5-93.1)	86.7 (77.6-96.9)	14	5195
	64.2 (51.3-80.2)	78.1 (69.2-88.3)	14	3217
	66.9 (55.0-81.3)	80.8 (70.3-92.8)	14	5247
	64.8 (49.8-84.4)	78.2 (70.8-86.3)	13	5256
	67.1 (53.7-83.8)	80.6 (72.2-90.0)	14	12203
	66.8 (52.7-84.7)	80.8 (72.2-90.3)	14	11426
Overall mean ± sd (n=11)	67.0 ± 2.26	80.7 ± 2.44	14	
	Transplant Clams from Long Island Sound			
	61.8 (52.6-72.6)	74.0 (63.5-86.2)	12	4484
	58.4 (49.2-69.4)	71.0 (60.1-83.8)	13	5641
Overall mean ± sd (n=2)	60.1 ± 2.40	72.5 ± 2.12	12	

0.01), evidenced by a shift in the distribution of eggs from late spawnings towards smaller sizes. Therefore, only eggs from the first spawning of each female were used in all comparisons between sites and size classes. The implications of this decrease in mean egg size as the spawning season progressed and its possible relation to a reduced viability or growth rate of larvae produced, have not been further investigated.

During 1978 clams were collected from western Long Island Sound, and transplanted into Great South Bay, as part of Brookhaven Town's shellfish management program. A few of these Long Island Sound clams were induced to spawn in the laboratory in late June, without previous conditioning. Egg size-frequency distributions were found to follow the same characteristic bimodal pattern. Analysis of variance indicated that the mean size of oocytes of the transplant clams (n = 2) was significantly smaller than that of Sexton Island clams (n = 29; $P \leqslant 0.005$). This was confirmed by comparing each normal component separately ($P \leqslant 0.025$). However, no significant difference ($0.1 < P < 0.05$) was found between the mean size of eggs spawned by clams from the two Bay sites.

A bimodal pattern of size of eggs spawned at one time has not been documented for other bivalves. However, a significant seasonal difference in the size of ripe eggs has been reported for the soft-shell clam, *Mya arenaria*, from Cape Ann, Mass. (Brousseau, 1978). The population was characterized by a biannual spawning cycle, with the same individuals spawning in spring and summer. The mean diameter (40-45 μm) of ova ready for release in the spring, was significantly smaller than that of summer oocytes (61-63 μm). Brousseau (1978) suggested that the spring cycle

might be a facultative event which takes advantage of the abundant food supply during the spring phytoplankton bloom, when a more rapid gametogenesis results in smaller oocyte size.

In venerid clams such as *Venus striatula* and *Venerupis pallustra*, unspawned ova persist through the winter and spring. Ansell (1961) suggested that in these species the older oocytes may be carried over into redeveloped follicles, so that spawned ova would consist of two separate year classes. In *M. mercenaria* residual oocytes are generally lost before the next spring (Loosanoff, 1937a; Porter, 1964), and their number varies greatly among individuals. However, each component in the size-frequency distributions obtained represents a large and relatively constant percentage of the total. Therefore, the possibility that one of the modal peaks could be attributed to unspawned ova from an old crop is discarded.

At present, no certain explanation can be advanced to explain the bimodal nature of the oocyte size-frequency distributions. The two modal size classes could reflect two peaks in oogenic activity, possibly characterized by different growth rates, occurring in early and late fall, or alternatively, in the late fall and late winter or early spring. In species such as *Venus striatula* and *M. mercenaria* there is little storage of food reserves, and gonadal development presumably takes place in response to food availability in the environment (Ansell, 1961; Ansell and Loosmore, 1963). Therefore, the two peaks in oogenesis might occur in response to an increase in the supply of phytoplankton or an increase in temperature leading to an increase in water filtration.

Larval development

One-way analysis of variance (Sokal and Rohlf, 1969, sec. 9.2) was performed on data of larval survival normalized by use of the arcsine transformation. No significant difference was found in percent fertilization or larval survival between clams of the three marketable size classes, or between clams from the two Bay sites. Differences within the same size group or location were as great as those among different size classes or locations. This is in agreement with the results of Loosanoff (1953), who noted, without describing his experimental design, that there was no difference in viability of spawn produced by clams of different sizes or ages. Culture of fertilized eggs from one sublegal clam (45.5 mm long) was attempted; it yielded a relatively high percent fertilization (92.8%) and percent larval survival (91.2%), indicating that seed clams are capable of producing viable eggs.

Temperature, egg density, sperm concentration, volume of incubation, and age of gametes were held constant in this study. However, water quality was an uncontrolled variable. Total percent larval survival ranged from 37.9 to 100%, and percent survival of straight-hinge larvae ranged from 0 to 86.0%. Such large differences are commonly observed when culturing bivalve larvae, and have been partly attributed to inherited characteristics from either parent (Loosanoff and Davis, 1963).

The parents for any mating experiment conducted were assumed to be a random sample of the breeding population. Since sperm for each mating was pooled from several males from the same site, it was also assumed that the progeny produced were fairly representative of parental combinations which might have occurred in the particular environment.

Sex ratios

The ratio of females to males from the random sample of Sexton Island clams (n = 178) was 1:1.2, and that for Carmans River clams (n = 128) was 1:1.1. These ratios show a slight excess of males, but do not differ significantly from the expected 1:1 ratio ($P > 0.1$). Twenty-one of the largest clams (chowders) were selected from a third site near Sexton Island characterized by a high density of chowders. The sex ratio (1:1.3) was again not significantly different from an equal sex ratio ($P > 0.1$). Therefore, an equal proportion of sexes also appears to be maintained among the older clams.

Age-size at first reproduction

The smallest female to spawn a measurable number of mature ova (in this case approximately 20,000), was sublegal, 33.1 mm in length and 17.6 mm in width. The smallest male to spawn was 36.7 mm in length and 19.2 mm in width. This indicates a slightly smaller size at first reproduction than that reported by Belding (1931), who noted that on the average New England clams became

sexually mature in their second year, when 1.5 in. (38.1 mm) in length. Loosanoff (1937b) determined that in *M. mercenaria* from Long Island Sound, ripe sperm were found within three to five months after setting, when clams were only 5-7 mm in length. Sperm could be discharged either at this time or at the end of the second summer. Most hard clams were protandric, and produced their first crop of ova in their second year. Histological analysis of *M. mercenaria* from South Carolina revealed that female clams were larger than males at the onset of gametogenesis, and that some clams smaller than 30 mm in length were ripe and spawning (Eversole et al., 1979).

Fecundity

As shown in Figures 3 and 4, there is no simple function relating size (measured by shell cavity volume or length), and egg production. Variability between individuals within the same size group was clearly extremely high.

In Figure 3 total egg production per female is plotted as a function of shell length. The linear correlation coefficient (r) for Sexton Island clams was 0.4998, and that for Carmans River clams was 0.3857, indicating that 25% and 15% of the respective variation in total egg production is attributable to the difference in size of clams. Since the data are not normally distributed, Spearman's rank correlation coefficient (r_s) was used; r_s was 0.3942 (n = 91), indicating that the association between length and number of eggs was significant at the 1% level. Lengths corresponding to the widths separating seed, littlenecks, cherrystones and chowders are marked in Figure 3. These limits represent only approximate values, calculated from a regression equation of shell length versus width based on combined data from both sampling sites (Table 1). However, clams from the two areas differ in their relative shell dimensions and length at which they reach legal width. Figure 3 also shows that though the scatter of the data is great, there is a noticeable rising and then leveling trend in maximum egg production as a function of increasing size.

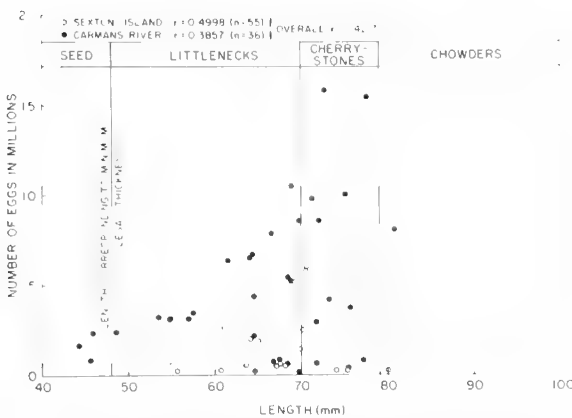

FIGURE 3. *Relationship between total egg production per female of* M. mercenaria, *in millions, and shell length, in mm. r: linear correlation coefficient.*

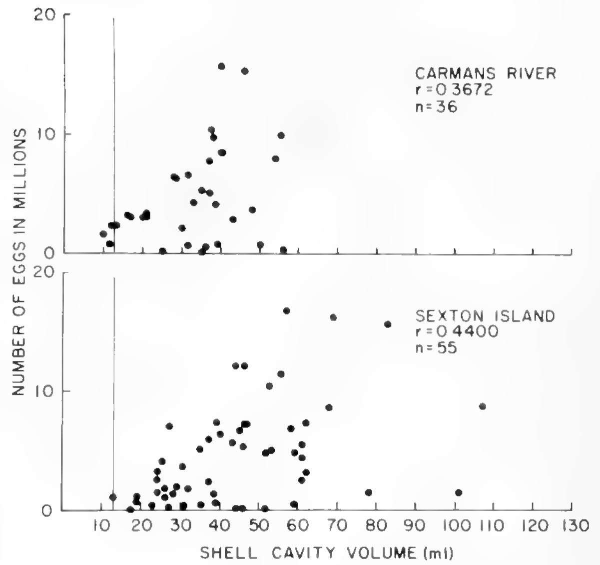

FIGURE 4. *Scattergram showing total egg production of* M. mercenaria, *in millions, as a function of shell cavity volume, in ml, obtained in this study. r: linear correlation coefficient. Vertical dividing lines indicate the volume corresponding to New York State's minimum legal thickness (width) as calculated from linear regression equations.*

The maximum number of eggs ($=15.8 \times 10^6$) for Carmans River clams was produced by a large cherrystone. However, only three chowders were represented in this sample. For Sexton Island, the maximum number of eggs ($= 16.8 \times 10^6$) was spawned by a cherrystone at the upper limit of its

TABLE 3. *Mean and range of egg production (in millions) of seed clams, littlenecks, cherrystones, and chowders from Sexton Island and Carmans River sampling sites in Great South Bay.*

	Sexton Island	Carmans River
Seed	(*)	$\bar{x}=1.614$ n=3 Range: 0.854-2.360
Littlenecks	$\bar{x}=2.390$ n=22 Range: 0.252-7.422	$\bar{x}=3.302$ n=15 Range: 0.197-7.879
Cherrystones	$\bar{x}=5.675$ n=17 Range: 0.291-16.795	$\bar{x}=7.088$ n=15 Range: 0.836-15.819
Chowders	$\bar{x}=6.323$ n=16 Range: 0.622-16.219	(**)

(*) No seed clams spawned.
(**) Values not included because sample size (n) is very small.

size category (classified as a cherrystone according to width, but with a length above the limit separating cherrystones from chowders). The second and third highest numbers of eggs (16.2×10^6 and 15.7×10^6) were produced by chowders. In both Davis and Chanley's (1956) and Ansell's (1967) experiments the maximum number of eggs was spawned by chowder clams.

Total egg production of the different size classes for both sites is shown in Table 3. Fecundity of clams from the two areas was compared using Wilcoxon's non-parametric two-sample test (Sokal and Rohlf, 1969, sec. 13.10). No significant difference ($P > 0.4$) was found between egg production of littlenecks from Sexton Island and Carmans River, or between that of cherrystones from the two areas ($P > 0.2$). Therefore, results from both sites were pooled to compare egg production of littlenecks, cherrystones and chowders, using Wilcoxon's test. Chowders and cherrystones produced a significantly greater number of eggs than littlenecks ($P < 0.01$ and $P < 0.001$ respectively). However, no significant difference ($P > 0.5$) was found between the fecundity of cherrystones and chowders.

In this study, shell length resulted in a slightly higher correlation with egg production than did total volume or shell cavity volume. The total number of eggs spawned versus shell cavity volume is plotted in Figure 4 in order to facilitate

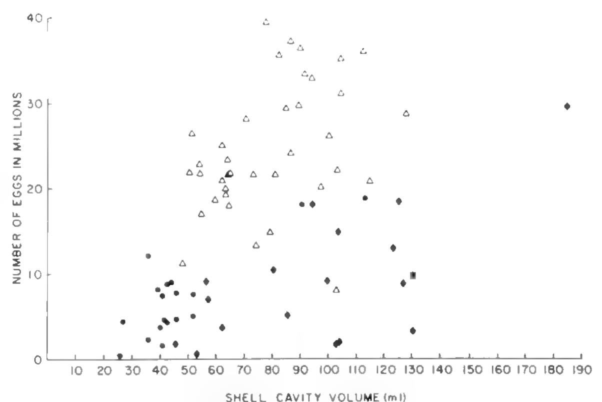

FIGURE 5. *Total number of eggs produced by* M. mercenaria, *in millions, as a function of shell cavity volume, in ml.* Δ: *from Davis and Chanley (1956);* ●: *from Ansell (1967), first experiment;* ♦: *from Ansell (1967), second experiment.*

TABLE 4. *Mean number of ripe oocytes per unit follicle area (0.391 mm²) of the gonad, remaining at the end of the spawning season. Range and standard deviation (sd) are indicated.*

Experimental Clams (n=10) Spawned in the Laboratory			Clams From Natural Populations in Great South Bay (n=10) Sampled 10/12/78		
$\bar{x}$(*)	sd	Range	$\bar{x}$(*)	sd	Range
1.3	2.25	0-13	0	0	—
40.0	6.80	24-52	14.2	4.23	5-22
26.3	6.39	12-36	29.5	4.70	21-41
31.6	4.39	24-40	15.8	3.46	11-23
25.5	4.37	17-33	42.4	8.19	30-59
23.7	6.62	10-34	1.3	0.99	0- 3
6.2	4.40	0-14	0.1	0.33	0- 1
13.3	5.94	6-27	7.0	2.17	3-12
24.1	7.34	6-33	0	0	—
15.5	4.98	6-27	0	0	—
Overall mean ± sd	20.8 ± 11.71		11.0 ± 14.73		

(*) Each mean was calculated from 25 replicate fields.

comparison with results of other investigators (Davis and Chanley, 1956; Ansell, 1967) shown in Figure 5. The linear correlation coefficient was 0.44 for the Sexton Island sample (composed of 22 littlenecks, 17 cherrystones and 16 chowders), and 0.37 for the Carmans River sample (3 seed clams, 15 littlenecks and 3 chowders). These values are consistent with an overall correlation coefficient of 0.38 obtained by Davis and Chanley (1956), which indicated that about 15% of the variation in fecundity was attributable to differences in size. Ansell (1967) found no significant correlation ($r = 0.26$) for his first experiment with animals collected in June, while finding a significant correlation ($r = 0.66$) at the 1% level for his second experiment with clams sampled in February and conditioned for one month.

Results of a quantitative comparison of gonadal histological sections of clams sampled in October from natural populations in the Bay, and clams spawned out in the laboratory for this study, are shown in Table 4. The data indicate that there is an extremely high variability in the number of residual oocytes among individuals. Further, out of a random sample of 13 clams spawned in the laboratory, only 15% had completely spent gonads, defined as those with less than two ripe ova per 0.39 mm² of follicle area. In contrast, 46% of the clams from natural populations evidenced spent gonads ($n = 13$). It appears that clams induced to spawn in the laboratory did not spawn as fully as those in the natural environment. Therefore, results of studies involving laboratory spawning probably underestimate natural fecundities. This method provides a better estimate of relative fecundities than of absolute fecundities expected in nature. However, since in *M. mercenaria* only partial evacuation of the gonad takes place during natural spawning, the alternative histological or stereological method will overestimate fecundities.

Histological analysis also revealed the presence of unspawned ripe ova surrounded and invaded by follicle cells and undergoing cytolysis in the gonad of a clam which had never been induced to spawn. This indicates that resorption constitutes a

possible mechanism of eliminating residual oocytes, though it is generally reported that these ova are lost by the process of extrusion (Loosanoff, 1937a).

The possible effects of stress conditions from long-term conditioning in the laboratory, on gametogenesis and fecundity, must be taken into consideration. Bivalves in an advanced stage of gonad development appear to be fairly tolerant to stress. Once gametogenesis is triggered, growth and maturation of gametes proceed in spite of marked temperature and nutritive stress, as long as there is a minimum amount of food or reserves present, and the temperature is not as high as to cause resorption of gametes (Bayne, 1975). The effects of stress conditions seem to depend on whether they occur at an early or late stage of the gametogenic cycle (Sastry, 1968). For example, starvation of *Ceratoderma (Cardium) edule* early in the resting stage prevents the initiation of gametogenesis, whereas starvation halfway through the resting stage has no effect (Gimazane, 1972). However, the fecundity of mussels *(Mytilus edulis)* held at a ration below the maintenance requirement for four weeks, was significantly reduced when compared with mussels provided with a high ration (Bayne et al., 1975). Conditioning of broodstock under stress conditions may also reduce the viability and vigor of the early stages of larval development (Bayne, 1972; Helm et al., 1973).

As determined from this study, approximately 15 to 25% of the variation in egg production of *M. mercenaria* is attributable to difference in size of clams. Therefore, female fecundity in shellfish such as the hard clam and the American oyster does not appear to be as strongly size dependent as in many fish populations (Bagenal, 1973). Although a dependence of egg production on food abundance over clam beds at some time prior to the spawning season has been suggested (Ansell, 1961), no significant difference in fecundity was detected in this study between two very dissimilar Bay habitats.

The large variability in fecundity within the same size group of clams may reflect genetic differences or differences in condition between individuals. A direct relationship between condition and "spawning potentiality" has been described in the hard clam (Ansell and Loosmore, 1963). Marked individual differences in spawning behavior and response to spawning stimuli, characteristic of this species (Loosanoff, 1937c), may account for a considerable proportion of the variation in egg production.

Davis and Chanley (1956) found that the mean egg production per female for clams collected from Long Island Sound in January, and spawned at intervals of 3, 7 and 14 days, was 24.6×10^6 (Range $= 8.0 \times 10^6$ - 37.3×10^6). Ansell (1967) estimated a mean fecundity per female of 7.11×10^6 (Range$=0.38 \times 10^6$ - 18.83×10^6) for his first experiment, and 9.28×10^6 (Range$=0.58 \times 10^6$-29.93×10^6) for his second experiment. He attributed this discrepancy with Davis and Chanley's results to differences in size of clams used, and to the fact that clams sampled for his study in winter had not undergone the main period of gonad proliferation.

Fecundity estimates reported here are considerably lower than those obtained by Davis and Chanley, and are more consistent with fecundities found by Ansell. Differences with Davis and Chanley's results could be partly ascribed to the use in this study of a longer conditioning period (May to early November) without supplementary feeding, and wider spawning interval. The discrepancy could also reflect differences in fecundity between Long Island Sound and Great South Bay populations. In addition, Davis and Chanley used a sample of clams dominated by chowders (82%), which included no littlenecks. In this study, a wide range of sizes was used, including many smaller clams (52% littlenecks).

It appears that the often quoted overall mean of 25 million eggs per female represents a rather high estimate of fecundity for Great South Bay populations, which are dominated by littlenecks under four years of age (Greene, 1978). Particularly in heavily exploited areas, clams are removed soon after they reach legal size and are therefore not allowed to grow to a larger size. It takes from 5.5 to 8 years for clams to reach cherrystone size, and over approximately 7 years to reach chowder size (Greene, 1978). Intensive harvesting has caused a sharp downward shift in size-frequency distributions. The evidence presented suggests that a continuing shift to smaller sizes could significantly reduce total egg production in the Bay.

The data presented suggests that chowders are at least as productive as cherrystones, and should consequently not be treated as an expendable group despite their low market value. There is no evidence to support a decline in egg production with increasing age. On the other hand, for use in spawner transplant programs or as parent stock for hatchery production of seed, large cherrystones or chowders may be used alternatively, since no significant difference in fecundities was found between the two size classes.

Results of this study suggest that the current New York State minimum size limit may be ineffective in protecting the breeding stocks in the Bay. Mean egg production by individual seed clams is only a small fraction, less than one third, of that produced by cherrystones. The maximum egg production of a large cherrystone is about eight times that produced by a seed clam. The State's minimum legal size should therefore be reexamined, or alternatively, regulatory efforts be directed to the preservation of selected clam beds dominated by larger clams which have the highest fecundities.

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LITERATURE CITED

Ansell, A.D. 1961. Reproduction, growth and mortality of *Venus striatula* (Da Costa) in James Bay, Millport. J. Mar. Biol. Assoc. U.K. **41**:191-215.

Ansell, A.D. 1964. Some parameters of growth of mature *Venus mercenaria* L.J. Cons. Int. Explor. Mer. **29**:214-220.

Ansell, A.D. 1967. Egg production of *Mercenaria mercenaria.* Limnol. Oceanogr. **12**(1):172-176.

Ansell, A.D. and F.A. Loosmore. 1963. Preliminary observations on the relationship between growth, spawning and condition in experimental colonies of *Venus mercenaria* L.J. Cons. Int. Explor. Mer. **28**(1):285-294.

Bagenal, T.B. 1973. Fish fecundity and its relations with stock and recruitment. Rap. p-v. Cons. Int. Explor. Mer. **164**:186-198.

Bayne, B.L. 1972. Some effects of stress in the adult on the larval development of *Mytilus edulis.* Nature **237**:459.

Bayne, B.L. 1975. Reproduction in bivalve molluscs under stress. In: Physiological Ecology of Estuarine Organisms, J. Vernberg (ed.), Univ. of South Carolina Press, Columbia, S.C.:259-277.

Bayne, B.L., P.A. Gabbott and J. Widdows. 1975. Some effects of stress in the adult on the eggs and larvae of *Mytilus edulis* L.J. Mar. Biol. Assoc. U.K. **55**:675-689.

Beech, G. 1975. Fortran IV in Chemistry. An introduction to computer-assisted methods. John Wiley and Sons:303 p.

Belding, D.L. 1931. The quahog fishery of Massachusetts. Marine Fish. Ser. 2, Div. Fish. Game, Dept. Cons. Comm. Mass. Boston.

Bricelj, V.M. 1979. Fecundity and related aspects of hard clam *(Mercenaria mercenaria)* reproduction in Great South Bay, New York. MS thesis, State Univ. of NY at Stony Brook.

Brousseau, D.J. 1978. Spawning cycle, fecundity, and recruitment in a population of soft-shell clam, *Mya arenaria,* from Cape Ann, Massachusetts. Fish. Bull. **76**(1):155-166.

Carpenter, E.J., Mar. Sci. Res. Ctr., SUNY at Stony Brook. Cessation of nutrient addition to a eutrophic estuary. Research proposal submitted to the Marine Water Quality Lab., Env. Protection Agency, June 1979.

Carriker, M.R. 1961. Interrelation of functional morphology, behavior, and autoecology in early stages of the bivalve, *Mercenaria mercenaria.* J. Elisha Mitchell Sci. Soc. **77**(2): 168-241.

Davis, H.C. and P.E. Chanley. 1956. Spawning and egg production of oysters and clams. Biol. Bull. **110**:117-128.

Eversole, A.G., W.K. Michener and P.J. Eldridge. 1979. Gonad development in relation to size and age of hard clams grown in a South Carolina estuary. Abstract in Nat. Shellfish. Assoc. Technical Sessions, Vancouver, B.C., June 1979.

Galtsoff, P.S. 1964. The American oyster *Crassostrea virginica.* Fish. Bull. **64**:48 p.

Gimazane, J.P. 1972. Etude experimentale de l'action de quelques facteurs externes sur la reprise de l'activite genitale de la coque, *Ceratoderma edule* L., Mollusque bivalve C.r. Seanc. Soc. Biol. **166**:587-589.

Glancy, J.B. 1956. Biological benefits of the Moriches and Shinnecock Inlets with particular reference to pollution and the shellfisheries. Rept. to the N.Y. Army Corps of Engineers: 18 p.

Greene, G.T. 1978. Population structure, growth and mortality of hard clams at selected locations in Great South Bay, New York, MS thesis, State Univ. of NY at Stony Brook:199 p.

Greene, G.T., A.C.F. Mirchel, W.J. Behrens and D.S. Becker. 1978. Surficial sediment and seagrasses of eastern Great Bay, NY, Mar. Sci. Res. Ctr., SUNY at Stony Brook, Sp. Report 12:30 p.

Helm, M.M., D.L. Holland and R.R. Stephenson. 1973. The effect of supplementary algal feeding of a hatchery breeding stock of *Ostrea edulus* L. on larval vigour. J. Mar. Biol. Assoc. U.K. **53**:673-684.

Heppell, D. 1961. The naturalization in Europe of the quahog, *Mercenaria mercenaria* (L.). J. Conch. **25**:21-34.

Jones, C.R. Distribution of surficial sediments and eelgrass in Great South Bay, New York. Unpublished manuscript.

Keck, R.T., D. Maurer and H. Lind. 1975. A comparative study of the hard clam gonad development cycle. Biol. Bull. **148**:243-258.

Loosanoff, V.L. 1937a. Seasonal gonadal changes of adult clams, *Venus mercenaria* (L.) Biol. Bull. **72**(3):406-416.

Loosanoff, V.L. 1937b. Development of the primary gonad and sexual phases in *Venus mercenaria* Linnaeus. Biol. Bull. **72**(3):389-405.

Loosanoff, V.L. 1937c. Spawning of *Venus mercenaria* (L.) Ecology **18**(4):506-515.

Loosanoff, V.L. 1953. Lack of relation between age of oysters or clams and quality of their spawn. Fish. Biol. Lab., Milford, Conn., Bull. **17**(4):1-2.

Loosanoff, V.L. and H.C. Davis. 1963. Rearing of bivalve molluscs. In: Advances in Marine Biology, Vol. 1, F.S. Russell (ed.), Academic Press.

McHugh, J.L. and R.B. MacMillan. 1976. Comprehensive report on the hard clam *(Mercenaria mercenaria)* industry in New York State. Unpublished manuscript. College of Marine Studies, Univ. of Delaware:1-57.

Orr, H., J.C. Marshall, T.L. Isenhour and P.C. Jurs. 1973. Introduction to computer programming for biological scientists. Allyn and Bacon, Inc. Boston.

Porter, H.J. 1964. Seasonal gonadal changes of adult clams, *Mercenaria mercenaria* (L.) in North Carolina. Proc. Natl. Shellfish Assoc. **55**:35-52.

Rose, D.C. and E. Heath. 1978. Viability of the American oyster, *Crassostrea virginica,* spermatozoa exposed to stress. Estuaries **1**(4):245-251.

Ryther, J.H. 1954. The ecology of phytoplankton blooms in Moriches Bay and Great South Bay, Long Island, New York. Biol. Bull. **106**:198-209.

Sastry, A.N. 1968. The relationship among food, temperature and gonad development of the bay scallops *Aequipecten irradians* Lamarck. Physiol. Zoology **41**(1):44-53.

Sokal, R.R. and F.J. Rohlf 1969. Biometry. The Principles and Practice of Statistics in Biological Research. W.H. Freeman and Co.:776p.

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GROWTH MODELS OF THE NORTHERN QUAHOG, *MERCENARIA MERCENARIA* (LINNÉ)

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ABSTRACT

The Gompertz growth equation provides an accurate model of ontogenetic growth in Mercenaria mercenaria *(Linné) from New Jersey waters. The equation yields a correlation coefficient (r) value of −0.982 when fitted to yearly height' data collected from sectioned valves of 277 specimens from death assemblages in Barnegat Bay. In addition, it predicts asymptotic height' values and growth curves that are realistic in comparison to those derived from the logistic and monomolecular growth equations. Height' (H') is defined as a dimension of the shell from the umbo to the ventral margin at a slight angle to the dorsal-ventral plane.*

Selection of the best fitting growth model for M. mercenaria *depends on the estimation of growth parameters in the Gompertz, logistic, and monomolecular functions. A new mathematical procedure is presented and applied in this study which allows for rapid calculation of these parameters. It requires only two steps: (1) the linearization of the growth functions; and (2) a linear regression analysis of the transformed data. This method of modeling has great potential application to ecological and paleoecological investigations of bivalve growth in general because most bivalves exhibit a growth rate that decreases according to a nonlinear function with increasing age. The Gompertz, logistic, and monomolecular equations accurately describe this type of growth.*

INTRODUCTION

Growth studies of the hard clam, *Mercenaria mercenaria* (Linné), have been of interest over the years as exemplified by the voluminous literature on the subject. However, with the exception of the work by Loesch and Haven (1973), there has been little effort at applying mathematical functions to describe ontogenetic growth of the bivalve. Most workers have performed growth studies on selected size and age ranges.

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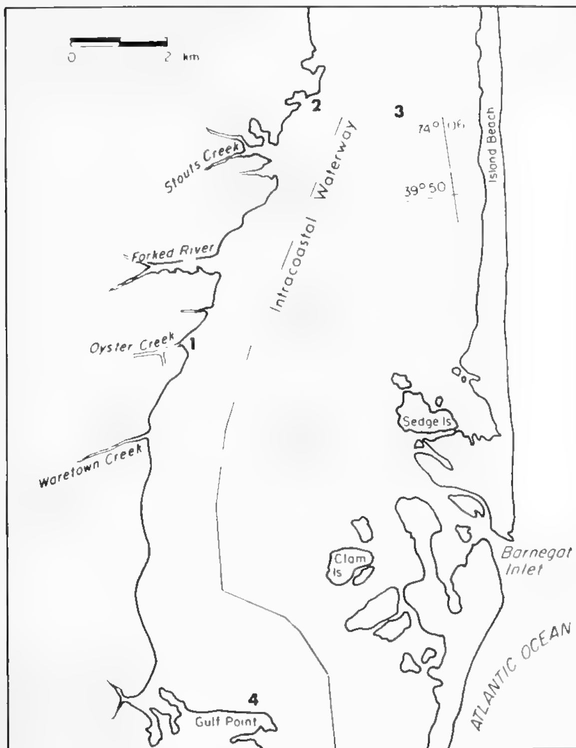

FIGURE 1. *Sampling sites (1-4) of Mercenaria mercenaria in Barnegat Bay, New Jersey.*

The objective of this paper is to generate growth models of *M. mercenaria* which will be of academic and economic importance. These models are used to describe and predict ontogenetic growth in the species. The mathematical equations employed in our investigation consist of the Gompertz, logistic, and monomolecular functions.

Mercenaria mercenaria lends itself ideally to ontogenetic growth studies because it secretes shell material daily (accretionary growth), which leaves a day by day history of its growth (Pannella and MacClintock, 1968; Rhoads and Pannella, 1970; Cunliffe and Kennish, 1974). This growth record can be discerned microscopically by examining valve cross sections of the bivalve. By counting and measuring daily growth increments and growth breaks from the umbo to the ventral valve margin, it is possible to accurately determine the size of the organism at any particular age (Kennish and Olsson, 1975; Kennish, 1978; Kennish, 1980).

MATERIALS AND METHODS

Growth rates of *M. mercenaria* were investigated in Barnegat Bay, a lagoon-type estuary located along the east coast of New Jersey (Figure 1). Kennish and Olsson (1975) and Kennish (1978) have reviewed the physical characteristics of the estuary. Death assemblages (assemblages of empty valves) (Johnson, 1960) of the clam were sampled at four sites in the bay during the summer of 1974 (Figure 1). Specimens were washed on 0.5 cm and 1 cm mesh screens, measured for size by vernier calipers accurate to 0.01 mm, and prepared for microscopic study.

Size measurements on the shells were made from the umbo to the ventral margin at a slight angle to the dorsal-ventral plane (Figure 2a). Therefore, the dimension measured only approximates the true height of the organism. This dimension has been labelled H′ to differentiate it from the true shell height (H). These two dimensions are related according to the following equation: $H' = 0.979\ H$.

Valves of dead specimens were studied using the shell microgrowth analysis technique of Barker (1964), Pannella and MacClintock (1968), and Rhoads and Pannella (1970). Following this method, valves were sectioned in a dorsal-ventral direction and acetate peel replicas of the valve cross sections were prepared (Kennish et al., 1980). A phase contrast petrographic microscope containing a graduated ocular was subsequently utilized to analyze microgrowth patterns in the peels.

Growth rates were determined by measuring annual increments of growth in valve cross sections from the umbo to the ventral margin. The basic microgrowth analysis employed to obtain these measurements were counts of daily growth increments and seasonal growth breaks (Figures 2b-i). Counts of daily growth increments were made across the shell, and daily growth increments were grouped into annual units and measured. Annual units were observed to correspond closely with seasonal growth breaks (Kennish, 1980).

GROWTH RATES AND GROWTH MODELS

A total of 277 specimens from the death assemblages at sites 1-4 in Barnegat Bay were examined microscopically. This included 101 valves from site 1, 74 from site 2, 64 from site 3, and 38

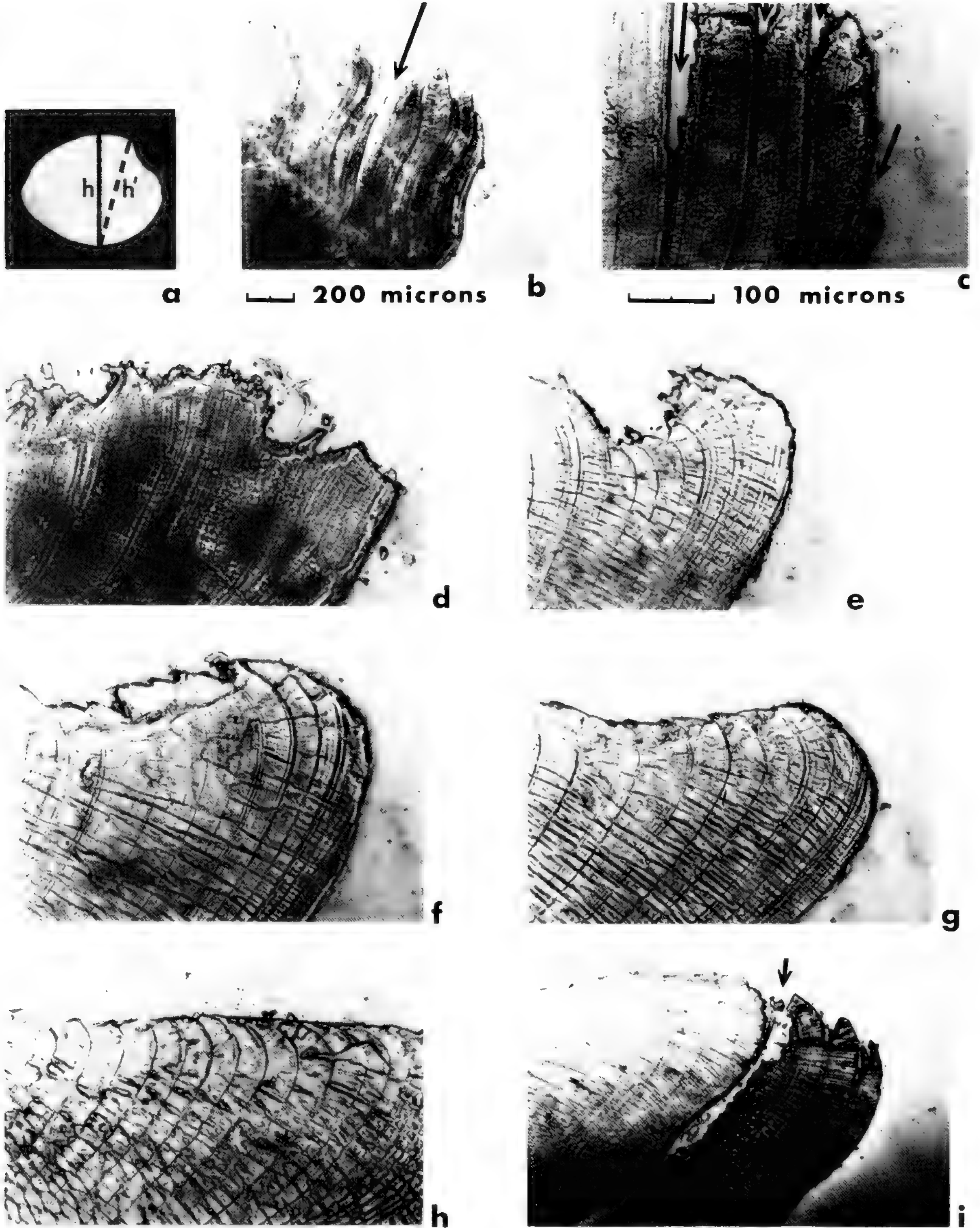
h
h'
a
200 microns
b
100 microns
c
d
e
f
g
h
i

FIGURE 2. *(a) Right hand valve of the northern quahog, M. mercenaria (Linné). The solid line (h) defines the true height of the organism. The dashed line (h') represents the dimension of height utilized in this study. (b-i) Microgrowth patterns in shells of M. mercenaria from Barnegat Bay. Two major types of microgrowth patterns occur in this species: (1) growth breaks; (2) daily growth increments. Growth breaks appear as V- or U-shaped notches in the outer shell layer (b, c, i). They form during periods of valve closure and anaerobic metabolism concomitant with periods of environmental or physiological stress. Seasonal growth breaks are used in age and growth rate determinations, serving as datums. Some growth breaks are annual. These can be counted and measured to establish the age and size of the organism at death and the season of death (Kennish, 1980). The basic unit of growth in the shell of M. mercenaria is the daily growth increment. A daily growth increment appears in cross section as a calcium-carbonate-rich layer between successive organic-rich lines (e-h). When the valves of the organism are open and water is freely pumped, aerobic metabolism occurs and calcium-carbonate-rich regions of the shell are deposited. When the valves of the clam are closed, anaerobic respiratory pathways are utilized, resulting in organic-rich growth lines. Because growth increments form on a daily basis, it is possible to determine the size of the organism at any particular age by counting and measuring the increments from the umbo to the ventral valve margin. (b-d) Shell microgrowth patterns in specimens that died in the early spring include thin growth increments (slow growth), high organic content, and growth breaks (arrows). Scale of (d) equals that of (c). (e) Ventral valve margin of a clam that died in late spring. Daily growth increments are thicker than those of clams that died in the early spring. High organic content is absent (see b-d). Scale equals that of (c). (f-h) Summer microgrowth patterns. Thick daily growth increments characteristic of rapid summer growth precede the shell margins. Scales equal that of (c). (i) A growth break (arrow) in the shell of* M. mercenaria *during the summer season. Note the deep notch in the shell and reduced growth subsequent to the break. Scale equals that of (b) (Kennish, 1978).*

from site 4; the valves ranged from 14-76.5 mm in height'. The mean age-height' statistics of each sample and the pooled samples are presented in Tables 1 and 2, respectively.

Inspection of the data reveals extremely uniform growth rates at all four localities, with growth being fastest and nearly linear for the first three years of life and dropping off gradually into the gerontic stage. Most of the clams are less than seven years of age. Apparently, growth begins to slow when the organism attains sexual maturity at age two (Carriker, 1961; Rhoads and Pannella, 1970), and energy is diverted into reproductive processes. Juvenile and adult age specific sizes are nearly identical at sites 1 and 2, slightly greater at site 4, and somewhat reduced at site 3, but these differences are minor. Furthermore, a tendency exists for clams to reach a common size in old age in all assemblages due to a reduction in growth rates and a decline in absolute and relative variability of growth.

The growth pattern exhibited by *M. mercenaria* in Barnegat Bay, in which the bivalve tends to undergo a gradual, more or less nonlinear decline of growth rate with age, can be described mathematically. Three of the most widely used equations in the study of metazoan growth are the logistic, Gompertz, and monomolecular (Price, 1970; Green, 1979). They all have one concept in common — the tendency for the size of the organism to reach an asymptotic limit in a finite period of time.

A major objective is to establish which of these three expressions best represents the growth of *M. mercenaria* in Barnegat Bay. This requires fitting each equation to the growth data and selecting the best fitting model. Predictive growth curves can be constructed once parameters are estimated and predictive functions formulated.

Since growth models are most often expressed as continuous or deterministic functions, then it is possible to algebraically manipulate either the

TABLE 1. *Mean age-height' relationships observed in death assemblages of clams at sites 1-4 (After Kennish, 1978).*

	Site 1, N* = 101			Site 2, N* = 74			Site 3, N* = 64			Site 4, N* = 38		
Age	N**	Mean height' (mm)	Std. dev.	N**	Mean height' (mm)	Std. dev.	N**	Mean height' (mm)	Std. dev.	N**	Mean height' (mm)	Std. dev.
1	101	12.39	3.76	74	11.36	4.16	64	7.04	2.71	38	12.72	5.15
2	89	23.00	4.49	68	23.51	4.96	64	17.70	4.24	35	25.61	6.19
3	65	32.91	4.49	63	35.01	4.89	63	29.54	5.22	25	37.30	6.42
4	50	42.36	5.26	44	43.78	4.15	54	39.55	5.23	19	46.97	5.25
5	28	49.37	6.27	19	49.89	3.01	28	47.75	5.81	12	55.58	7.05
6	16	57.61	7.76	9	56.18	3.94	10	55.11	4.05	6	62.13	8.88
7	6	59.52	5.41	6	63.60	1.70	7	63.30	4.56	3	65.80	3.04
8	0	——	—	4	70.60	1.75	3	69.13	5.95	0	——	—

N* = number of clams
N** = number of size measurements per age

function or its derivative to the extent that a linear form of the equation in two variables is generated. Conveniently, all three models chosen in this study are readily converted into a linear form. For example, the familiar logistic equation:

$$H_t = H_{max}\,[1+e^{-kt}\,.\,\frac{H_{max}-H_o}{H_o}]^{-1} \qquad (1)$$

where H_{max} is the maximum or asymptotic size, H_o is the initial size, and k is the intrinsic growth factor, can be expressed in differential form as:

$$\frac{dH_t}{dt} = kH_t - bH_t^2 \qquad (2)$$

where, $k/b = H_{max}$.

Dividing (2) by H_t to obtain:

$$\frac{dH_t}{dt \cdot H_t} = k - bH_t \qquad (3)$$

where (3) represents the simple linear decrease in the specific growth factor (Pielou, 1969), we have effectively linearized the logistic function.

For sufficiently close measurements of H_t, for example when Δt is short, dH_t/dt may be approximated (e.g., $(H_{t+1} - H_t)/\Delta t = \Delta H/\Delta t \approx dH/dt$). If one then divides by the average of H_t over the interval, then a reasonable estimate of instantaneous k can be determined (e.g., let $H_{avg} = (H_t + H_{t+1})/2$, then $dH_t/dt \cdot H_{avg} = (H_{t+1} - H_t)/H_{avg}$). Plotting all values of H_{avg} (X-axis) against $dH/dt \cdot H_{avg}$ (Y-axis) will yield a straight line of slope $= -b$ and Y-intercept of k.

Thus, by knowing only the actual values of H_t measured in field collections, it is possible to calculate a predicted value of H_{max} and k, where:

$H_{max} = k/b = $ (Y-intercept)/slope

The Gompertz growth equation:

$H_t = H_{max}\,.\,e^{-p\cdot e^{-kt}}$ (Ricklefs, 1967) (4)

is converted into its linear form:

$$\frac{dH_t}{dt \cdot H_t} = k \cdot \ln H_{max} - k \cdot \ln H_t \qquad (5)$$

TABLE 2. *Mean age-height' relationships for the pooled death assemblages of clams from sites 1-4 (After Kennish, 1978).*

	N* = 277		
Age	N**	Mean height' (mm)	Std. dev.
1	277	10.92	4.44
2	256	22.17	5.51
3	216	33.05	5.67
4	167	42.35	5.47
5	87	49.82	6.11
6	41	57.35	6.65
7	22	62.69	4.37
8	7	69.97	3.73

N* = number of clams
N** = number of size measurements per age

Plotting $\ln H_{avg}$ on the X-axis against $dH_t/dt \cdot H_{avg}$ on the Y-axis generates a linear data set with the slope yielding k and the Y-intercept yielding H_{max}. Again, only information regarding the actual values of H_t is necessary to estimate the parameters of the growth equation. For the Gompertz model, one must calculate the value of p which determines the initial size, H_0, after solving for k and H_{max}.

Finally, the monomolecular model, which is a more general form of the von Bertalanffy equation, albeit equivalent when c = 1, is given by:

$$H_t = H_{max} \cdot (1 - ce^{-kt}) \text{ (Price, 1970)} \quad (6)$$

and is converted into its linear form:

$$\frac{dH_t}{dt} = kH_{max} - kH_t$$

This differential form does not require any knowledge of H_{max} as postulated by Green (1979). A simple plot of H_{avg} against dH_t/dt gives very satisfactory estimates of the parameters and the asymptotic value, H_{max}. However, it is necessary to calculate the correct value of c which sets the initial condition of H_t $(= H_0)$.

In some cases where growth or size is being measured, it may be impossible to record every value of H_t for every time interval, Δt. We have found that linear interpolation of the missing data points will lower the accuracy of predicting both k and H_{max}, but the proposed technique still gives very high correlation coefficients irrespective of the missing data.

A machine language program is presented in the Appendix for use in estimating the growth parameters of the three models employed in this study. The program is specifically designed for a Texas Instrument TI-59 hand-held programmable calculator.

We have noted that the growth rates of *M. mercenaria* are remarkably consistent, with little deviation in growth measurements per age class from one individual to another. Thus, we were not concerned with the precision demanded by Knight (1968) because only height′ values are used in the parameter estimation technique. H_{max} is presumably not known in field studies and cannot, therefore, be programmed into the current method; but H_{max} can be reasonably predicted based on actual data. That is, if additional size data were available for clams from Barnegat Bay, then we could further refine our estimates of H_{max}.

We also disregard the reparameterization technique of Gallucci and Quinn (1979) because our method does not require apriori knowledge of H_{max}. Furthermore, in calculating the linear regression, best-fit for the transformed data set (H_{avg} and dH_t/dt), we automatically determine variance. For height′ measurements of clams in the present study, variance estimates were generally low for all models.

Our methodology requires only one iteration of the data set and, therefore, is far more economical than the trial-and-error methods previously suggested by Ricklefs (1967), Krebs (1978), and Poole (1974). It has been applied by Crossner (1977) in solving for the logistic parameters in the growth of starlings. Crossner contributed to the development of the technique in association with the second author of this paper.

Because growth of *M. mercenaria* was similar in all assemblages studied (Table 1), the logistic, Gompertz, and monomolecular equations were fitted to the pooled death assemblages of clams at sites 1-4. Initially, the linear regression yielded the predictive values of H'_{max}, k and b; the parameters p and c were subsequently calculated in order to determine the initial conditions. The growth equations were iterated based on the predicted parameters and initial conditions. Table 3 lists the parameters, constants, and correlation coefficients recorded for each equation. Predictive growth curves for the pooled death assemblages of clams are shown in Figure 3.

The parameters b, c, and p in the logistic, Gompertz, and monomolecular equations have no biological significance. Because of this, they cannot be compared across the models for the purpose of selecting the best growth model. Although the parameter k has biological significance, it cannot be compared across parameters of the different models because each model defines k differently. Statistically, all of the equations fit the growth data of *M. mercenaria* quite well as is evident from the high correlation coefficient values (≥ -0.914). The degree of fit is best for the Gompertz function, with a correlation coefficient value of -0.982. The monomolecular equation displays the poorest fit to the data ($r = -0.914$) and the logistic equation an intermediate fit ($r = -0.927$) between the Gompertz and monomolecular (Table 3).

TABLE 3. *Constants (H'_{max}), correlation coefficients (r), and estimated parameters (b, c, k, p) of the logistic, Gompertz, and monomolecular growth equations. The data represent the best fit of the linearized equations to the mean annual growth rates of 277 Mercenaria mercenaria from sites 1-4.*

	Constant	Correlation Coefficient	Parameter			
Equation	H'_{max} (mm)	r	b	c	k	p
Logistic	67.61	−0.927	0.011	-	0.745	-
Gompertz	74.22	−0.982	-	-	0.424	-1.79
Monomolecular	121.35	−0.914	-	1.01	0.109	-

DISCUSSION

In reality, the logistic equation is unacceptable as a model for *M. mercenaria* growth because it predicts a growth curve with an inflection later in ontogeny, and it underestimates the asymptotic height'. The observed growth curve for this species reveals no such inflection (Figure 3). If an inflection occurs during ontogeny, it may be confined to the meroplanktonic stage.

The monomolecular equation does not fit the growth data as well statistically as the logistic and Gompertz functions, and it greatly overestimates the asymptotic height', yielding an H'_{max} value of 121.35 mm (Table 3). No clams in the death assemblages at sites 1-4 exceed 76.5 mm in height'. Based on these factors, the monomolecular equation is also rejected as a model of growth of *M. mercenaria* in Barnegat Bay.

The Gompertz model closely fits the observed growth for the pooled death assemblages of clams. In addition, it predicts as asymptotic height' of 74.22 mm which is more realistic than those of the logistic and monomolecular models. In effect, it represents the most accurate model tested, and is useful for comparing and contrasting the growth of *M. mercenaria* throughout the estuary.

Among the oldest clams, the Gompertz model predicts low growth rates. This causes the projected growth curve to deviate slightly from the observed growth curve among the larger sizes (Figure 3). The same effect has been noted for other species of clams. For instance, when fitting the Gompertz equation to growth data of the razor clam, *Siliqua patula* (Dixon), Weymouth et al. (1931) became aware of "growth being maintained at a higher rate than the first part of the curve would lead one to predict." Laird et al. (1965) explained the deviation in old age experienced by Weymouth et al. (1931) as due to ad-

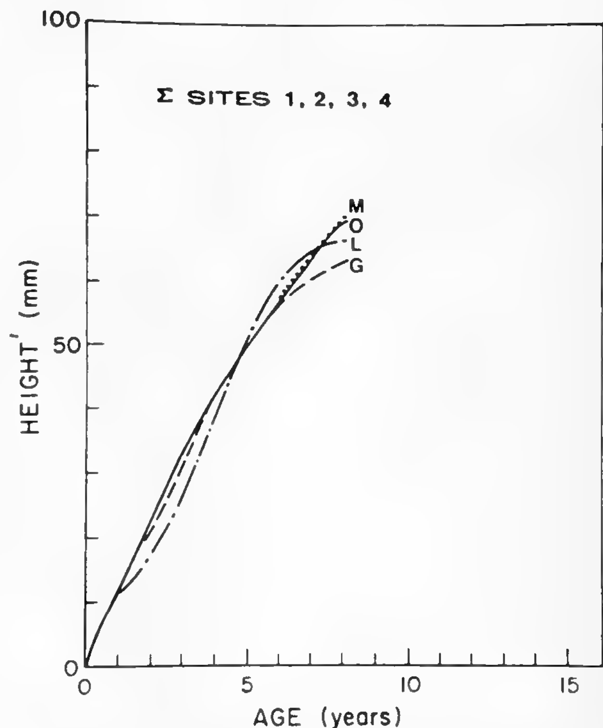

FIGURE 3. *Observed and predicted cumulative growth curves for the pooled death assemblages of clams at sites 1-4.*

O = observed growth

L = logistic model =

$$.745/.011\ [1 + e^{-.745t} \cdot (\frac{67.61-10.92}{10.92})]^{-1}$$

G = Gompertz model = $74.22e^{-1.79e^{-.424t}}$

M = monomolecular model =

$$121.35\ (1 - 1.01e^{-.109t})$$

ditional, accretionary growth that is nearly linear with respect to time.

Although the Gompertz model accurately models shell growth of *M. mercenaria*, the most precise mathematical model would appear to be one possessing a combination of the characteristics of the Gompertz and monomolecular functions. Such a model should eliminate anomalous estimates of growth rates in older clams. Hopefully, this problem will be investigated in future research.

SUMMARY

Growth rates of 277 *M. mercenaria* from four death assemblages in Barnegat Bay, New Jersey were examined by employing microgrowth analysis of shell cross sections. These specimens displayed similar growth rates, with growth decreasing with increasing age.

The logistic, Gompertz, and monomolecular equations were fitted to the growth data collected on the 277 clams, and predictive growth models were formulated. The Gompertz equation accurately modeled ontogenetic growth in the species.

ACKNOWLEDGEMENTS

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APPENDIX

A program is presented to calculate the parameters and constants of the logistic equation with allowance for missing data points. Codes are designed for a Texas Instrument TI-59 programmable calculator. User instructions follow:

1. Load program as listed (steps 000-203).
2. Press clear memory registers (CMs) and reset (RST) in order to zero all registers (this must be done for every new data set).
3. Press user defined key B:
   a) enter total number of data points (including missing data).
   b) press R/S then enter first data point and press R/S again.
   c) if missing data occur, press SET FLAG 1 before R/S, then enter next available data point and the number of missing data points. Calculator supplies linear estimates of missing data.
   d) after last data point is added, machine will not accept more data.
   This data set can now be used for any of the three models.
4. In this example, the calculations for the logistic parameters and constants are programmed. Simply press user defined Key E, and the intermediate linear data will be generated as well as the results of the linear regression analysis.

step	code	mnemonic	step	code	mnemonic	step	code	mnemonic	step	code	mnemonic	step	code	mnemonic
000	76	LBL	041	75	-	082	73	RC*	123	15	E	164	54	)
001	11	A	042	43	RCL	083	09	09	124	71	SBR	165	55	÷
002	36	PGM	043	20	20	084	85	+	125	11	A	166	43	RCL
003	01	01	044	95	=	085	43	RCL	126	05	5	167	17	17
004	71	SBR	045	67	EQ	086	14	14	127	09	9	168	95	=
005	25	CLR	046	13	C	087	95	=	128	42	STO	169	42	STO
006	92	RTN	047	71	SBR	088	99	PRT	129	09	09	170	18	18
007	76	LBL	048	37	P/R	089	69	OP	130	01	1	171	99	PRT
008	12	B	049	61	GTO	090	39	39	131	42	STO	172	43	RCL
009	05	5	050	00	00	091	72	ST*	132	15	15	173	17	17
010	09	9	051	28	28	092	09	09	133	99	PRT	174	32	X⇌T
011	75	-	052	91	R/S	093	97	DSZ	134	97	DSZ	175	43	RCL

012	91	R/S	053	69	OP	094	08	08	135	00	00	176	18	18
013	42	STO	054	29	29	095	14	D	136	10	E′	177	78	Σ+
014	00	00	055	42	STO	096	69	OP	137	19	D′	178	71	SBR
015	99	PRT	056	12	12	097	39	39	138	76	LBL	179	37	P/R
016	95	=	057	99	PRT	098	22	INV	139	10	E′	180	61	GTO
017	42	STO	058	91	R/S	099	86	STF	140	73	RC*	181	01	01
018	20	20	059	42	STO	100	01	01	141	09	09	182	34	34
019	01	1	060	08	08	101	71	SBR	142	42	STO	183	76	LBL
020	42	STO	061	99	PRT	102	37	P/R	143	16	16	184	19	D′
021	15	15	062	43	RCL	103	61	GTO	144	53	(	185	79	$\bar{x}$
022	99	PRT	063	12	12	104	00	00	145	24	CE	186	99	PRT
023	05	5	064	75	-	105	28	28	146	85	+	187	55	÷
024	09	9	065	73	RC*	106	91	R/S	147	69	OP	188	32	X⇌T
025	42	STO	066	09	09	107	76	LBL	148	39	39	189	99	PRT
026	09	09	067	95	=	108	37	P/R	149	73	RC*	190	69	OP
027	29	CP	068	55	÷	109	43	RCL	150	09	09	191	12	12
028	91	R/S	069	53	(	110	15	15	151	54	)	192	99	PRT
029	99	PRT	070	43	RCL	111	85	+	152	55	÷	193	32	X⇌T
030	72	ST*	071	08	08	112	01	1	153	02	2	194	99	PRT
031	09	09	072	85	+	113	95	=	154	95	=	195	69	OP
032	69	OP	073	01	1	114	42	STO	155	42	STO	196	13	13
033	39	39	074	54	)	115	15	15	156	17	17	197	99	PRT
034	91	R/S	075	95	=	116	99	PRT	157	99	PRT	198	22	INV
035	87	IFF	076	42	STO	117	92	RTN	158	53	(	199	79	$\bar{x}$
036	01	01	077	14	14	118	91	R/S	159	73	RC*	200	99	PRT
037	00	00	078	76	LBL	119	76	LBL	160	09	09	201	32	X⇌T
038	52	52	079	14	D	120	13	C	161	75	-	202	99	PRT
039	43	RCL	080	71	SBR	121	91	R/S	162	43	RCL	203	91	R/S
040	09	09	081	37	P/R	122	76	LBL	163	16	16			

LITERATURE CITED

Barker, R.M. 1964. Microstructural variation in pelecypod shells. Malacologia **2**:69-86.

Carriker, M.R. 1961. Interrelation of functional morphology, behavior, and autecology in early stages of the bivalve *Mercenaria mercenaria*. J. Elisha Mitchell Sci. Soc.**77**:168-241.

Crossner, K.A. 1977. Natural selection and clutch size in the European starling. Ecology **58**:885-892.

Cunliffe, J.E. and M.J. Kennish. 1974. Shell growth patterns in the hard-shelled clam. Underwater Nat. **8**:20-24.

Gallucci, V.F. and T.J. Quinn II. 1979. Reparameterizing, fitting, and testing a simple growth model. Trans. Amer. Fish. Soc. **108**:14-25.

Green, R.H. 1979. Sample design and statistical methods for environmental biologists. Wiley-Interscience, John Wiley & Sons, N.Y., 257 pp.

Johnson, R.G. 1960. Models and methods for analysis of the mode of formation of fossil assemblages. Geol. Soc. Amer. Bull. **71**:1075-1086.

Kennish, M.J. 1978. Effects of thermal discharges on mortality of *Mercenaria mercenaria* in Barnegat Bay, New Jersey. Environ. Geol. **2**:223-254.

Kennish, M.J. 1980. Shell microgrowth analysis: *Mercenaria mercenaria* as a type example for research in population dynamics, in D.C. Rhoads and R.A. Lutz, eds., Skeletal growth of aquatic organisms. Plenum Press, N.Y., 255-294

Kennish, M.J., R.A. Lutz and D.C. Rhoads. 1980. Preparation of acetate peels and fractured sections for observation of growth patterns within the bivalve shell, in D.C. Rhoads and R.A. Lutz, eds., Skeletal growth of aquatic organisms. Plenum Press, N.Y., 597-601.

Kennish, M.J. and R.K. Olsson. 1975. Effects of thermal discharges on the microstructural growth of *Mercenaria mercenaria*. Environ. Geol. **1**:41-64.

Knight, W. 1968. Asymptotic growth: an example of nonsense disguised as mathematics. J. Fish. Res. Board Can. 25:1303-1307.

Krebs, C.J. 1978. Ecology, the experimental analysis of distribution and abundance. Harper and Row, N.Y., 678 pp.

Laird, A.K., S.A. Tyler and A.D. Barton. 1965. Dynamics of normal growth. Growth **29**:233-248.

Loesch, J.G. and D.S. Haven. 1973. Estimated growth functions and size-age relationships of the hard clam *M. mercenaria* in the York River, Virginia. Veliger **16**:76-81.

Pannella, G. and C. MacClintock. 1968. Biological and environmental rhythms reflected in molluscan shell growth, in D.B. Macurda, ed., Paleobiological aspects of growth and development, a symposium. Paleont. Soc. Mem. 2 (J. Paleont. 42 (5) supp.) pp. 64-80.

Pielou, E.C. 1969. An introduction to mathematical ecology. Wiley-Interscience, John Wiley & Sons, N.Y., 279 pp.

Poole, R.W. 1974. An introduction to quantitative ecology. McGraw-Hill, Inc., N.Y., 532 pp.

Price, C.A. 1970. Molecular approaches to plant physiology. McGraw-Hill Book Company, N.Y., 398 pp.

Rhoads, D.C. and G. Pannella, 1970. The use of molluscan shell growth patterns in ecology and paleoecology. Lethaia **3**:143-161.

Ricklefs, R.E. 1967. A graphical method of fitting equations to growth curves. Ecology. **48**:978-983.

Weymouth, F.W., H.C. McMillin and W.H. Rich. 1931. Latitude and relative growth in the razor clam, *Siliqua patula*. J. Exp. Biol. **8**:228-249.

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FOOD OF THE KING CRAB, PARALITHODES CAMTSCHATICA AND THE DUNGENESS CRAB, *CANCER MAGISTER* IN COOK INLET, ALASKA[1]

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ABSTRACT

The three most frequently observed prey types in the stomachs of the king crab, Paralithodes camtschatica, *in Kamishak Bay, Cook Inlet, were barnacles, bivalves of the family Mytilidae and hermit crabs which appeared in 81, 13, and 12% of the stomachs, respectively. In Kachemak Bay, Cook Inlet, the clam,* Spisula polynyma, *was observed in 38% of the king crab stomachs. Barnacles and the snail* Neptunea lyrata *occurred in 14 and 11% of the stomachs, respectively.* Cancer magister, *the Dungeness crab, in Kachemak Bay, preyed primarily on small* S. polynyma *which occurred in 48% of the stomachs. Barnacles and amphipods were found in 11 and 6% of the stomachs, respectively.*

INTRODUCTION

The king crab, *Paralithodes camtschatica,* and the Dungeness crab, *Cancer magister,* are commercially harvested in Cook Inlet, Alaska (Figure 1). Approximately 60% of Cook Inlet king crabs are caught in Kamishak Bay, an additional 34% are taken in Kachemak Bay, and the remainder are captured near the mouth of the Inlet. Dungeness crab occur primarily in Kachemak Bay. Catches of king and Dungeness crabs from the Inlet averaged 1,860 and 141 metric tons, respectively, during 1971-1975 (Alaska Department of Fish and Game 1976 Catch and Production Statistical Leaflet No. 28). No information is currently available on the diet of these crabs in the Inlet.

[1] Contribution No. 390, Institute of Marine Science, University of Alaska.

METHODS

Paralithodes camtschatica and *Cancer magister* were collected on seven cruises from October 1976 to August 1978. Collections were made primarily with various size trawls. One collection of *C. magister* at Station D1 was made by SCUBA divers. The average carapace length of king crabs examined was 105 mm with a range of 35 to 150 mm. The average shell width of Dungeness crabs taken with trawls was 142 mm, ranging from 22 to 210 mm. On one occasion, 64 *C. magister* less than 50 mm carapace width were captured; these are treated separately. The location of the sampling stations are shown in Figure 1. Stomachs were removed and fixed in 10% buffered formalin for examination in the laboratory under a dissection microscope at 750 magnifications. Prey were identified to the lowest possible taxon and counted. Additional sampling with dredges,

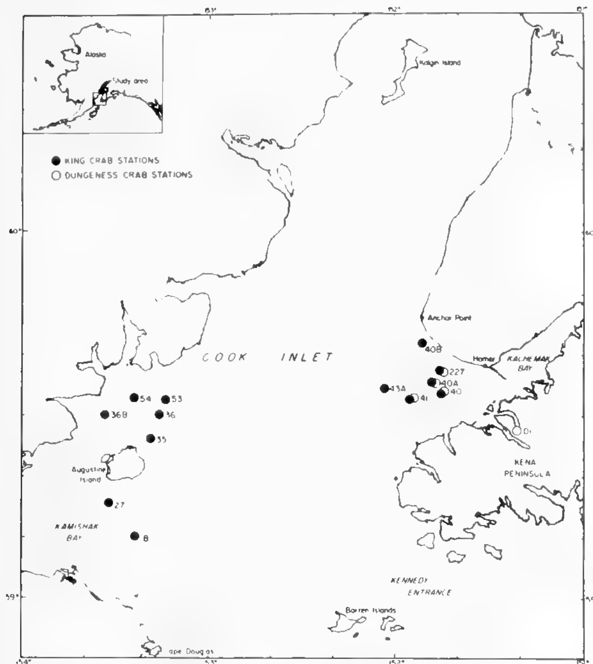

FIGURE 1. *The location of stations in Cook Inlet, Alaska where king crab,* Paralithodes camtschatica, *and dungeness crab,* Cancer magister, *were collected for this investigation.*

grabs, and fine-mesh trawls at each station made it possible to obtain information on potential prey, and facilitated identification of stomach contents. The contents of the stomachs of 18 king crabs feeding exclusively on barnacles at Station 35 in November 1977 were digested in KOH, rinsed with distilled water and the dry weight of barnacle shell determined. The total live weight of 100 barnacles taken from trawls at Station 35 was determined and the average wet meat weight calculated after KOH digestion. An estimation of the average number of barnacles, based on shell weight, in each stomach was made. By examining shell thickness and sizes of the resilium of the shells of the clam *Spisula polynyma* in stomachs, it was possible to estimate sizes and ages of the clams eaten (Feder et al., 1978).

RESULTS AND DISCUSSION

Paralithodes camtschatica

One hundred and seventeen king crabs were examined from Kamishak Bay; 90% of the stomachs contained food. The three most frequently observed prey were barnacles, mussels of the family Mytilidae, and hermit crabs which appeared in 81, 13 and 12% of the stomachs, respectively. In addition, 17 other categories of food items were observed; none occurred in more than 6% of the stomachs. Bivalves occurred in 27% of the stomachs, and gastropods were found in 12% of the stomachs (Table 1). In May 1978, 41% of the crabs with empty stomachs were newly molted or molting. Eighteen king crabs collected at Station 35 in Kamishak Bay in November 1977 had full stomachs, and were feeding exclusively on the barnacle *Balanus crenatus.* The stomachs contained the equivalent of 11.2 (SD = 7.4) barnacles per crab. The average wet meat weight for the eleven barnacles was 2 g.

In Kachemak Bay, 113 king crabs were captured, and 72% contained food. Bivalves occurred in 60% of the stomachs. The clam, *Spisula polynyma,* was the most frequently occurring prey species, and was observed in 38% of the stomachs. Barnacles were found in 14% of the stomachs. The snail *Neptunea lyrata* occurred in 11% of the stomachs (Table 2).

Spisula polynyma less than 10 mm in length, young-of-the-year or one year old clams, occurred in 30 of the 43 stomachs. Only 13 stomachs contained large *S. polynyma* meats with pieces of clam shell exceeding 1 mm in thickness. *Spisula polynyma* with shells of this thickness are approximately 80 mm in length or larger and at least 9 years old (Feder et al., 1978). Pieces of *Neptunea lyrata* opercula up to 15 mm in length were found in the stomachs of adult crab.

Most of the king crabs in Kachemak Bay, in contrast to Kamishak Bay, contained the remains of a variety of organisms. For example, one specimen contained 21 young-of-the-year *S. polynyma*, 2 *Solariella* sp., 1 *Oenopota* sp., and shells of *Balanus* sp.

Only 16 king crabs were captured at station 6 near the mouth of the Inlet. In the 12 specimens that contained food, 10 had eaten the protobranch clam, *Nuculana fossa.* Each of these stomachs contained between 10 and 25 of these small bivalves. The clam *Macoma* sp. occurred in 4 stomachs, and one stomach had unidentifiable crustacean remains (Feder et al, 1978).

TABLE 1. *Food of* Paralithodes camtschatica *from Kamishak Bay, Cook Inlet, Alaska.*

Station	Date month/year	No. stomachs examined	No. with food	Hydrozoa	Bryozoa	Polychaeta	*Solariella* spp.*	*Polinices* spp.	*Neptunea lyrata*	Unid. Gastropoda	Gastropod eggs	*Nucula tenuis*	*Nuculana fossa*	*Glycymeris subobsoleta*	Mytilidae	*Macoma* spp.	*Tellina nuculoides*	Unid. Bivalvia	*Balanus* spp.	Amphipoda	Paguridae	Unid. Crustacea	Plant material
18	6/78	5	5	-	-	-	4	1	-	1	-	2	1	2	-	1	-	-	-5	1	1	-	-
27	6/78	30	30	2	1	-	1	-	-	1	2	-	-	-	14	-	1	1	30	-	9	-	-
35	11/77	36	36	-	-	1	-	-	1	1	-	-	1	1	1	-	-	-	29	-	1	-	1
35	5/78	22	17	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	17	-	-	-	1
35	6/78	13	13	1	-	-	-	1	-	1	-	-	1	-	-	-	-	1	13	-	3	-	-
36	5/78	3	1	-	-		-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-
36B	5/78	2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
53	11/77	3	3	-	-	-	-	-	1	1	-	-	2	-	-	-	-	-	-	1	-	1	-
54	5/78	3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total frequency of occurrence		117	105	3	1	1	5	2	2	5	2	2	7	3	15	2	1	2	95	2	14	1	2
Percent frequency of occurrence			90	3	1	1	4	2	2	4	2	2	6	3	13	2	1	2	81	2	12	1	2

*The genus *Margarites* occurs in the area and may be included in this category.

TABLE 2. *Food of* Paralithodes camtschatica *from Kachemak Bay, Cook Inlet, Alaska.*

Station	Date month/year	No. stomachs examined	No. with food	Foraminifera	Hydrozoa	Bryozoa	Polychaeta	*Solariella* spp.*	*Neptunea lyrata*	*Oenopota* spp.	Unid. Gastropoda	Gastropod eggs	*Nucula tenuis*	*Nuculana fossa*	*Glycymeris subobsoleta*	*Modiolus Modiolus*	*Chlamys* spp.	*Clinocardium ciliatum*	*Spisula polynyma*	*Macoma* spp.	*Tellina nuculoides*	Unid. Bivalvia	*Balanus* spp.	Amphipoda	*Pandalus* spp.	Paguridae	Unid. Crustacea	Ophiuridea	Asteroidea	Unid. tissue	Plant material
40	6/78	1	1	-	-	-	-	-	1	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	5	-	-	-
40	7/78	35	29	1	-	1	3	6	2	9	1	-	3	3	-	2	-	-	26	2	-	-	2	1	9	1	1	-	-	-	-
40A	6/78	42	36	2	8	-	-	2	8	-	3	-	-	-	1	2	1	1	13	2	-	1	9	-	-	-	3	1	-	2	3
40B	6/78	3	2	-	1	1	1	1	1	-	1	1	-	-	-	-	1	-	1	-	-	1	1	-	-	-	-	1	-	-	-
41	6/78	2	2	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	1	-	-	1	-	-	1	-	-
43A	3/78	28	10	1	8	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	1	3	-	-	5	-	-	2	-	1
227	8/78	2	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-
Total frequency of occurrence		113	81	5	17	3	5	9	12	9	6	1	3	4	1	5	2	2	43	4	1	3	16	1	9	8	4	7	3	3	4
Percent frequency of occurrence			72	4	15	3	4	8	11	8	5	1	3	4	1	4	2	2	38	4	1	3	14	1	8	7	4	6	3	3	4

*The genus *Margarites* occurs in the area and may be included in this category.

TABLE 3. *Food of* Cancer magister *with carapace widths greater than 50 mm from Cook Inlet, Alaska.*

Station	Date month/year	No. stomachs examined	No. with food	Foraminifera	Hydrozoa	Bryozoa	Polychaeta	*Solariella* spp.*	*Natica* sp.	*Neptunea lyrata*	Unid. Gastropoda	*Nucula tenuis*	*Nuculana fossa*	*Glycymeris subobsoleta*	Mytilidae	*Chlamys* spp.	*Clinocardium ciliatum*	*Spisula polynyma*	*Macoma* spp.	*Tellina nuculoides*	Unid. Bivalvia	*Balanus* spp.	Amphipoda	*Pandalus* spp.	*Crangon* spp.	Paguridae	*Chionoecetes bairdi*	Unid. Crustacea	Ophiuridea	Teleostei	Unid. tissue	Plant material
40	7/78	25	18	-	-	-	-	-	1	-	1	-	6	-	-	-	-	11	1	1	8	1	-	-	1	1	-	-	1	-	-	-
40	8/78	52	40	-	-	-	-	-	-	1	-	10	7	-	-	-	-	33	-	-	6	1	1	1	1	1	-	-	2	1	-	-
40A	12/77	18	12	3	-	-	2	3	-	-	-	-	-	-	-	-	-	9	1	-	-	2	-	-	-	1	-	-	2	-	-	-
40A	6/78	132	104	5	1	1	5	2	-	-	-	-	-	3	-	6	-	80	3	-	5	2	10	10	-	-	1	6	3	4	2	-
40A	7/78	9	5	4	-	-	1	-	-	-	-	-	-	-	-	-	-	2	-	-	-	3	1	-	-	-	-	-	-	-	-	-
40A	8/78	6	5	1	-	-	1	1	-	-	-	-	-	-	-	-	-	2	-	-	-	-	2	2	-	-	-	-	-	1	-	-
41	6/78	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	7/78	22	21	1	-	-	1	-	1	-	-	-	-	-	-	-	-	9	-	1	-	10	1	-	-	2	-	-	-	-	-	-
41	8/78	13	8	2	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	1	3	-	-	1	1	-	-	-	-	-	-
227	8/78	6	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-
D1	8/78	63	33	1	-	-	6	-	-	-	-	-	-	-	7	-	4	13	-	-	-	17	6	-	-	9	-	4	-	2	6	4
Total Frequency of Occurrence		349	251	17	1	1	16	6	2	1	1	10	13	3	7	6	4	168	5	2	20	39	21	13	4	15	1	10	8	8	8	4
Percent Frequency of Occurrence			72	5	<1	<1	4	2	<1	<1	<1	3	4	1	2	2	1	48	1	<1	6	11	6	4	1	4	<1	3	2	2	2	1

*The genus *Margarites* occurs rarely in the area and may be included in this category.

TABLE 4. *Food of* Cancer magister *with carapace widths of 22 - 45 mm from Cook Inlet, Alaska.*

Station	Date month/year	No. stomachs examined	No. with food	Foraminifera	Polychaeta	*Solariella* spp.*	Unid. Gastropoda	*Nucula tenuis*	*Spisula polynyma*	*Tellina nuculoides*	Unid. Bivalvia	*Balanus* spp.	Amphipoda	Paguridae	Unid. Crustacea	Unid. tissue	Sand
40A	8/78	64	51	23	18	2	1	1	9	1	6	18	1	8	2	2	27
Percent Frequency of Occurrence			80	36	28	3	2	2	14	2	9	28	2	13	3	3	42

*The genus *Margarites* occurs rarely in the area and may be included in this category.

Tarverdieva (1976) provides information on feeding of king crabs from Bristol Bay, Alaska. There, echinoderms and molluscs were the predominant food items occurring in 50 and 35% of the stomachs, respectively. Feder and Jewett (1980) observed the cockle *Clinocardium ciliatum* in 67%, the snail *Solariella* spp. in 55%, *Nuculana fossa* in 50%, the polychaetous annelid *Cistenides* sp., and brittle stars of the family Amphiuridae in 35% of 124 king crab stomachs examined in the southeastern Bering Sea. Takeuchi (1968a, b) examined the food of king crabs from the Kamchatka region of Japan, and found that molluscs, crustaceans, and echinoderms were the main food items. Takeuchi (1968b) found that the frequency of occurrence of the above prey groups in crab stomachs corresponded to the relative abundance of these organisms. The barnacle, *Balanus crenatus,* and clams were important prey species of king crabs in shallow water areas of Kodiak Island, Alaska with cockles becoming more important in deeper water (Feder et al., 1979). In Cook Inlet, barnacles, clams, snails, and hermit crabs are widely distributed (Feder et al., 1978), and were fed upon in proportion to their abundance. Small echinoderms were relatively rare at the stations examined (Feder et al., 1978).

Cancer magister

Food occurred in 331 (80%) of the 413 Dungeness crab stomachs examined (Tables 3 and 4). Individuals over 50 mm carapace width preyed primarily on small bivalves (67%), barnacles (11%), and amphipods (6%). The clam *Spisula polynyma* was the most frequently occurring species, and was observed in 48% of the stomachs. Crustaceans occurred in 30% of the stomachs. All other prey species occurred in less than 5% of the stomachs examined.

In 93% of the *C. magister* stomachs containing *S. polynyma,* the intact shells and shell fragments were from clams less than 10 mm in shell length, young-of-the-year or one year old clams. The maximum number of young *S. polynyma* observed in a single stomach was 125. The meat of large *S. polynyma* and pieces of shell 1 to 2 mm thick were observed in 29 stomachs.

In small *C. magister,* 22 to 44 mm in carapace width (Table 3), the most frequently occurring animals were Foraminifera (36%), polychaetes (28%), barnacles (28%), and small clams (25%). Individuals with empty stomachs were generally in a newly molted or molting condition.

In a northern California study, the five most frequently observed categories of prey for *C. magister* were clams (35%), fishes (24%), isopods (17%), amphipods (16%), and razor clams *Siliqua patula,* 12% (Gotshall, 1977). Butler (1954) examined *C. magister* from British Columbia, Canada, and found that crustaceans (59%) and clams (56%) were the most frequently occurring food items. He reported fish remains in only 4 Dungeness stomachs. The results of Butler (1954) and Gotshall (1977) are similar to those for Cook Inlet crabs. All of the studies showed that clams and several kinds of crustaceans are important prey for *C. magister.* The major difference between the studies was the importance of fish in the California Dungeness diets as compared to the low frequency of occurrence of this prey in British Co-

lumbia and Cook Inlet crabs. Isopods were rarely encountered in grabs or dredges in Cook Inlet, and razor clams do not occur in the study area. Young *S. polynyma* are not commonly collected with grabs or dredges in Kachemak Bay (Feder et al., 1978). Therefore, the high incidence of predation on this clam is probably a reflection of prey preference by the Dungeness crab.

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REFERENCES

Butler, T.H. 1954. Food of the commercial crab in Queen Charlotte Islands region. Fish. Res. Bd. Can., Pacif. Prog. Rep. No. 99:3-5.

Feder, H.M., S.C. Jewett, M. Hoberg, A. Paul, J. McDonald, G. Matheke, and J. Rose. 1978. Distribution, abundance, community structure, and trophic relationships of nearshore benthos of the Kodiak Shelf, Cook Inlet, northeast Gulf of Alaska and the Bering Sea. Annual Report to NOAA. R.U. #281/5/303, Inst. Mar. Sci., Univ. of Alaska, Fairbanks. 296 p.

Feder, H.M., M. Hoberg and S.C. Jewett. 1979. Distribution, abundance, community structure, and trophic relationships of the nearshore benthos of the Kodiak shelf. Annual Report to NOAA. R.U. #5, Inst. Mar. Sci., Univ. of Alaska, Fairbanks. 113 p.

Feder, H.M. and S.C. Jewett. 1980. A survey of the epifaunal invertebrates of the southeastern Bering Sea with notes on the feeding biology of selected species. Inst. Mar. Sci. Rep. R78-5. Univ. of Alaska, Fairbanks. 105 p.

Gotshall, D.W. 1977. Stomach contents of northern California Dungeness crabs, *Cancer magister.* Calif. Fish and Game **63**(1):43-51

Takeuchi, I. 1968a. Food of king crab, *Paralithodes camtschatica,* off the west coast of Kamchatka in 1958. Fish. Res. Bd. Can. Transl. Ser. No. 1193: 21 p. From: Bull. Hokkaido Reg. Fish. Res. Lab. 33:23-44, 1959.

Takeuchi, I. 1968b. Food of king crab, *Paralithodes camtschatica,* off the west coast of the Kamchatka Peninsula, 1958-1964. Fish. Res. Bd. Can. Transl. Ser. No. 1194: 21 p. From: Bull. Jap. Sea Reg. Fish. Res. Lab. No. 33:32-44, 1967.

Tarverdieva, M.I. 1976. Feeding of the Kamchatka king crab, *Paralithodes camtschatica,* and tanner crabs, *Chionoecetes bairdi* and *Chionoecetes opilio* in the southeastern part of the Bering Sea. Soviet J. Mar. Biol. **2**(1):34-39.

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Cover
Scanning electron micrograph of fracture line in oyster *(Crassostrea virginica)* shell.
Courtesy M.R. Carriker, R.E. Palmer and R.S. Prezant. (See page 139).