





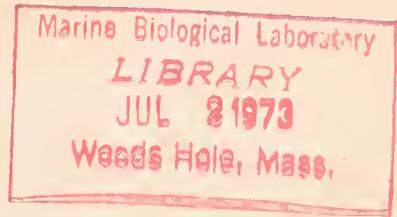








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THIS VOLUME IS DEDICATED TO  
THE MEMORY OF  
DR. TAKEO IMAI



*The inquiry, knowledge and belief of truth is  
the sovereign good of human nature.*

-Bacon

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ABSTRACTS OF TECHNICAL PAPERS PRESENTED  
AT THE 1972 NSA CONVENTION

LARVAL CESTODE INFECTIONS IN  
SEVERAL EDIBLE BIVALVE MOLLUSKS  
FROM THE VICINITY OF  
ST. TERESA, FLORIDA

Edwin Cake

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*Tallahassee, Florida*

Twenty-five specimens of three edible bivalves, Atlantic-Bay Scallops, *Argopecten irradians concentricus* (Say), Sunray Venus Clams, *Macrocallista nimbosa* (Lightfoot), and Atlantic Surf Clams, *Spisula solidissima raveneli* (Conrad) collected from the vicinity of St. Teresa Beach, Florida, were examined for larval cestode parasites. Phyllobothriid plerocercoids of the genus *Echeneibothrium* (Beneden) were found free in the stomach and digestive diverticula of *A. irradians* and *S. solidissima*. One immature phyllobothriid of the genus *Rhodobothrium* (Linton) was recovered from a capsule in the mantle cavity of one *M. nimbosa*. Encysted lecanicephalid metacestodes of the genus *Polypocephalus* (Braun) were found in the visceral masses of *A. irradians*, and of the genus *Tylocephalum* (Linton) in the visceral masses of all three species and in the foot musculature of the two clam species. Encysted plerocercoids of the trypanorhynch, *Parachristianella dimegacantha* (Kruse), were found in the intestine walls of all three bivalve species and in the foot musculature of the two clam species. All five cestode genera encountered have elasmobranchs as final hosts and are not known to be harmful to man. Quantitative data are presented on the cestode larvae from each species and some cestode-load and host-size relationships are discussed. Bivalve hosts of the same five cestodes are reported incidentally from a related, unpublished study of marine mollusks in the same area.

DISCOVERY OF DUCT SYSTEM IN  
ACCESSORY BORING ORGAN OF  
*UROSALPINX CINEREA FOLLYENSIS*  
BY SCANNING ELECTRON MICROSCOPY

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Accessory boring organs (ABO) of adult snails were excised, fixed slowly with agitation in increasing concentrations of glutaraldehyde, and prepared for examination in the scanning electron microscope by a freeze dry technique developed by T. Otaka and S. Honjo (SEM, 1972/II, Proc. Workshop Biol. Specimen Prep. Techn. SEM, III. Res. Inst., p. 359-363).

Examination of the exterior of ABOs with the scanning electron microscope revealed for the first time a large number of ducts which open conspicuously at the surface among the microvilli. Each duct, when dilated, was edged by a conspicuous flange. The ducts were traced into the interior of the ABO in fracture sections of the gland. Earlier studies with the transmission electron microscope (Nylen, Provenza, and Carriker, Amer. Zool. 9: 935-965) revealed star-shaped dilations among the groups of secretory cells. It is suggested these dilations may be a part of the duct system. The function of the ducts is still unclear.

THE MATERIALS, METHODS AND POLITICS  
OF OFF-BOTTOM HIGH DENSITY OYSTER  
FARMING IN CAPE MAY COUNTY,  
NEW JERSEY

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In 1966, *Minchina nelsoni* (MSX) - resistant oyster

seed on surf clam shell obtained from the Delaware Bay side of the Cape May peninsula was transferred to a natural shell bed in Holmes Creek, a tributary of the Great Sound, on the ocean side of the peninsula. All live oysters had disappeared from formerly abundant natural live beds in this area - possibly as a result of decades of pesticide effect upon larval stages. In 1967-68, two 1,000 x 50 ft lagoons were dredged in a tidal marsh at the entrance of Holmes Creek, and the growth of oysters in these lagoons, both in racks and on vertically suspended punched surf clam shell, was monitored. During 1968-72, a reusable cultch assembly of scrap tire beads strung in stacks was devised and a method for growing oysters using this material was tested and patented. Growth to market size of oysters on shell or tire beads was found to require two years from time of set.

In 1969, the local county government, with approval from the State Health Department, erected a sewage treatment plant and outfall pipe within one tidal cycle of this operation thereby causing it to be condemned for shellfish harvesting.

During 1970-71, 10% of the rafting of oysters originally planned for 1969-70 was completed, and the actual amount and potential loss due to condemnation was documented. These oysters are being maintained suspended from rafts. Documented actual production from 1970 set equals 95 bu, plus projected production from approximately 150,000 one year old oysters of the 1971 set equals 450 bu or a total of 545 bu. Potential yield per two year period from full rafting in the two lagoons equals 545 x 10, or 5,450 bu.

#### DESIGN OF AN EXPERIMENTAL SELF-SUPPORTING, CLOSED CYCLE OYSTER CULTURE SYSTEM

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Newark, Delaware*

The present study describes a unique system that has been designed, built and is under experimentation to grow oysters in closed cycle under controlled environment. The system essentially consists of two oyster growing tanks with one common biological-mechanical filter, charcoal-fiber filters, a bank of UV lights, water treatment system with ozone and algal culture system. Importance and use of ozonating recycling water in such a system are discussed. The system is unique in two respects. First, the oyster culture system is coupled with an algal culture system

so that a regulated amount of algae is fed to oysters and the algal culture tanks are refilled with sterilized sea-water from the ozone treatment tank. Second, the complete system has been automated with the help of electrical timers, pumps, solenoid valves, ball valves, etc. Only usual maintenance is required.

#### RECENT TRENDS IN THE EPIZOOTIOLOGY OF *MINCHINIA NELSONI* (MSX) IN DELAWARE BAY<sup>1</sup>

Susan E. Ford

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Activity of the oyster pathogen *Minchinia nelsoni* (MSX) has fluctuated markedly in lower Delaware Bay since it was first recorded there in 1957. Intense disease pressure and heavy mortalities associated with the onset of the epizootic in the late 1950's had dropped to almost negligible levels by the early 1960's. In 1963, MSX activity began to rise and from 1964 through 1967, disease levels were as high as those recorded during the first years of the epizootic. A downward trend began in 1968 and, except for a moderate resurgence in 1970, has continued. Disease levels resulting from the 1971 infection period were lower than at any time since the early 1960's. Widespread, heavy mortalities of the type experienced during the onset of the epizootic have not been repeated despite periods of high MSX activity, although populations of susceptible seed oysters continue to experience heavy losses when transplanted to epizootic areas. Additional evidence of resistance has been seen in an increased tendency to maintain infections at low, non-lethal levels. This has been noted in all oysters, but is particularly evident in oyster stocks native to the lower Bay, which have been exposed to heavy selective pressure for 15 years.

Two annual peaks in MSX prevalence levels, of approximately equal height, were seen in oysters in lower Delaware Bay during the high activity years of 1964-67. These occurred during the winter and late spring, were often in the 70 - 90% range, and were the result of early summer and late summer-fall infective periods respectively. Winter peaks just prior to and after the years of high disease activity rarely exceeded 50%; the spring peak was even more abbreviated and occasionally not seen, indicating an infective period restricted mainly to early summer.

Monitoring of upper Bay seed beds, whose normal mid-tide salinities range from 10 - 16 ‰, indicates

that MSX in low salinity areas has followed the same fluctuations as in higher salinity regions. The high activity years of the mid-1960's coincided with a severe drought when salinities throughout the Bay hit peaks 2 - 5‰ above normal, and when the duration of above average salinities was lengthy and coincided with the infective and immediate post-infective period. In the middle of the drought, MSX extended as far up bay as the upper-most of the productive seed beds where it had not been since the first years of the epizootic. At the same time, salinities on the lower seed beds had become high enough to permit MSX activity comparable to that in the lower Bay. When salinities are normal, disease levels on the seed beds are light to non-existent.

Not only was salinity implicated in heightened disease activity during a period of drought, but high salinity areas of the lower Bay continually sustain the highest levels of MSX. Nevertheless, salinity does not explain all the phases of MSX activity recorded during the past 15 years, particularly the early part of the epizootic when high disease activity coincided with normal salinity. To explain these fluctuations it will be necessary to look for factors other than salinity and in addition to resistance of the oyster population.

<sup>1</sup> Supported under PL 88-309 contract 3-3-R-7 with the National Marine Fisheries Service.

#### EARLY DEVELOPMENT IN THE OCEAN QUAHOG, *ARCTICA ISLANDICA* (L.)

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*U. S. Department of Commerce*

*National Oceanic and Atmospheric Administration*

*National Marine Fisheries Service*

*Middle Atlantic Coastal Fisheries Center*

*Laboratory for Experimental Biology*

*Milford, Connecticut*

The normal spawning season of ocean quahogs in southern New England waters is late summer. Attempts to ripen mahogany clams out of season in the laboratory produced limited success. Clams obtained from the field in late fall and subjected to a water temperature of 10°C and ample algal food for 10 weeks failed to ripen. However, clams obtained from the fishery in late winter and subjected to the same regimen ripened in about 5 weeks.

Ripe clams could not be induced to spawn by rapidly increased temperature, rapidly decreased temperature or a sperm suspension. A few untreated, stripped eggs were found to be fertilizable by stripped

sperm; however, fertilization and the percent development of stripped eggs to normal larvae were significantly increased when the eggs were exposed to dilute ammonium hydroxide before fertilization was attempted.

The earliest, fully developed, straight-hinge larvae are about 110 μ long and 80 μ wide and have an unusually long hinge line. Metamorphosis takes place when the larvae are approximately 200 μ long. Larvae were reared to metamorphosis at 10°C in about 60 days.

#### LABORATORY CONTROL OF PACIFIC OYSTER MORTALITY BY MANIPULATION OF TEMPERATURE AND NUTRIENT CONCENTRATION

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Temperature and nutrient were found to be critical environmental factors in abating or initiating a laboratory mortality of adult Pacific oysters. A significant mortality did not occur until the temperature of the seawater was 18°C and above. Prior conditioning of oysters at temperatures below 18°C resulted in a lowered mortality rate. Enrichment of the seawater with a nutrient medium increased the rate of death. Ultraviolet light treatment of the seawater reduced the mortality to the level of the control oysters. The research gives support to the contention that a microorganism is responsible for the mortality.

#### PRELIMINARY ESTIMATES OF GROWTH FUNCTIONS AND THE SIZE-AGE RELATIONSHIP FOR THE HARD CLAM, *MERCENARIA MERCENARIA*, IN THE YORK RIVER, VIRGINIA

Joseph G. Loesch and Dexter S. Haven

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Two groups of hard clams ranging from the smallest size practical for individual marking through the larger sizes (approximately 30 - 90 mm in length) were measured, code-marked and planted in similar natural substrates at two locations in the York River. Both groups have been harvested, remeasured and planted annually, and growth functions determined from length increments.

CYTOLOGY AND CYTOCHEMISTRY OF  
AMEBOCYTES OF *MERCENARIA MERCENARIA*

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Amebocytes of *Mercenaria mercenaria* were classified into cell types by a variety of microscopical and cytochemical procedures. Three different amebocyte types were identified: a small ( $28\mu$ ) motile granulocyte, a large ( $45\mu$ ) non-motile granulocyte and an agranulocyte ( $5\mu$ ). The small granulocyte comprised 61% of the total cell population; it had four distinct types of granules in the cytoplasm. The large granulocyte made up 37% of the cell population; this granulocyte possessed the same four types of granules but contained approximately one-third the number found in the smaller granulocyte. The agranulocyte had no visible granules with only a thin peripheral rim of cytoplasm surrounding the nucleus. The four types of granules observed in granulocytes in decreasing order of abundance were: (1) a large ( $1.5\mu$ ) blunt type, (2) a small ( $0.7\mu$ ) dot-like type, (3) a large ( $1\mu$ ) spherical refractile type and (4) a rod-shaped type approximately  $2\mu$  in length.

The nucleus of all cell types appeared morphologically similar having uniformly dispersed chromatin and a rim of chromatin lining the nuclear membrane.

Supravital studies with Janus Green B showed a preferential uptake by the large, blunt granules. Within 10 min the dye had been converted to the red-reduction product, diethyl safrin. When neutral red was applied supravitaly, both the large blunt granules and small dot-like granules took up the dye. The color changed from red to yellow in about one-half hour.

Studies with esterases indicated a strong non-specific esterase in the small granulocyte. Acid phosphatase and NADH dehydrogenase cytochemical studies are presently under investigation.

COMPARATIVE FUNCTIONAL MORPHOLOGY  
OF FEEDING MECHANISMS IN  
*RANGIA CUNEATA* (GRAY) AND  
*POLYMESODA CAROLINIANA* (BOSC)

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Preliminary investigations of the functional

morphology of feeding in two estuarine clams are discussed and compared. The clams, *Rangia cuneata* (Gray) and *Polymesoda caroliniana* (Bosc), were taken from the same location in the Ochlockonee River estuary, south of Tallahassee, Florida, on the north-west Florida Gulf coast. Both clams are morphologically filter feeders. *R. cuneata* is shown to have typical mactrid gill and palp morphology and ciliary currents similar to other mactrids. *P. caroliniana*, a corbiculid, is in the same general gill-palp articulation category as *R. cuneata*. Ciliary current pathways on the gills, visceral mass and mantle in each species are similar. *P. caroliniana*, however, appears to possess more ridge currents on the palps than *R. cuneata*. The *P. caroliniana* palps are also free dorsally, whereas those of *R. cuneata* are attached by most of their dorsal margins, thus limiting their movement.

*R. cuneata* does not possess a fourth pallial aperture as do some other members of the Matridae for the ejection of pseudofeces. The orientation of the waste canal and the siphonal membrane, however, is such that pseudofeces are extruded through the inhalent siphon upon quick closure of the valves. *P. caroliniana* has no waste canal, but pseudofecal material is ejected in a manner similar to that in *R. cuneata*.

Qualitative samples of stomach and gut contents of both clams from the same habitats are similar in composition, including many diatoms, algae and much unidentifiable material.

PREY SELECTION IN THE OYSTER LEECH,  
*STYLOCHUS ELLIPTICUS*<sup>1</sup>

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*Rutgers University*  
*New Brunswick, New Jersey*

Several different prey preferences have been previously reported for *Stylochus ellipticus*. In the present study, it was determined that preferences differed from worm to worm and were influenced by previous diet history. *Stylochus* when offered several prey showed the greatest preference for those on which the worms had been feeding when collected.

Diet patterns in adult *Stylochus* were very rigid and mature worms could not be induced to feed on certain prey although starved for as long as 30 - 40 days. Evidence was collected indicating selection patterns may be established very early in the life cycle of *Stylochus*.

An experiment was conducted for six weeks to determine if prey density could be correlated with food preferences shown in laboratory feeding experiments. A small population of *Stylochus* found at the mouth of Dias Creek in Delaware Bay was selected for the study. Each week 20 - 30 worms were collected and estimates were made of the density of four prey found with the worms: *Mya arenaria*, *Modiolus demissus*, *Nassarius obsoletus*, and *Odostomia impressa*. Worms brought to the laboratory were isolated individually in small aquaria and all four prey species given as food. Feeding rates were determined on each prey and an analysis of variance performed.

There was a significant difference between the feeding rates of the worms on each of the prey for all six weeks. Preference for *Mya* was high initially but decreased sharply. Initial preference for *Nassarius* was low but increased as the preference for *Mya* decreased. *Modiolus* and *Odostomia* were the least preferred of the prey. A relationship was noted between preference and density of the prey. As density of *Mya* decreased, the preference decreased. Similarly, an increase in the density of *Nassarius* was accompanied by an increase in preference. Densities of *Modiolus* and *Odostomia* remained low throughout the six-week period, as did the preferences for these prey.

It is suggested that planktonic *Stylochus* may be able to establish wherever suitable prey exist and that food selection patterns will be determined by the density of those prey. Several interesting questions are posed: (1) Can planktonic worms delay metamorphosis if suitable prey are not found; (2) Is it possible that food preferences are established even earlier than suggested, when both predator and prey are in the plankton?

<sup>1</sup>Supported under PL 88-309 contract 3-3-R-3 with the National Marine Fisheries Service.

#### A POTENTIAL USE OF THE WASTE HEAT BYPRODUCTS OF A STEAM TURBINE ELECTRIC GENERATING PLANT

Edwin H. Powell

*Windmill Point Oyster Company*

*Urbanna, Virginia*

and

*Potomac Electric Power Company*

*Washington, D. C.*

The basic concept under investigation was that warm water in the electric generating plant canal could be beneficially used to provide small cultch-free oyster spat with normal growth in advance of the regular season. As applied to the temperatures avail-

able at the generating plant on the Potomac River, seed oysters could be cultured in the canal beginning in early March for later planting on conventional oyster beds in the river during mid-April. This procedure could give up to three months early growth advantage thus allowing possible harvest the following November.

A full annual cycle test, utilizing over a thousand hatchery produced cultch-free seed oysters, (divided into 14 groups and counted and measured at appropriate intervals) showed no significant difference in survival of the groups located in the canal, the Potomac River and the Rappahannock River.

#### SURF CLAMS AND SOCIETY: A RATIONALE FOR SOUND MANAGEMENT

Jon Rittgers

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National Oceanic and Atmospheric Administration  
National Marine Fisheries Service  
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Biologists identify four phases in the historical development of a commercial fishery: 1) the early period when landings are low; 2) the developmental period when landings are growing rapidly; 3) the peak period when landings reach a high level and are maintained for a period of time; and 4) the period of decline when landings are falling due to reduction in stocks brought about by various causes which may or may not include overfishing. Economists identify three stages of production which are similar to the four biological phases of development: Stage I, when physical returns to investment in the firm (or industry) are increasing at an increasing rate; Stage II, when returns to investment are increasing but at a decreasing rate; and Stage III, when further investment will bring about a reduction in total output.

Some evidence suggests that the surf clam industry is reaching (and perhaps is well into) the third biological phase of development. The exact stage of economic development is not clearly defined, but it would appear that Stage III has not been reached. Thus the surf clam industry is in the enviable position of not having to reduce levels of employment and capital investments to ensure continuing vitality of the industry. It need only cope with the common property institution which will, if left unaltered, inevitably lead to operating biologically and economically in the final (and least desirable) stages of development.

To overcome the problems inherent in the common property institution, immediate steps should be

taken to develop a system of property rights designed to delineate resource tenure (ownership rights) and to ensure high levels of certainty in tenancy (use rights). State and Federal Government assistance may be required to solve the problems which will arise in developing such a system.

RESISTANCE OF *CRASSOSTREA VIRGINICA*  
TO *MINCHINIA NELSONI* AND  
*LABYRINTHOMYXA MARINA*<sup>1</sup>

George A. Valiulis and Harold H. Haskin

Oyster Research Laboratory  
N. J. Agricultural Experiment Station  
Rutgers University  
New Brunswick, New Jersey

Previous reports from this laboratory have indicated an innate resistance to mortality in certain stocks of *Crassostrea virginica* due to *Minchinia nelsoni* (MSX). Whether there is also a resistance in some stocks of *C. virginica* to *Labyrinthomyxa marina*, and whether this resistance (if present) can be correlated with that for MSX, was the objective of this experiment.

Four laboratory-reared stocks of *C. virginica* of known resistance to MSX were injected with 7 graded dosages of *L. marina* cells ranging from 10 to 100,000 cells/oyster. The source of infective inoculum was the minced, infected tissue of dead oysters collected from the field. Approximately 2,200 oysters were kept in aquaria in aerated, running sea water maintained at 28 - 30°C and about 20 ‰ salinity for a test period of 105 days.

The control groups of oysters (uninjected, and injected with uninfected oyster tissue mince) never showed infection with *L. marina*, as determined by fluid thioglycollate culture and sectioning. Of the oysters that died in the experimental groups 316 were examined for *L. marina* and 89% of these were found to be infected; of these 83% had heavy or very heavy systemic infections. Examination of oysters

still living at the termination of the experiment showed a trend of progressively higher incidence and weighted incidence of *L. marina* in each group receiving a higher dose regardless of stock.

Sections of the initial live samples and of live samples at the termination of the experiment showed that there was a 3 - 20% incidence of light MSX infection which was random and had no correlation with the dosage of *L. marina* injected. Mortality due to *L. marina* in each of the experimental groups of oysters was obtained by subtracting the highest control mortalities of the same stocks from the total mortalities of the experimental groups of the same stocks.

Comparisons of final percent cumulative mortalities of the four stocks indicated no distinct differences in resistance at higher doses of *L. marina* (500 - 100,000 cells/oyster). However, at lower doses (10 - 100 cells/oyster) one stock showed a consistently greater susceptibility (2.4 - 11.8 times greater) than the other three stocks.

Comparisons of the resistances of these stocks of oysters to *L. marina* under low dose, laboratory conditions with the resistances of these same stocks to MSX under field conditions showed that: (1) The two stocks that were most resistant to MSX were also resistant to *L. marina*; (2) The stock most susceptible to MSX was also the one most susceptible to *L. marina*; (3) The stock moderately susceptible to MSX was resistant to *L. marina*.

From the results of this experiment and from other field experiments (the results of which are not reported here) the trend seems to be emerging that stocks of oysters most susceptible to MSX are also the most susceptible to *L. marina*, those most resistant to MSX are also resistant to *L. marina*; however, those stocks which are moderately resistant (or susceptible) to MSX may or may not be resistant to *L. marina*.

<sup>1</sup>Supported under PL 88-309 contract 3-3-R-3 with the National Marine Fisheries Service.

## NSA PACIFIC COAST SECTION

SOME OBSERVATIONS OF CLAM  
DISTRIBUTION AT FOUR SITES  
ON HOOD CANAL, WASHINGTON

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Personnel of the Bureau of Sport Fisheries and Wildlife, Division of River Basin Studies, conducted a study of intertidal shellfish populations at 4 sites on Hood Canal in March and April, 1972. The purpose of the study was to determine whether bulkheads and attendant fill in the upper intertidal levels have an effect upon shellfish.

Samples were collected along 4 transects perpendicular to the shoreline at each site. Two transects were located in front of a bulkhead and 2 on an adjacent natural beach. Sampling stations were located at 10 ft intervals on the transects. A sample of substrate  $\frac{1}{4}$  m<sup>2</sup> and approximately 8 in deep taken at each station was sorted through 1 in and  $\frac{1}{4}$  in mesh screens, and all clams were saved for classification and measurement.

At 3 of the sites more than twice as many clams were found on natural beaches than on bulkheaded beaches. There was significant difference between bulkheaded and natural beaches at 2 sites in numbers of Japanese littleneck clams, *Venerupis japonica*, found in the upper intertidal area. There was also a trend toward differences in size and distribution. Clams inhabiting lower intertidal levels did not seem to be affected by bulkheads.

Several hypotheses for the differences were proposed. The most probable explanation is the change in current patterns associated with bulkheads which result in less favorable conditions for settling and survival of clam larvae. These conditions also may cause a reduction in availability of nutrients and food.

A PILOT ECONOMIC STUDY OF  
OYSTER RAFT CULTURE IN  
YAQUINA BAY, OREGON

Gary G. Gibson and Dennis S. Lund

*Fish Commission of Oregon and  
Newport Oyster Co.  
Newport, Oregon*

An oyster raft 12 x 20-feet was anchored in Yaquina Bay in June 1971. One hundred and

forty-six strings of unbroken Japanese oyster seed were suspended from the raft. Labor and material costs were recorded.

Costs of concrete anchors, piling, boom logs and equipment for harvesting on a larger scale were determined and added to the actual costs of construction, stringing and planting. The combination of these actual and estimated expenses amount to \$1.12/6 ft string. Other expenses such as transportation, tools, insurance, rent, attorney and accountant fees and administration were estimated to be \$.25/6 ft string (based on an average annual production of 80,000 strings per year).

Production from the raft after 6 months was 16.7 bu of cocktail-sized oysters (100/pint). The oysters brought \$20/bu in the shell or \$334. Potentially, a raft will support 204 6 ft strings or 34 bu worth \$680 or \$3.33 per string.

A gross profit of \$3.33/string, minus \$1.37/string for expenses, equals \$1.96 net profit per string or \$400 per raft per year.

CLAM DISTRIBUTION AND ABUNDANCE  
IN GRAYS HARBOR AS RELATED  
TO ENVIRONMENTAL FACTORS

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*Weyerhaeuser Company  
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From 1967 - 69 surveys were conducted in Grays Harbor to determine the distribution and abundance of native and introduced clams. Nine species including 4 softshell-type and 5 hardshell-type clams occur in the bay. The softshell clams, especially *Mya arenaria*, are the most numerous and have the widest distribution, occurring from within a mile of the bay mouth eastward to the mouth of the Chehalis River at Hoquiam. The hardshell-type clams occur mostly in the western portion of the bay. *Clinocardium nuttalli* has the widest distribution in this group, occurring from the mouth of the bay east to Johns River and Neds Rock.

Clam distributions are discussed in relation to seasonal levels of salinity and pulp mill effluents. Persistent conditions of low salinities throughout most of the bay in winter are thought more important in limiting the colonization of greater portions of the bay by hardshell clams. Summer pulp effluent levels have had little apparent effect on the colonization of softshell type clams in the eastern bay areas, where highest levels occur.

Densities of softshell type clams, excepting *Cryptomya californica*, were independent of substrate organic levels between 0.5% and 3.0% and moisture content between 10% and 50%. Densities of *Mya arenaria* were greater on coarser substrates while finer substrates had greater densities of *Macoma natsuta*, however. *Clinocardium nuttalli* and *Vernerupis japonica* colonized substrates with lower organic levels, 0.5% - 1.5%, and a particle size similar to that colonized by *Mya*.

#### CULTIVATION OF GREEN MUSSEL IN NEW ZEALAND

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University of the Pacific  
Greenbrae, California*

The article describes various methods of cultivation of the New Zealand green mussel, *Perna canaliculus*, which at present is grown on a relatively small scale in New Zealand, but the farming of which seems to offer many promising possibilities.

This mussel, which is native to both the North and South Islands of New Zealand, lives in water in environments closely resembling those of the Pacific Northwest and northern California shores.

Methods of cultivation, rate of growth under different conditions and other aspects of biology, ecology and cultivation of these bivalves were discussed.

#### FEEDING STUDIES WITH PACIFIC OYSTER LARVAE

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Marine Science Center  
Newport, Oregon*

Growth and setting of Pacific oyster larvae fed *Isochrysis galbana* were compared with larvae fed brewers yeast and 4 dry artificial diets prepared by Dr. Samuel Myers of the Louisiana State University Food Science Department. The dry diets most effective in promoting oyster growth were composed of single-cell protein (yeast), fish meal and solubles, soybean meal, whey, rice bran and vitamins. The components of the dry rations were bound with starch or alginate and dried to form particles of low solubility in seawater.

When 20,000 cells/ml of *Isochrysis* was fed as a supplement with the dry diets FDSC 1102-71 P. W.

Flake and TC 1119-71 2A for the last 10 days prior to metamorphosis, larvae set as well as those fed 80,000 cells/ml of *Isochrysis*. However, in the absence of the supplemental algae, larvae failed to grow or set well. Optimum feeding level of the dry rations appears to be 1-2 mg/l fed once per day. Concentrations of 4 mg/l and above greatly reduced setting of larvae.

Brewers yeast produced erratic results when fed to larvae for 10 days prior to setting. As in the case of the dry rations, 10,000-20,000 cells/ml of supplemental algae in addition to the yeast was necessary for larval growth. When fed brewers yeast at 50,000 cells/ml immediately before setting, however, larvae set much more densely than when fed 50,000 cells/ml of algae.

Larvae of less than 140  $\mu$  occasionally grew very well on the dry rations, but more often growth was considerably less and mortality significantly higher than in cultures fed *Isochrysis*. Brewers yeast was never a suitable food for larvae of less than 140  $\mu$ .

Larvae fed 100,000 cells/ml of *Isochrysis* for 3-5 days prior to setting set at least 3 times more densely than larvae fed 50,000 cells/ml, and 10 times more densely than larvae fed 25,000 cells/ml.

#### TEST FOR FLAVOR DIFFERENCES IN PACIFIC OYSTERS RELATED TO DIFFERENCES IN GROWING AREAS OR METHODS OF CULTURE

David Miyauchi, George Kudo and  
Max Patashnik

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National Oceanic and Atmospheric Administration  
Pacific Fishery Products Technology Center  
National Marine Fisheries Service  
Seattle, Washington*

It is a common opinion that Pacific oysters raised in Hood Canal have a milder flavor than those raised in other Washington waters, such as Southern Puget Sound, and that oysters raised "off bottom" have a milder flavor than those raised "on bottom." During the Winter of 1971 and the Spring of 1972, the Pacific Fishery Products Technology Center at Seattle in cooperation with the Washington State Department of Fisheries conducted sensory tests to compare the flavor of oysters grown near Quilcene in Hood Canal and in Southern Puget Sound. We also compared the flavor of oysters grown on the bottom and those grown off the bottom suspended from floats. In

triangle tests, our experienced panel was able to detect differences in flavor between "on bottom" oysters raised in Hood Canal and those raised in Southern Puget Sound. Based on the ability of the panel members to reproduce their results, the difference in flavor between these oysters was statistically significant but equivocal from a practical point of view. The panel could not distinguish flavor differences between "off bottom" oysters grown in Quilcene and in Southern Puget Sound.

When the flavors of Hood Canal oysters raised "on bottom" were compared with those raised "off bottom," the panel reported a detectable difference that was statistically significant but not clear-cut. The same was true of Southern Puget Sound oysters raised "on bottom" and "off bottom."

#### FISH PROTEIN USED TO BIND PIECES OF MINCED GEODUCK

David Miyauchi, Max Patashnik and  
George Kudo

*U. S. Department of Commerce  
National Oceanic and Atmospheric Administration  
National Marine Fisheries Service  
Pacific Fishery Products Technology Center  
Seattle, Washington*

In 1970 when the State of Washington started leasing subtidal geoduck beds for commercial harvesting, our laboratory in cooperation with the Washington State Department of Fisheries obtained yield data, palatability scores, and information on cold-storage characteristics of the various edible components of the geoduck.

The fledgling geoduck processing industry, which consists of 4 or 5 small processors, requested our aid in finding a suitable binder with which to make marketable patties out of the trimmings from their prime geoduck steaks. In response to this request, we prepared frozen blocks of minced geoduck, using our fish binder made from rockfish flesh and common food ingredients. Breaded portions prepared from ¼ and ½ in. thick slices from the blocks were judged to be an improvement over those now being prepared commercially. The experimental samples held together well during deep-fat frying and pan frying. Samples of experimental blocks have been given to the various geoduck processors for their evaluation and modification.

#### A CHEMICAL ASSAY FOR PARALYTIC SHELLFISH POISONING

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Douglas, Alaska*

Saxitoxin separated from contaminating similar substances on Amberlite XE-64 can be coupled with 2,4-dinitrofluorobenzene yielding a brilliant orange-yellow precipitate. This N-substituted, 2,4-dinitroaniline compound is solubilized in ethyl alcohol. Spectrophotometric analysis revealed a single, sharply spiked peak at 372 millimicrons. The test was developed on certified toxin provided through the courtesy of the U. S. Food and Drug Administration. The test has been carried out on toxic butter clams from Porpoise Island near Juneau, and on razor clams from beaches throughout Southeast Alaska, Prince William Sound, and Unmak Island in the Aleutians. A positive reaction was also observed using cultures of *Gonyaulax catenella* kindly provided by Dr. Ken Chew and Louisa Norris, Department of Fisheries, University of Washington. Negative results were observed on other planktonic species: *Prorocentrum micans*, *Ostreopsis menotas*, *Amphidinium operculatum* and *Peridinium trochodium*. The latter species were kindly provided by Dr. Richard Norris, Department of Botany, University of Washington.

#### PRELIMINARY REPORT ON GROWTH RATE AND REPRODUCTIVE CYCLE OF THE SOFT-SHELL CLAM AT SKAGIT BAY, WASHINGTON

Russell G. Porter

*Washington Cooperative Fishery Unit  
University of Washington  
Seattle, Washington*

Growth rate and the annual reproductive cycle of the soft-shell clam, *Mya arenaria* L., are being studied at the Skagit River delta in Puget Sound, Washington. A brief explanation of the research and methods is presented. Sampling began in November, 1970 and will continue through Spring, 1973. The annual reproductive cycle during 1971 is described and the various stages of gonadal development enumerated. Spawning commences a little later for smaller clams, but in general lasts from late May through early September. In 1971 peak spawning occurred at Skagit Bay during July. A general comparison between the spawning cycle at Skagit Bay and those from studies

along the east coast from Canada to Maryland is presented.

#### PRELIMINARY EVALUATION OF OYSTER SEED HOLDING-TRAYS

A. J. Scholz

*Washington State Department of Fisheries  
Brinnon, Washington*

Seed oysters (*Crassostrea gigas*) usually suffer 50-75% mortality within the first year of planting due to siltation, crowding and predation. Oyster seed held in trays for 4 months and then planted had twice the survival as oyster seed initially planted on the ground (evaluation made at 11 months). The growth of the tray-reared seed was the same as the ground-reared control seed.

#### LARVAL DEVELOPMENT OF THE PIDDOCK, *ZIRPHEA PILSBRYI* LOWE

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*Fisheries Research Board of Canada  
Nanaimo, British Columbia*

Larvae of the rough piddock, *Zirphea pilsbryi* Lowe, were cultured at 2 temperatures, 15 and 20°C. The larvae have a characteristic round or circular shape, a dark band around the margin of the shell, a purple color near the ventral margin and a pink umbone region. At 15°C, larvae had a mean shell length increment of 4.6  $\mu$ /day and settled in 35-40 days; at 20°C the mean shell length increment was 6.7  $\mu$ /day and settlement occurred in 25-29 days. Metamorphosis occurred when the larvae had a shell length between 240 to 300  $\mu$ .

#### THE JAPANESE OYSTER DRILL, *OCENEBRA JAPONICA* DUNKER, IN NETARTS BAY, OREGON

Douglas R. Squire

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Marine Laboratory  
Port Orford, Oregon*

A viable population of *Ocenebra japonica*,

localized on a sandy low-tide island in the tail of Netarts Bay, was the subject of a general investigation. The drills are inactive in the winter; sheltered beneath relict *Crassostrea gigas* left on the crown of the island by a defunct commercial operation. Egg capsules are deposited on the relicts in May and June. Prey items did not include oysters, but were chiefly cockles, *Clinocardium nuttalli*, and less abundant bivalves. Protoconch juveniles were first observed in August, 1971; *Macoma inconspicua* and juvenile *C. nuttalli* were their major prey. Data from spat-baited wire traps furnished a good index of adult distribution, and indicated a downshore postspawning movement followed by a return to the relicts in the fall.

Aquarium-held snails fed single-prey diets of oyster, cockle, and *Olivella biplicata* for 2 months were tested for prey preference, along with starved and naive (field) drills. Statistical comparison ( $X^2$  homogeneity) of these data demonstrated prey choice reflected dietary history (Ingestive Conditioning), and confirmed that the cockle was the most important prey item in the field.

Implimented control measures consist of the construction of oyster shell heaps at strategic points on the island followed by removal of the shell and predators in the late fall of 1972.

#### PRODUCTION OF SHELLFISH FEED BY CONTINUOUS ALGAL CULTURE

Frieda B. Taub, Kathleen Ballard and Fred Palmer

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College of Fisheries  
Seattle, Washington*

A continuous algal culture apparatus of 32 liters (8 gal.) was developed which was capable of a sustained daily yield of  $2.0 \times 10^{11}$  cells consisting of 2-5 g ash free-dry weight of *Monochrysis lutheri*. This is a considerably greater yield than could be realized from this amount of space or effort, had traditional batch cultures been used.

The protein content of the cells varied from 7-45% of dry weight but not in the relatively orderly manner shown in the one liter continuous culture experiments.

Culture units of this size produce enough cell material for feeding trials of millions of oyster or

clam larvae, thousands of seed animals, or a few adults.

Scaling up to full hatchery size represents a further stage of development.

#### EXPERIMENTS IN OYSTER RAFT CULTURE AT CLAM BAY, WASHINGTON

Christopher Weller and Kenneth Chew

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College of Fisheries  
Seattle, Washington*

Oyster raft culture was initiated at Clam Bay on Central Puget Sound in May, 1971. A smaller experimental operation was also set up at Seabeck Bay. Preliminary information obtained at these 2 sites is presented.

Spacings between oyster strings of 20, 30 and 40 cm did not appear to effect differences in growth through December, 1971 in Clam Bay. There was a significant growth difference of 1.7 cm in length between Clam Bay and Seabeck Bay by December.

The mussel, *Mytilus edulis*, and the barnacle, *Balanus glandula*, were the most important competitors with respect to effect upon oysters. Observations at Clam Bay show that barnacles may undermine the attachment of oysters to cultch. Mussels were severe competitors at Seabeck Bay. By April, 1972, fouling comprised principally of mussels, amounted to 89% of wet weight per cultch. Many oysters appeared stunted.

The seastar, *Evasterias troschelli*, set on oyster strings in the early summer of 1971 at Clam Bay. By July of 1972, average radius length was 7.9 cm. There was a significant difference between spacings in number of seastars per string. The numbers were 2.0 for the 20 cm, 0.5 for the 30 cm, and 0.4 for the 40 cm spacing. Damage to mussels and oysters related directly to numbers and distribution of seastars. In July,

oyster damage was not yet extensive. At the 20 cm spacing, 4 percent of the cultch demonstrated signs of attack upon oysters. Oyster damage at the other 2 spacings was negligible. Mussels were more severely affected. There was evidence of predation on 29, 5 and 4 percent of the cultch at the 20, 30 and 40 cm spacings respectively.

#### A PARTIAL REVIEW OF PROBLEMS AND PROSPECTS OF THE PACIFIC COAST OYSTER INDUSTRY

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Brinnon, Washington*

Some of the problems facing the Pacific Coast oyster industry are: obtaining an adequate supply of seed oysters at a feasible price; offsetting the problems of adult mass mortality; and culturing around oyster drills. General improvement in methods of oyster culture, particularly obtaining better first year survival of seed, is important. Increased competition for use of water areas may also cause future problems.

On the plus side, the vast supply of relatively unpolluted, nutrient-rich water gives this area a major advantage. Recent efforts locally to upgrade an oyster product based on *Crassostrea gigas*, and the tremendous interest in France for use of *C. gigas* as a gourmet oyster would suggest that we should take a second look at the different possibilities of using *C. gigas*.

In review of the problems of oyster production nationally, it would appear that, while the Pacific Coast area has problems, these have solutions more readily available than is the case in other areas of the country. It would appear that with proper organization and effort a substantial increase in oyster production could be made in this area.

## ECOMORPHISM AND SOFT ANIMAL GROWTH OF *CRASSOSTREA IREDALEI* (FAUSTINO)

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### ABSTRACT

Marked ecomorphism in *Crassostrea iredalei* (Faustino) has been measured and established for three groups of oysters grown by different methods of culture—stick, hanging and broadcasting. The indexes of ecomorphism developed by the author are 0.84, 0.87 and 0.76 for the groups mentioned in the above order.

Shell volume, which is greatly affected by ecomorphism, has a curvilinear relationship with the weight of the soft animal. From bimonthly samples taken over a two-year period the shell volume and dry meat weight ratios were established as 73.52, 69.59 and 58.31 mg/cc for stick, hanging and broadcast grown specimens, respectively. These values were taken as measures of the physiological well-being of the soft animal.

### INTRODUCTION

In general, oyster culture may be categorized into bottom and off-bottom techniques. The methods involved in either case influence the shell formation rather sharply and hence, the growth of the animal as a whole. Shaw (1965) and Shaw and Merrill (1966) observed that suspending oysters off the bottom helped improve the condition of their meats and promoted a faster growth rate.

Shells of oysters grown on the bottom in tidal flats were quite different from those of the same species cultured off-bottom. This ecomorphic tendency is particularly noticeable in *Crassostrea iredalei*, the most important commercial species in the Philippines, but not in *C. malabonensis* (Faustino) which are commonly found growing in the same areas.

Shuster (1957) stated that he "... believed that information on the relationship of growth patterns to environmental factors will give additional insight into the lives of these mollusks, and thus, may be of practical value in the management of shellfish crops." The subject of this paper is in consonance with Shuster's investigation; namely the intricate relationships between shell formation as affected by methods of culture (ecomorphism)<sup>1</sup>, and the growth of the soft animal.

### STUDY SITE — BACOR BAY

Bacoor Bay, approximately 10 km<sup>2</sup> in area, is 12 km southwest of Manila North Harbor and almost directly south of a former U.S. military naval restricted area at Sangley Point, Cavite Province, Philippines (Fig. 1).

Specimens were collected in the approximate center of the bay and within a 0.50 km radius. This area includes an oyster farm of the Philippine Fisheries Commission and a few private oyster beds. Rainfall, solar radiation and tides that occurred in the bay area at the time of study are given in the appendix.

### METHODS AND MATERIALS

Live specimens were usually collected at bi-monthly intervals from December 1969 - October 1971. Collections consisted of 12 samples each of oysters cultured by stick (S) and broadcast (B) methods and 10 samples of the hanging (H) oysters. Each sample averaged from about 50 - 100+ specimens.

Immediately after collecting, live oysters were scrubbed clean and classified according to Carreon

<sup>1</sup>Ecomorph: Intraspecific growth of species in response to special environment.

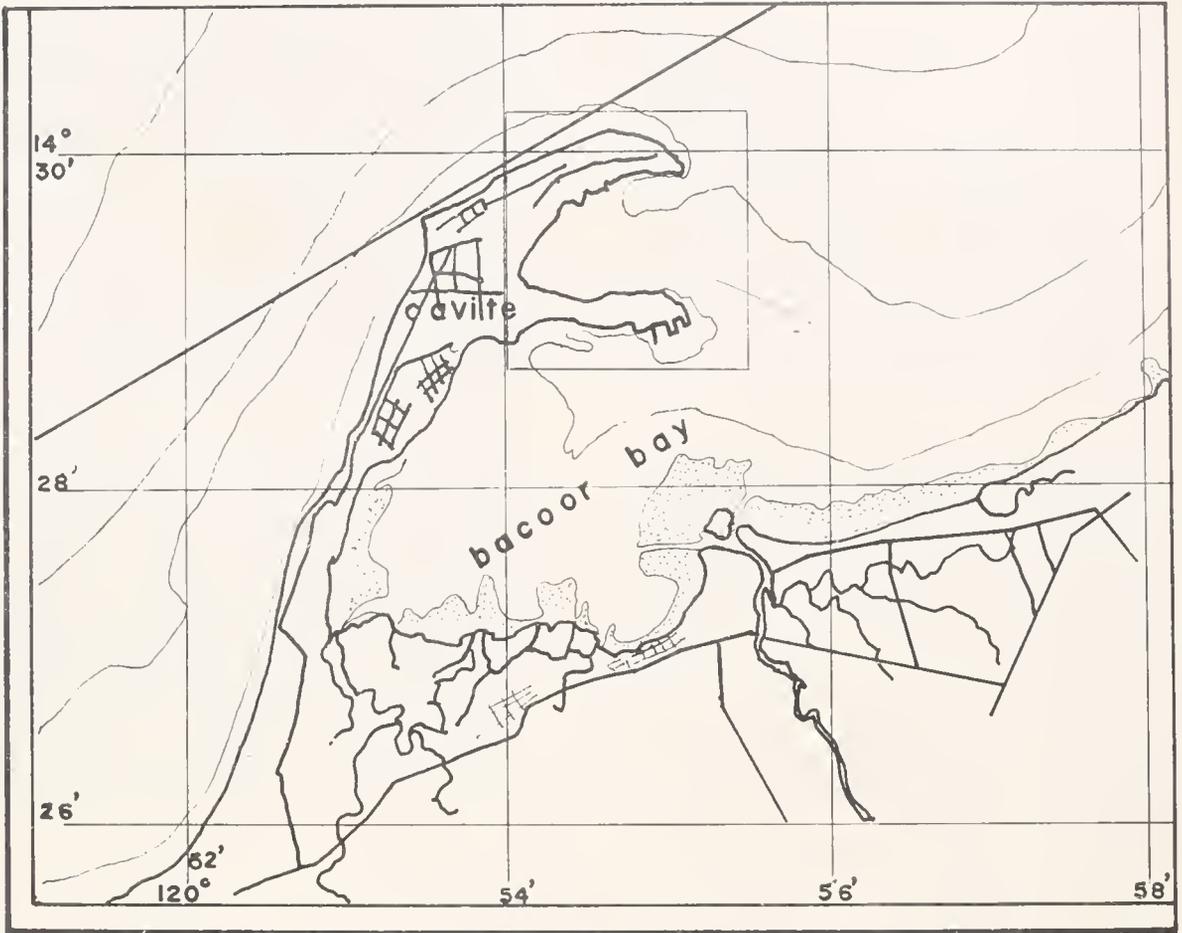


FIG. 1. Map portion of Manila Bay indicating location of Bacoor Bay. Scale 1:30,000 at Lat.  $14^{\circ} 32'$ ; traced from a map made by the Philippine Coast and Geodetic Survey.

(1969). The oysters were partly opened by carefully cutting through the hinge ligament. A syringe needle was then inserted through the opening and 2 - 3 ml of 10 - 15% formalin solution were injected. After the treated specimens had set overnight, they were shucked and the soft meat washed with dilute formalin to remove extraneous materials. The meats were drained for 2 hr and then individually weighed in tared paper boats. Specimens were dried in an oven at  $50 - 60^{\circ}\text{C}$  for 48 hr.

The shell measurements included longer and shorter axes of the left valve in centimeters (Fig. 2) and the shell volume in cubic centimeters. The oysters under study were equilateral, therefore, the longer and shorter axes of both valves were more or less equal.

Soft modelling clay was used to obtain volume measurements. Each shell was meticulously loaded so that the valves fitted together in a normal position. The volume of the molded clay was then measured by displacement.

## RESULTS

### *Ecomorphism in S, B and H Samples*

Generally, specimens from S samples were laterally concave on the left valve and closely described the curve of the cross-section of bamboo post used for attachment (Fig. 3). The shell was more or less dorso-ventrally equimorphic, rounded or blunt at the lip region, and with a moderate to very deep cavity near the hinge. Shell outline was less elongate than others, commonly oval to sub-quadrated.

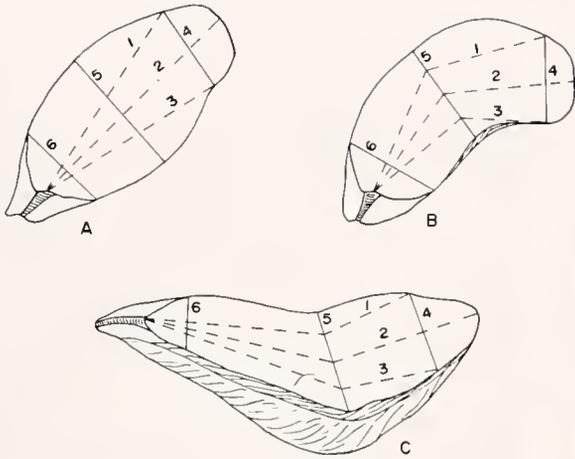


FIG. 2. Pattern for taking shell measurements to assume near elliptical outlines; A, in the case of shell with growth axis more or less straight; B, for shells dorso-ventrally deflected or vice-versa; C, for shells levo-dextro deflected or vice-versa. Longer axis = average measurements of 1, 2 and 3; shorter axis = average of 4, 5 and 6.

In contrast, B shells were regularly elongate, with older specimens wide, thin and flat at the lip region or posterior end, rarely deep near the hinge (Fig. 4). H shells were moderately elongate and normally subtriangular to oval in outline. The left valve was regularly deep at the hinge region. (Fig. 5).

#### The Index of Ecomorphism

The great individual diversity in the shell formation of *C. iredalei* makes it very difficult to establish the specific shell measurement which would give the best fitting measure of the degree of ecomorphism. Of the three dimensional relationships of bivalve shells cited by Galtsoff (1964), only the shell height-shell area regression measurement seemed to slightly differentiate the very apparent trend of ecomorphism in each of these three groups of oysters (Fig. 6). However, the information gathered did not yield clear-cut values that would comparatively distinguish one group from the other. For this reason, the author decided to use shell volume as a function of ecomorphism and to correlate observed volume to relative volume (volume of a sphere) whose surface area was equivalent to the plane shell area (assumed) with a configuration presumed nearly elliptical in outline. This assumed shell area was calculated from the observed dimensions of the shell's longer and shorter axes (Fig. 2).

Since a sphere contains the greatest volume

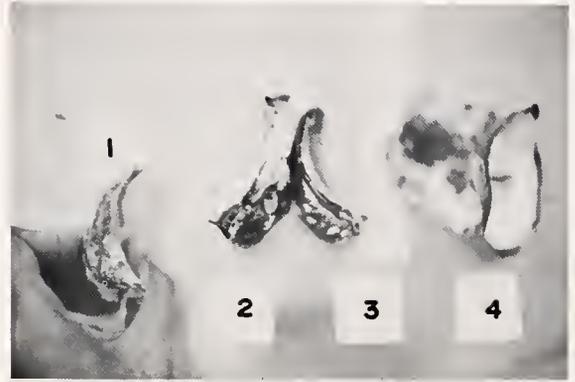


FIG. 3. Ecomorphic group of *C. iredalei* grown by the stick method. Shells above are in girdle view while below they are shown correspondingly in right valve view.

NOTE: All shells of Figures 3-5 show the weak edges of right valves slightly trimmed away to facilitate volume measurements by the use of modelling clay.

with the least surface area as compared to any other volumetric configuration, the ratio of observed shell volume to that of an assumed spherical volume will approach unity as the ecomorphic shell becomes deeper, and much lower than unity as the shell becomes more flat and shallower. Also, a shell that is flat and shallow has a greater surface area in proportion to its actual volume which in turn is much less than the volume of a spheroid assuming the same surface area. Based on this principle the indexes of ecomorphism of S, H and B specimens collected in this study were computed (Table 1).

#### Statistics of Shell Volume - Meat Weight Relationship

All the statistical analyses on the shell volume

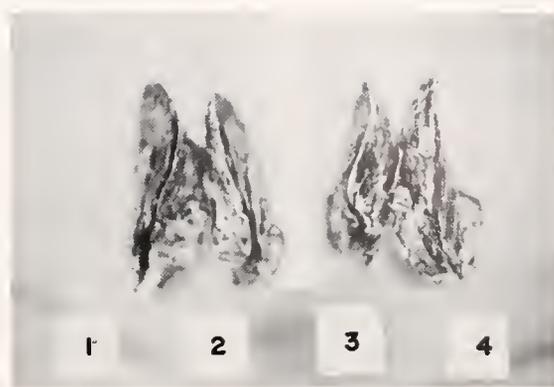
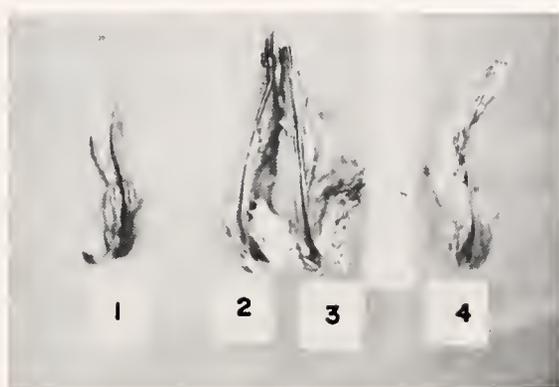


FIG. 4. Ecomorphic group of *C. iredalei* grown by the broadcast method. Shells above are in girdle view while below they are shown correspondingly in right valve view.

FIG. 5. Ecomorphic group of *C. iredalei* grown by the hanging method. Shells above are in girdle view while below they are shown correspondingly in right valve view.

TABLE 1. Shell areas, volumes and indexes of ecomorphism of *Crassostrea iredalei* grown by three different methods of culture. (All values tabulated are averages of total collections of each group.)

Sample Group	Area (cm <sup>2</sup> )	Volume (cm <sup>3</sup> )		Index of Ecomorphism <sup>c</sup>
		<sup>a</sup> V <sub>o</sub>	<sup>b</sup> V <sub>s</sub>	
Stick	25.0040	9.8653	11.7426	0.84
Hanging	24.4392	9.7544	11.2499	0.87
Broadcast	32.5206	13.3386	17.4820	0.76

<sup>a</sup>Observed shell volume content.

<sup>b</sup>Volume of sphere whose surface area = plane shell area assumed nearly elliptical in outline.

<sup>c</sup>The ratio of V<sub>o</sub> to V<sub>s</sub>.

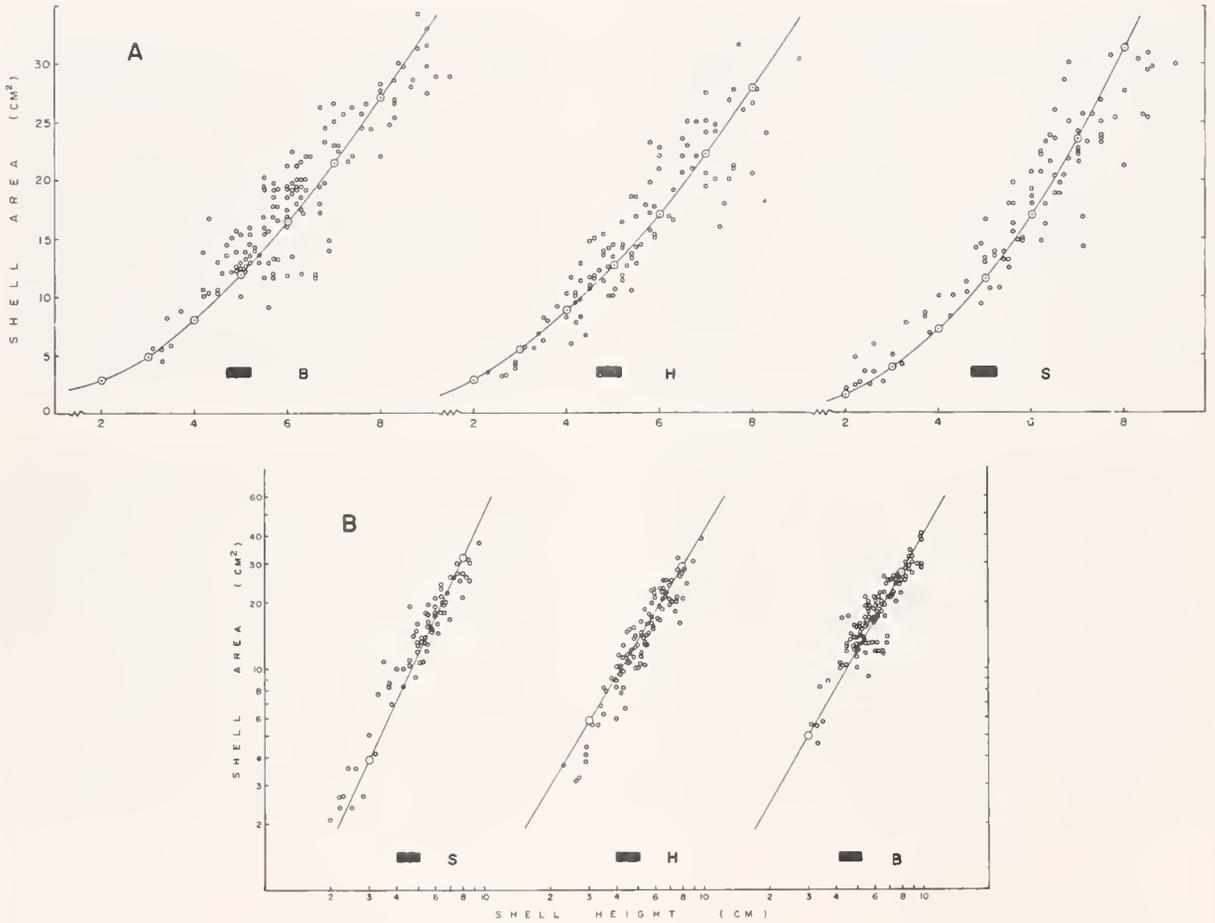


FIG. 6. A: Scatter diagram of shell area vs shell height of three ecomorphic groups of *C. iredalei*; B: Logarithmic regression of shell area on shell height of the same data in A.

and soft animal weight relationship were computed by the IBM System/360 in which the Xs and Ys represented the volumes (mm<sup>3</sup>) and weights (mgm), respectively. The nature of x and y relations was studied in two regression equations, namely

1) Rectilinear:  $Y = a + bX$

2) Exponential:  $Y = aX^b$ ; and based on the IBM output the best line of fit for the regression of meat weight on shell volume is curvilinear in each group, the degree of curvilinearity being different in each case (Fig. 7).

A comparative summary of the statistical parameters obtained by equations 1 and 2 above is presented in Table 2 (IBM output). From this Table, the respective exponential equations may thus be written as follows;

S Group:  $Y = -19.43 X^{1.03151}$

Log Y = 1.03151 Log X - 1.28847

H Group:  $Y = -30.43 X^{1.07673}$

Log Y = 1.07673 Log X - 1.48331

B Group:  $Y = -61.78 X^{1.13991}$

Log Y = 1.13991 Log X - 1.79082

Peters and Van Voohris (1940) recommended that the correlation coefficient, as computed from the actual sample, must be invariably shown to differ from 0, hence its standard error:  $\delta r = \frac{1}{\sqrt{N-1}}$ , and probable error: P.E.r = 0.6745 x  $\delta r$  must be computed for samples of the same size whose true  $r = 0$ . Using these formulas, highly significant values were obtained as tabulated:

Sample Group	$\delta r$	P.E.r
S	0.036273	0.024484
H	0.037087	0.025024
B	0.036394	0.024552

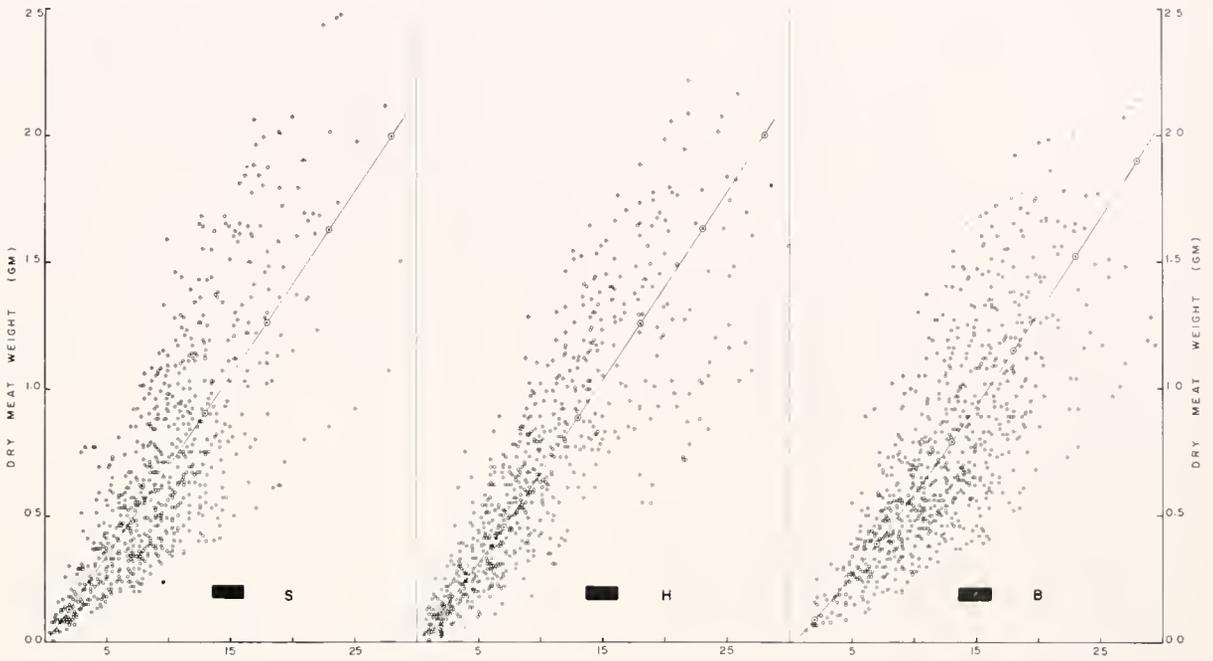


FIG. 7. The regression of dry meat weight on shell volume of three ecomorphic groups of *C. iredalei*.

TABLE 2. Comparative IBM statistical results between rectilinear and exponential regressions of meat weight on shell volume of *Crassostrea iredalei* (Faustino).

Statistical parameter	S		H		B	
	$Y = a + bX$	$Y = aX^b$	$Y = a + bX$	$Y = aX^b$	$Y = a + bX$	$Y = aX^b$
N	761	-do-	728	-do-	756	-do-
Mean	727.16162	2.74721	683.09058	2.69052	835.50244	2.83453
Std. deviation	513.97534	0.33972	493.68896	0.39975	552.70166	0.28881
Correlation						
X vs Y	0.83110	0.86776	0.85543	0.88861	0.82731	0.87189
Intercept of Y on X	-77.37354	-1.28847	28.69775	-1.48331	-105.56641	-1.79082
Regression coefficient	0.08267	1.03151	1.06822	0.07673	0.07319	1.13991
Std. error of reg. coef.	0.00196	0.02144	0.00153	0.02063	0.00181	0.02332
Std. error of estimate	281.34497	0.16895	255.86574	0.18347	310.68384	0.14152
Computed						
T value	42.15930	48.10270	44.50340	52.20300	40.44020	48.98010
F value	1,777.41	2,313.87	1,980.56	2,725.15	1,635.41	2,390.25

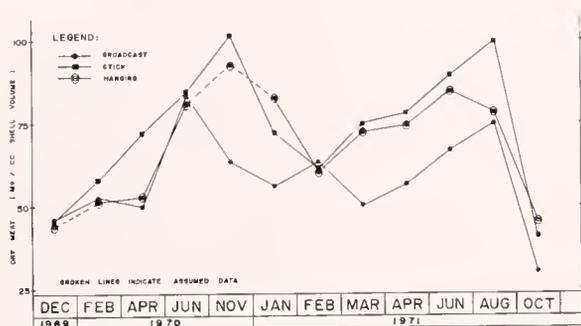


FIG. 8. Graphical comparison on the periodical condition of three ecomorphic groups of oysters grown by different methods. Condition of soft animal expressed in milligrams of dry meat per milliliter of shell volume.

The significance of the statistical parameters obtained for exponential regression of meat weight on shell volume were rather acceptable even at  $P \geq 0.001$  when referred to the tables of Fisher and Yates (1957).

#### *Ecomorphism and the Growth of the Soft Animals*

Shell volume is perhaps one of the primary factors affecting the physiology of the soft animal. When the valves are tightly closed for several hours at certain intervals within and/or between tidal cycles, the animal is protectively sealed within a limited amount of space. How much and to what extent this affects the soft animal is rather difficult to discern. The author believes that the meat weight, as a measure of growth, is greatly affected by all the biophysical activities of the animal in producing its shell. In one way or the other, the growth and general well-being of the soft animal may be related to some parameters of the shell, particularly shell volume and variations in shell form as a result of ecomorphism. Following this belief, it may be further stated that ecomorphic groups within the same species which attain greater volume with least shell surface area will tend to exhibit better meat growth than those animals with a lower volume and greater shell surface.

As a result of the data thus gathered, and supported by the statistical parameters shown in Table 3, an analysis of variance was conducted to measure the significance of the differences in meat

weights of the three ecomorphic groups. The variance ratio was,  $F = 16.6789$  which exceeded the table value of 6.91 when  $n_1 = 2$  and  $n_2 = \text{infinity}$ , with  $P \geq 0.001$  (Fisher and Yates, 1957).

From all observations, it appears that in terms of meat weight, oysters of the S ecomorphic group were in better condition than those of the other two groups. Oysters of the broadcast method were the poorest in weight throughout most of the study as shown in Figure 8. This graph is based on the computed well-being of the soft animal per sampling time expressed in milligrams of dry weight per cubic centimeter of shell volume. The annual values obtained are as follows:

	1970	1971
S . . . . .	70.65 mg/cc	79.19 mg/cc
H . . . . .	.66.74 mg/cc	70.70 mg/cc
B . . . . .	.59.65 mg/cc	54.90 mg/cc

For all the samples gathered throughout the period, the average shell volume-meat weight ratios are 73.52 mg/cc, 69.59 mg/cc and 58.31 mg/cc for S, H and B groups, respectively.

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## APPENDIX

*Major ecological factors in Bacoor Bay.*

Month	Rainfall <sup>a</sup>		Solar Radiation		Tide (feet) <sup>c</sup> Ref:MLLW			
	(mm)		gm cal/cm <sup>2</sup>		1970		1971	
	1970	1971	1970	1971	Highest	Lowest	Highest	Lowest
Jan	25	13	342	360	4.5	-1.7	4.3	-1.3
Feb	1	0.5	488	346	4.2	-1.5	3.9	-1.0
Mar	4	43	464	415	3.8	-1.1	3.7	-0.6
Apr	38	7	474	466	4.1	-0.8	4.2	-0.6
May	14	215	490	416	4.6	-1.1	4.5	-0.6
Jun	256	368	366	401	5.0	-1.2	4.7	-0.8
Jul	284	296	351	391	5.1	-1.2	4.7	-0.8
Aug	222	135	319	448	4.9	-1.0	4.8	-0.7
Sep	347	132	308	408	4.3	-0.6	4.6	-0.4
Oct	277	451	223	321	4.2	-0.3	4.5	0.0
Nov	347	132	308	408	4.2	-0.3	4.5	0.0
Dec	277	451	223	321	4.3	-0.3	4.5	-0.2
	390	97	221	324	4.3	-0.3	4.7	-0.2
	46	155	244	352	4.4	-0.9	4.6	-0.8
						-1.6		-1.2

<sup>a</sup>As recorded in Bacoor, Cavite.<sup>b</sup>As recorded by the nearest weather station located at latitude 14°39' north and longitude 121°04' east.<sup>c</sup>As predicted by the Phillipine Coast and Geoditic Survey with reference to the station at Manila.

## A STUDY OF CHEMO-RECEPTORS ON LABIAL PALPS OF THE AMERICAN OYSTER USING MICROELECTRODES<sup>1</sup>

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### ABSTRACT

*Tungsten microelectrodes, insulated except for their tips, were used to pick up receptor potentials from chemical receptors on labial palps of the American oyster. A functional criterion was used to determine when a microelectrode had penetrated a receptor cell. The receptor cell responded with differential sensitivity in response to four major taste substances. An equation was derived which defined the relationship between taste receptor potential and strength of chemical stimulant.*

### INTRODUCTION

The two pairs of labial palps of the oyster which lie at the anterodorsal side of the body under the mantle hood are joined together into a single unit which serves primarily for the final sorting of food particles and for the delivery of the food to the mouth (Galtsoff, 1964). Researchers in the past have shown that an oyster may reject food which has no value to it (Lotsy, 1895; Grave, 1916; Loosanoff, 1949; Ukeles, 1970). They speculated that the labial palps possess chemical receptors. A previous study by the author confirmed this speculation by demonstrating electrophysiologically the existence of chemical sensors on the palps (Dwivedy, 1972)<sup>2</sup>. The objective of the present study was to further the previous study in order to define characteristics of these chemical sensors.

### ANATOMY AND HISTOLOGY OF THE PALPS

#### *Anatomy*

A detailed study of structures of the labial palps has been made by Galtsoff (1964). A synopsis of his work is quoted in part as follows:

“The four soft flaps which lie at the anterodorsal side of the body under the mantle hood are labial palps (Fig. 1). The two pairs of palps, one on each side, are joined together into a single unit. Each pair consists of one external and one internal palp. The two external palps join together above the mouth where they form the upper lip; the two internal palps are united below the mouth into a lower lip. As a result of this arrangement, the mouth is an irregularly shaped, narrow, curved slit. Both lips are arched; the lower one is shorter and its edge is thicker than that of the upper lip.”

#### *Histology*

Galtsoff (1964) has also studied the histology of the labial palps in detail. A synopsis of his work is quoted in part below:

“Each labial palp consists of a layer of connective tissue covered on both sides by columnar ciliated epithelium set on a basement membrane. The epithelium of the smooth surface of the palp consists of almost cubical cells with relatively large nuclei and small cilia (Fig. 2). Cell boundaries are distinct, the cells themselves are crowded and compressed, and there is a very thin and transparent cuticle on the periphery. In the subepithelial layer

<sup>1</sup>Published as Miscellaneous Publication No. 658 with the approval of the Director of the Delaware Agricultural Experiment Station. Publication No. 2 in the Department of Agricultural Engineering.

<sup>2</sup>Dwivedy, R. C. 1972. Instrumentation and technique of electrophysiological studies of chemoreceptors on labial palps of the American oyster. Paper #72-513, American Society of Agricultural Engineers, St. Joseph, Michigan.

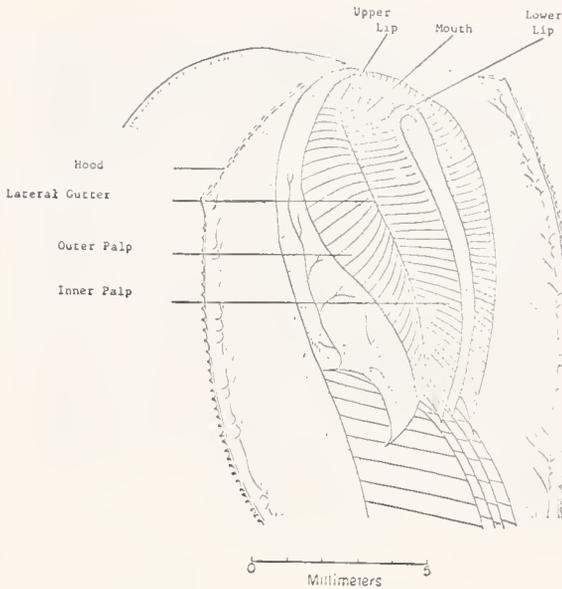


FIG. 1. Labial Palps of the American Oyster (Reprinted from Galtsoff, 1964).

large eosinophilic cells and mucous cells are very abundant. The palps are innervated by the nerve emerging from the cerebral ganglion and entering the anterior end of the junction between the paired lobes."

#### METHODS

The labial palps and part of the gills of adult American oysters, *Crassostrea virginica*, were exposed by drilling or breaking through the anterior and posterior portions of the flat valve of a specimen; this was done without causing injury to the underlying tissues. A recording microelectrode was inserted in one of the labial palps and a reference electrode was inserted in the gill. Two Narsheage MM3 micromanipulators were used to hold the electrodes and to regulate the depth of probing. An optical microscope with a magnification of 100x was used during probing of the electrodes.

Tungsten microelectrodes were manufactured by using technique described by Hubel (1957). Tungsten wires were electropolished until a final tip diameter of about  $1\mu$  was achieved. The electrodes were then washed in detergent and were insulated, except for their tip, with a clear stone-mudge coating material. The impedance of these electrodes, measured in preparation at about  $70^{\circ}\text{F}$ , was approximately 75 megohms.

Electrical responses of the labial palps were fed

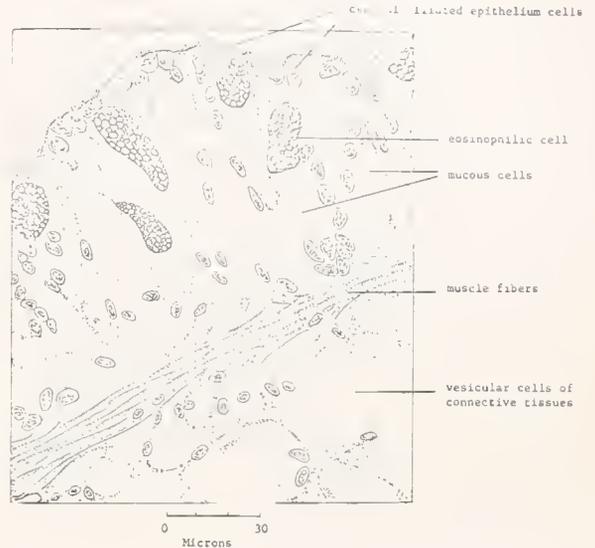


FIG. 2. Cross section of the smooth side of labial palp of the American Oyster (Reprinted from Galtsoff, 1964).

to a Tektronic Dual Trace Oscilloscope, through a Model P16 D.C. microelectrode amplifier manufactured by the Grass Instrument Co. The oyster-electrode preparation was housed in a copper Faraday cage to prevent stray electrical pick up by the electrodes. The recording of electrical responses of the labial palps upon their chemical stimulation has been termed as Electropalpusgram, hereafter referred to as EPG.

The recording set up is shown in Figure 3. The oyster was probed using the technique described previously. The electrodes were connected through

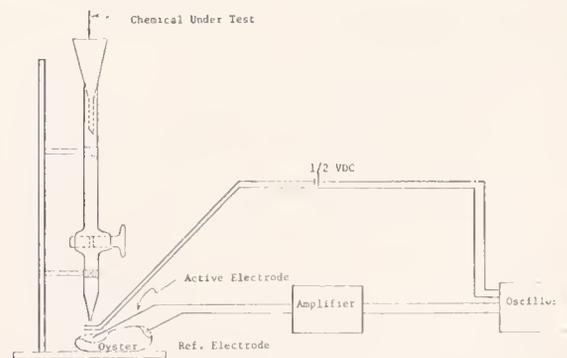


FIG. 3. Schematic diagram showing set-up for simultaneous recording of EPG and latency period.

the amplifier to the upper beam of the oscilloscope. A burette was used to drop liquid stimulants over the oyster palps. The open and uninsulated ends of two stiff copper wires were placed just underneath the burette outlet but above the oyster palps. These two wires were connected through a 0.5 volt dry cell to the lower beam of the oscilloscope. During passage, the chemical drop completed the open D.C. circuit resulting in a signal of the oscilloscope just before stimulating the palps. The time difference between this signal from D.C. circuit and the onset of electrical response from labial palps was the sum of the two time components, time for the chemical drop to travel from the D.C. circuit to the palps and the latent period of electrical response of the palps. The first component was measured by replacing the oyster preparation by another similar D.C. circuit which was subtracted from the total time lag to obtain actual latent period.

It is a standard practice to make functional identification of the particular type of cell or receptor that initiates an observed electrical response to a chemical stimulus. For example, a single nerve fiber dissected free from the chorda tympani nerve is assumed to be a taste fiber (and not a temperature, tactile, pain etc., which are also found in the same nerve bundle) if it responds to low or moderate concentrations of appropriate taste stimuli applied to the surface of the tongue. Since it was not possible to see taste cells at the surface of the palps, a similar functional criterion was established for the purpose of this study. The palps were traversed until a sudden decrease in D.C. potential was measured; this indicated that the electrode had penetrated a cell. If the D.C. potential did not change upon chemical stimulation, then another location was sought. On the other hand, if the potential did change upon stimulation, then the penetrated cell was assumed to be an active taste cell. The sudden decrease in D.C. potential upon penetration of the electrode was considered to be the resting potential of the cell and depolarization of the cell acted as receptor potential. A large number of attempts were made usually before such a cell was found. Furthermore, no appreciable response has ever been observed when the microelectrode was penetrated into other parts of the body, such as gills. To verify that the electrical responses were not just the result of artifacts of electrode, the oyster was killed by injecting NaCN into its body after responses had been recorded. The responses ceased completely when the oyster was dead.

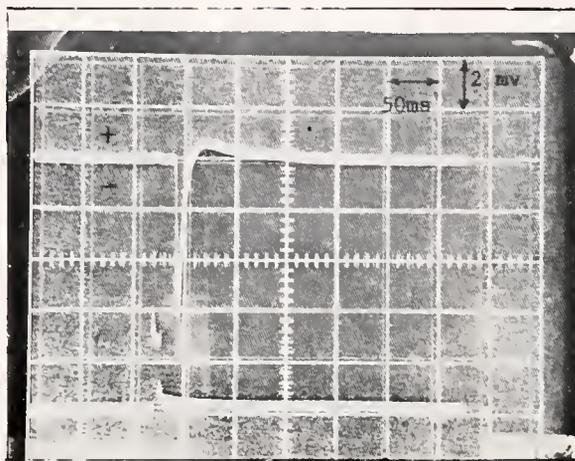


FIG. 4. A typical electrical response of the chemical sensors in labial palps of the oyster, referred to as *Electropalpusgram* (EPG). Upper trace is EPG, lower trace is signal from DC circuit for latency measurement.

Receptor potential of the chemo-sensor in labial palp was measured in response to distilled water which served as a response to solutions with zero molarity. Larger responses were observed as the concentration was increased. The strength of the test solution was increased until the receptor potential (magnitude of negative wave at the onset of EPG) ceased to increase. Distilled water rinses were applied to the labial palp between stimuli. Taste receptor potentials were plotted against molar concentrations of solutions.

## RESULTS

The recording of a typical electrical response (EPG) of the chemo-sensors of labial palps is shown in Figure 4. The sensors respond to chemical stimulation by a sharp negative wave followed by a slow positive wave (with respect to the reference electrode in the gills). The D.C. circuit responds with rather a sharp spike (lower trace in Fig. 4) upon contact of a chemical drop to the open ends of wires (Fig. 3). The time that it took the chemical drop to travel from the ends of the wires to the labial palps was measured as about 25 milliseconds. This time period was subtracted from the total time lag between the two signals in Figure 4 to obtain actual latent period of the receptors. The usual magnitude of latent periods was about 50 milliseconds for the several chemicals that were tested in this study.

Four distinct taste submodalities are recognized in human; sweet, salt, bitter and sour. These sub-

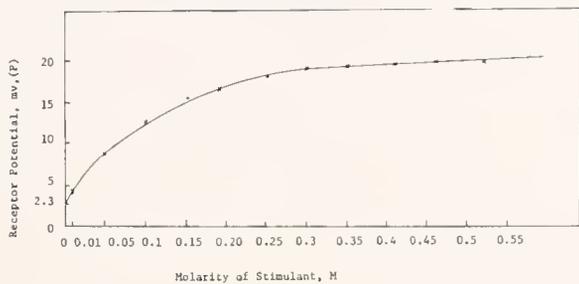


FIG. 5. Taste receptor potential plotted as a function of molar concentration of sucrose.

modalities are associated with four major substances, which in the same order are: sucrose, sodium chloride, quinine sulfate, and hydrochloric acid. Pure solutions of these four major substances were tested in this study. Resulting curves are given in Figures 5, 6, 7 and 8. Each point in these curves represents an average value of three recordings from individual oysters. Variation within corresponding individual readings was insignificantly small for a given solution. The receptor potential obtained in response to sodium chloride diminishes as the concentration of solution is increased until it reaches the saturation point. Conversely, the receptor potentials with other test chemicals increase as the concentration of the solution is increased up to saturation.

Slopes of the curves represented in Figures 5, 6, 7 and 8 were measured at several points by using a half-silvered mirror. Perpendiculars to the curves were drawn by positioning the mirror so that the portion of the curve reflected in the mirror matched the curve behind the mirror. The slopes of these curves were plotted against molarity of test solution on semi-logarithm graphs. The

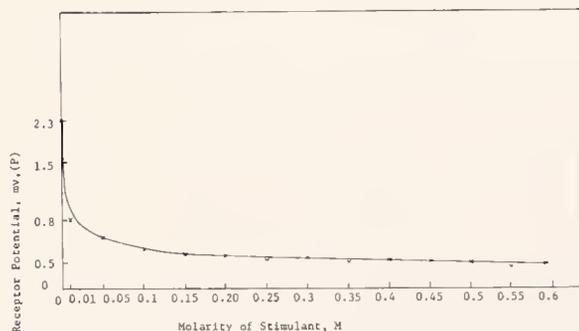


FIG. 6. Taste receptor potential plotted as a function of molar concentration of sodium chloride.



FIG. 7. Taste receptor potential plotted as a function of molar concentration of quinine sulfate.

resulting plots, as shown in Figures 9, 10, 11 and 12 are straight lines with negative slopes. The magnitude of the slope of the straight line multiplied by 2.3026 to convert to naperian logarithms is denoted by  $K$  and is shown in each plot.

#### DISCUSSION

It is reasonable to assume that the magnitude of negative wave at the onset of EPG (Fig. 4) is a measure of sensitivity of the sensor cell. On this basis, it is evident by examination of curves represented in Figures 5, 6, 7 and 8 that the sensitivity of the sensor cell differs for chemicals tested in this study. Differential sensitivity of the receptors indicates the possibility that an oyster is probably able to discriminate between different chemicals. Further studies are required to prove or disprove this assumption.

The cubical ciliated epithelium cells (Fig. 2) are the only cells that are probably sensory cells. By using a histological technique as described by Bultitude (1958), attempts were made without success to localize the electrode tip after recording

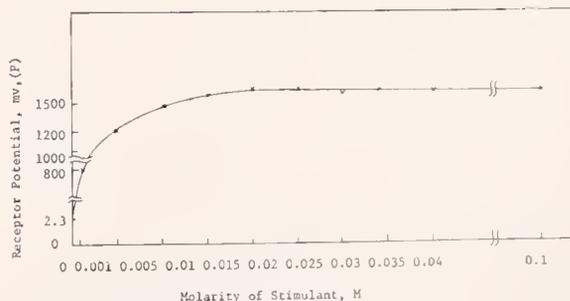


FIG. 8. Taste receptor potential plotted as a function of molar concentration of hydrochloric acid.

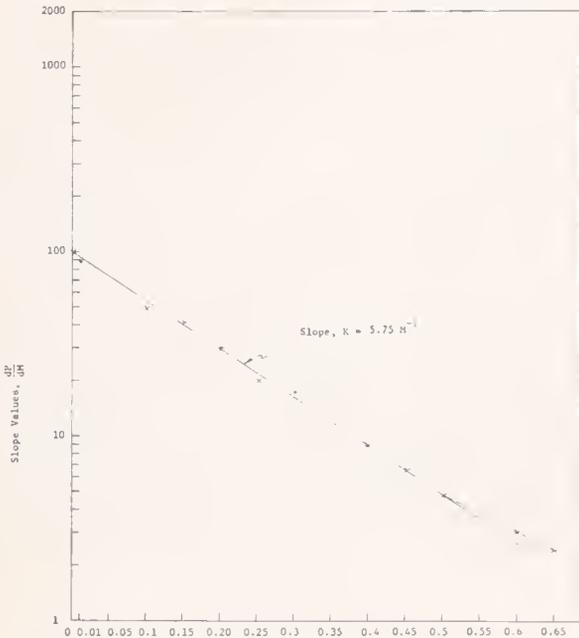


FIG. 9. Relationship between logarithm of slope values and molar concentration of sucrose.

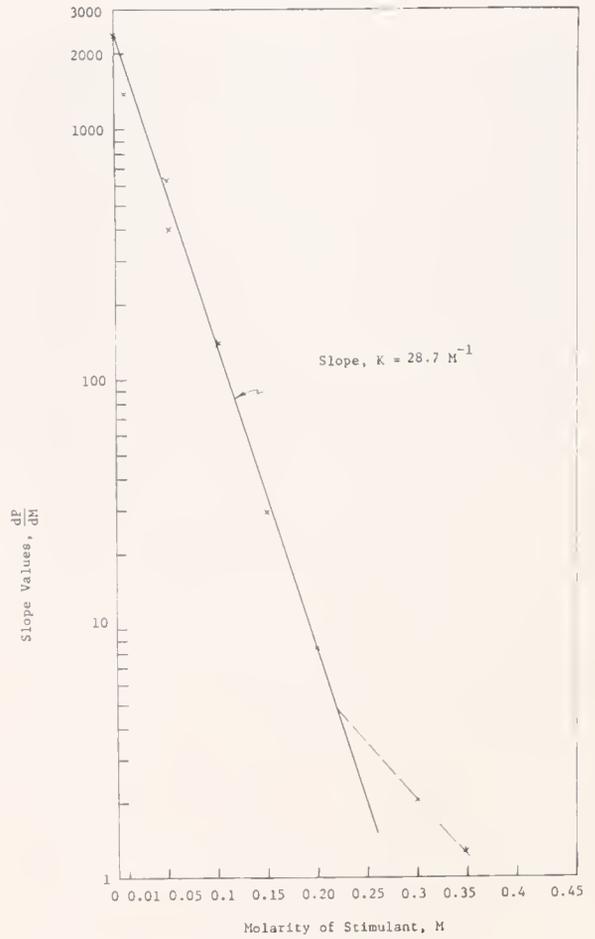


FIG. 10. Relationship between logarithm of slope values and molar concentration of sodium chloride.

the responses. The reason for this failure was that the diameter of the colored spot was about  $20\mu$  whereas the size of the cells is much smaller. Moreover, a slight movement of the tip of the electrode during this experiment caused widespread marking. For this reason, any definite statement about the origin of the electrical responses recorded from labial palps cannot be made. However, there is a high probability that these electrical responses were the result of depolarization of the membrane of the ciliated epithelium cells (Fig. 2).

*Characteristic Equation for Taste Receptors of the Oyster*

It was found that a straight line relationship exists between the molarity of the chemical stimulant and logarithm of the slopes of the curves of taste receptor potential versus molarity of stimulant. In other words,  $\log \frac{dP}{dM}$  and  $M$  are related by

a straight line where  $P$  is the receptor potential and  $M$  is the molarity of the stimulant.

A typical equation of the straight lines shown in Figures 9, 10, 11 and 12 is

$$\text{Log } \frac{dP}{dM} + \text{Log } C_1 = -KM$$

where  $\log C_1$  is a constant equal to the ordinate-intercept of the straightline plot and  $K$  is another experimentally determined constant ( $K$  is

equal to slope of straight line multiplied by 2.3026 to convert to naperian logarithms). The above equation can be solved for  $P$  as follows:

$$\text{Log } \frac{dP}{dM} + \text{Log } C_1 = -KM \dots \dots \dots (1)$$

$$\text{Log } \left[ \left( \frac{dP}{dM} \right) C_1 \right] = -KM \dots \dots \dots (2)$$

$$\frac{dP}{dM} = \frac{e^{-KM}}{C_1} \dots \dots \dots (3)$$

Where  $e$  is the base of naperian logarithm:

$$\frac{dP}{dM} = C e^{-KM} \dots \dots \dots (4)$$

where  $C = \frac{1}{C_1}$

Integration of the equation (4) results into:

$$P = \frac{C}{K} e^{-KM} + C_2 \dots \dots \dots (5)$$

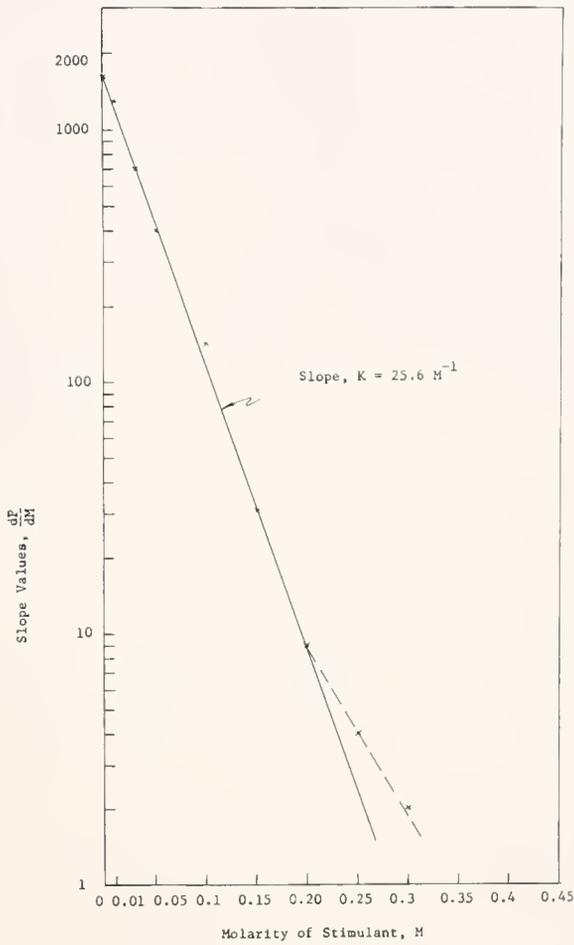


FIG. 11. Relationship between logarithm of slope values and molar concentration of quinine sulfate.

When the molarity of the test solution was zero, i.e., distilled water, then the receptor potential was experimentally obtained as 2.3 mv. (Figs. 5, 6, 7 and 8).

$$\text{Therefore } C_2 = 2.3 + \frac{C}{K}$$

$$P = 2.3 + \frac{C}{K} (1 - e^{-KM}) \dots \dots \dots (6)$$

Where P is the taste receptor potential in mv, C and K are experimentally determined constants and M is molarity of the test solution.

Equation (6) governs the relationship between taste receptor potentials and molarity of solutions for any chemicals tested in this study except NaCl. For NaCl, since magnitude of the receptor potential diminishes as the concentration of solution goes up until saturation the characteristic

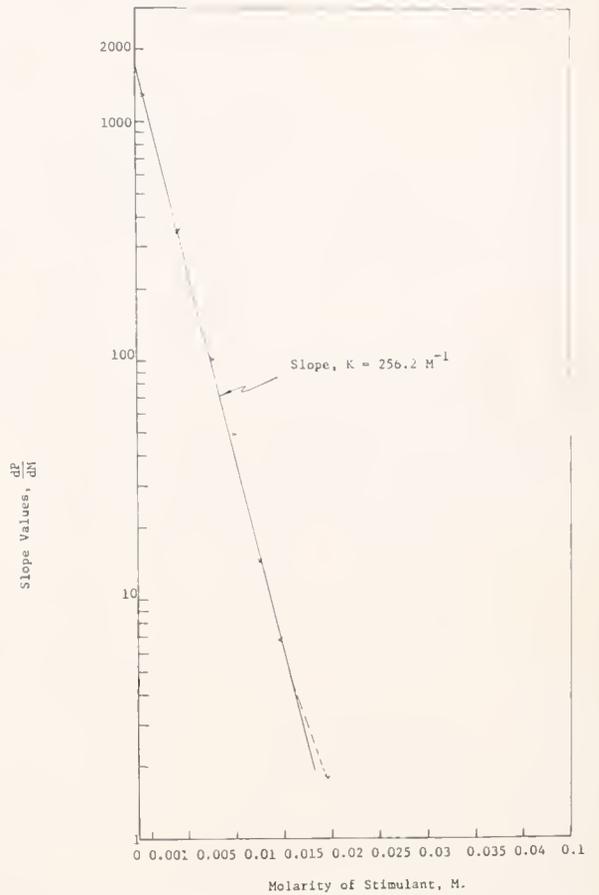


FIG. 12. Relationship between logarithm of slope values and molar concentration of hydrochloric acid.

equation would be as follows:

$$P = 2.3 - \frac{C}{K} (1 - e^{-KM}) \dots \dots \dots (6a)$$

For large values of the molarity of solutions M, the factor  $e^{-KM}$  becomes very small (K being constant) and can be practically neglected. The equations (6) and (6a) then reduce to:

$$P = 2.3 + \frac{C}{K}$$

and

$$P = 2.3 - \frac{C}{K}$$

respectively.

The pattern of diminishing receptor potentials in response to increasing concentrations of sodium chloride is compatible to the fact that the oyster lives in saline water and therefore, its sensory system may not be aroused when exposed to

changes within the natural environment.

#### ACKNOWLEDGMENT

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## CONCENTRATIONS OF FIVE TRACE METALS IN THE WATERS AND OYSTERS (*CRASSOSTREA VIRGINICA*) OF MOBILE BAY, ALABAMA

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### ABSTRACT

*From January 1968 through June 1969, samples of water and oysters were collected at monthly intervals from eight locations in Mobile Bay, Alabama. These samples were analyzed for cadmium, chromium, copper, lead and zinc by atomic absorption spectrophotometry and the results analyzed statistically.*

*Oysters from Mobile Bay contained less cadmium, copper and zinc than the average concentrations reported for Atlantic Coast oysters. The concentration of chromium was approximately the same while the lead concentration was about two times that of the average value for Atlantic Coast oysters.*

*Oysters collected from the western side of the Bay were found to contain a significantly greater concentration of copper and zinc than oysters collected from the eastern side. These differences were attributed to differences in river systems that contribute the fresh water discharge and runoff to opposite sides of the Bay.*

*Although concentrations of the trace metals investigated were  $10^3$  -  $10^5$  higher in oysters than the concentrations in the environmental water samples, poor correlation was observed between the two sets of data.*

### INTRODUCTION

Marine organisms have the ability to accumulate trace elements from the environment (Vinogradov, 1953). Hiltner and Wichmann (1919) demonstrated that metallic wastes in industrial effluents could be responsible for abnormally high concentrations of copper and zinc in oysters. Hunter and Harrison (1928) reported detecting lead and arsenic in oysters growing in industrially polluted waters. The potential danger to public health that could arise from the consumption of shellfish contaminated with heavy metals was discussed at the National Shellfish Sanitation Workshop held in Washington, D. C. in 1961 (McFarren, Campbell and Engle, 1961). Concern continued to grow as the coastal

waters became more heavily industrialized, and the U. S. Public Health Service initiated a program to provide information on the relationship of trace metal levels in the environmental waters and the levels in oysters.

The purposes of this study, performed at the Gulf Coast Water Hygiene Laboratory<sup>2</sup>, were (a) to provide data to serve as background concentrations of cadmium, chromium, copper, lead and zinc in oysters in Mobile Bay for future reference; (b) to determine if the trace metal concentrations in samples of shellfish growing waters could be correlated with the trace metal concentrations in oysters and (c) to determine the variations that can occur in trace metal concentrations in both oyster and water samples from different localities in a relatively small area such as Mobile Bay.

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<sup>2</sup> Former name of the Gulf Coast Water Supply Research Laboratory, Dauphin Island, Alabama.



FIG. 1. Location of sampling sites in Mobile Bay, Alabama.

#### FIELD SAMPLING PROCEDURES

Monthly collection of oyster and water samples began during January of 1968 from the eight locations in Mobile Bay shown in Figure 1. Both oyster and water samples were collected through January 1969. From February 1969 through June 1969 when sampling ended, only oyster samples

were collected. Ten or 12 oysters, collected by dredging, were used as a sample from each location. Since stratification in the relatively shallow waters over the oyster reefs in Mobile Bay is not pronounced (Austin, 1954; McPhearson, 1970), the water samples from each location were collected by submerging a one gallon polyethylene bottle below the surface.

## LABORATORY PROCEDURES

All laboratory glassware was washed in a detergent solution and rinsed in tap water followed by rinsing in deionized water. The glassware was then rinsed in dilute nitric acid (1:4) and finally rinsed, three times in glass-distilled water.

A Perkin Elmer Model 303 atomic absorption spectrophotometer<sup>3</sup>, with instrument settings recommended by the manufacturer, was used to determine the metal concentrations in the prepared samples. The samples were prepared for analysis as described below.

*Oyster Samples*

The shells of the oysters were scrubbed with a stiff brush under running tap water to remove mud. The oysters were then shucked, and the pooled meats were drained and homogenized for three minutes at high speed in a Sorvall Omni-Mixer. Duplicate 10 - 20-gram aliquots of each homogenate were weighed to the nearest 0.01 g into 300 ml tall form beakers. Twenty ml of concentrated reagent grade nitric acid was added to each

<sup>3</sup> Mention of commercial products does not necessarily imply endorsement by the U. S. Government.

beaker. To prevent foaming, two drops of a dilute aqueous suspension of Dow Antifoam C were added to each sample. The beakers were covered with watch glasses and the contents heated to boiling on a hot plate. Gentle boiling was continued until the tissue had been completely digested, about 4-6 hr. Each digested sample was filtered through glass wool into a 50-ml volumetric flask and diluted to volume with distilled water.

Preliminary studies indicated that the solids content of the prepared samples did not interfere with the analysis and also that the iron content was not of sufficient magnitude to interfere with the determination of chromium as described by Giammarise (1966). Recoveries of the five elements from fortified samples ranged from 95-103%.

*Water Samples*

A 2-liter water sample was filtered through a 0.45 $\mu$  membrane filter. The sample was placed in a 3-liter beaker and concentrated to 200 ml by gentle boiling under a stream of clean, dry air. The concentrated sample was adjusted to pH 3 with HCl and transferred to a separatory funnel. One ml of 2% aqueous solution of ammonium pyrrolidine dithiocarbamate was added, and the

TABLE 1. Summary of cadmium concentrations in oyster and water samples from Mobile Bay.

Sampling Location	Cadmium concentrations			Total Samples	Number Quantifiable
	Range	Median	Mean $\pm$ S.E.		
Oyster samples (mg/kg wet weight)					
50	0.07-1.61	1.00	1.04 $\pm$ 0.07	14	14
89	0.05-1.16	0.65	0.60 $\pm$ 0.08	13	13
118	0.03-1.16	0.68	0.70 $\pm$ 0.07	13	13
119	<0.05-1.20	0.50	0.65 $\pm$ 0.10	12	11
83	0.20-1.30	0.47	0.53 $\pm$ 0.08	13	13
92	0.10-0.80	0.51	0.52 $\pm$ 0.08	15	15
104	<0.05-0.60	0.45	0.49 $\pm$ 0.04	15	14
112	0.05-0.75	0.49	0.46 $\pm$ 0.04	15	15
Water samples ( $\mu$ g/l)					
50	<0.1-1.4	0.6	--	13	8
89	<0.1-1.5	0.4	--	12	8
118	<0.1-1.1	0.3	--	12	8
119	<0.1-9.1	0.6	--	11	8
83	<0.1-1.8	0.5	--	12	8
92	<0.1-1.3	0.5	--	11	8
104	<0.1-1.2	0.6	--	12	8
112	<0.1-1.4	0.5	--	12	9

TABLE 2. Summary of chromium concentrations in oyster and water samples from Mobile Bay.

Sampling Location	Chromium concentrations			Total Samples	Number Quantifiable
	Range	Median	Mean $\pm$ S.E.		
Oyster samples (mg/kg wet weight)					
50	0.70-3.30	0.28	0.52 $\pm$ 0.20	14	14
89	<0.10-0.63	0.24	0.27 $\pm$ 0.04	14	13
118	<0.10-0.83	0.25	0.33 $\pm$ 0.06	14	12
119	<0.10-0.65	0.23	0.30 $\pm$ 0.05	13	12
83	0.12-0.70	0.34	0.34 $\pm$ 0.04	14	14
92	0.12-0.80	0.37	0.38 $\pm$ 0.04	16	16
104	<0.10-1.00	0.28	0.38 $\pm$ 0.06	16	14
112	<0.10-0.58	0.26	0.29 $\pm$ 0.03	16	14
Water samples ( $\mu$ g/l)					
50	<0.1-0.8	<0.1	--	13	4
89	<0.1-1.4	<0.1	--	12	3
118	<0.1-2.3	<0.1	--	12	4
119	<0.1-1.0	<0.1	--	11	3
83	<0.1-3.7	0.2	--	12	7
92	<0.1-2.6	0.2	--	11	6
104	<0.1-2.9	0.2	--	12	7
112	<0.1-2.9	0.8	--	12	8

funnel was shaken and then allowed to stand for several minutes to chelate the metal ions. The chelated metals were then extracted with methyl isobutyl ketone (MIBK). The MIBK fraction containing the metal chelates was placed in a 50 ml beaker and carefully evaporated to dryness. The residue was taken up in 10 ml concentrated nitric acid and heated until the solution was clear. The sample was then made to an appropriate volume for analysis (10-25 ml) with distilled water.

Recoveries for cadmium, copper, lead and zinc from fortified estuarine water were found to range from 91-100%. Results of the sample analyses were not corrected for recovery.

## RESULTS AND DISCUSSION

The results of the analyses of the water and oyster samples are summarized in Tables 1 through 5. The values for the median and mean concentrations in oysters are in fair agreement. Since the concentrations of the elements were below detectable quantities in many of the water samples and occasional samples contained extremely high concentrations, the median and mean concentrations were quite different; the median values were believed to reflect more accurately the conditions over an extended period of time.

The data obtained were statistically analyzed in the following manner. Analysis of variance was used to compare concentrations of each of the metals in oyster samples from the eight stations. The relationship of each metal concentration in the oyster samples to the overlying water at eight sampling stations were determined by calculating correlation coefficients. Results of these analyses of the data were compared for statistical significance at the 5% probability level.

### Water Samples

The concentration of each metal in the water samples varied highly from month to month, and no seasonal trends were readily observable. The ranges of concentration ( $\mu$ g/l) of the metals in all water samples were: cadmium, <0.1 - 9.1; chromium, <0.1 - 3.7; copper, <0.1 - 15.0; lead, <0.3 - 29.4; zinc, <0.1 - 25.0 (Tables 1-5). Many of the water samples contained concentrations of metals too low to quantify, notably chromium with 45% of the samples indeterminate.

When the data for cadmium were examined no obvious differences were apparent among the concentrations in the water samples from the eight stations. Similar observations were made for copper, lead and zinc. However, twice as many sam-

TABLE 3. Summary of copper concentrations in oyster and water samples from Mobile Bay.

Sampling Location	Copper concentrations			Total Samples	Number Quantifiable
	Range	Median	Mean $\pm$ S.E.		
Oyster samples (mg/kg wet weight)					
50	27.0-78.3	37.8	43.2 $\pm$ 4.2	16	16
89	13.0-57.6	21.0	24.1 $\pm$ 3.0	15	15
118	10.8-36.7	15.2	20.0 $\pm$ 2.3	15	15
119	10.1-54.1	15.4	22.7 $\pm$ 4.1	14	14
83	3.7-17.5	9.0	9.6 $\pm$ 1.1	14	14
92	5.7-17.8	10.0	10.7 $\pm$ 0.8	16	16
104	5.0-20.0	10.8	10.9 $\pm$ 0.9	16	16
112	5.0-33.0	13.0	15.2 $\pm$ 1.7	16	16
Water samples ( $\mu$ g/l)					
50	0.1-13.0	1.0	--	13	13
89	<0.1-15.0	1.0	--	12	10
118	<0.1-6.0	1.2	--	12	11
119	<0.1-7.0	1.7	--	11	10
83	<0.1-7.2	1.7	--	12	11
92	0.2-3.0	1.8	--	12	12
104	0.5-8.0	1.4	--	12	12
112	<0.1-7.1	1.8	--	12	11

TABLE 4. Summary of lead concentrations in oyster and water samples from Mobile Bay.

Sampling Location	Lead concentrations			Total Samples	Number Quantifiable
	Range	Median	Mean $\pm$ S.E.		
Oyster samples (mg/kg wet weight)					
50	0.17-1.59	0.76	0.80 $\pm$ 0.11	14	14
89	<0.10-1.79	0.70	0.83 $\pm$ 0.12	14	13
118	<0.10-1.22	0.70	0.72 $\pm$ 0.09	14	12
119	<0.10-1.50	0.67	0.84 $\pm$ 0.12	13	12
83	0.13-1.60	0.88	0.85 $\pm$ 0.11	13	13
92	<0.10-1.50	0.80	0.92 $\pm$ 0.10	14	13
104	<0.10-1.70	0.78	0.86 $\pm$ 0.10	14	13
112	0.17-1.75	0.68	0.77 $\pm$ 0.11	14	14
Water samples ( $\mu$ g/l)					
50	<0.3-11.8	0.5	--	13	10
89	<0.3- 7.8	1.0	--	12	9
118	<0.3-10.2	1.2	( $\mu$ g/l)	12	8
119	<0.3- 7.2	2.0	--	11	7
83	<0.3-16.4	2.7	--	12	8
92	<0.3-13.2	2.0	--	11	7
104	<0.3-14.0	2.2	--	12	8
112	<0.3-29.4	3.0	--	12	8

TABLE 5. Summary of zinc concentrations in oyster and water samples from Mobile Bay.

Sampling Location	Zinc concentrations			Total Samples	Number Quantifiable
	Range	Median	Mean $\pm$ S.E.		
Oyster samples (mg/kg wet weight)					
50	925-3800	1980	2200 $\pm$ 194	16	16
89	350- 911	603	611 $\pm$ 38.2	15	15
118	250- 702	478	496 $\pm$ 33.8	15	15
119	235- 900	478	497 $\pm$ 46.8	14	14
83	140- 600	319	350 $\pm$ 29.1	14	14
92	238- 529	350	366 $\pm$ 29.2	16	16
104	200- 540	371	364 $\pm$ 24.5	16	16
112	140- 678	412	436 $\pm$ 33.8	16	16
Water samples ( $\mu$ g/l)					
50	<0.1-17.0	2.3	--	13	12
89	0.1- 9.8	2.6	--	12	12
118	<0.1- 7.7	2.8	--	12	11
119	0.2-25.0	2.2	--	11	11
83	<0.1-21.2	2.4	--	12	11
92	0.6-12.0	2.5	--	11	11
104	0.3- 9.1	2.5	--	12	12
112	<0.1-11.2	3.6	--	12	11

ples from eastern stations contained detectable concentrations of chromium and the median concentrations were substantially higher at those stations (Table 2).

#### Oyster Samples

The average concentrations of the trace metals in all oyster samples were compared with concentrations in oysters from the Atlantic Coast (Table 6). The most pronounced differences were that the Atlantic Coast oysters contained approximately five fold more cadmium and copper and twice as much zinc. The chromium content was about the same, and the Mobile Bay oysters contained almost twice as much lead.

The data for the metal concentration of the oyster samples were subjected to statistical analysis to determine if the oyster populations at the various stations were homogeneous with respect to each element. No significant difference ( $P>0.05$ ) was found among the stations with respect to chromium and lead concentrations and none for cadmium concentrations, except those from station 50 which were significantly higher. Oysters from station 50 also contained significantly more copper and zinc than those from the other stations.

Copper and zinc concentrations in oysters from the other stations followed a common pattern.

The concentrations of each of these metals in oysters from stations 83, 92 and 104 on the eastern side of the Bay were not significantly different ( $P>0.05$ ) and the concentrations of each metal in oysters from stations 89, 118 and 119 on the western side of the Bay were not significantly different ( $P>0.05$ ). The concentrations from stations 89, 118 and 119 were, however, significantly higher than those in oysters from 83, 92 and 104 ( $P<0.05$ ). Oysters from station 112 also contained significantly ( $P<0.05$ ) more copper and zinc than those from the other eastern stations.

Hugget, Bender and Sloan (In Press) reported that as the freshwater source of an estuary is approached the oysters contain increasing amounts of copper and zinc. This may be responsible for the copper and zinc levels in oysters from station 50 being significantly higher than the levels in oysters from stations 89, 118 and 119 in lower western Mobile Bay. Although the levels of these two elements in oysters from the latter stations were significantly higher than those in oysters from the lower eastern section (stations 83, 92 and 102), it has been calculated from the data of McPhearson (1970) that the salinities in those two areas of the Bay are not significantly different.

Austin (1954) has shown that because of the prevailing circulation in Mobile Bay, oysters at

TABLE 6. A comparison of trace metal concentration in Mobile Bay oysters with levels reported for Atlantic Coast Oysters.

Element	Concentration (mg/kg wet weight)	
	<sup>a</sup> Mobile Bay	<sup>b</sup> Atlantic Coast
Cadmium	0.62	3.10
Chromium	0.35	0.40
Copper	19.5	91.50
Lead	0.82	0.47
Zinc	665	1482

<sup>a</sup>Average values of all samples in this study.

<sup>b</sup>Pringle, Hissong, Katz and Mulawka (1968): Oysters from Maine to North Carolina.

stations 83, 92 and 102 would receive the discharge and runoff from Fish River and Bon Secour River whereas those on the western side would receive water from the river system at the head of the Bay. The differences observed in the zinc and copper levels in these two groups of oysters is most certainly influenced by differences in the copper and zinc concentrations in the different rivers flowing into Mobile Bay. Whether the significantly higher copper and zinc burdens in the oysters from the western side represent industrial pollution or naturally higher copper and zinc content in the river system is not known.

#### COMPARISON OF SAMPLES

When the data were arrayed so that the concentrations of each metal in companion oyster and water samples from each station could be compared, little correlation existed. The interdependence of the two sets of data was further examined by calculating the correlation coefficients for each metal in oyster and water samples when both contained a quantifiable concentration of the element. Correlation coefficients for the chromium data were not calculated since the method used to determine chromium in water detects only hexavalent chromium (Midget and Fishman, 1967), and the total chromium concentration was determined in the oysters; thus correlation would not be expected.

The correlation between the concentration of copper or cadmium in oysters and the concentration of these elements in the water samples was not significant at the 5% probability level at any station, and correlation between the zinc concentrations of oyster and water samples existed only at station 50. Correlation at the 5% probability

level existed between lead concentrations in the oyster and in the water samples at five of the eight stations. Correlation was observed at the three southernmost stations (89, 118 and 119) on the western side of the Bay and at stations 83 and 104 on the eastern shore.

Shuster and Pringle (1969) exposed oysters to various levels of lead, cadmium, chromium, copper and zinc under controlled conditions. They reported that the rate of accumulation of each metal occurred in three phases and that an approximate doubling of metal concentration occurred in the tissue upon doubling the concentration of the metal in the water. Since their data indicate that lead is concentrated in a manner similar to the other metals, the reasons for correlation only between oyster and water lead concentrations observed in this study are not apparent. The poor degree of correlation observed here agrees with the findings of Ikuta (1958) who demonstrated the difficulty of correlating levels of copper and zinc in the Pacific oyster with the levels in the environmental waters. The mechanism of trace element concentration by shellfish is not well understood. The poor degree of correlation observed between trace metal concentrations of companion oyster and filtered water samples suggests that such concentration may occur through particulate ingestion of suspended material from seawater or ingestion of elements via their pre-concentration in algae or other food material as proposed by McFarren, *et al.* (1961) and Brooks and Rumsby (1965).

Since trace metal concentrations in estuarine waters will fluctuate with the tidal stages, amount of fresh water runoff and variations in discharges containing trace elements, the metal levels in the

shellfish, regardless of the mechanism of concentration, reflect differences in the long-term levels of the trace metals in the water better than the data obtained by direct analysis of water samples themselves.

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## PREY PREFERENCE OF *STYLOCHUS ELLIPTICUS* IN CHESAPEAKE BAY

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### ABSTRACT

*Flatworms, Stylochus ellipticus, collected from two locations in Chesapeake Bay and randomly offered oysters and barnacles exhibited similar feeding behavior, preying on both species. However, when the flatworms were segregated, based on known prey at the time of collection, they exhibited marked prey preference. These experiments support the hypothesis of "ingestive conditioning" in S. ellipticus as proposed by other investigators.*

### INTRODUCTION

Several investigators have documented predation by *Stylochus ellipticus* and other polyclad flatworms on various marine organisms. These accounts have been reviewed by Hopkins (1949, 1950), Provenzano (1961), Landers and Rhodes (1970) and Christensen (1971). *S. ellipticus* has been described as either an oyster predator, barnacle predator or both. Extensive predation on raft-caught seed oysters at the Oxford Laboratory prompted an investigation of the feeding habits of *S. ellipticus*. Flatworms with known feeding habits from two areas in Chesapeake Bay were offered various combinations of oysters, barnacles or both, and their predatory activity was monitored.

### METHODS AND MATERIALS

Flatworms, barnacles and oysters were collected during the spring and summer of 1969 from oyster shells suspended from rafts in the Tred Avon River and Harris Creek, two streams located on the Eastern Shore of Chesapeake Bay. Shells or shell fragments bearing oysters, barnacles or both were cleaned of other fouling organisms, flatworms, debris and barnacles or oysters in excess of the numbers chosen for the experiment. The barnacles and oysters were held in laboratory tanks provided with running seawater at ambient river temperature for several days to detect mortality due to handling, and then placed in con-

tainers to condition them to room temperature. Shells bearing known numbers of barnacles or oysters were then placed in appropriate containers with room conditioned worms. Controls containing only prey were included in all experiments.

Experiments were conducted in containers compatible in size to the predators and prey being used. Glass petri dishes filled with water were used in experiments involving very small flatworms measuring 1.0-2.0 mm in length; glass finger bowls holding 250 ml of water were used with flatworms from 2.0-4.0 mm; and glass beakers holding 900 ml of water were used with worms larger than 4.0 mm. The water was changed five times a week in the petri dishes and bowls and twice a week in the beakers. Only the beakers were aerated.

In all experiments, Tred Avon River water was used. During the period of these experiments, the salinity varied from a low of 11.8‰ on 23 July 1969 to a high of 14.9‰ on 28 October 1969. According to Landers and Rhodes (1970), a salinity difference from 7.5‰ to 27-28‰ has no effect on initiation or rate of predation of *S. ellipticus* on oysters.

Room temperatures during the experiment ranged from 20-22°C. Landers and Rhodes (1970) found that at temperatures from 10 - 22°C there was no difference in time of initiation or rate of predation of *S. ellipticus* on oysters or barnacles.

The water used for the first three sets of experi-

ments involving very small worms and barnacles was centrifuged and autoclave-sterilized to prevent introduction of larval worms or barnacles. Water used in the beakers was only centrifuged, since the chance of mistaking a recently set individual from a larger one used in the experiment was negligible.

The worms and their prey were counted at varying intervals depending on the length of the experiment. In experiments involving large numbers of small barnacles, the shells were marked off into grids with the number of barnacles per grid section recorded to facilitate future counting. When prey mortality exceeded 50%, they were replaced with the original number of new individuals. The same worms were used during an experiment. An exact count of worms was made with a dissecting microscope at the termination of each experiment or when it was necessary to replace the original prey stock.

Predation rate, in each given series of experiments, is expressed as the number of oysters or barnacles killed per worm per week.

## RESULTS

### *Predation on Barnacles*

The first three series of experiments involved only worms and barnacles collected from the Tred Avon River. Ropes and bags bearing oyster shells were suspended from a raft in late April 1969. Initial setting of barnacles, *Balanus* sp., began during the first week in May and by the end of May a density of approximately 600 individuals per 100 cm<sup>2</sup> was observed. Setting of *S. ellipticus* also began during the first week of May and approximately 40 worms per 100 cm<sup>2</sup> accumulated on the shells by the end of May. No oyster setting occurred during this period; therefore,

all flatworms used for the first three series of experiments had never eaten any oysters.

In the first series of experiments with very small worms (1.0-2.0 mm) and barnacles, predation rates in five separate glass petri dishes were observed to be .16, .16, 1.01, 1.40 and 2.50 barnacles killed per worm per week. None of the barnacles in either of two controls died during the two-week experimental period. In a second series of experiments conducted in eight glass finger bowls, predation rates were .70, .80, 1.00, 1.25, 2.00, 2.10, 2.10 and 2.40 barnacles killed per worm per week. None of the barnacles in the four controls died during the two-week experimental period. In the third series of experiments conducted in seven glass beakers, predation rates were .95, .95, 1.11, 1.15, 1.25, 1.75 and 2.00 barnacles killed per worm per week. Mortality in the seven controls was negligible. The mean predation rate for the three series of experiments was 1.34 barnacles killed per worm per week.

### *Predation by Unselected Worms*

Flatworms were collected from two different areas. Tred Avon River specimens were obtained from the same source as those used in the previous three experiments. Harris Creek specimens were obtained in a similar manner from shells suspended from a raft on 26 June. Both oysters and barnacles were setting at the time the shells were suspended. Two weeks after suspension the shells contained an average of 61 oyster spat, 26 barnacles and 3 flatworms. The results of the prey-preference experiment series with worms from the two areas are presented in Table 1. Mortality was negligible in the 12 control experiments used in these experiments.

A comparison between Tred Avon River worms

TABLE 1. Results of feeding experiments using flatworms from two different areas in Chesapeake Bay.

Experiment	Number of Replicates	Oyster Mortality		Barnacle Mortality	
		Range of Predation Rate	Mean Predation Rate	Range of Predation Rate	Mean Predation Rate
Tred Avon River Worms					
Oysters	5	.00-.90	.37	-	-
Barnacles	2	-	-	1.15-1.60	1.38
Both	5	.00-.50	.19	.40-.70	.54
Harris Creek Worms					
Oysters	10	.00-.90	.45	-	-
Both	5	.14-.27	.21	.50-.86	.68

TABLE 2. Results of feeding experiments using worms selected on the basis of prey utilization at time of collection.

Experiment	Number of Replicates	Oyster Mortality		Barnacle Mortality	
		Range of Predation Rate	Mean Predation Rate	Range of Predation Rate	Mean Predation Rate
Oyster Worms					
Oysters	5	.40-.80	.55	-	-
Barnacles	5	-	-	.08-.21	.14
Both	2	.22-.29	.25	.03-.04	.04
Barnacle Worms					
Barnacles	5	-	-	.07-.21	.15
Oysters	5	0	0	-	-
Both	5	0	0	.00-.14	.07

and Harris Creek worms indicates that they have very similar predatory activities. Regardless of the worm source, the mean predation rate on barnacles was about three times the mean predation rate on oysters if both were offered. If only oysters were offered, regardless of the worm source, the mean predation rate was double that on oysters in experiments where both prey species were available. When both oysters and barnacles were offered to Harris Creek worms, they preyed on oysters in all five experiments. Tred Avon River worms, however, preyed on oysters in only three of five experiments. The mean predation rates on oysters were essentially the same regardless of worm source. When only barnacles were offered to Tred Avon River worms, the mean predation rate (1.38) was consistent with that found in the previous experiments (1.34).

#### Prey Preference With Selected Worms

In other experiments involving prey choice, worms taken from the same sources as in previous experiments were segregated on the basis of prey utilization at the time of collection; i.e. flatworms found feeding on oysters were separated from those found feeding on barnacles. Flatworms found in empty oyster or barnacle boxes or on the substrate were not used.

The results of these experiments are presented in Table 2. In no experiment did known barnacle eating worms prey on oysters, even when an alternate food source was not offered. Known oyster eating worms preyed on oysters and barnacles in all experiments. However, predation by oyster eating worms on oysters was 3.5 times greater than on barnacles, whether they were offered together or separately. Barnacle predation rate (.14-.15) was considerably less than in

previous experiments. However, this phenomenon is probably due to the size of the barnacles used which were relatively larger in proportion to the worms than those used in prior experiments. Negligible mortality occurred in the 10 controls.

#### DISCUSSION

These experiments lend further support to the "ingestive conditioning" hypothesis offered by Wood (1968) and later supported by Landers and Rhodes (1970). When the predatory activities of *S. ellipticus* from seven different sources were compared, Landers and Rhodes (1970) found that worms from six sources preyed on either barnacles or oysters but not on both, while worms from only one source attacked both prey. In the two instances where worms preyed on oysters alone, the worms were obtained from raft-caught suspended seed. In the one case where both oysters and barnacles were preyed on, the worms were obtained from a recently planted oyster seed bed (Landers, personal communication). It is possible that worms obtained from the recently planted bed may have included individuals which had previously fed on barnacles and then moved into the oyster plant area.

Worms collected from rafts in the Tred Avon River and Harris Creek included individuals which had access to either prey species, as both oysters and barnacles were present on the rafts at the time worms were collected for prey preference experiments. This might account for the similarity in predatory activity of worms from both rivers (Table 1); that is, their predation on both oysters and barnacles. However, when

worms were segregated based on prey at the time of collection rather than by source, prey selection was different (Table 2) and suggested "ingestive conditioning." Known barnacle-eating worms appeared to have established a preference for barnacles. Known oyster-eating worms had not established the same preference and fed on barnacles when oysters were not available, yet only rarely did oyster-eating worms feed on barnacles when oysters were available.

It is interesting to note that, in all cases of high oyster mortalities caused by *S. ellipticus*, the oysters were crowded either as raft-suspended seed or dense bottom beds. Although Webster and Medford (1961) saw *S. ellipticus* in fresh spat boxes in Chesapeake Bay and suggested the worms killed oysters, no extensive mortalities on natural oyster bars caused by *S. ellipticus* have actually been observed. If oysters are the usual prey species, observations of naturally occurring mortalities of these important commercial bivalves should have been reported. In the experiments described here, the worms all had opportunity to feed at an earlier stage on either oysters or barnacles. Some worms became conditioned to the lack of oysters but not the lack of barnacles. It would appear, therefore, that barnacles are the preferred prey of *S. ellipticus* under most conditions. However, if barnacles are not available, as under certain aquaculture situations, *S. ellipticus* may become conditioned to feed heavily, and perhaps exclusively, on oysters.

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## CARDIAC EDEMA ASSOCIATED WITH *VIBRIO ANGUILLARUM* IN THE AMERICAN OYSTER

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### ABSTRACT

During a survey for parasite distribution in Chesapeake Bay oysters (*Crassostrea virginica*), sporadic cases of greatly enlarged and edematous pericardia were noted. Prevalence of the edematous syndrome, which we have termed "cardiac vibriosis", was estimated at 0.04%. Examination of aseptically aspirated pericardial fluid showed heavy concentrations of gram-negative motile rods which proved morphologically and culturally compatible with *Vibrio anguillarum*, an organism implicated in diseases of fishes and larval bivalve mollusks. Except for pericardial enlargement, the animals appeared to be grossly and histologically normal. Attempts to reproduce the pericardial edema experimentally by injection of *V. anguillarum* proved unsuccessful.

### INTRODUCTION

Oysters fall prey to many protozoan parasites and metazoan predators (Sindermann and Rosenfield, 1968; Farley, 1968). While a bacterial disease of larval oysters, called bacillary necrosis, has been described (Tubiash, Chanley and Leifson, 1965) no bacterial diseases of adult *Crassostrea virginica* have been reported. In our experience, adults of this species and other bivalve mollusks are also refractory to experimental bacterial infection.

Toward the end of the 1950's a fast-spreading, highly lethal oyster epizootic of unknown etiology appeared in productive areas of Delaware Bay, then rounded the Virginia Capes into the lower half of Chesapeake Bay (Haskin, Canzonier and Myhre, 1965; Wood and Andrews, 1962). Since the center of the fishery was threatened, a multi-agency, multidisciplinary study was launched.

By 1966 the etiology was established as a haplosporidan parasite, *Minchinia nelsoni* (Haskin,

Stauber and Mackin, 1966), whose proliferation was evidently mediated by intrusion of high salinities, concurrent with an extensive deficiency of rainfall.

Careful resource management, and more importantly, the return of normal rainfall, have contained the epizootic, but biologists continue to monitor the prevalence of the disease in enzootic and disease-free areas of the Chesapeake. For example, in Maryland this surveillance consists of gross and histologic examination of 25-50 oysters from 24 locations semi-annually.

In the course of this routine survey a previously undescribed syndrome was discovered. Out of more than 10,000 oysters examined during four years (1967 - 1970), four animals (0.04%) were found with grossly enlarged hearts and pericardial chambers (Fig. 1). Affected animals were found during 1969 and 1970 from the Manokin, St. Marys and South Rivers, which are Maryland tributaries of Chesapeake Bay.

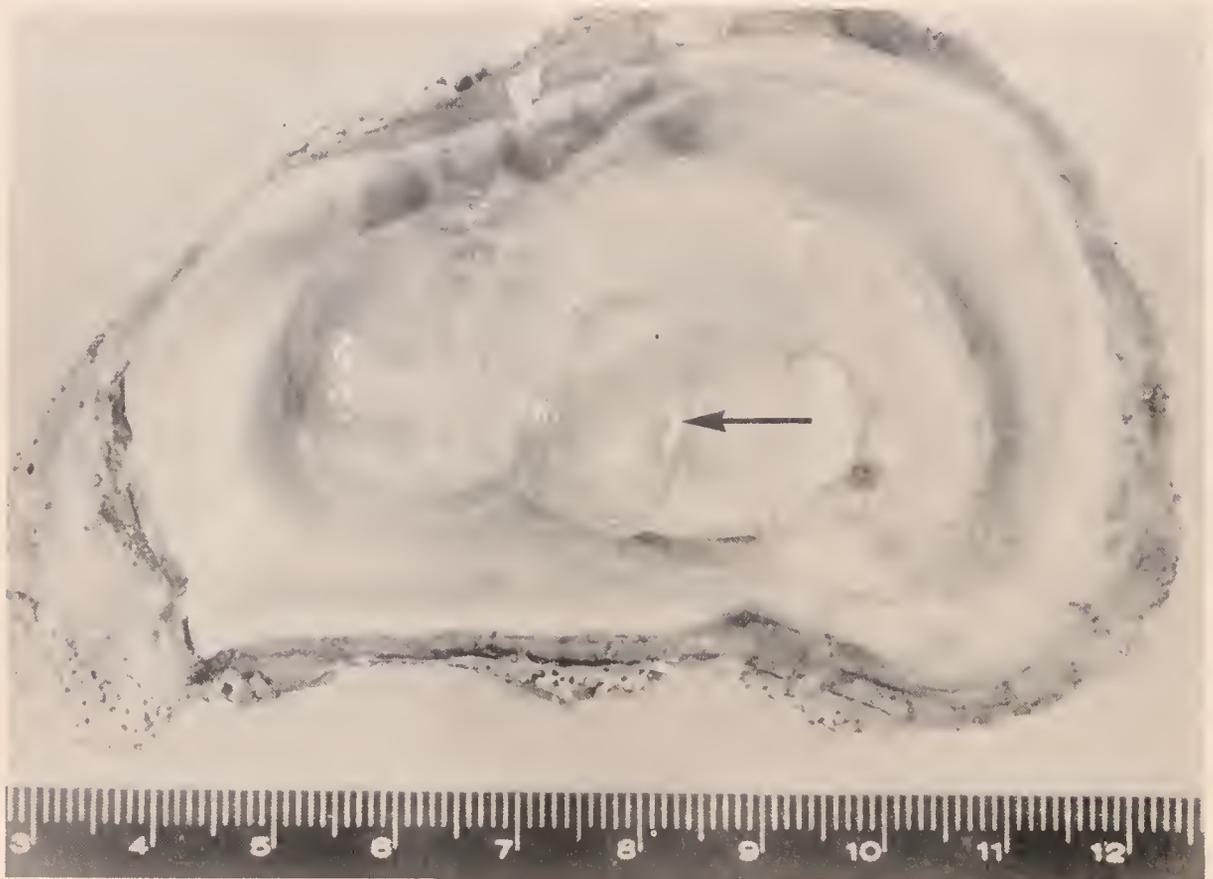


FIG. 1. American oyster, *Crassostrea virginica*, with greatly-enlarged, fluid-filled pericardial chamber (arrow). Darkened areas in center of upper shell margin mark old and new invasion by *Polydora* sp.

### OBSERVATIONS

*Oyster No. 1* - This oyster was collected in October 1969 slightly upstream from the mouth of the South River and was one of a large collection for a disease resistance study. It was 16.5 cm long, had a light infection of *Polydora* sp. inside the shell and was judged to be in "medium" market condition. The heart and cardiac chamber were greatly enlarged and obviously gorged with fluid (Fig. 1).

*Oyster No. 2* - This oyster was collected from the Manokin River in November 1969 as one of a routine sample of 25. It was 8 cm long with ripe gonads, a light invasion of *Polydora* sp. and was judged in "medium" condition. The heart was swollen and the cardiac chamber was enlarged, containing a jelly-like fluid.

*Oyster No. 3* - The animal was collected from the St. Marys River during March 1970. It was 11 cm long and was judged in "medium" condition.

Mantle recession, usually indicative of pathology or physiological stress (Farley, 1968) was evident. The heart and pericardial chamber were both greatly enlarged and fluid-filled.

*Oyster No. 4* - This oyster was collected from the Manokin River in September 1970. It was 9 cm long and in "watery" or "poor" market condition. Holes caused by the oyster drill, *Urosalpinx cinerea*, were present on the shell and invasion by *Polydora* sp. and mantle recession were seen in the shell interior. The heart was swollen and the pericardial chamber greatly distended.

### PROCEDURE

#### *Bacteriological*

Pericardial fluid was aspirated aseptically from each animal for microbiological study before the oysters were processed for histological examination. Gram stains were prepared from the peri-

cardial haemolymph and blood agar plates were streaked. Samples (0.2 ml) of the pericardial fluids were diluted serially in Tryptose-Glucose-Yeast extract (TGY) broth prepared in seawater (Tubiash, et al., 1965) to estimate bacterial counts and to isolate the predominant organisms. Incubation was at 28°C for 48 hr. Blood agar plates were streaked from the highest dilutions showing growth, and isolations made of the predominant organisms. Isolates were initially transferred to Eosin Methylene Blue agar (EMB), Krumweide's Triple Sugar agar slants prepared with 1% NaCl and Difco MOF fermentation medium with 1% glucose. Determinative tests were performed as shown in Table 1.

#### Histological

After macroscopic examination was completed and pericardial fluid had been aseptically aspirated from the affected oysters, they were rinsed in membrane-filtered seawater to remove as much mud, sand, shell fragments and detritus as possible. Two transverse cuts were made just posterior to the palps to prepare a section about 10 mm thick. The heart, pericardial cavity and surrounding tissue were also removed intact, placed in Davidson's fixative and refrigerated for at least 48 hours. The tissues were then run through successive changes of ethyl alcohol and embedded in paraffin. Six micron sections were cut, stained with Harris hematoxylin and alcohol-soluble eosin, and mounted in Permount.

### RESULTS

The gram stains revealed heavy to moderate concentrations of small gram-negative rods and on culture the predominant organisms also proved to be gram-negative rods. Bacterial concentrations on the three cardiac haemolymphs successfully cultured were estimated between  $10^6$  and  $10^7$  per ml. The fourth fluid was lost through contamination. Growth on Krumweide's Triple Sugar agar with 1% NaCl showed acid slants and butts, with no gas. MOF glucose medium showed acid production in the open and sealed tubes, hence glucose was fermented. The bacteria failed to grow on EMB. These findings led us to suspect that we were dealing with strains of a motile marine vibrio and determinative tests were performed as listed in Table 1. Isolates were forwarded to Dr. Riichi Sakazaki at the National Institute of Health in Tokyo, who confirmed our identification of *Vibrio anguillarum*.

*V. anguillarum* is one of the etiologic agents of bacillary necrosis in larval bivalve mollusks (Tubiash, Colwell, and Sakazaki, 1970). The three

TABLE 1. *Cardiac Vibrio Characteristics*

Gram-negative polar monotrichous rods. . . . .	+
Acid, no gas produced from:	
Glucose (fermentative) . . . . .	+
Sucrose . . . . .	+
Maltose . . . . .	+
Trehalose . . . . .	+
Mannitol . . . . .	+
Lactose . . . . .	-
Inositol . . . . .	-
Citrate Utilization . . . . .	+
Gelatin Liquefaction . . . . .	+
Production of:	
Indole . . . . .	+
Catalase . . . . .	+
Oxidase . . . . .	+
Arginine dihydrolase . . . . .	+
Lysine decarboxylase . . . . .	-
Ornithine decarboxylase . . . . .	-
Phenylalanine deaminase . . . . .	-
Growth in:	
0% NaCl Broth . . . . .	-
1% NaCl Broth . . . . .	+
6% NaCl Broth . . . . .	+
7% NaCl Broth . . . . .	-

cardiac isolates were therefore used to challenge week-old oyster larvae using methods described by Tubiash, et al. (1965). Forty-eight hour larval mortalities averaged 92, 87 and 96% respectively, while controls exposed to *Escherichia coli* averaged only 11%.

Histologic examination revealed no abnormalities other than the cardiac involvement. Microscopically the tissues appeared normal.

In October, 1970, 24 oysters were injected intracardially, via a small trocar puncture between the shell valves, with 24-hour vibrios washed from the surface of TGY agar. The 0.1 ml injection consisted of a suspension of about  $8 \times 10^7$  viable organisms. The oysters were maintained in flowing water throughout the winter. Five months later, in March, 1971, they were sacrificed and examined. Aside from mud blister formation at the injection sites, the animals were in good condition and the hearts appeared normal. Reproduction of the cardiac enlargement syndrome was therefore not achieved.

### DISCUSSION

Histologic examination of the four affected oysters revealed no abnormalities other than the cardiac involvement, except that the oyster rated

in "poor" condition was also parasitized by *Nematopsis ostrearum*, a gregarine protozoan parasite and the shell had been invaded by *Polydora* sp., an annelid blister-forming worm. This combination of parasitic stress, recovery from a possible infection with *M. nelsoni* (as evidenced by mantle cell recession and pigment cell infiltration) added to the cardiac infection, could well be responsible for the poor condition of the animal.

After studies of the oysters exhibiting cardiac enlargement was completed, 0.2 ml of pericardial fluid was aspirated and similarly cultured from each of six aseptically-opened normal oysters. Only a scattering of colonies appeared on the blood plates streaked with cardiac fluid from five of the six oysters, but a count estimated at 150 per ml was obtained from the fluid of the sixth oyster. The predominant organisms proved to be indistinguishable from and apparently identical to *V. anguillarum*.

Vibrios are known to be pathogenic to many species of finfish and larval bivalve mollusks (Anderson and Conroy, 1970; Tubiash, et al., 1965) but we have isolated *V. anguillarum* previously from apparently normal Chesapeake Bay oyster tissue (Tubiash, et al., 1970). The significance of our present finding is moot. Perhaps the lesson to be relearned is that all potential pathogens need not necessarily be associated with overt pathology and that in the present case, the vibrios are probably opportunists which may be eliciting a host-response bordering on pathology.

We hesitate to categorize this syndrome as a "disease", but are designating the condition "cardiac edema".

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LABYRINTHOMYXA-LIKE ORGANISMS ASSOCIATED WITH  
MASS MORTALITIES OF OYSTERS,  
*CRASSOSTREA VIRGINICA*, FROM HAWAII

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ABSTRACT

In July 1972, a massive mortality ravaged oyster stocks (*Crassostrea virginica*) in West Loch, Pearl Harbor, Hawaii. Oyster tissues cultured in fluid thioglycollate medium were found to be infected with a fungal parasite similar to *Labyrinthomyxa marina* (= *Dermocystidium marinum*). Examination of histological sections revealed hyphospore stages which had enlarged, formed presporangia and were believed to be forming planonts of developing zoospores.

INTRODUCTION

During July 1972, Eastern oysters, *Crassostrea virginica*, in West Loch, Pearl Harbor, Hawaii, experienced a massive mortality of 90-99%, or approximately 30-34 million oysters. With the possible exception of barnacles, no other forms of marine life appear to have been affected. No apparent environmental anomaly was found to be associated with the mortality. A less intense mortality occurred during the month of June in Middle Loch, Pearl Harbor. This mortality involved not only mollusks but crustaceans and polychaetes as well and could be directly related to low levels of dissolved oxygen. Oyster tissues from West Loch and Middle Loch were examined to determine if the mortality was of biotic etiology. In our examination of oysters from both areas, a fungus parasite similar to *Labyrinthomyxa marina* (Mackin, Owen and Collier, 1950; Mackin and Ray, 1966) was found.

*L. marina* has been extensively studied and documented as a serious oyster pathogen (Mackin et al., 1950; Mackin, 1952; Ray, 1954; Andrews and Hewatt, 1957; Sindermann and Rosenfield, 1967; Quick and Mackin, 1971). Its reported range appears limited to the Atlantic and Gulf coasts of

the United States and Mexico (Quick and Mackin, 1971). Its presence in oysters from Hawaii forms the basis of this report.

MATERIALS AND METHODS

Fifty oysters (*C. virginica*) from West Loch and 25 oysters from Middle Loch were examined 2 weeks after the initial report of the West Loch mortality. Each oyster was coded, opened at the hinge and examined for gross abnormalities. Rectal tissues from 25 of the West Loch oysters and 15 of the Middle Loch oysters were cultured in fluid thioglycollate medium, incubated at room temperature for 72 hrs, and examined after staining with Lugol's iodine solution (Ray, 1966).

A cross section of tissue approximately 6 mm thick was cut from the visceral mass of each oyster, fixed in Davidson's fixative (Shaw and Battle, 1957), dehydrated in ethanol and embedded in paraffin. Sections were cut at 6 $\mu$  and stained with periodic acid Schiff with Weigert's acid iron chloride hematoxylin as a counterstain (PASH).

In September 1972, in order to determine whether the fungus parasite was present in other mollusks, additional samples of 50 Eastern oysters (*C. virginica*) from West Loch and 50 each of

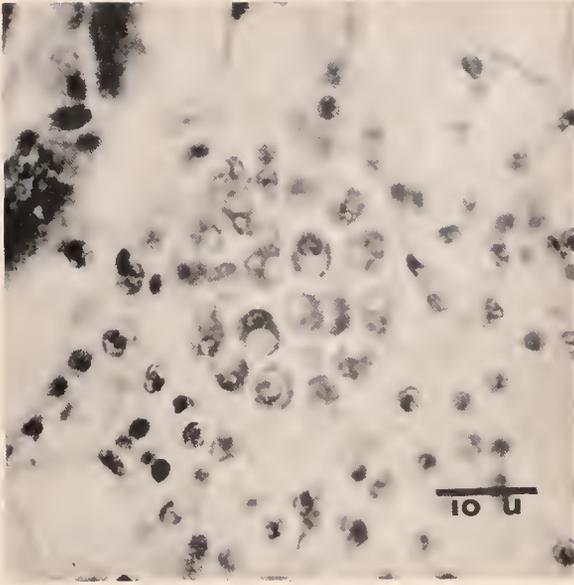


FIG. 1. Sporocyst of developing hyphospores. (PASH) 1000X.

Pacific oysters (*C. gigas*) and Manila clams (*Tapes philippinarum*) from Kaneohe Bay were processed and examined by both of the methods described above. Percentages of fungal infections reported are based on the thioglycollate technique.

## RESULTS AND DISCUSSION

Fifty-two percent of the West Loch oysters and 27% of the Middle Loch oysters were found to be infected with a fungal parasite similar to *L. marina*. All oysters were alive at the time of processing and without apparent tissue degeneration, and, based on gross observations, the physical condition of the infected oysters varied from medium to poor, depending on the extent of the fungus infection.

Additional samples of *C. virginica* taken in September 1972 from West Loch confirmed the continued presence of the fungus pathogen in 44% of the oysters surviving the original mortality. Samples of *C. gigas* and *T. philippinarum* taken at the same time from Kaneohe Bay were found to be free of this pathogen.

*Labyrinthomyxa*-like organisms have been reported in a wide variety of mollusks (Ray, 1954; Andrews, 1955; Andrews and Hewatt, 1957). Ray (1954) demonstrated fairly rigid host specificity and was unsuccessful in establishing cross infec-

tions. This possibly is why only *C. virginica* was found infected.

Hyphospores observed in thioglycollate-cultured tissues of Hawaiian oysters were considered comparable to those from *L. marina* infections of Gulf coast oysters described by Ray (1966). No abnormal increase in size or unusual staining characteristics were observed in any of the stained preparations. Examination of histological sections revealed hyphospores measuring 4-17 $\mu$ , 58% larger than the 3-10 $\mu$  hyphospores described from stained sections and fresh preparations by Mackin, et al. (1950). Hyphospores and sporangia possessed PAS-positive walls. Small hyphospores were generally intracellular; larger hyphospores and sporangia were frequently seen encapsulated by oyster hemocytes. Sporangia of hyphospores were numerous and often quite large (Fig. 1).

Enlargement of *L. marina* spores in thioglycollate media (Ray, 1966) is believed to represent an exaggerated developmental stage which occurs naturally in degenerating post-mortem oyster tissue (Mackin, 1962). Ray (1954) reported spore enlargement on rare occasions in living oyster tissue and believed the large spores to be abortive sporangial bodies. Perkins and Menzel (1966) believed that enlargement signaled the formation of

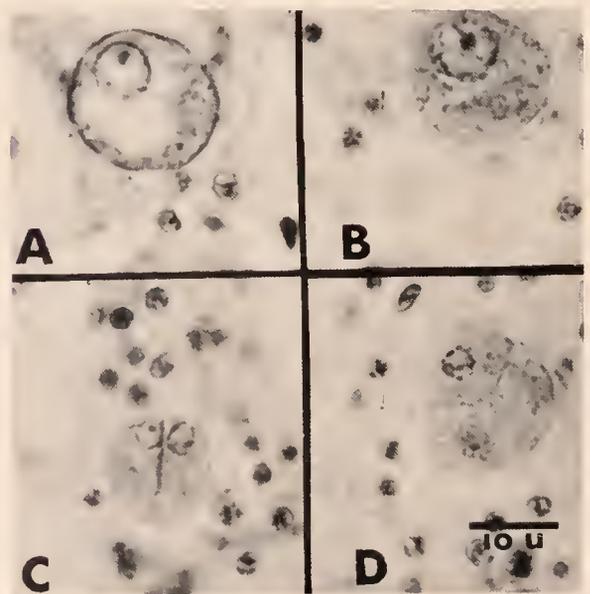


FIG. 2. A. Enlarged hyphospore; B. Presporangia; C. First cleavage two cell stage; D. Three-four cell stage. (PASH) 1000X.

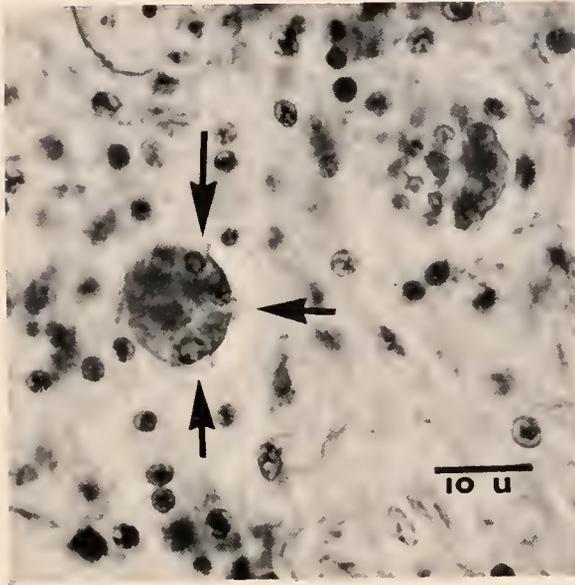


FIG. 3. Sporangium of presumptive planonts with suspected areas of discharge tube formation (arrows). (PASH) 1000X.

presporangia. They were able to induce sporulation in *L. marina* which, through successive bipartition of the protoplast, resulted in the formation of motile biflagellated zoospores. A similar sporulative process has been reported in *Labyrinthomyxa* sp. from the clam *Macoma balthica* (Perkins, 1968; Valiulis and Mackin, 1969).

The large spores found in the tissues of the Hawaiian oysters were accompanied by early division stages (Fig. 2) similar to those described by Perkins and Menzel (1966). Sporangia of what appear to be developing planonts occasionally had areas corresponding to the discharge pore and associated tube (Fig. 3) described from *L. marina* in oysters (Perkins and Menzel, 1967) and from *Labyrinthomyxa* sp. in *M. balthica* (Valiulis and Mackin, 1969). We were unable to determine from the examination of fixed tissue whether the planonts completed their development and formed motile biflagellated zoospores.

The similarity of the Hawaiian parasite to *L. marina* and the unusually high prevalence of the parasite suggests that it was the etiological agent responsible for the mortality which occurred in West Loch and possibly, to a lesser extent, for the mortality which occurred in Middle Loch.

As an incidental observation, the parasitic cestode *Tylocephalum* sp. was found in 18% of the

West Loch oysters and in 24% of the Middle Loch oysters. No histopathology, other than cyst formation, is associated with *Tylocephalum* sp. infections (Sparks, 1963).

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## AN APPRAISAL OF THE ALTERNATIVE EARNING POWER OF THE MARYLAND OYSTERMEN<sup>1</sup>

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### ABSTRACT

*Information collected from personal interviews with oystermen is used to determine labor market participation potential. Data presented indicate that the oystermen living in two communities located on Maryland's Eastern Shore would have more difficulty finding employment outside the fishing industry than their counterparts living in two western shore communities.*

### INTRODUCTION

For many years, the Chesapeake Bay and its tributaries have supported one of the United States' major commercial fisheries, the oyster fishery. It has been said that the watermen who participate in the fishery may very well be the last living specimens of an almost extinct species: the independent, the individual man (Lang, 1961). In the aftermath of Hurricane Agnes' destruction, interest has surfaced in the waterman's job mobility. The objective of this study was to assess the employment mobility of the Maryland oysterman.

### SCOPE

The investigation was designed to serve as a limited effort pilot study. Consequently, instead of encompassing the entire State of Maryland, four communities, Shady Side, Rock Hall, Crisfield-Smith Island and Avenue, were selected for analysis. Selection of these communities was made on the basis of geographical considerations, the overall number of licensed fishermen and the importance of the oyster industry to the local economies.

In the selection process, the existence of some contrasts in the structure of the local economy and relative importance of fishing activities were heavily weighted. Avenue, the smallest of the four communities with a population of 600 people, represents

an isolated economy where farming and fishing are the predominant activities. Of the cities selected, Crisfield's economy, while isolated, is the most diversified. Economic activity in Rock Hall and Shady Side is centered around the fishing industry with a limited amount of manufacturing activity located in each community. Because of its close proximity to Washington, D. C. and Annapolis, Maryland, Shady Side offers the greatest number of job alternatives.<sup>2</sup>

### PROCEDURE

Numerous factors serve to determine the employability and alternative earning power of an individual. Of the various factors that influence job mobility, age, level of education and amount of vocational training were considered critical. Skills acquired from part-time, off-season and other miscellaneous job experience also contribute to a person's mobility. The current and projected demand for and supply of individuals with various skills were viewed as important.

The data required for the investigation were generated by selecting a stratified random sample of

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<sup>2</sup>It is difficult to say how representative these four communities are of the entire oyster fishery. Sufficient information is not readily available to identify the characteristics of the population of oystermen in Maryland and relate them to those of the sample fishermen in these communities.

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<sup>1</sup>This work was supported by the National Marine Fisheries Service, Contract No. N-043-7-71.

TABLE 1. *Age frequency of licensed oystermen.*

Location	Age in Years							
	1-19	19-24	25-29	30-34	35-44	45-54	55-64	65+
Shady Side	0	1	5	6	7	5	2	3
Rock Hall	5	0	4	6	5	8	5	8
Crisfield- Smith Island	0	4	3	4	7	7	7	1
Avenue	0	4	3	2	5	7	4	5
Percent of Total	4	7	11	14	18	20	14	13

133 oystermen. Forty-one oystermen were interviewed in Rock Hall. This represented the largest number of interviews taken in a single community. Crisfield-Smith Island, Avenue and Shady Side followed with 33, 30 and 29 interviews, respectively.<sup>3</sup>

Taking into account information collected on employment, related variables such as skills obtained either from currently held part-time jobs or alternative jobs held in the past, level of education, age and the labor market in the four communities, a labor mobility table was constructed. Oystermen interviewed were classified as being either "potentially employable," "potentially trainable," "potentially hardcore unemployed" or "not in the labor force."

Individuals who had either sufficient educational training or marketable skills which matched the demand in the local labor market were classified as "potentially employable." The category "potentially trainable" included persons capable of participating in a training program. Age and level of education were used to get a first approximation of an individual's suitability for further training. Oystermen who had no marketable skills, who fell into the age bracket, 45-65, and who had completed less than 6 years of education were classified as being potentially hard-core unemployed. These individuals in all likelihood would find it difficult to make vocational

re-adjustments. The last category, "not in the labor force," included oystermen who were either over 65 years of age or students.<sup>4</sup>

## RESULTS

### *Age of the Oystermen Interviewed*

The average ages of the interviewees from Shady Side, Rock Hall, Crisfield-Smith Island, and Avenue were 41.3, 44.8, 42.2 and 45.8 years, respectively. Statistical analysis failed to reveal any significant differences between the averages. Further summarization of the survey information revealed that Rock Hall had the largest number of older oystermen (Table 1).

### *Level of Education*

Table 2 summarizes the educational data obtained from the interviews. Of the oystermen interviewed from Shady Side, 21% had an eighth grade education or less, whereas in each of the remaining communities, the percentage of watermen having the same level of formal schooling was substantially higher. Specifically, 42% of the oystermen living in Rock Hall had an eighth grade education or less. The percentage of watermen having no more than 8 years of formal education jumped to 60% in Avenue and 67% in Crisfield-Smith Island.

The average level of education completed for each of the communities was: Shady Side - 9.9 years, Rock Hall - 9.4 years, Crisfield-Smith Island - 7.8 years and Avenue - 8.3 years. Statistical analyses indicated that the average level of education for watermen living in Crisfield-Smith Island was significantly lower than that completed by oystermen based in Rock Hall and Shady Side.

### *Cross-Tabulation of Age Versus Education*

Cross-tabulation of the age and level of education data revealed that 28% of the watermen interviewed

<sup>3</sup>The validity of the actual sample sizes was verified by making comparisons with optimal sample sizes calculated from the information obtained in the preliminary interviews.

<sup>4</sup>It should be borne in mind that the above classification is only a preliminary step in identifying the differences in labor market participation potential.

TABLE 2. *Level of education.*

Location	Educational level (in years)							
	1-5	6	7-8	9	10-11	12	13-15	16
Shady Side	3	0	3	4	5	13	1	0
Rock Hall	5	2	10	5	4	15	0	0
Crisfield- Smith Island	4	2	16	3	6	2	0	0
Avenue	5	2	11	1	5	5	0	1
Percent of Total	13	5	30	10	15	26	1	1

had a high school education (Table 3). The majority of oystermen in this group (32%) were between 19-45 years of age. However, 48% of those interviewed had an eighth grade education or less, and 68% of this group were 46 years old or older.

#### *Vocational Training*<sup>5</sup>

To delve further into the educational level of the oystermen interviewed, each was asked whether he had ever received any vocational training and if so, had he used it within the past five years. Twenty-three percent of the licensed oystermen interviewed stated that they had received some type of

vocational training. Only 26% of the oystermen that responded "yes" to the vocational training question also stated that they had not used the acquired skills within the last five years (Table 4). The number of watermen receiving some vocational training ranged from none in Crisfield-Smith Island to 12 of the 29 fishermen interviewed in Shady Side.

#### *Part-time and/or Off-season Job Status*

Table 5 summarizes how interviewed oystermen allocated their working time. Of the watermen interviewed, 63 reported that they spent all of their time fishing. Out of the 63, 38 reported spending all their time harvesting oysters and crabs. A majority, 29, of the crabbers/oystermen lived in Crisfield-Smith Island. Fishermen located in the remaining communities concentrated more on clams and finfish.

<sup>5</sup> Vocational training was defined as schooling received either on the job or from a trade school.

TABLE 3. *Ages of licensed oystermen versus the level of education of oystermen interviewed.*

Age	Years of education								Percent of Total
	1-5	6	7-8	9	10-11	12	13-15	16	
1-19	---	---	1	1	2	2	---	---	5
19-24	---	---	2	3	---	3	---	---	6
25-29	---	---	2	2	5	6	---	---	11
30-34	---	---	4	1	4	8	1	---	14
35-44	1	1	9	1	4	7	---	---	18
45-54	4	3	10	---	5	8	---	---	23
55-64	6	---	6	3	---	---	---	1	12
65 +	6	2	6	2	---	1	---	---	13
Percent of Total	13	5	30	10	15	26	1	1	100

TABLE 4. *Percentage of oystermen who have received vocational training.*

Location	Have received vocational training	If so, have you used it within the last five years
Shady Side	42%	69% responded yes
Rock Hall	20%	67% responded yes
Avenue	7%	50% responded yes
Crisfield-Smith Island	0%	0% responded yes

Only 17 out of the 133 oystermen interviewed reported that they oystered full-time during the oyster season and held down a full-time job outside the fishing industry during the off-season. Those interviewed in this group were concentrated in Shady Side.

When asked if they held a non-fishing job in addition to their oystering activities, 49 of the 133 oystermen interviewed responded "yes." Seventy-eight of

those interviewed stated that they did not participate in other employment activities (Table 6). Of the four communities, Crisfield-Smith Island had the smallest number of oystermen who stated that they did hold either part-time or off-season jobs in addition to their oystering activities.

#### *Recognized Employment Alternatives*

In order to gauge the degree of recognition of employment alternatives, the oystermen were asked

TABLE 5. *Allocation of working time for interviewed oystermen.*

Allocation of Working Time	Shady Side	Rock Hall	Crisfield-Smith Island	Avenue	Total
Finfish-Oyster full-time	0	5	0	3	8
Crab-Oyster full-time	1	6	29	2	38
Finfish-Crab-Oyster full-time	0	4	0	4	8
Crab-Oyster-outside employment full-time	0	0	1	0	1
Clam-Oyster full-time	4	2	0	1	7
Finfish-Clam-Oyster full-time	0	0	0	1	1
Oyster with outside employment in off-season	11	1	1	3	16
Oyster part-time with outside employment	12	8	1	8	29
Retired	0	9	1	4	14
Other	1	6	0	4	11

TABLE 6. *Response to the question, "Do you hold a non-fishing job in addition to oystering?"*

Response	Shady Side	Rock Hall	Crisfield- Smith Island	Avenue	Total
Yes	23	11	3	12	49
No	6	27	29	16	78
No response	0	3	1	2	6
TOTAL	29	41	33	30	133

what they would do to support their families if the oyster supply failed due to pollution and/or disease. The responses given to this question are summarized in Table 7. Out of the 133 oystermen interviewed only 23 (17%) were unsure as to what they would do if the oyster fishery failed. However, 13 watermen who were undecided as to what type of work they would do stated that they would actively seek employment. Only 6 of the interviewees stated that they

would have to go on welfare.

#### *Labor Market Participation Potential*

Taking into account information collected on employment related variables such as skills obtained, past job experience, level of education, age, and the current labor market in the four communities, a labor mobility table, (Table 8) was constructed. Oystermen interviewed were classified as being either "poten-

TABLE 7. *Types of action that would be taken if there was a failure in the oyster fishery.*

Response	Shady Side	Rock Hall	Crisfield- Smith Island	Avenue	Total
Undecided or No Response	0	2	7	1	10
Undecided as to what type of work, but would try to find a job	1	2	7	3	13
Welfare	0	1	1	4	6
Stay on in some other aspect of watering	2	11	5	4	22
Retire or Retired	3	9	1	2	15
Construction	9	5	4	4	22
Farm	1	2	0	8	11
Mechanic	3	0	4	0	7
Bricklayer	2	0	0	0	2
Painter	1	0	1	0	2
Fireman	1	2	0	0	3
Management	1	0	1	0	2
Electrical Work	2	0	1	0	3
Iron Work	1	3	1	0	5
Police	1	0	0	1	2
Other	1	4	0	3	8

TABLE 8. *Labor market participation potential.*

Community	Oystermen Interviewed	Potentially Employable <sup>a</sup>	Possibly Trainable <sup>b</sup>	Potential Hard-Core Unemployed <sup>c</sup>	Not in Labor Force <sup>d</sup>
Avenue	30	25 <sup>d</sup>	15	2	3 <sup>d</sup>
Rock Hall	41	10	25	8	8
Shady Side	29	25	23	1	3
Crisfield- Smith Island	33	5	21	6	1

<sup>a</sup> Those having marketable skills or sufficient education.

<sup>b</sup> Those capable of participating in a training program. Age and level of education were used as a first approximation of trainability.

<sup>c</sup> Those having no marketable skills between the ages of 45 and 65 who have completed less than 6 years of school.

<sup>d</sup> Students and those over 65 years.

tially employable," "potentially trainable," "potentially hard-core unemployed," or "not in the labor force."

Of the four communities surveyed, Avenue and Shady Side were found to contain the largest number that were "potentially employable." The booming construction industry in these two areas served as one of the major explanatory factors. The large number of fishermen in these two communities with employment either part-time or during the off-season contributed to their mobility. Employment opportunities in Rock Hall and Crisfield-Smith Island were found to be limited. Further, the jobs available required educational training beyond that received by many of the surveyed watermen. The employability dilemma was indicative of the possible need for training programs and possible relocation of Rock Hall and Crisfield-Smith Island watermen, if a major disaster were to occur. Results reported in Table 8 indicate that a large number of the watermen located in these two communities were potentially trainable.

The relatively large concentration of potential hard-core interviewees in Rock Hall and Crisfield-Smith Island reflected the high concentration of older and less educated watermen located in these two communities, and the demand for individuals with high school educations and beyond.

#### SUMMARY

Information obtained from 133 interviews with

oystermen located in four Maryland communities indicated that the labor market participation potential was significantly higher for the two western shore communities of Avenue and Shady Side than for the eastern shore communities of Rock Hall and Crisfield-Smith Island. Vocational training and outside employment coupled with an expanding construction industry were found to contribute greatly to the potential employability of the interviewed watermen living in Shady Side. The employability of Avenue oystermen was found to be aided greatly by the strong demand for unskilled labor. Potential vocational readjustment for oystermen living in Rock Hall and Crisfield-Smith Island was found to be hampered by the lack of jobs for people with limited educational and/or vocational training, indicating a possible need for training programs and potential relocation if a serious disaster were to occur. The high concentration of potentially hard-core unemployed watermen located in Rock Hall and Crisfield-Smith Island could necessitate additional assistance programs. Age, limited educational training and additional job experience were factors that led to the classification of approximately 20% of the interviewees in the two eastern shore communities as being "potentially hard-core unemployed."

#### LITERATURE CITED

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GROWTH OF OYSTER LARVAE, *CRASSOSTREA VIRGINICA*,  
OF VARIOUS SIZES IN DIFFERENT CONCENTRATIONS  
OF THE CHRYSOPHYTE, *ISOCHRYSIS GALBANA*

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ABSTRACT

*Oyster larvae (Crassostrea virginica) of seven different size groups were fed different concentrations of Isochrysis galbana. The optimum concentration of I. galbana for each size group was determined by measuring the increase in mean length of larvae during the 48 hr test period. The optimum concentration increased with increasing larval size and ranged from 2.5  $\mu$ l of packed cells per liter of larval culture for larvae 74  $\mu$  long to 32.5  $\mu$ l of packed cells per liter of larval culture for larvae averaging 246  $\mu$  in length. It was found to be more efficient to increase the Isochrysis concentration as the larvae grew than to feed the larvae at constant rates.*

INTRODUCTION

There is much current interest in the culture of oyster larvae by large privately owned hatcheries and other organizations raising oyster larvae for their research programs. One of the most important factors in bringing large numbers of oyster larvae successfully to metamorphosis is the type and amount of food used during the rearing procedure.

Most studies of the food requirements of oyster larvae have been concerned primarily with the relative growth achieved with particular microorganisms. Cole (1937) was the first to demonstrate that pure cultures of naked flagellates could be used to produce significant growth of *Ostrea edulis* larvae under laboratory conditions. Bruce, Knight and Parke (1940) cultured six species of flagellated algae and found two, *Isochrysis galbana* and *Pyraminomonas grossi*, that were good foods for *O. edulis* larvae. Walne (1963) reported that *I. galbana*, among other algal species, was an acceptable food for *O. edulis* larvae. Davis (1950, 1953) tested a number of potential foods for *Crassostrea virginica* larvae and found that five flagellated species and *Chlorella* sp. were utilized. Later, 10 genera of microorganisms were tested by Davis and

Guillard (1958) and they found that the chryso-phytes, *I. galbana* and *Monochrysis lutheri*, were of approximately equal value and the best single foods for *C. virginica* larvae.

Some information is available on the quantitative aspects of feeding shellfish larvae. Loosanoff, Davis and Chanley (1953, 1955) studied the larvae of *Mercenaria mercenaria* and reported that heavy concentrations of *Chlorella* sp. killed larvae, that larval growth was abnormally slow when an insufficient amount of food was present, and that the optimum larval growth over a 12-day period occurred at concentrations of 50,000 large (8  $\mu$ ) or 400,000 small (4  $\mu$ ) *Chlorella* sp. cells/ml. Davis and Guillard (1958) found the optimum concentrations of *I. galbana* and *M. lutheri* for *M. mercenaria* larvae to be 200,000 and 250,000 cells/ml, respectively, with little difference in growth occurring over a wide range of concentrations.

Bayne (1965) reported that *Mytilus edulis* larvae exhibit a general increase in growth rate with increasing *I. galbana* concentrations up to 100,000 cells/ml, the highest cell concentration tested. Bayne's data also showed that the grazing rate and the number of cells caught per larva in 24 hr increased with an increase in larval size.

Walne (1956, 1963, 1965, 1966) investigated the quantitative aspects of feeding *O. edulis* larvae. Walne (1965) reported a rapid increase in assimilation of radioactively labeled *I. galbana* as food concentrations increased until at 50,000 cells/ml about 70% of the maximum assimilation is obtained. He further showed that at cell densities over 100,000/ml the increase in assimilation is slight for substantial increases in cell density. Walne also performed experiments which indicated that, as larval sizes increase from about 170 - 260  $\mu$ , the numbers of cells assimilated by a larva in 24 hr increase from 6,000 - 15,000. At larval densities of 1.0 - 1.5/ml Walne (1966) reported that it was necessary to add food to cultures more frequently than every 24 hr to maintain cell concentrations high enough for optimum growth of the grazing larvae.

Davis and Guillard (1958) reported some information on the relationship between algal concentration and the growth of *C. virginica* larvae. These workers fed five different concentrations of *I. galbana* and *M. lutheri* to oyster larvae. They found that a concentration of 250,000 *M. lutheri* cells/ml was optimum at each sampling in a 14-day experiment. With *I. galbana* young larvae grew best at 100,000 cells/ml, whereas older larvae grew fastest at 400,000 cells/ml. The data of Davis and Guillard, however, do not reveal the quantity of *I. galbana* to feed to larvae of specific sizes to obtain maximum growth.

Ukeles and Sweeney (1969) also reported some food concentration data for *C. virginica* larvae. They fed  $^{14}\text{C}$ -labeled *M. lutheri* to straight-hinge *C. virginica* larvae and found that retention is most efficient at a food concentration of about 200,000 cells/ml or 13,000 cells/larva. At these food concentrations approximately 150 - 250 *M. lutheri* cells were taken up and retained per larva in 24 hr. No data are reported for older larvae.

In the present work the concentrations of *I. galbana* necessary to effect maximum growth of *C. virginica* larvae of various sizes are reported, and some comparisons are made between feeding at constant rates and feeding on a graduated schedule according to larval size.

## METHODS

### Algal Culture

*I. galbana* was chosen for this study because it has been found to be one of the best foods for *C. virginica* larvae (Davis and Guillard, 1958) and because similar studies have been performed using

this species with *O. edulis* larvae (Walne, 1956, 1963, 1965, 1966). The *Isochrysis* used in these experiments was grown in semicontinuous unialgal cultures (not bacteria free) in a heat-sterilized, enriched seawater medium following the methods described by Ukeles (1971). The *Isochrysis* required was harvested daily and the density of the culture determined by centrifuging a 10-ml sample in a Hopkins tube for 15 min at 1,000 g. The resulting packed cell volumes were used to determine the appropriate quantities of algal suspension to feed to the larval cultures. The food concentrations reported, therefore, are expressed as microliters of packed cells per liter of larval culture.

### Feeding Concentration Experiments

All of the feeding concentration experiments were short-term, acutely measured tests, molded after the methods of Walne (1965). For each series of experiments a stock population of oyster larvae, consisting of the pooled progeny from a number of Long Island Sound parents, was reared according to the methods of Loosanoff and Davis (1963). The stock populations were reared at 28°C in 15-liter polyethylene containers containing filtered seawater to which 100 ppm sodium sulfamethazine (Sulmet, American Cyanamid Co.)<sup>1</sup> had been added, and were fed exclusively on a diet of *Isochrysis*. To obtain larvae of a uniform size for an individual experiment and to make the results more applicable to commercial hatcheries where larvae are separated and grown by size, the entire stock population was screened through a series of nylon mesh screens and the desired size group selected. The nylon screens used had square openings of 54, 75, 100, 135, 151, 180 and 216  $\mu$ . Mesh size refers to the screen in this series which retained larvae after a 3 min seawater rinse. Straight-hinge larvae were not screened for size, but were rinsed on a 36  $\mu$  nylon screen before use in the tests.

In the first series of experiments eight groups of *C. virginica* larvae in four basic size categories were tested in duplicate 1-liter cultures to which *Isochrysis* concentrations of 0, 2.5, 5.0, 10.0, 20.0 and 40.0  $\mu\text{l/l}$  were added daily. The cultures of about 15,000 larvae each were maintained in Pyrex glass beakers at 28°C in filtered and ultraviolet-treated seawater to which 100 ppm of

<sup>1</sup>Trade names mentioned in this paper do not imply endorsement by the National Marine Fisheries Service.

TABLE 1. Average optimum *Isochrysis* concentrations ( $\mu$ /l) for maximum growth of oyster larvae of various sizes in 48 hr.

Average Initial Larval Size ( $\mu$ )	Initial Larval Mesh Size	Average Optimum Feeding Concentration
74.4	S-H	2.5
80.1	54	5.6
107.0	75	13.8
139.8	100	17.5
170.0	135	22.5
200.4	151	32.5
246.4	180	32.5

sodium sulfamethazine had been added. The experiments were sampled and terminated at 48 hr. Growth data consisted of 100 larval measurements for each sample. Larval lengths were measured to the nearest 5  $\mu$  with an ocular micrometer.

Using the results from the first series of experiments, series of seven *Isochrysis* concentrations were selected for testing each of seven larval size groups. Each size group was tested in two 48 hr experiments, and duplicate 1-liter cultures were used at each of the seven concentrations of food tested in each experiment. Experimental methods were identical to those above. In the two experiments involving larvae larger than 237  $\mu$  in initial length a clean oyster shell was added to each beaker to provide a suitable substrate for larvae that might attain a size sufficient for metamorphosis.

#### Feeding Schedule Experiments

After the feeding concentration experiments had revealed the optimum feeding rate for the seven sizes of larvae, two experiments were performed in which the growth of oyster larvae using a graduated feeding schedule and several constant feeding rates were compared. The average optimum food concentrations, which comprised the graduated feeding schedule, are indicated in Table 1. The constant feeding rates were 0, 2.5, 5.0, 10.0, 20.0 and 40.0  $\mu$ /l. The tests were set up with straight-hinge larvae and were terminated when eyed larvae were observed. Larvae in all treatments were screened from the cultures and resuspended in clean seawater every two days. Larvae in one set of cultures in the first feeding schedule experiment were separated by size every two days, set up in beakers according to mesh size at a density of 15 larvae/ml, and the beakers fed at the concentrations of *Isochrysis* indicated in Table 1. All other methods and materials were identical to those above.

## RESULTS AND DISCUSSION

#### Food Concentrations and Larval Growth

The results of the first series of experiments are presented in Table 2. These data show generally the concentrations of *Isochrysis* necessary for good growth of oyster larvae of various sizes. However, in the first and seventh experiments reported in Table 2 the maximum growth was achieved in the lowest and highest food concentrations tested, respectively, making it necessary to expand the ranges of concentrations used in later tests. Because our initial food concentrations were widely spaced, we also wanted to test some intermediate concentrations. Therefore, for the remainder of the

TABLE 2. The average growth increments ( $\mu$ ) of oyster larvae of various sizes after being fed different concentrations of *Isochrysis* in Experimental Series 1.

Food Concentration ( $\mu$ /l)	Initial Larval Length ( $\mu$ )							
	77.1	77.7	104.1	104.2	139.4	145.8	200.6	204.2
40.0	3.3	10.1	17.1	22.0	5.9	31.9	30.9	8.4
20.0	4.1	13.1	19.2	33.4	3.4	41.0	19.9	27.0
10.0	7.9	14.9	19.5	38.4	11.8	20.9	8.0	14.6
5.0	11.7	18.8	15.1	28.8	9.0	7.1	3.8	4.9
2.5	13.8	19.3	9.1	16.1	4.7	1.4	0.0	4.2
Unfed	3.9	4.6	2.7	5.5	0.0	0.0	0.0	1.2

TABLE 3. The average growth increments ( $\mu$ ) of oyster larvae of various sizes after being fed different concentrations of *Isochrysis* in Experimental Series 2.

Food Concentration ( $\mu$ l/l)	Initial Larval Length ( $\mu$ )						
	74.2	80.1	104.6	137.1	168.1	200.4	255.0
60.0							9.7
50.0							17.1
45.0						38.1	16.8
40.0						38.1	11.9
35.0					20.5	35.6	16.3
30.0					27.6	38.1	17.6
25.0				43.1	30.6	37.7	11.1
20.0			41.9	42.9	34.2	26.7	
17.5			43.8	41.9	33.6	28.0	
15.0		17.0	45.1	43.6	33.2		
12.5		13.2	41.7	39.3	27.5		
10.0	11.7	14.7	39.0	25.8			
7.5	12.3	17.7	34.7	15.5			
5.0	15.0	14.2	18.6				
3.8	15.6	12.3					
2.5	14.6	12.7					
1.2	7.5						
0.6	5.6						
Unfed	2.2	0.0	0.0	0.0	2.3	3.4	0.0

food concentration experiments reported here we used the data in Table 2 to select a series of food concentrations to be tested against larvae of specific sizes.

Tables 3 and 4 present the results of the second and third series of experiments. In Figure 1 the *Isochrysis* concentrations which produced the most rapid growth of larvae of various sizes are plotted against initial larval length. Duncan's multiple range tests (Steel and Torrie, 1960) were performed to determine in each experiment which growth increments were not significantly different from the maximum increment obtained (95% confidence level), and these are indicated as vertical lines in Figure 1. The average optimum *Isochrysis* concentrations for larvae in the seven size groups tested are presented in Table 1.

These feeding experiments indicate that, as oyster larvae grow, their food requirements increase substantially. A 13-fold increase in *Isochrysis* concentrations was found necessary to support maximum growth of the larvae over the range of sizes tested. Straight-hinge larvae 74  $\mu$  in length grew

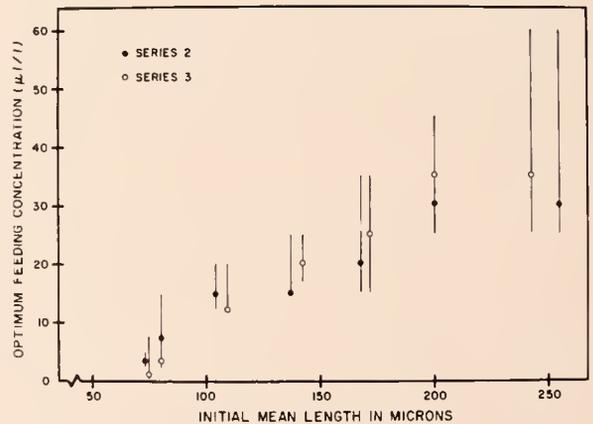


FIG. 1. The optimum *Isochrysis* concentrations for *C. virginica* larvae of various initial mean lengths. Points indicate concentrations in which the greatest growth increment was obtained in the raw data. Vertical lines indicate concentrations in which growth increments were not statistically different from those obtained in the concentration producing the greatest growth increment.

TABLE 4. The average growth increments ( $\mu$ ) of oyster larvae of various sizes after being fed different concentrations of *Isochrysis* in Experimental Series 3.

Food Concentration ( $\mu$ /l)	Initial Larval Length ( $\mu$ )						
	74.7	80.1	109.4	142.4	172.0	200.4	237.7
60.0							0.0
50.0							0.0
45.0						20.7	0.5
40.0						22.9	0.0
35.0					20.4	25.3	4.5
30.0					20.4	22.4	0.0
25.0				36.8	23.3	21.6	1.3
20.0			22.6	38.1	22.6	15.1	
17.5			24.7	37.7	20.2	11.2	
15.0		17.3	26.2	28.1	18.8		
12.5		18.8	27.4	23.6	14.1		
10.0	6.7	20.6	20.5	19.1			
7.5	7.9	19.8	18.6	11.0			
5.0	9.4	14.0	14.8				
3.8	9.5	21.6					
2.5	8.5	17.7					
1.2	10.3						
0.6	8.5						
Unfed	3.7	4.7	1.7	0.0	0.0	0.0	0.0

fastest at an average concentration of 2.5  $\mu$ /l, while larvae 200  $\mu$  in length required an average *Isochrysis* concentration of 32.5  $\mu$ /l for maximum growth.

Larvae longer than 237  $\mu$  grew slower than the other groups tested (Tables 3 and 4). There are no data in the literature to suggest that oyster larvae grow more slowly as they approach metamorphosis in the presence of a substrate suitable for setting. We suspect that this slow growth was due to a toxic substance associated with the *Isochrysis* cultures used for these tests since the larvae in the fed beakers failed to swim actively, while the larvae in the unfed beakers did swim actively.

The experiments of Davis and Guillard (1958), although not specifically designed to reveal optimum feeding concentrations for larvae of various sizes, did indicate a general increase in food requirements as oyster larvae grow. The data of these workers showed the optimum *Isochrysis* concentration for 75  $\mu$  larvae to be 10  $\mu$ /l, whereas 140  $\mu$  larvae grew best at 40  $\mu$ /l. These concentrations are somewhat higher than those found to be optimum in the present study. For the most part, larvae in the present study grew faster and at lower concentrations of food than did those of

Davis and Guillard. The lower temperatures (21 - 23°C) used by Davis and Guillard and differences in the quality of the *Isochrysis* cultures used may account for this discrepancy.

Guillard (1958) considered the data of Davis and Guillard (1958) and the levels of food organisms encountered by shellfish larvae in natural situations and suggested that an algal concentration of 10  $\mu$ /l be used as a guide in feeding oyster larvae at densities of 3 - 15/ml. The data from the present study show that this concentration of cells is less than optimum for larvae over 100  $\mu$  long.

The studies of Ukeles and Sweeney (1969) showed that about 150 - 250 *M. lutheri* cells/larva were taken up and retained by straight-hinge larvae in 24 hr at a concentration of 13,000 cells/larva, the most efficient feeding concentration. Assuming that 100,000 *Isochrysis* cells/ml is equal to 10  $\mu$  of packed cells/liter (Davis and Guillard, 1958), in our study only about 1,600 *Isochrysis* cells were available to each straight-hinge larva in a 24-hr period at the concentrations that produced the most rapid growth. Although we made no final algal counts, significant clearing of the cultures was observed and most of the those cells available

were probably utilized. The slower growth of straight-hinge larvae which we obtained at higher food concentrations (Table 2) shows that under the conditions in the present experiments the lower feeding rate is superior.

Walne (1956, 1963, 1965, 1966) provided much information on the feeding behavior of *O. edulis* larvae. Because this species is larviparous and releases larvae averaging  $170\ \mu$  in length, no comparisons of food requirements are possible for small larvae, but some can be made for larger ones. Walne (1965) reported that *O. edulis* larvae averaging  $219\ \mu$  in length catch an average of 24,000 *Isochrysis* cells in 24 hr. The growth data in the present study indicate maximum growth of similar size *C. virginica* larvae at a concentration of about 20,000 cells/larva in 24 hr. Walne (1965) also reported that, as *O. edulis* larvae grow from about  $170 - 260\ \mu$  in the planktonic phase, the assimilation of *Isochrysis* cells increases 2.5 fold. The data presented here indicate an approximate doubling of optimum cell concentrations for *C. virginica* larvae of similar sizes.

One of the prime considerations in evaluating a feeding schedule is the number of larvae that can be reared per unit volume. The present experiments indicate that acceptable growth can be achieved with proper feeding concentrations over a wide range of larval sizes at a density of 15 larvae/ml. Walne (1965) obtained rapid growth at a density of 140 *O. edulis* larvae/l and a cell concentration of 123,000/ml; but to get similar growth at a larval concentration of 5,000/l the *Isochrysis* concentration had to be tripled. In the first case the small number of larvae grazing did not significantly reduce the *Isochrysis* concentration, while at the higher larval density food became a limiting factor. Davis (1953) observed an inverse relationship between larval density and growth at various *Chlorella* sp. concentrations for *C. virginica* larvae. Loosanoff *et al.* (1955) who fed various amounts of *Chlorella* sp. to clam larvae, *M. mercenaria*, concluded that an increase in larval densities beyond a certain limit cannot be compensated for by a proportionate increase in the quantity of food. The limits in this situation appear to result from the mechanical interference with feeding at high algal concentrations (Loosanoff *et al.*, 1955), the possible occurrence of toxins produced by the algal cells or present in the algal suspension from some other source, and the accumulation of inhibiting quantities of metabolic wastes from the larvae at high densities.

At the larval density of 15/ml used in the

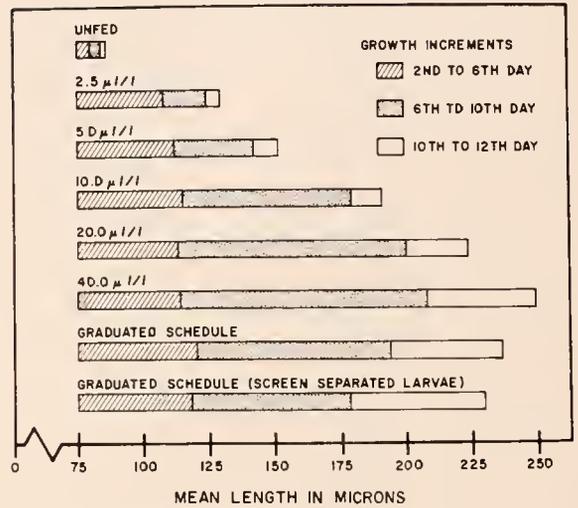


FIG. 2. The growth of *C. virginica* larvae on different feeding schedules.

Experiment 1.

present study the *Isochrysis* concentrations in the larval cultures were substantially reduced by grazing in the 24 hr between feedings. The ideal feeding situation should probably include provisions for continuous feeding so that an optimum concentration of cells would be present in the culture vessel at all times.

#### Feeding Schedules and Larval Growth

The results of the first feeding schedule experiment are presented in Figure 2. From 65 - 90% of the original larval population were alive in the different treatments on the twelfth day. A Duncan's multiple range test of the data from the final

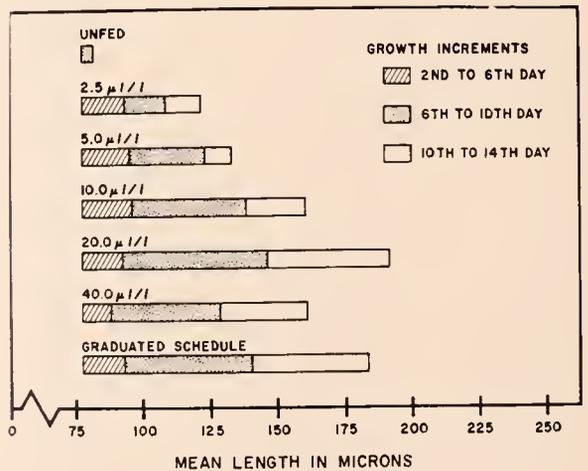


FIG. 3. The growth of *C. virginica* larvae on different feeding schedules. Experiment 2.

sampling date showed all treatment means to be significantly different from each other (95% confidence level), except between the means representing 20  $\mu\text{l/l}$  and the graduated feeding schedule for screened larvae. The best growth occurred at 40  $\mu\text{l/l}$ , but the cultures fed according to the graduated feeding schedule produced larvae only 5% smaller and required only 46% of the total *Isochrysis* used to feed the 40  $\mu\text{l/l}$  cultures. The larvae that were screen-separated into size groups, adjusted to 15/ml, and fed according to size, were fed 56% of the food required to maintain the larvae in the fastest growing treatment.

The growth data from the second feeding schedule experiment are presented in Figure 3. All treatment means for the final sample are statistically different from each other (Duncan's multiple range test, 95% confidence level), except those representing 10  $\mu\text{l/l}$  and 40  $\mu\text{l/l}$ . On the 14 day of the experiment 70% of the original larval population were alive in the best two treatments. The larvae at 20  $\mu\text{l/l}$  were 4% larger than those fed according to the graduated feeding schedule, but to effect this increase 63% more *Isochrysis* was required. These results show that using constant feeding rates *Isochrysis* concentrations of 20 to 40  $\mu\text{l/l}$  are required to effect maximum growth rates of *C. virginica* larvae at densities of 10 - 15/ml. Similar high rates of growth can be achieved by starting at much lower feeding rates and then increasing the *Isochrysis* concentration as the larvae grow. This latter method requires a smaller volume of algae than the constant concentration method and could yield significant savings to organizations rearing substantial numbers of oyster larvae.

#### ACKNOWLEDGMENTS

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## A NEW TECHNIQUE FOR MEASURING THE OXYGEN CONSUMPTION OF LARVAE OF THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA*

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### ABSTRACT

The oxygen consumption rates of larvae of the American oyster, *Crassostrea virginica*, were determined using an all-glass differential microrespirometer. Oxygen uptake was found to increase logarithmically as the larvae grew in size. This rate ranged from less than 0.2  $\mu\text{l O}_2/\text{hr}/1000$  larvae for individuals 60  $\mu$  in length to 20  $\mu\text{l O}_2/\text{hr}/1000$  larvae for individuals 200  $\mu$  in length.

### INTRODUCTION

The American oyster, *Crassostrea virginica*, is an estuarine bivalve that is naturally exposed to a wide range of environmental conditions. The development of techniques for rearing bivalve mollusks (Loosanoff and Davis, 1963) has prompted a number of studies on the effects of environmental changes (temperature, salinity, pH, industrial pollution) on the survival of bivalve embryos and larvae (Woelke, 1967; Calabrese and Davis, 1970; Calabrese, Collier, Nelson and MacInnes, 1973). Oxygen consumption is a parameter often used as an indicator of sublethal environmental stress on the metabolism of the organism studied. The small size and low respiration rate of bivalve larvae, however, have made accurate determinations of larval oxygen consumption extremely difficult. Conventional respirometers, such as the Warburg apparatus, are not sufficiently sensitive to determine a precise oxygen uptake rate. Vernberg and Costlow (1966), using a differential microrespirometer developed by Grunbaum, Siegel, Schulz and Kirk (1955), were able to measure the oxygen consumption of fiddler crab larvae (*Uca*, various species). Recently, Sastry and McCarthy (1972)<sup>1</sup> were able to measure oxygen consumption rates of larvae of two brachyuran crabs, *Cancer irroratus* and *C. borealis*, using the same type of microrespirometer.

The present study was designed to evaluate the use of a similar microrespirometer in measuring normal oxygen consumption rates of oyster larvae during the period of development from several

hours after fertilization to metamorphosis two or three weeks later. This evaluation may prove valuable in future studies using oxygen consumption of bivalve larvae as an indicator of stress induced by abnormal environmental conditions.

### MATERIALS AND METHODS

Oyster eggs were obtained following the procedure described by Loosanoff and Davis (1963). Adult oysters were induced to spawn by thermal stimulation and by addition of sperm stripped from a sacrificed male. The eggs were collected from more than one female to insure a heterogeneous sample. The number of fertilized eggs per unit volume was determined by microscopic examination of a subsample and approximately 500,000 eggs were then transferred to a 15 liter container maintained in a water bath at 26°C. The larvae were reared in natural seawater (salinity-25‰) that had been circulated through 15  $\mu$  and 1  $\mu$  Orlon filters, an ultra-violet light sterilization unit and an activated charcoal filter. The water was changed every other day, and the larvae were fed laboratory grown phytoplankton cultures of *Isochrysis galbana* and *Monochrysis lutheri*. A sample

<sup>1</sup> Sastry, A. N. and J. F. McCarthy. 1972. Oxygen consumption of larval stages of two sympatric species of brachyuran crabs, *Cancer irroratus* and *C. borealis*. In Abstracts of Papers Submitted for the 35th Annual Meeting, American Society of Limnology and Oceanography, Inc., Tallahassee, Florida.

of larvae was taken prior to each oxygen consumption experiment and the mean length of 50 larvae was determined. The all-glass microrespirometer used in this study was essentially that described by Grunbaum *et al.* (1955) and consisted of a capillary with a 0.3 mm bore and two 5 ml flasks; a respiration flask containing oyster larvae and a control or compensation vessel containing seawater but no organisms. Glass loops were formed on each end of the capillary to hold a carbon dioxide absorbent; a filter paper disc soaked with 1% KOH and tied in place. Several hundred larvae, in 2.0 ml seawater, were placed in each respiration flask and then immersed in a constant temperature (26°C) water bath. The amount of oxygen consumed by the larvae was determined by measuring the movement of the red distilled kerosene indicator in the capillary bore. The change in oxygen is given by K times h where h is the distance covered by the indicator and the proportionality factor is derived from the equation:

$$K = \frac{273}{T} \left( \frac{P - P_w}{P_o} \right) \left( \frac{V_g + 1}{V_g^1} \right)$$

in which:

- T = absolute temperature  
 P = atmospheric pressure (mm Hg)  
 P<sub>w</sub> = vapor pressure of water at T (mm Hg)  
 P<sub>o</sub> = standard atmospheric pressure (mm Hg)  
 A = cross section of area of capillary bore  
 V<sub>g</sub> = volume of respiration chamber  
 V<sub>g</sub><sup>1</sup> = volume of compensation chamber

Readings were recorded every 15 min during experiments of at least 2 hr in duration. The respiratory rate was calculated as microliters of oxygen consumed per hour per thousand larvae.

## RESULTS AND DISCUSSION

The results of this study are presented in Figure 1. Since the relationship between mean length ( $\mu$ ) of oyster larvae and the oxygen consumption rate is curvilinear, it is best expressed logarithmically by the equation:

$$10 (\log Y) = -4.556 + 2.941 (\log X)$$

where X = mean length of larvae sampled on the day of the experiment

and Y = consumption rate of ( $\mu$  O<sub>2</sub>/hr/1000 larvae)

The high correlation coefficient of the relationship ( $r = 0.875$ ) indicates a rise in oxygen consumption rate as the larvae grew in size. This rate increased from less than 0.2  $\mu$  O<sub>2</sub>/hr/1000 larvae for individuals 60  $\mu$  in length to 20  $\mu$  O<sub>2</sub>/hr/1000 larvae

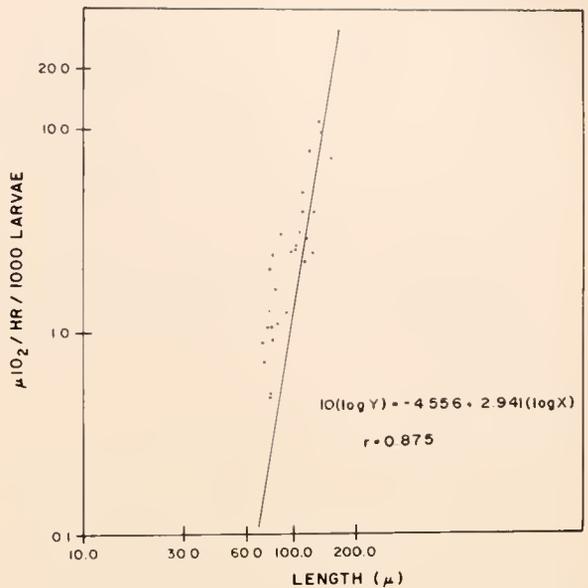


FIG. 1. Oxygen consumption rates of American oyster larvae ( $\mu$ l O<sub>2</sub>/hr/1000 larvae) vs size (length in  $\mu$ ) at 26°C and 25‰ salinity.

for individuals that had grown to 200  $\mu$  in length. Other workers have noted similar increases in oxygen consumption of developing oyster eggs (Black, 1962; Cleland, 1950), but little information is available on the normal oxygen consumption of 60-200  $\mu$  larvae.

This study has demonstrated the suitability of this microrespirometer in measuring oxygen consumption of larval bivalves. The small size of this instrument allows great sensitivity and performs well if operated under constant temperature conditions. The results, however, are valid only under the conditions described in this study and may not represent the actual respiratory rates in the environment or in a hatchery situation. The relative values, however, are valuable and should prove useful in future studies of the effects of environmental changes on bivalve larvae.

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## SHELLFISH MARICULTURE IN AN ARTIFICIAL UPWELLING SYSTEM<sup>1</sup>

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### ABSTRACT

A mariculture system was established on St. Croix, U. S. Virgin Islands, using "artificial upwelling" to obtain deep water, rich in nutrients necessary for plant life. Water from 870 m depth in the sea was pumped into 45,000-liter pools in which species of three diatoms, *Bellerochea* sp., *Chaetoceros simplex* and *Thalassiosira pseudonana* (= *Cyclotella nana*) were grown to feed shellfish. The St. Croix site was chosen because the ocean reaches a depth of 1000 meters approximately 1.6 km offshore.

Initially, 100,000 juvenile *Crassostrea virginica* (Gmelin) and 100,000 juvenile *Mercentaria mercenaria* Linné, from Long Island Sound were put into the system. The oysters grew very well during the first few months but died rapidly thereafter. While experiments indicated that clam survival and growth were good, they ceased feeding for 24-48 hr after handling. Clams in sediment grew faster than those in wire trays.

Later introductions of  $F_1$  hybrid (or racial cross) clams (male *M. mercenaria* X female *M. campechiensis* Say), grew far more rapidly than *M. mercenaria* and appeared very well suited to the conditions of our system.

Comparative growth studies of *C. virginica*, *C. gigas* (Thunberg) and *Ostrea edulis* Linné are underway.

### INTRODUCTION

Deep ocean water is cold and rich in nitrates, phosphates, silicates and other dissolved nutrients necessary for plant life. In our artificial upwelling system on the north shore of St. Croix, water from 870 m is pumped into 45,000-liter concrete onshore pools where planktonic algae are grown as food for shellfish in a controlled food chain (Roels, Van Hemelrijck, Gerard and Worzel, 1971).

The accumulation of nutrients in the deep water results from photosynthesis occurring in the euphotic zone which is limited to a depth of ap-

proximately 100 m. Unicellular phytoplankton converts solar energy, carbon dioxide, water, nitrate, phosphate and minor mineral elements into protoplasm for their cells, the first link in the food chain. As a result of this photosynthetic process in the upper layer of the seas, carbon, nitrogen and phosphorus are extracted from solution thereby depleting the surface waters of these nutrients.

Particulate organic material resulting from dead and disintegrating phytoplankton and the excreta from zooplankters which have grazed on the phytoplankton, sinks through the water column. Bacterial and chemical activity eventually oxidizes the organic matter to inorganic nitrate, phosphate and silicate, etc., resulting in the high dissolved nutrient content of deep water. Table 1 compares some parameters for surface water and water from 870 m depth pumped up through our deep-water

<sup>1</sup>This work was supported by Sea Grant 1-36119 from the U.S. Department of Commerce. Lamont-Doherty Geological Observatory Contribution No. 1919; City University Institute of Oceanography Contribution No. 11.

TABLE 1. Comparison of properties of deep and surface water.

	In Situ Temperature C	Salinity ‰	<sup>a</sup> NO <sub>3</sub> <sup>-</sup>	<sup>a</sup> PO <sub>4</sub> <sup>---</sup>	<sup>a</sup> SiO <sub>4</sub> <sup>---</sup>
Surface Water	26-29	35.83	2.3	0.1	0
Deep Water	7	34.87	32.5±0.7	2.15±0.09	26.5±0.5

<sup>a</sup> Values in microequivalents/liter.

pipe in St. Croix.

The cold temperature of deep ocean water can be used for a wide variety of cooling applications and for sea thermal power production by the "Claude" process (Claude, 1930) in areas where the temperature differential between the surface and the deep water is great enough. Some of the possible cooling applications are air conditioning, ice making, cooling for electrical power generating plants and desalination plants (avoiding thermal and brine pollution) and condensing atmospheric moisture for fresh-water production. The discharge water from these cooling systems would be a valuable resource for mariculture since its nutrient content, essential for algal growth, is much higher than that of surface water.

The advantages of using deep water over in-shore or estuarine surface waters are: (1) its relative sterility, i.e., lack of human disease-producing organisms, shellfish parasites, predators and fouling organisms; (2) its negligible content of oxygen-consuming dissolved organic matter and suspended sediment, especially pesticides and other man-made pollutants; and (3) the constancy of its chemical and thermal characteristics.

Progressive closing of shellfish beds due to: (1) increased coliform counts in near-shore waters; (2) mass shellfish mortality resulting from low salinity caused by the 1972 hurricane "Agnes", floods in the Chesapeake Bay System; (3) the September 1972 disastrous *red tide* in New England and (4) the destruction of shellfish beds due to a variety of pollutants, could all be avoided by use of this mariculture system.

This paper reports results of growth experiments with juvenile oysters (*C. virginica*, *C. gigas* and *O. edulis*) and clams (*M. mercenaria* and *M. mercenaria* X *M. campechiensis* F<sub>1</sub> hybrids) in our system.

## MATERIALS AND METHODS

Deep ocean water was pumped from 870 m depth into 1.2 m deep pools of 45,000 liter capacity. The pools were inoculated with planktonic diatoms *Bellerochea* sp. clone STX-114, *Chaetoceros simplex* clone STX-105 or *Thalassiosira pseudonana* clone 3H. Details of the algal mass culture system will be reported in a later publication. The algae grown in the pools to a concentration of 10<sup>4</sup> - 10<sup>5</sup> cells ml<sup>-1</sup> were pumped continuously at metered rates to a series of epoxy-coated plywood 750 liter shellfish tanks measuring 2.4 x 0.6 x 0.6 m (Fig. 1).

Water temperature in these tanks varied between 22° and 29° C. Cell concentrations in the water entering and leaving the shellfish tanks were monitored twice daily by counting in a Spiers-Levy eosinophil counter under 200-power phase illumination. Flow rates of the phytoplankton suspension to the tanks were based on growth rates of shellfish in each tank. Flow-rates to the different tanks were set according to the following formula:

$$\frac{\text{weight gain of the shellfish in that tank per 24 hr}}{\text{weight gain of the shellfish in all the tanks per 24 hr}} \times \text{total flow rate of phytoplankton suspension available}$$

The shellfish were grown in wire trays (0.6 x 0.5 x 0.1 m) stacked in the tanks. Effluent from the tanks was used as a nutrient source to grow carageenan-producing seaweed. Effluent from the seaweed tanks was filtered through sand to avoid pollution and introduction of new species in the natural environment.

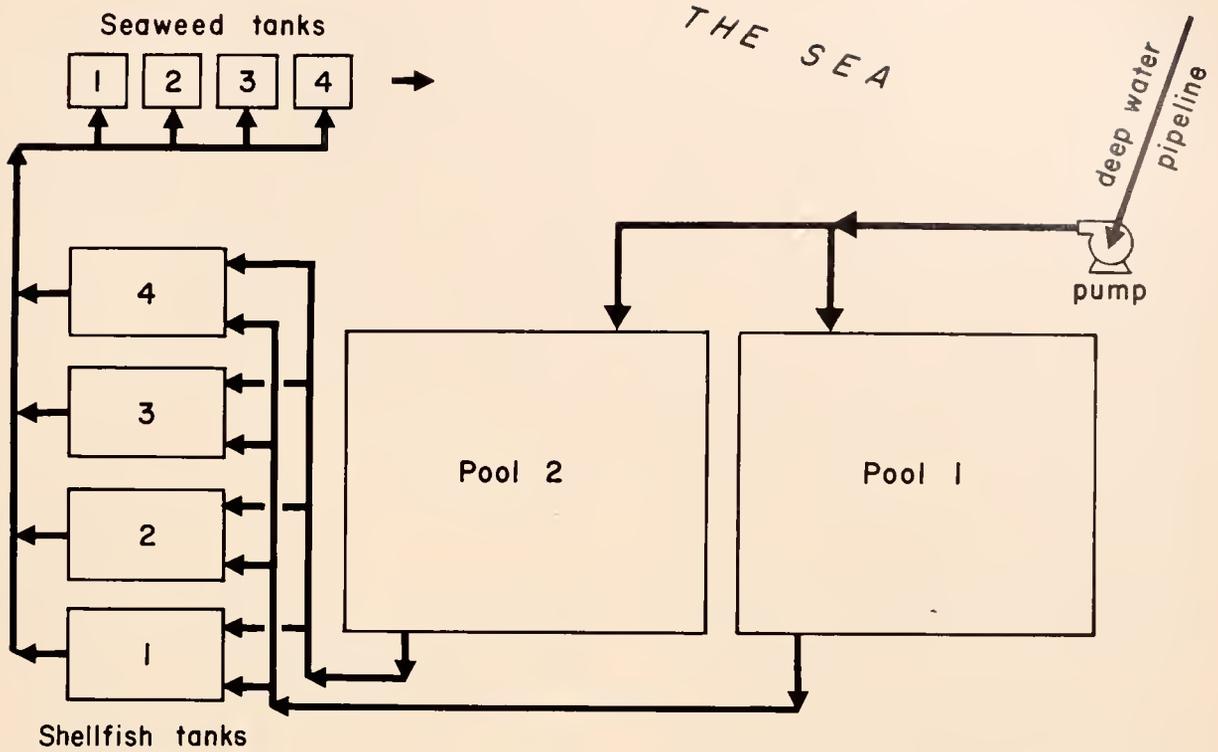


FIG. 1. The flow of deep water and phytoplankton suspension through the mariculture system of St. Croix.

Hatchery-reared shellfish were used in all growth experiments. The first populations of 100,000 juvenile *C. virginica* and 100,000 juvenile *M. mercenaria* were kindly supplied by Long Island Oyster Farms, Inc., in December, 1970. Juvenile oysters (50,000 each of *C. virginica*, *C. gigas* and *O. edulis*) obtained from Pacific Mariculture, Inc., and F<sub>1</sub> hybrid clams supplied by Dr. R. Winston Menzel, were used in later growth studies. Wet weight, linear dimensions and stacked and displacement volumes were measured regularly. Wet weight was determined by placing the shellfish in a pre-weighed wet net bag, draining for one minute and weighing on a Chatillon autopsy scale. Linear growth was determined by measuring extreme width, length and/or thickness with calipers. Stacked and displacement volumes were determined by immersing the shellfish in a graduated cylinder filled to the brim with seawater and measuring the volume of seawater displaced from the cylinder (displacement volume) and the volume occupied by the shellfish in the cylinder (stacked volume).

The effect that cleaning the animals had on the filtering efficiencies (per cent cells removed from

the incoming phytoplankton suspension by the animals) was determined. Comparable groups of *M. mercenaria* were cleaned at one, two or three-week intervals and their filtering efficiencies were compared. The growth of clams in sediment (grain size less than 0.821 mm) was compared to that of clams kept in wire trays.

The growth of the F<sub>1</sub> hybrid clams was com-

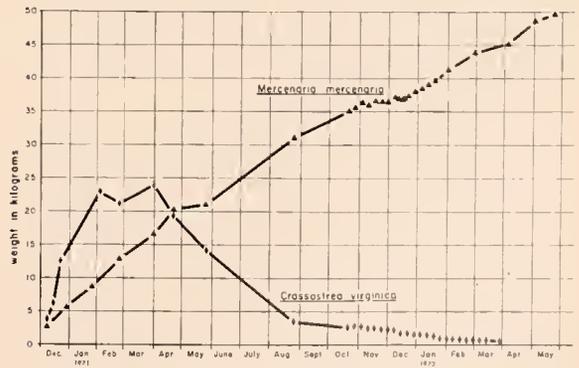


FIG. 2. Growth of *C. virginica* and *M. mercenaria* over an 18-month period.

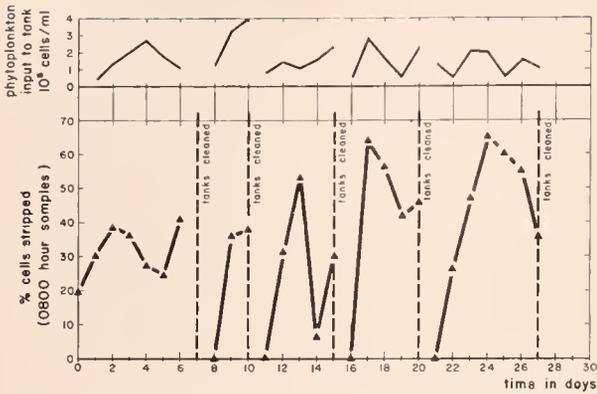


FIG. 3. Phytoplankton filtering efficiencies of *M. mercenaria* as a function of frequency of cleaning and cell density.

pared with that of the northern clam, *M. mercenaria*.

The growth rates of three different species of juvenile oysters (*C. virginica*, *C. gigas* and *O. edulis*) were compared.

#### RESULTS AND DISCUSSION

The first populations of *C. virginica* and *M. mercenaria* were directly influenced by difficulties encountered in the early stages of our mariculture system. Figure 2 illustrates the growth of both populations. It is clear from the growth curves that, compared to *C. virginica*, *M. mercenaria* grew fairly well in the system. *C. virginica* grew well initially but were subject to high mortality rates afterward. The definite cause of this mortality is unknown. A possible cause of death may have been the high temperatures combined with high salinities. E. Mandelli (personal communication) found that salinities above 35 ‰ were lethal to juvenile and adult *C. virginica* at 28-32°C.

Initially, the *M. mercenaria* were cleaned and measured and their tanks scrubbed weekly. The

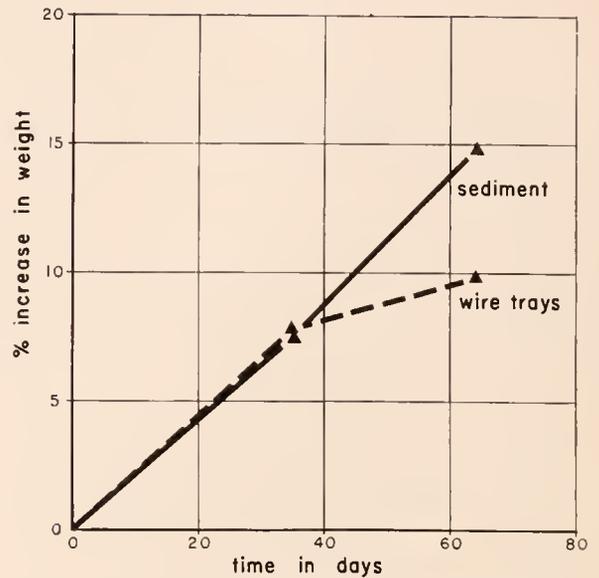


FIG. 4. Increase in weight over 64 days of *M. mercenaria* grown in sediment or in wire trays.

filtering efficiencies of the clams dropped off severely for 24-48 hrs after cleaning. This decrease in filtering did not appear to be correlated with changes in phytoplankton density flowing into the shellfish tanks (Fig. 3).

To determine whether this frequent cleaning of tanks and shellfish slowed down the growth of *M. mercenaria*, we monitored populations of clams in three tanks and varied the interval between cleanings. There were no differences between growth rates and filtering efficiencies of the clams in the tanks cleaned weekly and every two weeks. The clams in the tank cleaned every three weeks filtered cells more efficiently and had a higher growth rate (Table 2).

Clams growing in wire trays required frequent cleaning to remove fouling organisms: such as bryozoans (*Bowerbankia gracilis* Leidy) and epi-

TABLE 2. Comparison of growth rates and filtering efficiencies of three populations of *M. Mercenaria* cleaned and measured weekly, every two weeks or every three weeks.

Frequency of tank cleaning	Average cell filtering efficiency for 29 days	% increase in weight in 29 days
Weekly	34.4%	4.8%
Every two weeks	44.9%	5.2%
Every three weeks	57.8%	9.5%

phytes (*Enteromorpha* spp.). By allowing the clams to bury in sediment we avoided fouling and eliminated the need for cleaning, thus improving their growth rate (Fig. 4). However, cleaning of the tanks at 4-6 week intervals was continued and scheduled to coincide with routine measurements of the shellfish.

When phytoplankton production had been stabilized, experiments were undertaken to find the most suitable shellfish species for our system. Juvenile  $F_1$  hybrid clams grew five times faster than the *M. mercenaria*, as shown in Figure 5. These results confirm the potential use of *Mercenaria* hybrids in mariculture systems (Menzel, 1971). Comparative growth studies of the second series of juvenile oysters (*C. virginica*, *C. gigas* and *O. edulis*) indicate that the survival and growth rate of *O. edulis* were far better than of *C. virginica* and *C. gigas*. (Fig. 6).

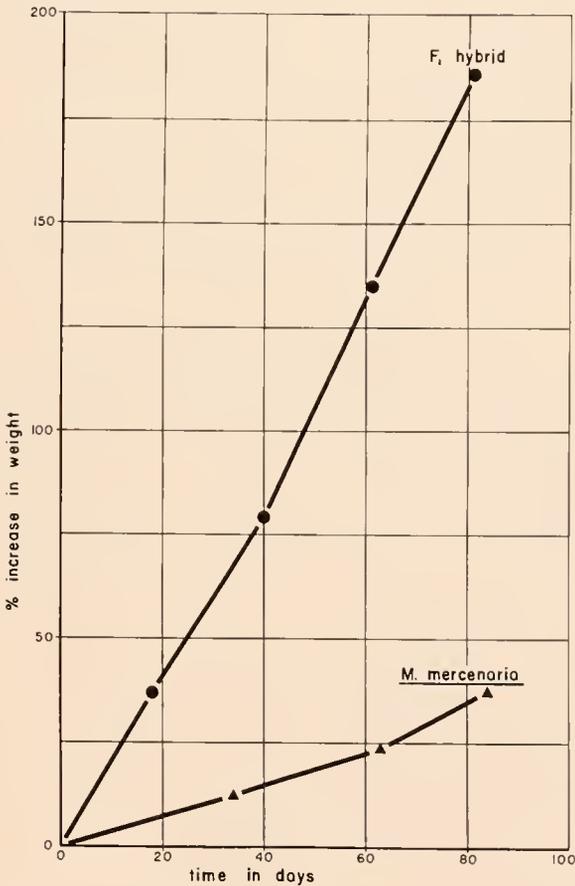


FIG. 5. Comparison of the growth of  $F_1$  hybrids of ♂ *M. mercenaria* x ♀ *M. campechiensis* with *M. mercenaria*. (The average length of the clams at the beginning of the experiment was 34 mm).

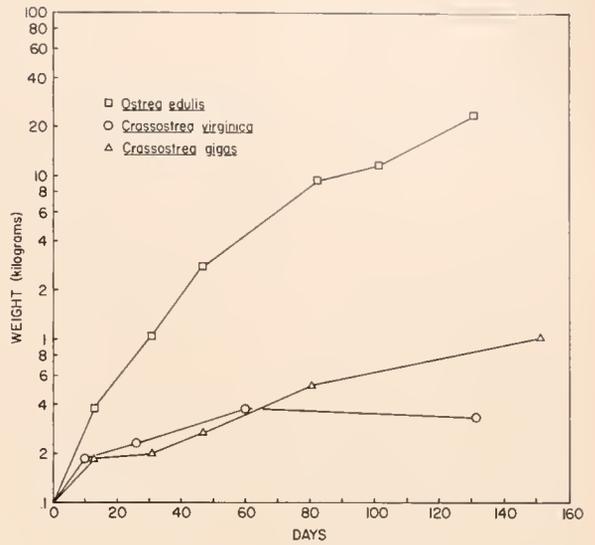


FIG. 6. Growth of 3 species of oysters: *O. edulis*, *C. virginica* and *C. gigas* (size of oysters at beginning of experiment was 2 to 3 mm).

Thus, we feel that this system offers a unique opportunity to optimize shellfish growth under managed conditions with controlled phytoplankton and water flow, low fluctuations in temperature and salinity and absence of predators. An economic and engineering study (unpublished) of clam production based on present small-scale results and extrapolated to a commercial scale indicates high profit potential for "artificial upwelling" mariculture.

ACKNOWLEDGMENTS

We wish to thank the following for their assistance with various aspects of this study: L. Aust, M. Bishop, C. Carson, L. Fick, W. Green, M. Lombard, P. McDonald, W. Tobias and L. van Hemelrijck.

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## GROWTH AND SURVIVAL OF THE BAY SCALLOP, *ARGOPECTEN IRRADIANS*, AT VARIOUS LOCATIONS IN THE WATER COLUMN AND AT VARIOUS DENSITIES

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### ABSTRACT

*Two experiments were carried out in 1971 using bay scallops spawned in August and September of 1970. Four groups of 150 scallops were held at the surface, one meter below the surface, two meters off the bottom, and one meter off the bottom, to find the effects of depth on growth and survival. Scallops were held in surface enclosures at four different densities (100, 75, 50 and 25/ft<sup>2</sup>) to find out the effects of crowding.*

*In the depth experiment growth was approximately equal throughout the water column. Mortality decreased with increasing depth with the exception of those held at one meter off the bottom.*

*In the density experiment growth up to a height of 27.0 - 28.0 mm (1.1 in) was approximately equal at all densities. Above this size, growth decreased with increasing density. Mortality was low and about equal at all densities for the first two months but increased with increasing density during the last two months.*

### INTRODUCTION

The bay scallop is a likely species for mariculture. It grows rapidly, has a high market value, can be readily conditioned and induced to spawn and its larvae are amenable to mariculture (Wells, 1927; Loosanoff and Davis, 1963; Sastry, 1965; Castagna and Duggan, 1971).

The Virginia Institute of Marine Science Eastern Shore Laboratory began investigating the possibility of culturing the bay scallop in 1968. Work completed up to 1971 established the biological feasibility of culturing this species from egg to market size. The purpose of the present study is to show how growth and survival are affected by the location of the scallops in the water column and by the density at which the scallops are held.

This work is a result of research sponsored by NOAA Office of Sea Grant, Department of Commerce under grant number NG572. The U. S. Government is authorized to produce and distribute reprints for government purposes not withstanding any copyright notation that may appear hereon.

### DESCRIPTION OF AREA

Experiments were carried out in Finney Creek in front of the Virginia Institute of Marine Science Eastern Shore Laboratory. Tidal amplitude is 1.2 - 1.5 m (3.6 - 4.9 ft). Water depth is 5-6 m at high tide. Temperatures ranged from 17.2 - 28.7°C and salinities from 20.8 - 31.6 ‰ during the experimental period. The bottom is soft mud. Tidal currents average approximately 30.0 cm/sec throughout the entire water column (Joseph and Van Engle, 1967).<sup>1</sup>

### MATERIALS AND METHODS

Juvenile scallops used in these experiments were spawned in the laboratory from brood stock in late summer of 1970 and tray reared in Finney Creek until May and June 1971 when the

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<sup>1</sup>Joseph E., and W. A. Van Engle. 1967. Nursery ground study. Bureau of Comm. Fish., Comm. Fish. Res. & Devel. Act. Funded under 88-309, Suppl. Rep. Vol. e. (Unpublished manuscript).

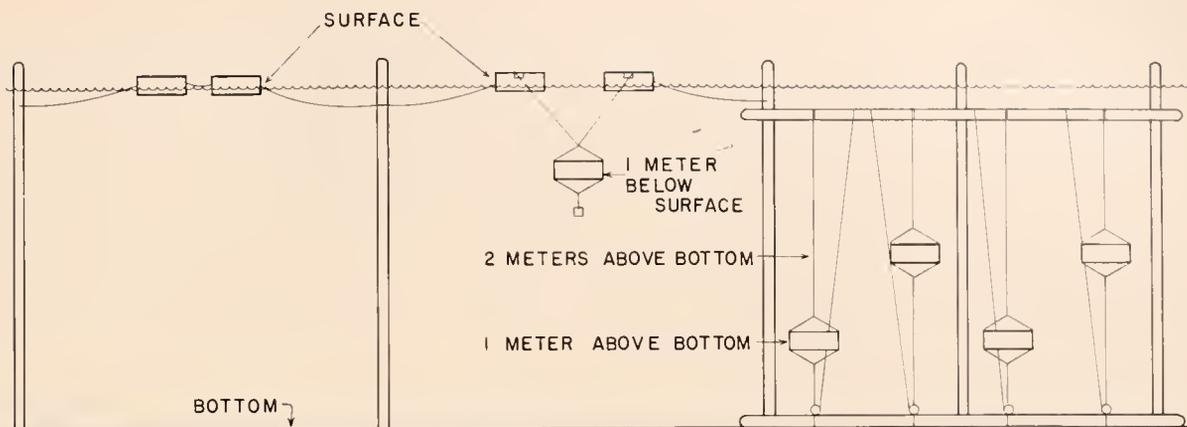


FIG. 1. Enclosures held at the surface, one meter below the surface, and one and two meters off the bottom.

experiments began.

Enclosures used in each experiment were constructed of  $\frac{3}{4}$  in. pine covered top and bottom with plastic screen (mesh opening 7.0 mm). Those used in the depth experiment measured 64.0 x 55.5 x 15.0 cm while those in the density experiment measured 122.0 x 56.5 x 15.0 cm. The surface enclosures used in the density experiments had 14.5 x 1.9 cm boards added on each side for stabilizing wings. Enclosures held at the surface were tied to stakes and maintained at the surface by their own buoyancy. Enclosures held below the surface were either suspended from surface floats or secured to poles at the appropriate depth (Fig. 1).

Experiments were run in duplicate. Mean growth measurements and mortality counts were averaged from duplicate enclosures at two-week intervals and enclosures were cleaned of the mud and fouling organisms that had accumulated during that period.

All enclosures were held in a line parallel to the tidal flow. All measurements of scallops refer to the height or distance from the hinge to ventral edge.

## RESULTS

### Depth Experiment: Growth and Mortality

This experiment ran from 10 June, 1971 - 7 October, 1971. One hundred and fifty scallops

with a mean size of 14.4 mm had been placed in each enclosure held at the surface, 1 m below the surface and 1 and 2 m above the bottom (Fig. 1).

At the end of this experiment scallops averaged 44.7 mm at the surface, 44.6 mm at 1 m below the surface, 47.0 mm at 2 m above the bottom and 42.7 mm at 1 m above the bottom, indicating approximately equal growth at all depths (Fig. 2).

With the exception of those scallops held at 1 m above the bottom, total percent mortality decreased with increasing depth: 16.5% at the

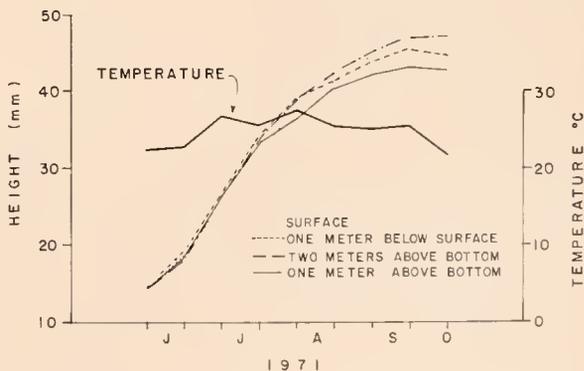


FIG. 2. Growth data for scallops held at various locations in the water column (surface, one meter below the surface, one and two meters above the bottom.)

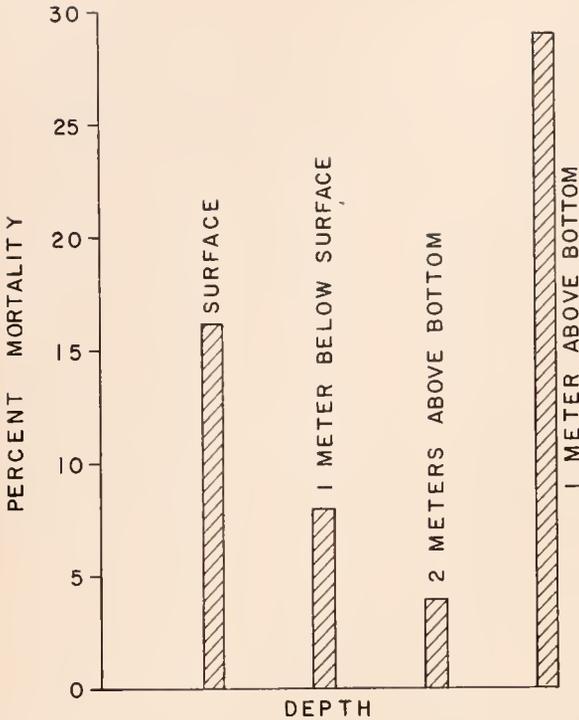


FIG. 3. Total percent mortality of scallops held at the four experimental depths.

surface, 8.0% at 1 m below the surface and 4.0% at 2 m above the bottom. Mortality at 1 m above the bottom was 29.0% (Fig. 3).

#### Density Experiment: Growth and Mortality

This experiment ran from 12 May, 1971 - 20 September, 1971. Initial densities of 100, 75, 50 and 25/ft<sup>2</sup> were tested.

Figure 4 indicates approximately equal growth at all densities until the scallops reached 27.0 - 28.0 mm. Above this size growth decreased with increased density.

Figure 5 indicates low mortality at all densities during the first two months and increased mortality during the last two months with higher densities having the greater mortalities. Mortalities began to increase when the scallops were about 37.0, 39.0, 43.5 and 46.2 mm at densities of 100, 75, 50 and 25/ft<sup>2</sup> respectively.

Total mortality at the end of the experiment averaged 35.0, 16.0, 6.2 and 3.2% at densities of 100, 75, 50 and 25/ft<sup>2</sup> respectively (Table I).

#### DISCUSSION

Although scallops grew and survived best at a

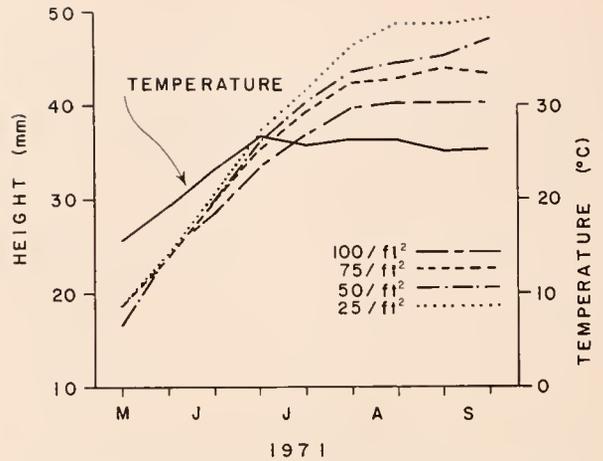


FIG. 4. Growth data for scallops held at the four experimental densities (100, 75, 50 and 25/ft<sup>2</sup>).

density of 25/ft<sup>2</sup>, the data suggests that densities as high as 60-65/ft<sup>2</sup> could be used (Table I). Control of factors mentioned below would probably allow scallops to grow and survive equally well throughout the water column.

Those factors which affected growth and survival in both experiments were: (1) heavy fouling of the screen meshes with hydroids, mud and/or algae resulting in poor water circulation; and (2) mechanical disturbance of enclosures due to boat wakes, wave action and/or tidal currents. The effect of these factors seemed to depend on the location of the enclosures in the water column, the density at which the scallops were held, the size of the scallops and stability of the enclosure.

In the density experiment fouling and mechani-

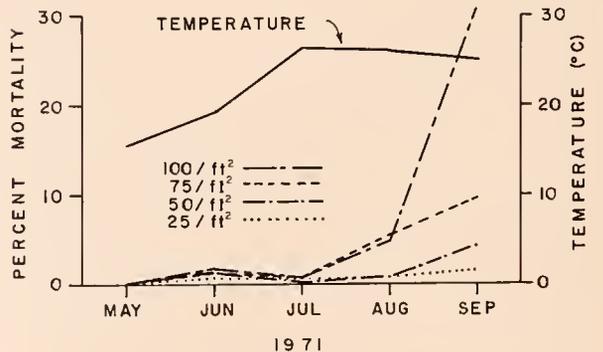


FIG. 5. Average monthly mortality of scallops held at the four experimental densities (100, 75, 50 and 25/ft<sup>2</sup>).

TABLE 1. *Initial densities, total percent mortality and final densities of scallops in density experiment.*

Initial Densities scallops/ft <sup>2</sup>	Total Percent Mortality	Final Densities scallops/ft <sup>2</sup>
100	35.0	65
75	16.0	63
50	6.2	47
25	3.2	24

cal disturbance were common to all enclosures. Decreased circulation due to fouling probably resulted in a greater competition for food which became more intense as the scallops increased in size. This was particularly true at the higher densities indicated by the decreased growth rate and increased mortality. Mechanical disturbance of the enclosures probably disturbed the scallops feeding behavior. Occasionally scallops were washed to one end of the enclosure causing some smothering of the scallops. Again this was more intense at the higher densities.

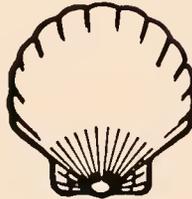
In the depth experiment the high mortality of scallops at one meter off the bottom is believed due to heavier accumulations of silt or mud in these enclosures than in the enclosures just above them. Scallops were frequently found buried.

Fouling was common to all the enclosures in this experiment and undoubtedly had an adverse affect on growth and survival.

The decrease in mortality from the surface to two meters off the bottom (Fig. 3) is believed due to the decreased effects of wave action and other surface turbulences with increased depth. The relatively stationary position in which the enclosures at two meters off the bottom were held helped reduce disturbances at this depth and probably accounts for the slightly higher mean size and percent survival attained by the scallops held here. The effects of the mechanical disturbance of the enclosures in this experiment were similar to those described for the density experiment.

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## LARVAL CULTURE OF THE CALICO SCALLOP, *ARGOPECTEN GIBBUS*<sup>1, 2</sup>

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### ABSTRACT

Mature calico scallops, *Argopecten gibbus*, collected from the grounds off Cape Kennedy, Florida, were induced to spawn in the laboratory. Fertilized eggs were reared to postlarvae in sea water of  $23^{\circ} C \pm 2.0^{\circ} C$  at a salinity of 35 ‰. The external morphology of eggs and developing larval stages are described.

### INTRODUCTION

The calico scallop, *Argopecten gibbus* (Linné), (Fig. 1)<sup>3</sup> is a commercially valuable shellfish which supports a developing fishery off the southeastern coast of the United States and in the Gulf of Mexico. Large concentrations of this benthic marine pelecypod occur on the continental shelf in the area of Cape Kennedy, Florida, in depths from 9-74 m (Drummond, 1969). Concentrations also occur south of Cape Hatteras off North Carolina in depths from ca. 13 m (Bullis and Thompson, 1965) to at least 94 m (Cummins, Rivers and Struhsaker, 1962). The general distribution of this organism is given by Allen and Costello (1972).

The National Marine Fisheries Service (NMFS)

initiated a life history study of calico scallops in 1969. A portion of the study was concerned with the early life history of this mollusk. The purposes of this paper are: (1) to present illustrations of the gross morphology and time sequence of larval development so these stages may be readily identified in plankton samples, and (2) to make available procedures for the mass culturing of this species.

Previous works on larval development of mollusks of the genus *Argopecten*<sup>4</sup> are by Belding (1910), Gutsell (1930) and Sastry (1965). These papers deal with a closely related species, the bay scallop, *Argopecten irradians*.

### MATERIALS AND METHODS

Techniques to induce spawning and rear molluscan larvae suggested by Loosanoff and Davis (1963) were modified at the Virginia Institute of Marine Science (VIMS) in rearing calico scallop larvae. Mature calico scallops (shell width 55 - 65 mm) were collected by otter trawl from the grounds off Cape Kennedy, Florida. They were transported to the NMFS Laboratory in Miami, Florida, in insulated containers of aerated sea water maintained at 20 - 23° C. At the laboratory, scallops were held on water tables and/or troughs of running sea water. Subsequently, a portion

<sup>1</sup>Contribution No. 225, Southeast Fisheries Center, National Marine Fisheries Service, NOAA, Miami, FL 33149.

<sup>2</sup>Contribution No. 478, Virginia Institute of Marine Science, Gloucester Point, VA 23062.

<sup>3</sup>Two terms are used in this paper to define shell dimensions. They are: (1) Length (L), a straight line measurement of the greatest distance between the anterior and the posterior shell margins; (2) Width (W), a straight line measurement of the greatest distance between the umbo and the ventral shell margin. Several authors use the term "height" for the dimension we define as width.

<sup>4</sup>Waller (1969) rejected the generic name *Aequipecten* and suggested *Argopecten*, the name currently in use.



FIG. 1. The right valve of a mature calico scallop, *Argopecten gibbus* - shell width 56 mm.

of these mature scallops was air-shipped to VIMS at Gloucester Point, Virginia, where spawning and larval rearing to setting were accomplished.

All culture techniques and most of the morphology were described from specimens, photomicrographs and information obtained from induced spawning and larval rearing at VIMS.

#### Induction of Spawning

Ovarian color is a reliable index of sexual maturity in calico scallops (Miller, Hudson, Allen and Costello, 1972).<sup>5</sup> Before we attempted to induce spawning, scallops were selected that showed orange-red ("ripe") ovarian color. The ovarian color was easily observed as the scallops gaped in the troughs of running sea water. Preliminary observations indicated that induced spawning in ripe calico scallops is easily achieved. We induced spawning several times in less than one hour by raising the water temperature from ca. 20 - 25° C. To trigger spawning, in addition to raising the water temperature, it was occasionally necessary to strip gametes from one mature calico scallop specimen and, with a pipette, introduce them gently into the water containing gaping scallops.

Calico scallops are hermaphrodites. Sperm cells are

usually extruded first when spawning is induced in the laboratory. After sperm cells have been discharged for 30 min to an hour, discharge of eggs begins. Once spawning begins, it may continue for several hours.

When techniques to induce spawning were established, 10 ripe scallops were selected. Their shells were carefully scrubbed to remove a variety of encrusting invertebrates which are frequently affixed to the outer shell (Wells, Wells and Gray, 1964). If these fouling organisms, e.g., the serpulid polychaete, *Pomatoceros caeruleus*, are not removed, they may spawn when spawning is induced in the scallops and contaminate the larval culture.

After cleaning, the scallops were placed, one to a dish, in 3"x5"x9" Pyrex glass containers, each  $\frac{3}{4}$  filled with filtered 20° C sea water at a salinity of 32.1 ‰. The containers were then placed on a water table. A black cloth was placed between the glass containers and the table top to aid in observing when spawn was first extruded. Temperatures in the dishes containing scallops were raised from 20 - 25° C by flowing warm tap water around them. In two of the dishes, sperm cells stripped from another mature calico scallop were introduced with a pipette. The scallops in these two dishes began to spawn 78 min after the water temperature reached 25° C. Six additional scallops spawned at various intervals in the next hour.

When the water in each dish became clouded (opaque) with suspended sperm, the scallop was removed and placed in a clean dish of 25° C filtered sea water. This procedure was continued until the scallop began to discharge only eggs. The scallop was then placed in a clean dish of 25° C filtered sea water where it was kept until spawning was completed. Dishes containing mixed sperm and eggs were discarded.

Since the eight scallops induced to spawn began extruding sperm and then eggs at various times over ca. a 2-hr period, we had available, simultaneously, dishes containing freshly spawned, unmixed suspensions of sperm cells, and freshly spawned, unmixed suspensions of eggs. A light suspension of sperm (35 cc) was added to each of the dishes containing eggs, and the mixtures were gently agitated. Following fertilization, the eggs were washed in a stainless steel screen (152  $\mu$  openings) to remove debris that accompanies spawning. We followed the washing procedure described by Loosanoff and Davis (1963).

After the fertilized eggs were washed, they were added to a container of filtered sea water and the number of eggs per unit of sea water was determined with a Sedgwick-Rafter cell. A sufficient quantity of

<sup>5</sup> Miller, G. C., J. H. Hudson, D. M. Allen, and T. J. Costello. 1972. Ovarian color changes in calico scallops, *Argopecten gibbus*. Unpublished manuscript filed at the National Marine Fisheries Service, Southeast Fisheries Center, Miami Laboratory, Miami, Fla.

the washed egg suspension was added to a 20-liter container<sup>6</sup> of filtered sea water to provide 25 eggs/ml. This concentration was reduced to ca. 10 larvae/ml at the straight-hinge stage.

Temperature in the culture was maintained at  $23^{\circ}\text{C} \pm 2.0^{\circ}\text{C}$  throughout larval development. To simulate conditions in the calico scallop's natural offshore spawning area, salinity was adjusted to 35 ‰ immediately after fertilization and held at this concentration. The culture was not aerated, and no illumination was provided. Water was changed every other day by straining the entire 20 liters through a stainless steel screen. A screen with mesh openings of  $50\mu$  was used initially; larger openings were used as the larvae increased in size. Larvae retained on the screens were returned to clean 20-liter containers of filtered sea water. Following the first two water changes, 0.2 cc of "twin biotic" (a mixture of streptomycin and penicillin) was added per liter of culture to retard bacterial growth. Feeding of the larvae was initiated 30 hr after fertilization. Unialgal cultures of *Monochrysis lutheri* were fed in quantities sufficient to provide, initially, concentrations of ca. 60,000 cells/ml. As the larvae grew, adjustments to concentrations of food were made to quantities where observations showed complete utilization.

#### EMBRYONIC DEVELOPMENT

Embryonic development of *A. gibbus* is similar to that described by Sastry (1965) for *A. irradians*. A detailed study of early cleavage was not made; therefore, the times that are reported for early embryonic development are approximations based on the most

<sup>6</sup> Plastic garbage can

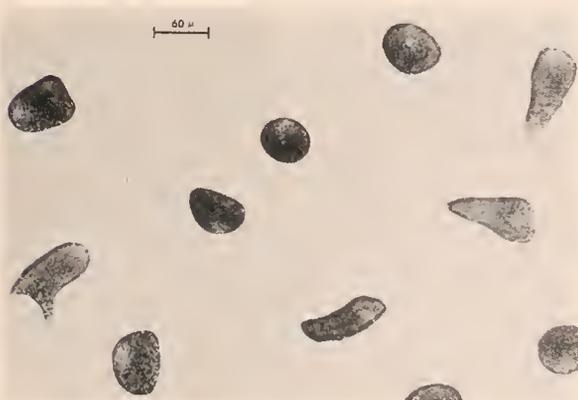


FIG. 2. *Argopecten gibbus* eggs ca. 35 min after spawning. Note irregular shape of most eggs.

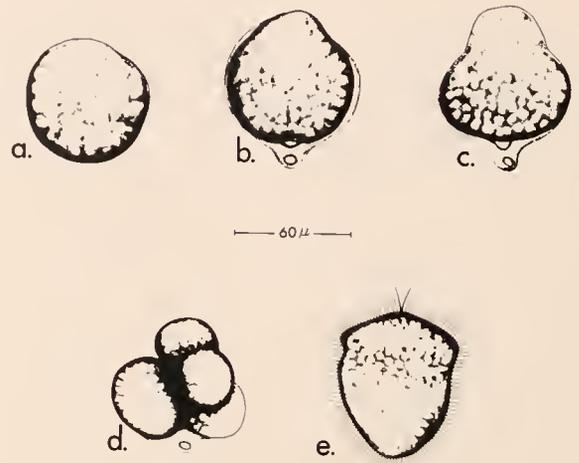


FIG. 3. Embryonic development of *Argopecten gibbus*: a) unfertilized eggs; b & c) zygotes 40-60 min after fertilization showing polar bodies; d) cell division ca. 100 min after fertilization; e) a ciliated trochophore 24 hr after fertilization.

typical stage represented in the culture samples observed. Developing zygotes from a single spawning showed considerable disparity in rates of development during the first 24 - 36 hr. Newly spawned eggs of *A. gibbus* were asymmetrical (Fig. 2), though observations of *A. irradians* eggs observed after spawning also appeared similarly asymmetrical.

Unfertilized eggs, measured with an ocular micrometer, averaged  $60\mu$  in diameter (Fig. 3a). Approximately 40 min after fertilization, two polar bodies formed as the zygote gradually modified to form a polar lobe (Figs. 3b and 3c). In most cultures discernible cleavage began 70 min after fertilization. As in the embryonic development of many other molluscs, unequal blastomeres were noted in all early cleavages, and micromeres proceeded with more rapid division than macromeres during the first 8 hr of development. Figure 3d depicts typical cell division 100 min after fertilization. Active ciliated trochophores were observed 24 hr after fertilization (Fig. 3e).

Shell secretion began during the early trochophore stage. The shell gradually enveloped the body and an active straight-hinge veliger was formed before the larvae were 48 hr old.

#### Larval Culture

Under our laboratory conditions, the larval period of the calico scallop was 16 days. Figure 4 is a composite made from photomicrographs taken every 24 hr. The larvae, items B through J in Figure 4, repre-

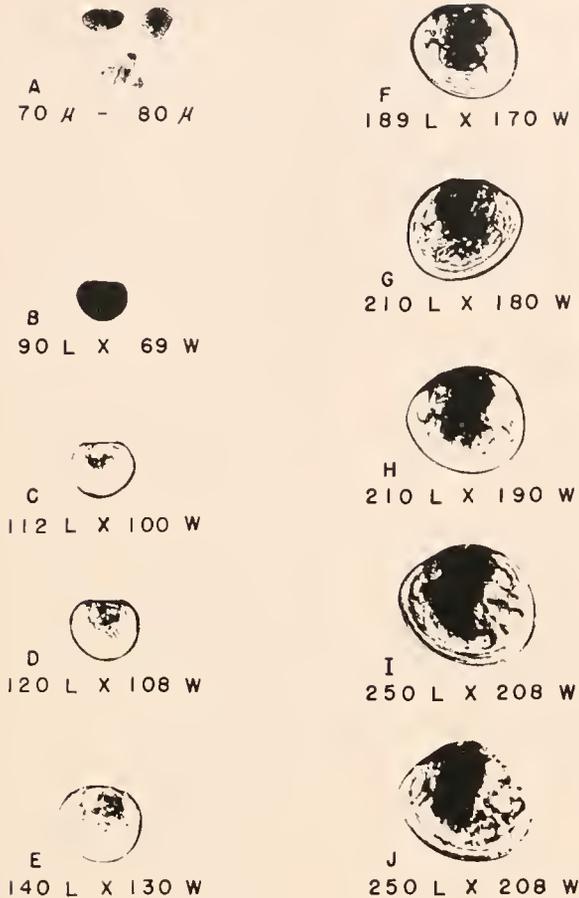


FIG. 4. Composite photomicrograph of larval *Argopecten gibbus*. Age in days: a) 1; b) 2; c) 4; d) 5; e) 7; f) 9; g) 11; h) 13; i) 15; j) 16. Length x width dimensions are given in microns.

sent the average sizes for each time stage obtained by measurement of 25 larvae from several photomicrographs of each 24-hr period. The early straight-hinge larvae appeared to be chopped off at one point along the hinge line. The umbo appeared at about 140 μ, rounded and poorly defined. It remained inconspicuous throughout larval development. Figures 5 and 6 show typical morphological features in the latter stages of larval development and just prior to setting. Chanley and Andrews (1971) made effective use of hinge line shapes in describing 23 species of bivalve larvae. The hinge line shape of the calico scallop larvae (Fig. 7) is distinctive but very similar to *A. irradians*. The toothed area is comprised of three taxodont teeth at each end of the hinge line. The central hinge area is undifferentiated. Other identifying characteristics of calico scallop larvae are their

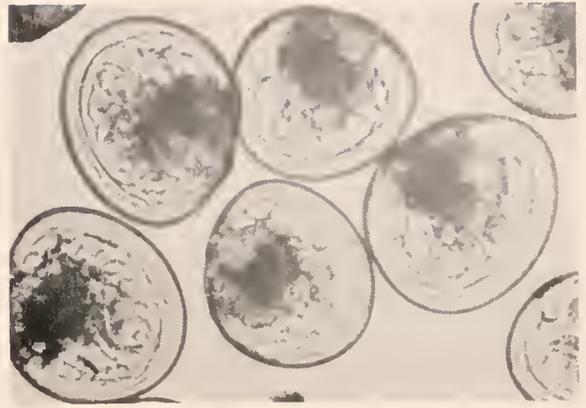


FIG. 5. Photomicrograph of live 12-day-old larvae of *Argopecten gibbus* length 210 μ.

pale color and development of an inconspicuous eye spot when the larvae reach a length of ca. 250 μ.

SUMMARY

Calico scallops, *A. gibbus*, have been induced to spawn in the laboratory and the larvae have been reared to setting. Development, on the basis of external morphology, is quite similar to that recorded for a closely related form, *A. irradians* (Sastry, 1965).

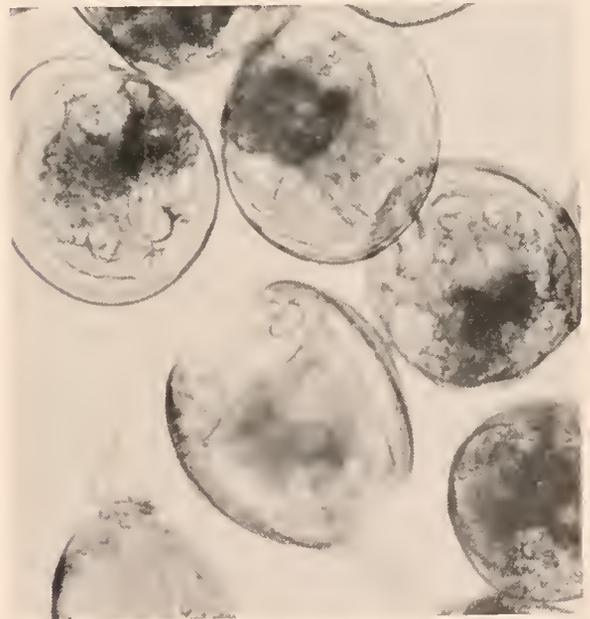


FIG. 6. Photomicrograph of live 16-day-old larvae with foot extended, showing anatomical relationship and early structure of gill and foot.



FIG. 7. Dorsal view of hinge of the larval *Argopecten gibbus*.

The major difference is that *A. gibbus* has a much larger pediveliger or newly set larvae which ranges in length from 235-270  $\mu$ . The difference is significant when compared to the bay scallop, *A. irradians*, which sets at a length of from 170-190  $\mu$ .

#### ACKNOWLEDGMENTS

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REPRODUCTIVE BIOLOGY OF YOUNG ADULT KING CRABS  
*PARALITHODES CAMTSCHATICA* (TILESIIUS) AT KODIAK, ALASKA

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ABSTRACT

*King crab mating was studied in the natural environment while simultaneously conducting mating experiments in undersea pens in an adjacent location. Pubescent females, 86-119 mm in carapace length, began mating 14 February, 1971, a month earlier than adults. The majority of females were mature at a length of 111 mm. The smallest adult was 96 mm. In nature pubescent females averaging 99 mm in carapace length mated with males averaging 142 mm in length. The smaller and more abundant males (90-109 mm) molted at the same time pubescent females were molting, and mated with experimental females when placed in undersea pens. Males mated with females larger than themselves but appeared to be incapable of mating during the 10-day interval bracketing the male molt.*

*Average growth of pubescent females is similar to that of juveniles and 3 mm more than that of small adult females. Males were found to attain sexual maturity at a smaller size than females.*

INTRODUCTION

King crab harvest in the Kodiak Island area increased slowly through 1958 when 5 million pounds were landed. Annual production increased rapidly after 1958, peaking in 1966 at 91 million pounds (Powell and Gray, 1969)<sup>1</sup>. Since 1966 average catch per effort has declined steadily and in 1971 landings were down to 12 million pounds. This was the lowest in the last 12 years.

Kodiak's fishing grounds have yielded 438 million pounds of male king crabs since 1950. Female king crabs have always been protected by regulation and the intense fishing pressure on males greater than 7 in. in shell width (6½ in. prior to 1963) has caused marked changes in the composition of the brood stocks. Tagging studies in the

upper Gulf of Alaska have shown king crabs to have a longevity of about 14 years and attain legal size in 7 or 8 years (Powell, 1967). Male crabs are, therefore, susceptible to harvest for as many as 8 years. As early as 1960, annual fishing mortality was a minimum of 33% in areas of fleet concentration (Powell, 1964) and average size and proportion of aneuviant males in the stocks declined as a result of fishing (Nickerson, Ossiander and Powell, 1966).

Trawl fishing studies during the 1962 mating season, when sexes are congregated, revealed four times as many females as males (Gray and Powell, 1966). During 1967 trawling, 11 times more females were captured than males (McMullen, 1967).

Scuba surveys of natural mating areas during 1963 and 1964 revealed that all males grasping females were larger than 119 mm and were just one molt away from commercial size (Powell and Nickerson, 1965). Further, that the male mates with the female within hours after she molts. In 1970, mating studies illustrated that recently molted males, just under legal size, could mate as many as 13 successive times but that mating

<sup>1</sup> Powell, G. C. and P. L. Gray, 1969. A study of the king crab fishery (*Paralithodes camtschatica*, Tilesius) within Kodiak Island Management Unit, Alaska, with emphasis upon recent catch statistics, 1960-1968. Typewritten manuscript, 140 pp.

ability decreased after the sixth mating (Powell, James, and Hurd, 1972)<sup>2</sup>.

The present investigation was initiated to study the adequacy of the current 7-inch size limit in providing adequate protection to male king crab brood stocks (legal carapace width of 178 mm converts to a carapace length of 145 mm)<sup>3</sup>. Males were believed to attain sexual maturity at the same or at a larger size than females because growth of the latter decreases markedly at sexual maturity. Quantitative data for sizes of females at maturity were lacking but it had been learned that adult females were as small as 96 mm and that many females attained adulthood at the length of 108 mm (Powell, 1958). Determining proportions of various size king crabs which are sexually mature became vital to insure proper management of the resource. The primary objective of the 1971 mating study, therefore, was to obtain qualitative data regarding the reproductive ability of small male and female king crabs in the size range 90-109 mm.

#### METHODS

Pubescent<sup>4</sup> crabs were located in Middle Bay (Fig. 1) by fishing with pots and then subsequently monitored for several months until mating was completed. Simultaneously, specimens were obtained for examination and for controlled studies in undersea pens. The controlled study allowed determinations of mating ability of individual small males in the absence of competition from larger males.

##### *Exploratory pot fishing*

Continuous fishing for 4 months with 18 pots enabled us to locate and study a school of small crabs before, during and after the molting and

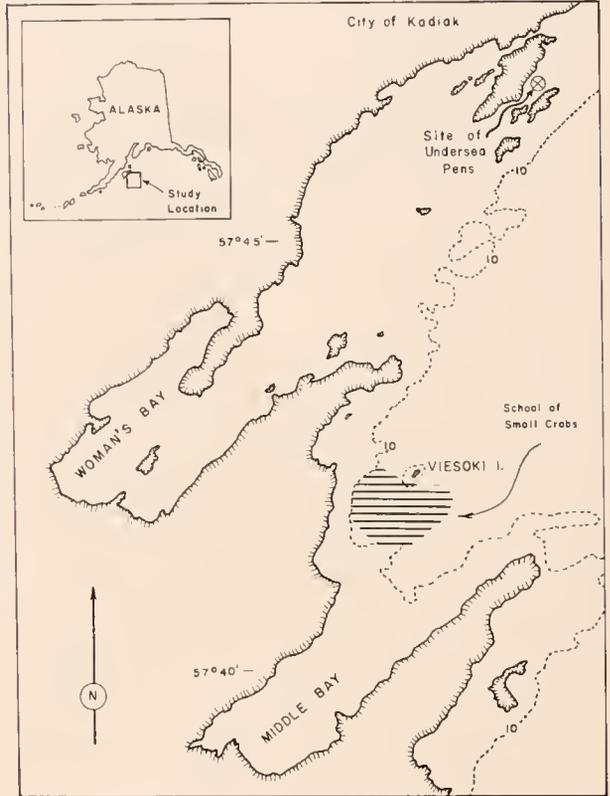


FIG. 1. Location of crab school in Middle Bay and site of undersea pens.

mating season. Scuba diving was employed to supplement pot fishing and to capture grasping pairs from natural mating areas. Scuba diving also confirmed the continued presence of the crabs during the molt when they could not be captured by pot fishing.

Seven different vessels ranging in length from 30 - 90 ft were used at various times throughout the study. Two pot sizes were used: 6-foot square by 3-foot high, and 4-foot square by 2-foot high. Stretched mesh was 2¾ in. on all pots to insure retention of small crabs. Pots were lifted at our convenience and as weather permitted.

A random sample of the catch from each pot was measured and studied to determine composition by size, sex, shell-age and ovigerousness. Partial clutches of eggs, matted abdominal setae and stage of molt were also recorded.

Crabs of the size and condition needed for the controlled study and for dissections were kept alive and brought back to Kodiak for these purposes. Crabs were handled carefully to avoid injury.

<sup>2</sup>Powell, G.C., K. E. James and C. L. Hurd, 1972. Ability of male king crabs (*Paralithodes camtschatica*, Tilesius) to mate repeatedly, Kodiak, Alaska, 1973. In preparation, Fish. Bull.

<sup>3</sup>Carapace length is used exclusively throughout the manuscript because it is the standard measurement used by researchers (see page 13 of Powell, 1967).

<sup>4</sup>For convenience in writing, the authors are using the term pubescent to refer not only to the female about to mate for the first time but also to that same female soon after molting and mating. In this way we can separate pubescent crabs that have just become adults from those that have been adults previously.

### *Dissections*

A separate group of small male and female crabs were dissected every 5 or 6 days so that the newly developing exoskeletons could be examined to predict the advent of the molting season. Abdominal cavities of various sized juvenile females were opened and oviducts examined to determine the presence or absence of internal eggs. A length frequency distribution was prepared showing the proportions of the various size females which were pubescent. Microscopic examination of reproductive tracts of seven males was also undertaken. Knowledge obtained from dissections was used to help determine size of experimental crabs for the controlled study, and beginning date for the study.

### *Controlled study*

Experimental crabs were housed in four undersea pens which were each subdivided into four separate compartments (total of 16), each a 4-foot cube, 64 cubic feet in size. Undersea pens were made of steel bars welded together and covered with small mesh web. Pens were bottomless enabling crabs to dig into the substrate as they would do in nature thus creating as near natural conditions as possible. Since pens could not be lifted to the surface, they were tended daily throughout the study by divers. Activities of experimental crabs were recorded underwater on bakelite slates. Pens were placed in 35 feet of water at Near Island Basin. This area is adjacent to the natural mating grounds and is one-half mile east of the City of Kodiak (Fig. 1). Several Dungeness pots situated alongside the pens served as temporary crab storage facilities.

New-shell males, 90-109 mm, were used in the controlled study (old-shell males this size are rare). One male and several females were placed into each of the 16 compartments. As soon as one of the females molted and mated (ovulation occurred) both she and her partner were removed from the compartment and placed in separate storage. A new male and female were added to the compartment shortly thereafter. As many individual matings as possible were arranged during the early mating season before mating of small females ended. Mated females were kept in storage from 8 - 14 days, depending upon water temperatures, until eggs had time to develop to at least the 8-blastomere stage. After eggs had adequate time to cleave, several hundred were collected from numerous locations among the egg mass and preserved in Bouin's solution. A sample of 100 eggs was taken from the Bouin's solution and examined microscopically for final determinations as to

whether or not mating had been successful.

In cases where ovulation did not occur during the first 4 days after female molting, the male was considered to have had adequate opportunity to mate, and was removed as a failure. Additional males were introduced and if ovulation still did not occur the female was dissected to determine if she was pubescent or still juvenile. If internal eggs were absent; i.e. the female was juvenile, males were given another opportunity to mate and were not considered unsuccessful in their initial attempt.

An interval of 8 or more days after molting was adequate for shell hardening. Both males and females were measured after an interval of this magnitude to determine growth increment and were subsequently released as soon as mating had been proven successful.

Bottom water temperatures were collected at the pen site using Ryan thermographs (Model F, fast response, waterproof). Divers also recorded temperatures with hand-held mercury thermometers.

### *Experimental animals*

Experimental animals used in the undersea pen study were all tagged with permanent loop tags so that individual crabs could be readily identified. Details on tagging procedure are presented by Gray (1965). Crabs were fed sea urchins, shrimp and fish every 5 or 6 days.

Graspee<sup>5</sup> females were preferred because of their impending molt and because males were attracted to them; however, some non-graspees, i.e.; females not ready to mate, were used successfully during intervals when graspees were scarce. Practically all of the experimental females were pubescent except for those molting and mating in late April. The majority of the graspees were captured from natural mating areas by scuba divers, three were captured in pots as were all of the non-graspees and males.

## RESULTS AND DISCUSSION

### *Mating Season of Pubescent Females and Location of Mating*

Exploratory pot fishing and scuba diving disclosed a large school of both juvenile and pubescent crabs in the vicinity of Viesoki Island, Middle Bay, 7 miles south of the city of Kodiak, Alaska

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<sup>5</sup>Graspee refers to a female crab being grasped by a male. Adult females become attractive to males prior to the molt and are grasped by a male and held until molting and copulation are completed.

TABLE 1. Size of mating crabs in the 35 grasping pairs which contained pubescent females, captured from natural mating areas, Kodiak Island, Alaska, 1971.

Grasping Pair No.	Carapace length (mm) <sup>a</sup>		Shell age of grasper males (months)	Size differences male length less female length (mm)	Grasping Pair No.	Carapace length (mm) <sup>a</sup>		Shell age of grasper males (months)	Size differences male length less female length (mm)
	Graspee	Grasper				Graspee	Grasper		
1	94	85	11	-9	19	104	145	11	41
2	95	119	11	24	20	94	145	11	51
3	98	123	10	25	21	104	146	22	42
4	106	123	11	17	22	104	147	24	43
5	103	124	8	21	23	91	147	23	56
6	103	129	11	26	24	94	148	11	54
7	101	135	11	34	25	102	148	11	46
8	90	136	11	46	26	113	148	11	35
9	106	137	11	31	27	97	149	12	52
10	97	138	11	41	28	107	150	11	43
11	88	138	11	50	29	100	152	11	52
12	104	139	11	35	30	106	154	12	48
13	98	140	22	42	31	102	155	12	53
14	95	140	11	45	32	97	155	12	58
15	88	143	12	55	33	99	159	12	60
16	95	143	11	48	34	108	160	12	52
17	96	145	11	49	35	111	177	24	66
18	89	145	11	56					
						3,479	4,967		

<sup>a</sup> Graspees are the females and graspers are the males.

(Fig. 1). Crabs were abundant in depths ranging from 7 - 16 fathoms and generally remained within this area throughout the study period, December - March.

The 65 pubescent females that were captured from this school and placed in undersea pens molted and mated during the period 14 February - 19 April the majority (85%) doing so prior to 21 March. Scuba divers swimming in the vicinity of Viesoki Island on 26, 27 February and 7 March observed thousands of shed exoskeletons lying on the bottom, confirming that molting of juvenile

and pubescent crabs was well underway. Grasping pairs were also more abundant at this time than on previous dives. Molting was further implicated by the disinterest in feeding evident by the sharp decline in average catch per pot lift (from 60/pot to 6) which occurred 22 February and continued through 7 March.

No pubescent graspees were captured after 13 March. Continued search effort disclosed adult females being grasped, indicating that pubescent females had mated first. Molting of adult females in undersea pens complemented observations in the

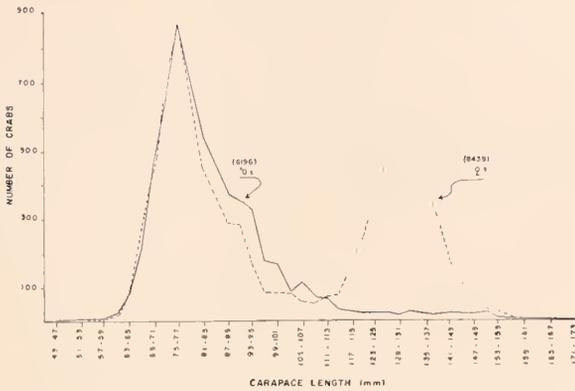


FIG. 2. Size distribution of 14,635 king crabs captured in Middle Bay, 17 December, 1970 - 31 March, 1971, Kodiak Island, Alaska.

natural environment. Adult females began molting and mating 23 April in the same general depth and locality as the pubescent females before them. *Size of Males Grasping Pubescent Females in Natural Mating Areas*

The majority (65%) of graspee females captured from natural mating areas were pubescent (35 of 54) primarily because we stopped searching for mating crabs shortly after adults began molting. Average length of males grasping pubescent females was 142 mm, 19 were legal size (54%). Average size of females was 99 mm (Table 1). Males averaged 42 mm larger than their female mates. The only male smaller than his partner was 85 mm, next smallest male was 119 mm. The majority of males (89%) ranged from 123-159 mm with the largest male 177 mm.

Exploratory fishing and diving revealed that males of the size which were grasping in the natural environment (123 mm and larger) were relatively scarce, and yet it was this scarce group of males that was mating rather than the smaller males (63-100 mm) which were abundant (Fig. 2).

A size relationship between mates opposite to that occurring naturally was created in the controlled undersea pen study because we wanted to test mating ability of small males. There, every male except one, was smaller than his female partner. Pubescent females averaged 14 mm larger than their mates but in spite of the difference, males mated successfully. Had the thousands of small males less than 100 mm participated in mating in nature, they would have been found grasping females to a greater extent than they were. The relative absence of crabs ranging in length from

102-116 mm is unexplained, but may represent the void between two age classes.

Between 1963 and 1971, 3,402 grasping pairs have been captured from natural mating areas. Only 6 males were smaller than 110 mm (Powell, Rothschild and Buss, 1972)<sup>6</sup>.

Small males may mate in the absence of large males or when a surplus of pubescent females exists. Since the majority of small newshell males, 90-109 mm, used in the undersea pen study mated successfully, the absence of this size from grasping pairs captured in natural mating areas suggests that they are not aggressive and do not compete with larger males.

During the last 6 days of fishing, 1,035 new-shell females were captured, 41 were ovigerous and 994 were non-ovigerous. Non-ovigerous females ranged from 59-106 mm while the ovigerous females ranged from 96-122 mm. The 994 non-ovigerous females were considered juveniles rather than unmated pubescents because most of them were too small to be adults; partial clutches and unfertilized eggs commonly found among unmated females were completely absent from those of adult size, and none of the ovaries examined contained developed eggs. A total of 1,673 males ranging in length from 47-186 mm were captured during the same period and in the same area indicating the presence of an adequate number of males to service all pubescent females. Unmated females commonly extrude and carry infertile eggs while females that mate with males that have mated repeatedly have partial clutches of eggs. Neither partial clutches nor infertile eggs were observed.

#### *Molt Increment of Females and Males*

Average growth increment of pubescent female crabs was greater than that of either small or large adult females. The average growth of 59 pubescent, 11 small adult, and 2 large adult females was 11, 7, and 4 mm respectively (Table 2). Average growth of 3 juvenile females was similar to that of pubescent females (11 mm). Another estimate of the growth of pubescent females was obtained by comparing the size difference between the smallest pubescent and smallest adult female (Fig. 3). Growth estimated in this way was 10 mm, the

<sup>6</sup> Powell, G. C., B. J. Rothschild, and J. Buss. 1972. A Study of King Crab (*Paralithodes camtschatica*, Tilesius) Brood Stocks, Kodiak Island, Alaska, 1963-1971. 30 pp. Typewritten manuscript.

TABLE 2. Growth increment per molt of experimental female and male king crabs used in undersea pen study, 1971. <sup>a</sup>

Carapace length (mm)	No. of pubescent females	Increment per molt		No. of adult females	Increment per molt		No. of males	Increment per molt	
		range average			range average			range average	
72-77	-	-	-	-	-	-	1	12	12
78-83	-	-	-	-	-	-	1	14	14
84-89	-	-	-	-	-	-	9	10-16	13
90-95	-	-	-	-	-	-	4	10-17	14
96-101	7	8-12	11	1	8	8	5	13-18	15
102-107	13	5-12	9	6	4-9	7	9	12-16	14
108-113	24	6-14	10	4	4-8	7	-	-	-
114-119	14	6-14	10	-	-	-	-	-	-
120-125	1	9	9	-	-	-	-	-	-
126-131	-	-	-	-	-	-	-	-	-
132-137	-	-	-	1	4	4	-	-	-
138-143	-	-	-	1	4	4	-	-	-
	59			13			29		

<sup>a</sup>Three juvenile females 96, 108 and 109 increased 7, 14 and 11 mm respectively.

same as the average for those which actually molted. Average increment for males of comparable size was greater than that for either pubescent or adult females. The 29 males which molted in undersea pens averaged 14 mm. This data compares favorably with and complements that presented by Powell (1967).

#### Size at Which Females Attain Sexual Maturity

##### Small females without externally developing

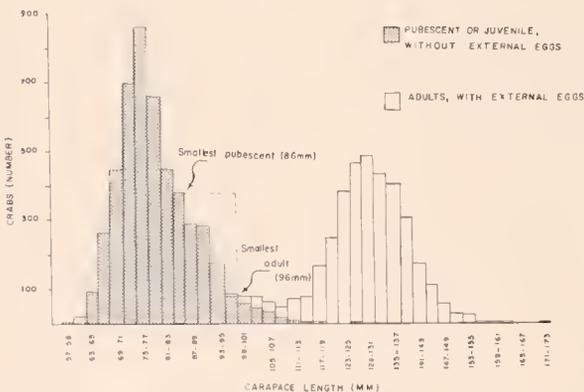


FIG. 3. Size distribution of juvenile and adult females in a sample of 8,439 king crabs, 17 December, 1970 - 31 March, 1971, Middle Bay, Kodiak Island, Alaska.

eggs possess silky incubatory setae and are either juvenile or pubescent<sup>7</sup>. Dissection is necessary to make the determination; oviducts full of eggs reveal that females are pubescent.

During exploratory fishing in Middle Bay, 8,439 female king crabs were captured, measured and examined for presence or absence of external eggs. Juvenile and pubescent females combined totaled 4,856, adults numbered 3,583. The smallest adult female captured was 96 mm in carapace length.

Dissections of 180 pre-molt females including juveniles, pubescents and adults revealed that all females less than 86 mm were juvenile, i.e., had empty oviducts; and that all pubescent and adult females had ripe eggs in their oviducts (Fig. 4).

Absence of both external and internal eggs indicates females are juvenile and will remain as such for at least another year, being incapable of ovulating in the ensuing mating season. Figure 4 shows that pubescent females ranged from 84-119 mm but that at the size 111-113 mm the majority were adults. It is interesting to note that the majority of females 90-92 mm were juveniles and, that the majority of females 108-110 mm were

<sup>7</sup>An exception to this statement could exist if a small adult female were not mated. Unmated small females are uncommon.

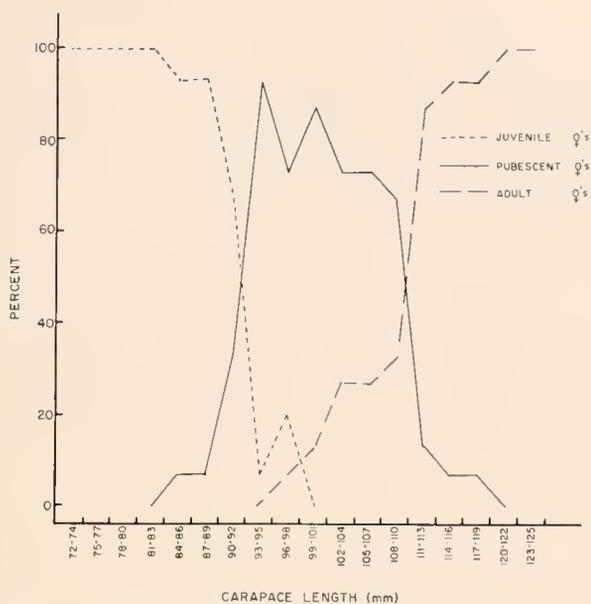


FIG. 4. Size at sexual maturity of female king crabs, December 1970, Middle Bay, Kodiak Island, Alaska (10 crabs in each size group).

pubescent and would become 118-120 mm through molting before having their first opportunity to mate.

#### Size at Which Males Attain Sexual Maturity

At present, no easy method to determine maturity of male crabs exists. An attempt to determine maturity by microscopic observation of reproductive tracts from 49 males, 71-160 mm carapace length, revealed no apparent difference except size of the tract. All tracts were similar in color and convolution and all contained spermatophores. Non-motile spermatozoa similar to those illustrated by Marukawa (1933) were found within spermatophores of two crabs checked, 79 and 132 mm in carapace length. Smaller crabs probably should have been included in the examination to insure the inclusion of juveniles. More research of male reproductive tracts is needed.

Placing males in pens with ripe females proved to be a direct, practical and dependable approach for determining sexual maturity of male crabs.

Experimental new-shell males within the 100-109 mm size class were the first group to be placed with females in the undersea pens. Each of the 18 males within this class mated successfully. Egg clutches on all females were large and no attempt was made to differentiate between their relative sizes. Four failed to mate their first opportunity, but their failure may be attributed to

the fact that their females had molted 10-17 days earlier. Many females attempting to mate 10 days after molting are unsuccessful (McMullen, 1969; Kurata, 1961)<sup>8</sup>. When introduced to females which had just molted, males mated quickly at the second opportunity.

Each of the males in the 100-109 mm size class except one was smaller than his female mate. Males averaged 11 mm smaller, two males successfully mated females 38 and 40 mm larger than themselves (Table 3). Males which failed to mate for reasons other than their own inability, such as a male with a juvenile female, are omitted from the comparison since no useful purpose would be served by including them. One such male is believed to have failed because of his impending molt.

Witnessing the success of the 100-109 mm males, the smaller second group, 90-99 mm, was subsequently incorporated into the experiment. Of the 18 within this class, 16 mated successfully and two failed. It is believed that the two were immature. One of the 16 failed on his first opportunity but succeeded when given another try (Table 3). Each of the 16 males was smaller than their mates, averaging 14 mm less. One male mated with a female 26 mm larger than himself. All egg clutches were large and no attempt was made to differentiate between their relative sizes. The proportion of males mating successfully in this group was .875 with a standard error of .08.

In supplemental experiments, 6 new-shell males from 84-89 mm in size were tested for mating ability. Three were successful and three failed. Two possibly were immature, and the third is believed to have failed because of an impending molt. The two males that were considered juveniles were 20 and 23 mm smaller than their female partners. The authors would like to emphasize that the experimental males were necessarily smaller than the females because adult females have never been found smaller than 96 mm and small adult females are scarce.

In other supplemental experiments, 10 mature males larger than 109 mm had opportunities to mate. The only ones which failed were those preparing to molt or completing their molt.

Males attain sexual maturity a year or two before females and at a smaller size. These smaller

<sup>8</sup>Kurata, H. 1961. King crab investigations in the eastern Bering Sea in 1961. International North Pacific Fish. Comm. Prelim. Transl. Doc. 481. 6 p.

TABLE 3. *Size relationships of mating partners within the 38 mating pairs tested experimentally.*<sup>a</sup>

File	Size of new-shell males within the two experimental groups (carapace length (mm))							
	90-99				100-109 <sup>b</sup>			
	Male tag #	Carapace length (mm)		Size difference (mm)	Male tag #	Carapace length (mm)		Size difference (mm)
	Male	Female <sup>c</sup>			Male	Female <sup>c</sup>		
1	849	90	116	26	851	100	109	9
2	841	92	109	17	830	100	112	12
3	829	92	105	13	863	100	108	8
4	824	93	115	22	871 <sup>d</sup>	101	141	40
5	842	93	101	8	871	101	112	11
6	826	95	108	13	-	-	-	-
7	855	95	109	14	860	101	114	13
8	856	95	113	18	845	101	118	17
9	850 <sup>d</sup>	95	113	18	866	103	118	15
10	840	96	113	17	866	103	107	4
11	854	97	112	15	867 <sup>d</sup>	103	116	13
12	857	98	112	14	766	104	104	0
13	665	98	109	11	862 <sup>d</sup>	104	142	38
14	770	99	104	5	864	105	110	5
15	847	99	108	9	827	106	116	10
16	846	100	108	8	859	106	114	8
17	825 <sup>e</sup>	92	111	19	869	106	111	5
18	839 <sup>e</sup>	99	111	12	731	107	104	-3
19	-	-	-	-	870	108	112	4
20	-	-	-	-	861	108	112	4
21	-	-	-	-	865 <sup>d</sup>	109	115	6

<sup>a</sup>Pairs in which the male failed to mate for reasons other than immaturity are omitted.

<sup>b</sup>Males 871 and 866 mated 2 females within a 3-day period of time.

<sup>c</sup>Sizes given are those for new-shell females after molting.

<sup>d</sup>Indicates those males which failed to mate at their first opportunity; they are listed with the female with which they mated.

<sup>e</sup>Indicates those males which failed to mate.

males have growth rates equal to or greater than the pubescent females. The average size difference between partners was equal to one year's growth.

The size difference between a 90 mm experimental male, such as those which mated in the undersea pens, and a male just smaller than legal size (144) is 55 mm. The smaller 90 mm male would have to molt four times to attain commercial size, taking 4 years, illustrating that some males may have five opportunities to mate before becoming available to the commercial fishery; the last opportunity to mate occurs just before the fishing season opens but after the crab has molted

to legal size. Caution is recommended, however, because 90-109 mm males which mated under controlled experimental conditions seldom are found mating in nature, suggesting that behavior in undersea pens may be limited in its applicability to conditions existing in nature.

#### *Grasping as an Indicator of Mating Ability*

Grasping activity of small males (100-109 mm) used in the 1971 pen study was compared with that of 24 large males (138-193 mm) used in a 1970 pen study. Only males with graspee females are included in the comparison.

Small males were observed by divers to be grasping their female partners 56% of the time as compared to 80% for large males. Small males grasped their female partner only 34% of the time when total time together is used in the calculation rather than just the last 6 days prior to copulation. Regardless of how the 2 groups were compared, smaller males consistently grasped less than larger ones. No relationship between grasping and the time of day that observations were made was apparent. No diving was conducted after dark.

All males in the comparison mated successfully. Less grasping or lack of aggression might lead to reduced mating in nature by small males not only because they would often be coexisting with large males, but also because interruptions in grasping activity could allow females to escape. In the undersea pens, females were unable to leave the proximity of the males even if not grasped.

Data suggest that only adult males grasp females, but data also reveal that small adult males do not grasp to the extent that large males do, and therefore lack of grasping cannot be interpreted as a sign of immaturity.

Of the 7 males which failed to mate, (those marked d & e in Table 3), 56% (4 crabs) were never seen grasping during a combined observation total of 23 days. The three unsuccessful males in supplemental studies were seen grasping 10 of 21 days observed or 48% of the time. The relative amount of time each male was seen grasping was similar to that of the successful males.

Males have not been known to grasp juvenile females. In the present pen study the only three females that were not grasped were found upon dissection to be juveniles. These three females were with their male partners for a combined total of 98 days, and were observed for a total of 70 days.

#### *Interval of Time for Copulation and Ovulation*

Copulation and the deposition of sperm on the female's gonopores can occur only after the female molts, and precedes ovulation. It was hypothesized that longer intervals of elapsed time for completion of copulation and ovulation might be indicative of reduced male mating ability, especially since females ovulate soon after sperm deposition has occurred. Total days elapsed time for completion of copulation and ovulation, recorded during the 1970 and 1971 mating studies, were compared.

Small males during 1971 copulated almost as quickly as the larger males which mated in 1970. In 1970, all 74 females had been mated within

two days after molting. In most of these matings, copulation and ovulation probably occurred within 24 hr after molting. In all 10 cases where females were examined within 24 hr after molting, eggs were present indicating that mating had occurred.

In 1971, 45 of 47 females were mated within two days after molting, only two required more than two days. Observations were made more frequently than during 1970 and several females examined 24 hr after molting still had not ovulated, however, colder temperatures during 1971 may have retarded ovulation.

The two males which required more than two days for mating were not typical individuals. One was the smallest male that mated during the study (85 mm length) and the other was the first new-shell male captured after the molt in Middle Bay.

Apparently, the 10 - day interval of time bracketing the male molt is a period during which the male is limited in his ability to mate. Some males may be limited for a greater period of time. Numerous males had opportunities to mate just before and after their molt but the closest to the molt that any male mated was five and six days respectively (Table 4). One of the males that mated seven days after molting actually molted the same day as his female partner and chose not to mate with her until the seventh day afterwards. Some of the males that failed to mate could have been juveniles but it is not likely that many were.

#### *Effects of Temperature on Mating*

Bottom water temperatures of Near Island Basin in 1971 were 3-6°F lower than during the same period in 1970. Temperatures in 1971 were approximately 35°F on 14 February when the first female molted and mating began. Temperatures gradually declined for the next month and were approximately 31.5°F on 10 March. Most of the pubescent females in the pens were molting and mating while water temperatures were declining. Small crabs in nearby Middle Bay were molting at the same time and probably under the similar temperature conditions. Temperatures increased after 10 March and 1 April were back to 35°F. Adults mating later in April were mating during rising temperatures. Data suggest that molting and mating are not closely regulated by declining or rising temperatures of this magnitude.

Maximum daily fluctuations of 2°F were associated with "spring" tides. Temperature would rise quickly, remain level for 6 hr, and then decline to the original temperature. Cold air temperatures (0-20°F) cooling exposed inter-tidal

areas during low tides probably had a marked effect in reducing sea water temperatures each time the tide would rise.

Colder temperatures slowed egg development in

1971. McMullen (1970) reported the 8-blastomere stage was attained 5-7 days after fertilization at temperatures of 37-40°F. During this study, the 8-blastomere stage was attained 10-14 days after

TABLE 4. *Effect of Male Molting Upon Mating Ability.*

<u>Premolt Male Crabs</u>			<u>Postmolt Male Crabs (Cont.)</u>			
<u>No. of Males</u>						
No. of days before molting	Failed to mate	Mated	Comments - how soon males mated after having the opportunity <sup>a</sup> (first comments refer to the males which failed to mate)			
			4	2	-	One had opportunity for 2 days, other had opportunity for 4 days.
			5	1	-	Had opportunity for 5 days.
			6	-	1	Male mated right away.
			7	1	2	Had opportunity for 7 days. One mated right away, the other had opportunity for 7 days. <sup>b</sup>
1	1	-	10	1	1	Had opportunity for 1 day. One male had opportunity for 8 days, the other mated right away.
3	2	-	11	1	1	Both had opportunity for 3 days. Male had opportunity for 8 days, the other mated right away.
5	1	2	12	1	-	Had opportunity with 2 females for 5 days. Both mated right away. Male had opportunity for 8 days, the other mated right away.
7	-	1	13	-	1	Male mated right away. Male had opportunity for 8 days.
9	-	1	16	-	1	Male mated right away. Male mated right away.
14	-	1	17	1	-	Male mated right away. Male mated on 4th day. One male had opportunity for 6 days.
Tot.	4	5	20	-	1	Mated on the 8th day.
<u>Post molt Male Crabs</u>						
<u>No. of Males</u>						
No. of days after molting	Failed to mate	Mated	Comments - how soon males mated after having the opportunity <sup>a</sup> (first comments refer to the males which failed to mate)			
			22	1	1	Male had opportunity for 3 days, the other mated right away.
			27	-	1	Male mated on the 3rd day.
			Tot.	10	10	
3	1	-				Had opportunity for 3 days.

<sup>a</sup>Each day the male is with the new-shell female constitutes one opportunity.

<sup>b</sup>This male molted the same day as the female.

fertilization at temperatures of 32-35°F.

### SUMMARY

Male king crabs attain sexual maturity at a smaller size and at a younger age than females. The crucial question is whether or not these small mature males are functioning as brood stock. Pubescent males and females are congregated in schools along with juveniles of similar age and size with each group molting prior to adults in late February and March. The approximate 10 - day interval bracketing a male's molt is a period during which males are incapable of mating, therefore many pubescent males are unavailable for mating with pubescent females. This partially accounts for larger males, many of which molt a month later and/or are aneuvivants, being available to mate with pubescent females. Larger adult females segregate in separate schools located in similar depth but molt and mate in April and May. Since small males mated with females considerably larger than themselves in the pen studies, it is likely that some would also mate in the natural environment if they had the opportunity. The degree to which they attempt to mate and their ability to compete remains unknown.

The 7-inch size limit appears to protect most brood stock males from commercial harvest for two or more years, especially when used simultaneously with a quota and closed season, but intense harvest on some grounds, if allowed to persist, may still create undesirable sex ratios. A few young males probably have four seasons to mate before attaining legal size, and many at least three seasons. Past intensive commercial harvests (prior to quotas and extended closures) in locations where schools of older males and females were segregated from those of younger crabs, particularly off-shore areas, has resulted in the occurrence of unmated females as high as 30% (Powell, 1969; Powell and Davis, 1969)<sup>9</sup>. Harvest of legal-size males must be regulated in areas inhabited only by older crabs if full participation by females is to be obtained. Abundance of unmated females is much less in shoreward areas where undersize males are abundant.

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## THE FEASIBILITY OF CLOSED SYSTEM MARICULTURE: PRELIMINARY EXPERIMENTS WITH CRAB MOLTING<sup>1</sup>

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### ABSTRACT

*A recirculation system for inducing shedding in the blue crab, Callinectes sapidus Rathbun, and preliminary experiments on crab molting are described. The most important result was the inducement of out of season molting (January-March) in the Delaware Bay area. It appeared that temperature was a key factor in promoting out of season molting. Regardless of its present limitations, year round crab molting and growth may be feasible in a closed recirculation system.*

### INTRODUCTION

Since 1968 this laboratory has been engaged in research directed toward developing facilities for a completely enclosed, environmentally controlled pilot shellfish hatchery (Harman and Maurer, 1971; Price and Maurer, 1971; Maurer, 1972). An important by-product of the research was consideration of new species for inclusion in closed system mariculture. The blue crab, *Callinectes sapidus* Rathbun, was one species which was studied. To accomplish this research it was necessary to design and construct a recirculation system.

One objective of this paper is to report results of an experiment concerning growth and molting in the closed system. Factors such as temperature, salinity, photophase, nutrition, season, privacy, moisture and hormone concentrations influence molting in reptantian decapods. The purpose of the experiment was to determine which factors are necessary for inducing molt in blue crabs out of season, in this case January through March. Shedding of blue crabs in Delaware waters under natural conditions normally occurs from April -

November. By developing methods to molt blue crabs in the winter the soft shell industry can be pursued throughout the year. In addition, by inducing molting the year round, faster growth rates are realized and market size crabs can be obtained much quicker.

### MATERIALS

#### *Closed System Design*

The recirculating seawater system is housed in a 1.52 x 2.74 m insulated room and consists of a reservoir, filtering unit, pump, eight trays, lights, an air cooling unit and two timers (Fig. 1). The volume of the entire seawater system is 1,135 liters. The reservoir conveniently holds 680 liters with dimensions of 1.82 x 1.23 x 0.33 m. The trays (Fig. 2-1) which are 2.74 x 0.36 x 0.17 m are divided into 26 cubicles. Dividers are made from asbestos boards in which slots are cut in the center and cross pieces such that they interlock forming cubicles (0.15 m on all sides). Clearance of 3.2 cm is provided on the bottom of all cross pieces to reduce accumulation of waste material. Water also flows through holes drilled in the cross pieces at the water line. Water depth in the trays is 6 cm.

The filtering unit is 0.46 x 1.22 x 0.46 m and is divided into three compartments (Fig. 3). The pump draws its water from the middle compart-

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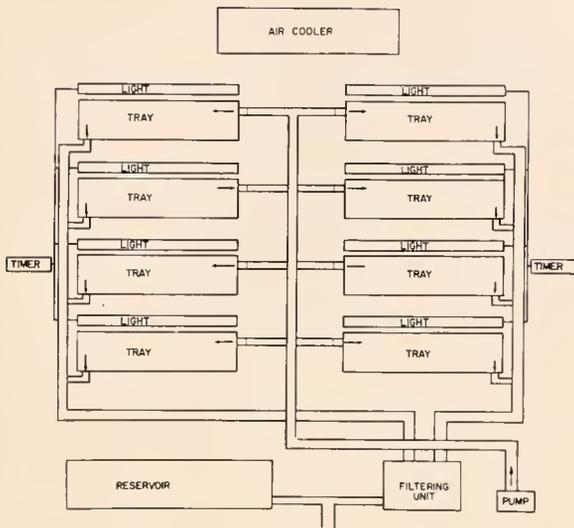


FIG. 1. Schematic of recirculating seawater system.

ment (0.46 x 0.30 m) through a 2.81 cm PVC pipe in which a foot valve is installed for convenience in priming (Fig. 3-1). A float switch is also installed to protect the pump in case of line obstruction or breakage. The lateral compartments are 0.46 x 0.46 m, each with four 2.54 cm PVC pipes spaced equally 1.27 cm from bottom. Each pipe (Fig. 3-2) leads to the middle compartment. Small slits are cut into these pipes allowing water to flow into the pipe and then into the center compartment. The lateral compartments are half filled with crushed clam shells, approximately 1.22 cm in diameter, which serve as filtering and buffering agents. All wooden components are made from 1.91 cm marine plywood coated with fiberglass.

Each tray is equipped with a light fixture consisting of a F-72 cool white fluorescent bulb enclosed in a moisture proof plastic cover (Fig. 2-2). Lights for each bank of trays are controlled separately by a time clock which allows simultaneous testing of two photoperiods. To avoid interference from other light sources, opaque curtains are installed in front of each bank. Heat from the lights is modified by a Tecumseh cooling compressor, model No. C2516 MTK, which enables a constant temperature to be maintained.

Water is pumped to a head tank and remains in it several days to facilitate sedimentation. From there the water is pumped through a heat exchanger into the reservoir which is closed from the rest of the system by a rubber stopper inserted in a 30 cm long, 3.81 cm PVC connecting pipe (Fig.

3-3). After salinity and temperature are adjusted the rubber stopper is pulled and the water fills the filtering unit. The pump 20 gpm (Fig. 3-4), is turned on and the water flows to the individual trays. At each tray there is a valve that controls the rate of flow (Fig. 2-3). The water then fills the tray to the desired depth determined by the length of stand pipe inserted in the drain hole (Fig. 2-4). When the water level reaches the top of the stand pipe it flows into the return line via flexible pipe (Fig. 2-5) and is returned to the filtering unit. At that point the cycle is completed. The stand pipes are pulled out, and the drain valve is opened to flush the system.

Water was changed every two weeks during the molting experiments. Temperature and pH remained stable and salinity increased about 2 ‰ in the same period. The holding capacity of this system is 200 crabs. The entire system including construction of two insulated rooms cost \$3,500.

## METHODS

Crabs were collected from the field in late

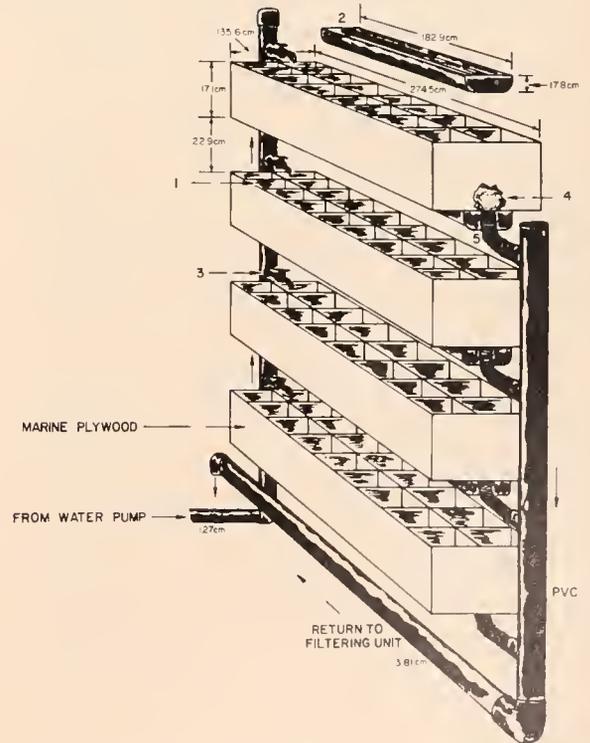


FIG. 2. Schematic of crab tray holding facilities for recirculating seawater system.

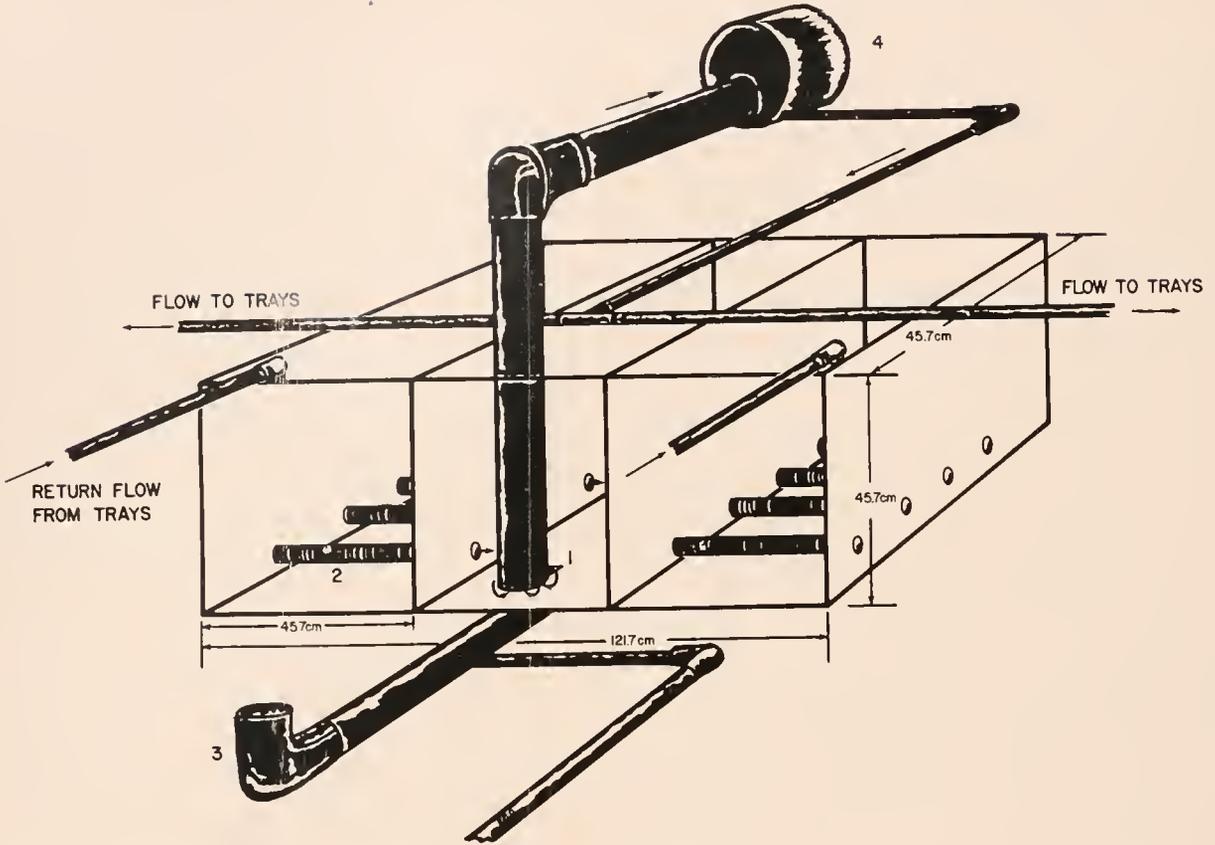


FIG. 3. Diagram of filtering unit for seawater recirculating system.

December 1970 (3-6° C). They were placed in ambient, still, aerated sea water in the laboratory. The water was gradually raised to 20-22° C over a period of two days. After acclimation each crab was weighed and measured and placed into a compartment (Fig. 2-1) within the recirculation system. Throughout the experiment the temperature and salinity were 25° C and 25 o/oo respectively. Crabs were fed silversides, *Menidia menidia* Linné, five days a week. Based on Aiken's (1969) research concerning molting in crayfish, two photophases were established. Thirty-four crabs were exposed to a 16 hr day and thirty were exposed to an 8 hr day. The dates crabs entered the system and molted or died were recorded together with growth determinations (weight, length, width). All measurements were recorded during the C<sub>4</sub> molt stage (Drach, 1939). The experiments were terminated in late March.

#### RESULTS AND DISCUSSION

For the 16 hr and 8 hr photophases, 20 of 34

crabs (59%) and 16 of 30 crabs (53%) molted respectively. The average time to first molt was 27.3 days (16 hr photophase) and 28.6 days (8 hr photophase). Crabs in the 16 hr phase grew (average increase in width - 19.3%, length - 22.3% and weight - 104%) slightly more than crabs in the 8 hr phase (average increase in width - 17.4%, length - 19.1% and weight - 83.1%). The Mann-Whitney Test, a non parametric statistic (Conover, 1971), indicated no significant difference between photophases ( $P > 0.05$ ) in width, length, weight and days to the first molt. Survivorship in the 16 and 8 hr photophase was 68% and 70% respectively. In addition, 5 crabs in the 16 hr photophase molted a second time; on the average the second molt occurred 23 days after first molt. There was essentially no difference in length between the first molt (average increase of 22.3%) and second molt (average, 22.6%), a slight increase in average width from 19.3 to 24.6%, and a reduction in average weight from 104 to 94.6%. Initiation and completion of a complete molt cycle in a closed sys-

tem was an encouraging result.

The most important result was the inducement of out of season molting. It appears that temperature is a key factor in promoting out of season molting. Experiments in progress also confirm this (Epifanio, personal communication). Field survey data show that blue crabs in a local thermal effluent (Island Creek, Indian River Bay, Delaware) were molting in January. The water temperatures in the effluent may be 7-8° C higher than ambient sea water. No statistics on the frequency of shedders per month are recorded for Delaware, but molting of this species does not normally occur in winter waters of the Delaware Bay region. In other laboratory experiments out of season molting has been induced in blue crabs from Virginia waters (Haefner, personal communication). Temperatures ranging from 18 - 25° C were used in these experiments. Haefner (1971) found the incidence of mortality among peeler crabs higher in recirculated water (55%) than in new seawater (38%) and highest (65%) in artificial seawater.

Based on the present experiments the effect of photophase on molting is statistically insignificant. However, it would be premature to discount the biological significance of photophase on molting particularly in view of the small number of crabs used in the experiments and initial mechanical problems with the recirculation system. Research on other reptantian decapods has demonstrated that photoperiods affect molting and breeding (Little, 1968; Aiken, 1969). Refinements in this system together with larger numbers of crabs under combinations of temperature, photophase, nutrition and seasonality must be pursued to determine optimum relationships. For example, water purity may be improved by filtration through a 5  $\mu$  (AFCO) filter bag or by chlorination. After this, the water is dechlorinated, ozonized or passed through an ultraviolet radiation treatment to kill bacteria and viruses. Sanders and Fryer (1972) recommended combinations of the above procedures to control fish pathogens in hatcheries.

Regardless of its present limitations, this recirculation system has some advantages. The estuarine waters of the Delaware Bay region are extremely turbid (Secchi readings less than 0.5 m) which makes laboratory work difficult. It is imperative to have particulate free water in controlled laboratory experiments not only for water quality control but to prevent clogging water passages through the partitions, making isolation of individual crabs possible. Past work in the labora-

tory has shown that heavy mortality of crabs is caused by cannibalism. Since this was a molting study the effect of cannibalism became even more serious during intermolt stages. Isolation was important because it provided privacy eliminating fighting and cannibalism. Without controlling siltation and cannibalism these experiments could not have been conducted. Fouling of seawater systems in Delaware waters can also be a serious problem particularly during the summer. With improved filtration the present system would essentially avoid this problem. In research on disease of blue crabs Cook (1972) faced similar problems and was obliged to design and construct a recirculation system to hold large numbers of crabs in a healthy environment. His crabs were held over several months in the system. In the present work water was changed every two weeks. The senior author found that water quality in closed systems can reduce ingestion rates on the horseshoe crab, *Limulus polyphemus* Linné, in a month (unpublished data). This demonstrates that water quality in closed systems for mariculture must be improved. Our work together with Haefner's (1971) and Cook's (1972) leads us to believe that year round crab molting and growth is definitely feasible in a closed recirculation system.

#### ACKNOWLEDGMENTS

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## EFFECTS OF SALINITY AND TEMPERATURE ON EMBRYOS OF THE GEODUCK CLAM (*PANOPE GENEROSA* GOULD)<sup>1</sup>

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### ABSTRACT

*Combined effects of salinity and temperature on embryos of geoducks were examined. Results indicate narrow salinity and temperature limits for geoduck embryos. For optimum development to the straight-hinge larval stage salinities must remain between 27.5 and 32.5 ‰, and temperatures between 6 and 16°C. Environmental requirements delineated by these experiments agree with the natural distribution of adult geoducks.*

### INTRODUCTION

Recent findings by the Washington State Department of Fisheries of large populations of geoducks in the subtidal zones of Puget Sound have led to a commercial fishery for these large clams. This new fishery is restricted to divers who harvest geoducks with small hand held water nozzles. Landings from the first year's fishing exceeded 400,000 lb. Annual yield could increase considerably because estimated standing crops are well over 100 million lb (Goodwin, 1973)<sup>2</sup>;

The increased interest in this species requires detailed ecological information upon which management decisions can be based. Objectives of this study on the effects of salinity and temperature on embryonic<sup>3</sup> stages are to supply some of this needed information. The work was conducted at the Washington State Department of Fisheries Shellfish Laboratory located at Point Whitney on Hood Canal, Washington.

### METHODS

The bifactorial approach of testing two environmental parameters simultaneously in many different combinations was used in this study (Brenko and Calabrese, 1969). The general methods reported in this paper were developed by Woelke (1968)<sup>4</sup> in oyster embryo bioassays. Spawning were induced by thermal stimulation and normally occurred at temperatures between 12-14°C. Fertilized eggs (30,000/liter) were held at different temperature and salinity ranges in one-liter polyethylene beakers. After allowing the embryos to develop into straight-hinge larvae, samples of about 250 larvae from the cultures were preserved and later counted to determine the number which developed normally. The percentage of embryos which developed normally to the straight-hinge stage was used as a measure of the stress of the culture medium.

Salinities were determined by a hydrometer. Accuracy of this method was within  $\pm 1.0$  ‰ as verified by chemical titration. Culture temperatures were maintained in water baths within  $\pm 1^\circ\text{C}$  of the designated temperatures.

A preliminary test was conducted on the rate

<sup>1</sup>The work reported here was partially financed by the National Marine Fisheries Service, Fisheries Research and Development Act, PL 88-309.

<sup>2</sup>Goodwin, C. L. 1973. Subtidal geoducks of Puget Sound, Washington, Technical Report, Wash. State Dept. of Fish., in preparation.

<sup>3</sup>Fertilized egg to straight-hinge stage = embryo; straight-hinge stage to setting size = larvae.

<sup>4</sup>Woelke, C. E. 1968. Development and validation of a field bioassay method with Pacific oyster, *Crassostrea gigas*, embryo. Ph. D. Thesis, Univ. of Wash., Coll. Fish., 140 p.



TABLE 3. *The combined effects of temperature and salinity on geoduck embryos; percentage of normal straight-hinge larvae (each figure represents the mean of triplicate cultures).*

Salinity (‰)	Temperature (°C)			
	10	14	16	18
22.5	2	0	1	0
25.0	16	59	59	5
27.5	60	82	75	13
30.0	88	85	63	5
32.5	67	17	3	0
35.0	1	1	0	0
Salt water concentrate and Jackson Creek (30‰)				
	-	65	70	-
Frozen seawater control (30‰)				
	-	78	59	-
Seawater control (29.1‰)				
	89	85	68	8

detrimental to geoduck embryos and 6°C appears to be the lower temperature threshold. Survival and normal development were low at salinities below 25‰ and above 32.5‰ regardless of temperature.

Results of the second bifactorial experiment were similar to the first. The percentage of normal larvae was slightly higher at 14°C compared to 16°C in comparable salinities which indicates that 16°C is the upper tolerance limit for geoduck embryos.

The experiments suggest that geoduck embryos have relatively narrow salinity and temperature limits. For satisfactory percentages (70% or above) of embryos to develop into straight-hinge larvae, salinities must remain between 27.5 and 32.5‰ and temperatures between 6 and 16°C. Salinity limits are comparable with two previous experiments conducted on the effects of salinity on geo-

duck embryos held at a constant 14°C. Salinity and temperature limits for geoduck embryos are narrower than those of the coot clam, *Mulinia lateralis* (Calabrese, 1969). For the development of a satisfactory percentage of coot clam embryos, the salinity must remain between 20 and 30‰, and the temperature from 12.5 - 27.5°C.

The low percentage of normal development of embryos in the controls with Rila Marine Mix and freshwater of the first experiment and those with concentrated salt water (freezing method) mixed with freshwater of the second experiment reduces, somewhat, the reliability of the results. These control cultures were slightly toxic to geoduck embryos. This may have artificially narrowed the tolerance limits established by the experiments. The freezing and thawing of seawater apparently lowered the water quality as shown by the lower percentage of normals in the frozen seawater controls of the second bifactorial experiment. Calabrese (1969) and Brenko and Calabrese (1969) did not include controls needed for a comparison of my results. My preliminary experiments indicate that controls other than those prepared from unaltered seawater are needed to properly interpret the results of these types of experiments.

Salinity tolerance limits suggest that the geoduck is an estuarine animal which cannot tolerate salinities found in the open ocean or to prolonged exposures of water less than 25.0‰. Temperature requirements show that they prefer cold water and would not be expected to be found in areas where water temperatures are above 16°C during their spring and early summer spawning season. These requirements agree with the known distribution of geoducks in the State of Washington (Goodwin, 1973)<sup>2</sup>. Tolerance limits of larvae and older stages of geoducks are probably wider than those of embryos. Larval stages in some of my earlier feeding experiments and adults held in the laboratory have survived prolonged temperatures of 18°C and short-term exposures of 20°C.

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## HERMAPHRODITISM IN TWO SPECIES OF PELECYPOD MOLLUSKS

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ANNAPOLIS, MARYLAND

### ABSTRACT

Five additional hermaphroditic soft shell clams (*Mya arenaria*) were found in samples from several beds in Chesapeake Bay in 1971. With these new findings, a total of ten hermaphrodites, 7 bilateral and 3 mixed, have been found among 1,311 specimens examined. These are the only known cases of soft clam hermaphroditism ever reported for Chesapeake Bay. One bilaterally hermaphroditic hard clam (*Mercenaria mercenaria*) was found from 546 examined in Chincoteague Bay. This is the first known case of hermaphroditism in hard clams in Chincoteague Bay. A total of 520 hard clams were also examined from Chesapeake Bay, but no hermaphrodites were found.

### INTRODUCTION

Otto (1972) reported the discovery of five hermaphroditic soft shell clams (*M. arenaria*) collected from various areas of Chesapeake Bay. These clams were considered as "accidental functional ambisexual" by the classification of Coe (1943). Four of the clams were bilaterally hermaphroditic and the fifth was of the mixed type wherein the alveoli contained both male and female gametes. To my knowledge this was the first report of hermaphroditism in *Mya* collected from Chesapeake Bay tributaries. Since the publication of the earlier report, five more hermaphroditic *Mya* and one hard clam (*M. mercenaria*) have been found. All the *Mya* were collected from Chesapeake Bay tributaries, while the single *Mercenaria* was part of a sample collected from Chincoteague Bay. Table 1 details the results of macroscopic and microscopic examinations. The hard clam specimen will be discussed separately.

The specimens were from samples collected regularly in a project (#3-131-R under P.L.88-309) undertaken by the State of Maryland, Department of Natural Resources. This project consists of the collection and examination of mollusks (*Crassostrea virginica*, *M. arenaria*, *M. mercenaria*, *Tagelus* sp., and others) to determine parasite prevalences, distribu-

tions and pathological conditions. *Mya* were collected during 1971 only. Collection of *Mercenaria* is a continuing part of the project.

### HISTOLOGICAL METHODS

All specimens in our samples are processed with the same methods. Samples are individually coded when received. The animals are scrubbed, measured and examined macroscopically before and after opening. They are graded according to condition: Fat, Medium or Watery. A transverse section 10 mm thick is taken from each animal through the visceral mass, gonad, gills and kidney. The tissues are placed in Davidson's fixative for at least 48 hr, dehydrated in successive changes of ethanol and xylene and embedded in paraffin. Sections 6u thick are permanently stained with Harris hematoxylin-eosin for examination.

### HERMAPHRODITISM IN *M. ARENARIA*

#### *Results of Examinations*

Table 1 details pertinent information related to samples where hermaphroditic *Mya* were found. In all cases the clams were in developmental phase. The total number of *Mya* examined in the project was 1,311. The percent prevalence of the 10 herma-

TABLE 1. Summary of data related to samples where hermaphroditic *Mya* were found in Chesapeake Bay.

Sample Site	Sample Date-1971	% Sex ratio		Av. Shell Length(cm)	% Hermaphroditic		Number in Sample	Salinity ‰	Temp. °C
		Female:Male			Bilateral	Mixed			
Chester River	20 May	46	: 50	7.0	4	-	24	6.76	17.41
Potomac River	11 Jun	36	: 60	7.0	4	-	25	3.00	23.22
Corsica River	30 Jun	52	: 44	6.0	4	-	25	7.80	27.46
Corsica River	2 Sep	48	: 48	6.0	4	-	25	7.46	26.00
Chester River	2 Sep	40	: 56	6.5	-	4	25	10.23	25.49
Chester River	16 Sep	44	: 52	6.5	-	4	25	9.70	26.42
Corsica River	4 Oct	40	: 52	6.5	8	-	25	9.89	21.82
Eastern Bay	4 Oct	36	: 56	6.0	4	4	25	13.80	22.64

phroditic clams found was 0.76% (Bilateral type: 0.53%; mixed type: 0.23%).

#### HERMAPHRODITISM IN *M. MERCENARIA*

This hermaphroditic clam was found in a sample of 20 clams collected from Chincoteague Bay on 26 May 1972. Up to and including this sample of clams, a total of 1,066 were examined. The incidence of this condition in the hard clam samples was 0.094%. A total of 546 clams were collected from this area (0.18% incidence). The other clams were collected from various areas in Chesapeake Bay. A review of the literature indicates that this condition is rare in *M. mercenaria*. Loosanoff (1936) stated that he found only two cases of functional hermaphroditism among several hundred adult clams. He also stated that there was strong evidence of protandry in young, immature clams.

#### Results of Macroscopic Examination

The average size of the clams in the sample was 8.5 cm, while the hermaphrodite was 8.0 cm. The range of physical conditions was as follows: Fat - 3 (15%); Medium plus - 7 (35%); Medium - 9 (45%); and Medium minus - 1 (5%). The hermaphrodite was Medium minus. No abnormalities were noted in this clam.

#### Results of Microscopic Examination

The following sex ratio was noted: 5 males, 14 females, and the single hermaphrodite. The hermaphrodite and the rest of the sample was free of parasites.

The clam was bilaterally hermaphroditic. Both sperm and eggs appeared to be of mature size; the development being parallel (Fig. 1). The physical condition of the clam, in addition to the sexual anomaly, appeared to be slightly abnormal in that

phagocytic infiltration was heavy throughout the animal; an indication of physiological stress.

#### DISCUSSION

The reason or cause for the relatively high level of hermaphroditism in the *Mya* samples can be only speculated upon at this time. That the condition is rare, according to the literature, cannot be disputed. The discovery of ten such endowed animals in a small number of areas (5) in Chesapeake Bay would remove this condition from that category; at least in Chesapeake Bay. That environmental conditions or stresses on these *Mya* may be one of the causative agents is very possible. Since *Mya* here are near the southern limit of their geographical distribution, any change, however slight, in their environment probably affects

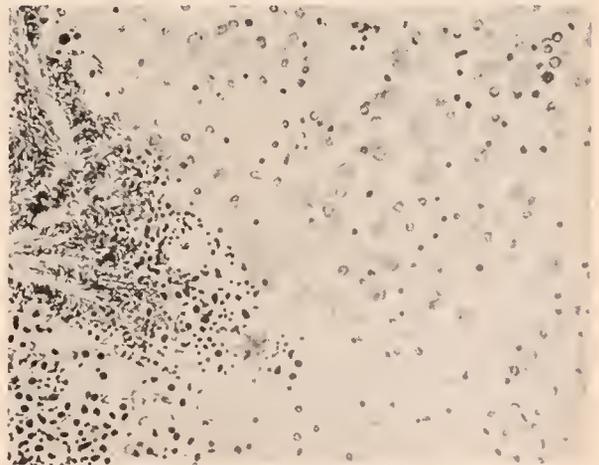


FIG. 1. Bilaterally hermaphroditic gonads of *M. mercenaria*. Sperm are small, dark-staining bodies. (About 430X).

them greatly. It could be that gonad development is also affected by subtle changes that are not reflected in the Bay's hardier species such as the oyster (*C. virginica*).

As far as concerns the *Mercenaria* case, this clam, moreso than the *Mya*, deserves the classification of Coe (1943) as an "accidental functional ambisexual" (italics mine). Loosanoff (1936) noted the presence of small ovocytes along the walls of adult male alveoli and stated that "this may be the potentiality of changing sex even in the adult condition." In this case, as with *Mya*, environmental conditions, may, in part, effect this phenomenon.

#### ACKNOWLEDGMENTS

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## ABUNDANCE OF THE LOW SALINITY CLAM, *RANGIA CUNEATA* IN SOUTHWESTERN LOUISIANA

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### ABSTRACT

The low salinity clam *Rangia cuneata*, was found to be very common in oligohaline waters of southwestern Louisiana, discontinuously distributed across a band over 100 miles long and 10 miles wide, occurring in tidal creeks, lakes and bays from the shoreline to at least 4 m in depth. It was replaced in the intertidal zone by *Polymesoda caroliniana*, in saltier, deeper waters by *Tagelus plebius* and *Macoma mitchelli*, and in fresh water by unionids. It was inexplicably absent or rare in many areas, showing no correlation with total sediment carbon, except for being very rare in very highly organic sediments rich in plant detritus.

Populations were usually composed of uniformly sized animals ranging from means of 28 mm in Grand Lake to 57 mm in one tidal creek in Vermilion Bay. Large populations of juveniles were rare although recently metamorphosed juveniles were sometimes taken. It is estimated that southwestern Louisiana has a minimum standing crop of between 24 and 48 billion clams based in part on an average of 11.1 clams/m<sup>2</sup> found over the whole study area.

### INTRODUCTION

Although perhaps as common in their habitat as oysters are in their's, the moderate sized Louisiana road clam or *rangia*, *Rangia cuneata* Gray, long utilized by prehistoric man for food (McIntire, 1958), has received little interest until very recently. Indian mounds composed largely of *rangia* provide part of the basis of an extensive mudshell industry, which in 1966-67 (2 years) removed nearly 9½ million cubic yards of shell. Louisiana is the only state with large enough fossil populations to support such an industry, although *rangia* is now being considered in much of its range as a possible source of food. However, suspected slow growth rates (Fairbanks, 1963; Wolfe and Peteway, 1968; Gooch, 1971<sup>1</sup>) may render this clam less amenable to harvest than oysters, which reach market size very rapidly in Louisiana (Hopkins,

Mackin and Menzel, 1953).

Nevertheless, southwestern Louisiana probably contains more *R. cuneata* than any other comparable area of the world, except perhaps Lake Ponchartrain, and the animal is undoubtedly of enormous significance to the ecology of the area. To this end, this study was devoted to determining the distribution and abundance of *R. cuneata* and associated mollusks from about the Atchafalaya River mouth to near but not including Sabine Lake (Fig. 1).

### METHODS

Clams were collected in deep water with an angle iron frame dredge 85 x 20 x 93 cm long, pulled behind either a 40 ft or 18 ft boat at about 3 kn for 3 min at each station. The bag was constructed out of 1 in stretched mesh which retained clams as small as 25 mm, with a few down to 15 mm. Shallow waters (less than 2 m) were sampled with two random square meter frames thrown from a small boat. Clams were then removed from the quadrat by diving. Juvenile

<sup>1</sup>Gooch, D. M. A study of *Rangia cuneata* Gray in Vermilion Bay, Louisiana. M. S. thesis, USL: 50 pp.

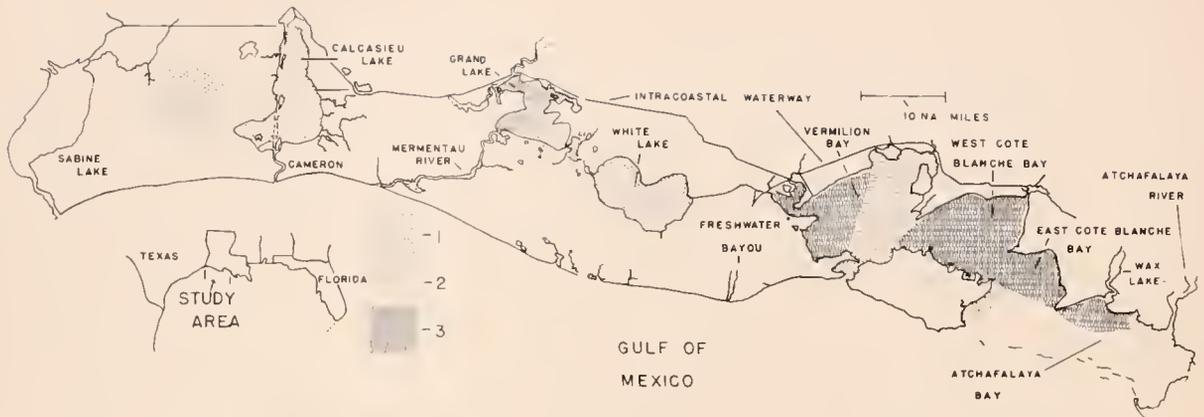


FIG. 1. Map of study area showing estimated concentrations of clams. 1 = 0 clams, 2 = less than  $10/m^2$ , 3 - over  $10/m^2$ . For more details, see Figure 2. White areas not sampled.

clams were collected with a 2 or 5 m long cylinder of fiberglass or plexiglass with diameters of 56 or 63 mm. Two cores were taken at each station, sieved and examined for small mollusks, but large amounts of plant fiber at some stations undoubtedly obscured some of the clams. At each station salinity was measured by a Beckman RS5-3 conductivity meter, pH by meter or Hach color kit, oxygen with YSI model 54 m and temperature by thermistor.

Ninety-three shallow water stations were spaced three nautical miles apart around major water bodies, with some sampling elsewhere. Thirty-nine deep water stations were laid out in a grid separated by three nautical miles. Some areas could not be sampled due to shallow water and other problems.

Sediment samples were collected along with juvenile clams. Organic matter was measured by loss on ignition and is expressed in percent total carbon, including a small amount of carbonate carbon.

#### DESCRIPTION OF AREA

The area of study includes a very old reworked delta of the Mississippi, now known as the chenier plain region (Russell and Howe, 1935; Van Lopik, 1955). Cheniers are low, sandy intrusions above an otherwise flat marshland composed of several species of fresh and brackish water plants, with true salt marsh plants rare (O'Neil, 1949; Chabreck, 1970<sup>2</sup>). Degradation of these plants with other allochthonous sources results in high concentrations of plant detritus or peat mixed in with clays and silts. In addition mud is being

added continually from the rivers and is reworked with the detritus (Coleman, 1966).

From Sabine Lake to Vermilion Bay (Fig. 1) the marsh is nearly continuous except for numerous tidal creeks and ponds and the estuaries of two rivers, the Calcasieu and the Mermentau. Grand Lake, associated with the latter and White Lake, with no apparent river system, are oblong ovate "lakes" roughly parallel with the shoreline. These lakes are isolated on all three sides from salt water by control structures completed in 1951. From Vermilion Bay to the Atchafalaya River mouth there is a system of shallow bays (2-3 m) separated from the Gulf of Mexico by marsh on the western end (Marsh Island) and dead oyster reefs on the eastern end (Point au Fer). These reefs have been killed by the increasing flow of the Atchafalaya, which has been capturing much of the Mississippi River flow (Gunter, 1952; Thompson, 1955) and now is building its own delta in Atchafalaya Bay (Shlemon, 1971).

#### HYDROGRAPHY

Except for Vermilion Bay there is relatively little hydrographic data on the area, although the mouth of the Atchafalaya River has attracted some interest due to the increased flow. Salinities there have been very low, usually within the range of fresh water through Atchafalaya Bay into West Cote Blanche Bay. Salinities increased to an aver-

<sup>2</sup>Chabreck, R. H. 1970. Marsh zones and vegetative types in the Louisiana coastal marshes. Ph.D dissertation, LSU. 113 pp.

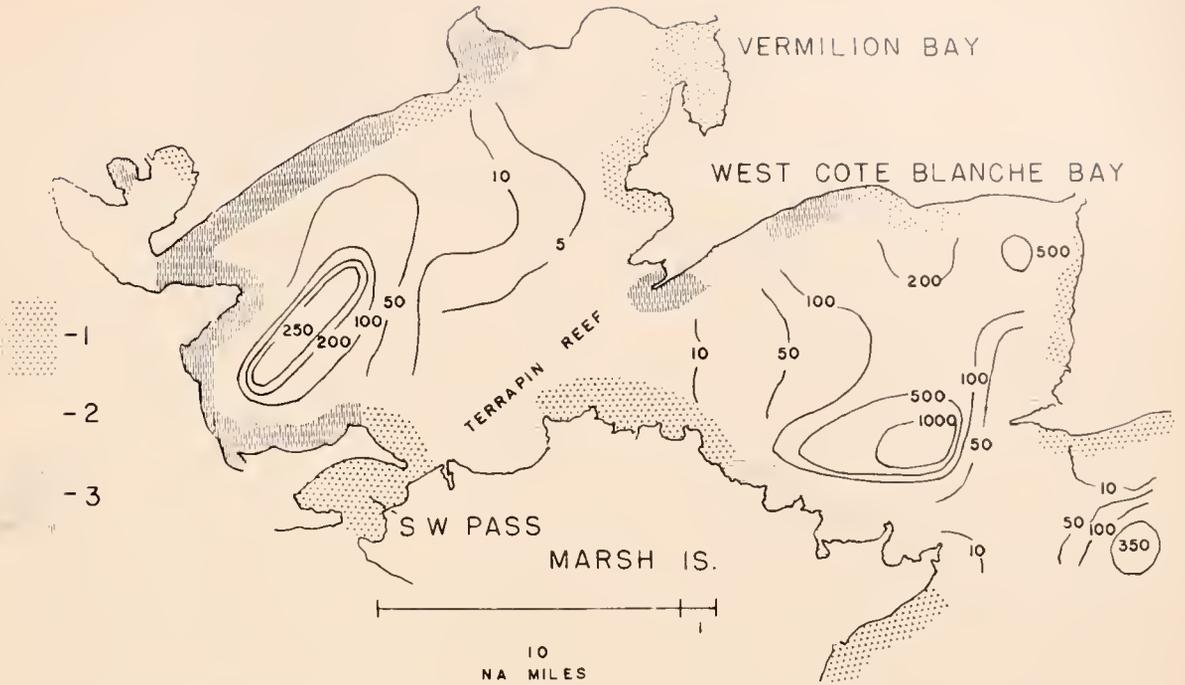


FIG. 2. Area of highest concentrations. Shoreline concentrations in nos./m<sup>2</sup>. 1 = less than 1. 2 = 1 -10. 3 = more than 10. Offshore are lines of equal density. Numbers are clams caught per 3 minute dredge haul. (To estimate numbers/m<sup>2</sup> divide by 6)

age of 3.7 ‰ in Vermilion Bay. Although this is about the same as reported by Dugas (1970)<sup>3</sup> for 1969, it is 2-3 ‰ lower than that observed in 1963-64 (Fontenot, 1967<sup>4</sup>).

Westward through the marsh salinities decrease to near fresh water in Grand and White Lakes. Data given by Gunter and Shell (1958) showed similar salinities for this area although they noted some as high as 2.7 ‰. Calcasieu Lake has been reported to be somewhat saltier (Kellogg, 1905) and the highest salinities (15.5-26.0 ‰) in the study were found there. Probably the Lake Charles Ship Channel has caused an increase in the average salinity of the Lake.

DISTRIBUTION AND ABUNDANCE

*Rangia* was not continuously distributed across

southwestern Louisiana. It was absent in much of the shallow water of Atchafalaya Bay, at Terrapin Reef between Vermilion and West Cote Blanche Bays, White Lake, Calcasieu Lake and most of the northern marsh area between Calcasieu and Sabine Lakes. Its center of abundance lies in western Vermilion Bay (the area studied by Gooch, 1971<sup>1</sup>), central and eastern West Cote Blanche Bay, with lesser concentrations in parts of Grand Lake, central East Cote Blanche Bay and western Atchafalaya Bay (Fig. 2, Tables 1 and 2). In Vermilion Bay clams appeared equally abundant along the shoreline and in deep water. However, in West and East Cote Blanche Bays clams were scarce along much of the shoreline while reaching high densities in deeper water.

The highest density of clams found in shallow water in a single sample was 238/m<sup>2</sup> in Vermilion Bay. Doubtlessly higher densities could be found by further searching since Gooch (1971)<sup>1</sup> reported concentrations up to 756/m<sup>2</sup>. Nevertheless, our data indicates an average concentration in shallow water of 11.1/m<sup>2</sup> with highest numbers in Vermilion Bay to none found in White and Calcasieu Lakes (Table 1). In core samples covering 1.5 m<sup>2</sup>, an average of 14/m<sup>2</sup> was taken for clams over 10

<sup>3</sup>Dugas, R. J. 1970. An ecological study of Vermilion Bay, 1968-69. M. S. thesis, USL:107 pp.

<sup>4</sup>Fontenot, B. J. 1967. Seasonal relative abundance and distribution of postlarval white and brown shrimp in Vermilion and Cote Blanche Bay. M.S. thesis, USL: 77 pp.

TABLE 1. Average numbers/m<sup>2</sup> at shallow water stations in several Louisiana bays.

	Highest concentration	No. Stations	Avg. Abundance <sup>a</sup>
Atchafalaya Bay	69	8	6.1
East Cote Blanche Bay	34	6	7.0
West Cote Blanche Bay	130	11	8.5
Vermilion Bay	238	18	26.6
White Lake	0	11	0
Grand Lake	116	13	16.9
Calcasieu Lake	0	13	0
Miscellaneous	97	12	11.8
Total (all stations)		92	11.1

<sup>a</sup>Lowest sample in all bays was 0.

mm while for clams under 10 mm the rate was 28/m<sup>2</sup>. The number of clams taken by core in shallow and deep water were exactly the same (0.08/core). While this does not constitute proof that deep and shallow water samples are comparable there are no data refuting this hypothesis. Various estimates of abundance based on our data, based on weights given by Hopkins (1970) and based on the acreages given by Chabreck (1971)<sup>5</sup> and Perret, et al. (1971) are shown on Table 3.

Accepting the slow growth rate of rangia as suggested by previous workers (Fairbanks, 1963; Wolfe and Petteway, 1968; Gooch, 1971<sup>1</sup>), it

might be prudent to harvest no more than 5% of the population annually until more information is gathered about the actual deep water concentrations, the effect of harvesting, recruitment, possible culture methods and the importance of the clam to the ecology of the bays. This should still give a potential annual harvest of about 2 billion clams, at a wet meat weight of 22 million pounds (45.5 million kg.).

Regardless of the precise figure, rangia populations between Sabine Lake and Atchafalaya Bay must number in the tens of billions, with total weights in the billions of pounds (85% is shell weight). Based on our recommendations a few billion rangia could be harvested each year. However, current harvest is about 8-9 billion pounds of shell a year, which exceeds the replacement amount by a factor greater than 18, assuming the whole Louisiana coast is producing the same amount of rangia as the western part.

<sup>5</sup>Chabreck, R. H. 1971. Ponds and lakes of the Louisiana coastal marshes and their value to fish and wildlife. 25th Ann. Conf. S. E. Assoc. Game and Fish Comm. (mimeo. 19 pp.).

TABLE 2. Average numbers/3 min haul at deep water stations by dredge.

	Highest concentration	Lowest	No. Stations	Avg.
Atchafalaya	233	4	3	143.7
East Cote Blanche	352	6	5	83.4
West Cote Blanche	1458	22	10	37.8
Vermilion	273	0	15	53.5
White Lake	0	0	2	0
Grand Lake	190	0	2	95.0
Total (all stations)			37	60.0

TABLE 3. Total amounts of *Rangia cuneata* in southwest Louisiana study area based on various means of estimation.

Total Study Area (Chabreck, 1971 <sup>5</sup> )			
Total from	Number (in millions)	Shell Weight (lbs. in millions)	Wet Meat Weight (lbs. in millions)
Shallow water avg. (11.1/m <sup>2</sup> )	38,457	1,864	390
Tube samples (14.0/m <sup>2</sup> )	48,504	2,350	491
By Bay System (Perret et al., 1971)			
Bay	Number (in millions)		
Atchafalaya	3,325		
East Cote Blanche	2,332		
West Cote Blanche	3,092		
Vermilion	13,091		
Grand	2,170		
Total of above	24,011		
Total based on total acreage	32,332		

Most of the clams lie in the area from Vermilion to western Atchafalaya Bay (Fig. 2). Within this area, which covers about 330 thousand acres (133.5 thousand hectares), there is an estimated standing crop of 23 billion clams.

Several workers have suggested that *rangia* abundance might be correlated with sediment type or amount of organic matter. Figure 3 shows organic matter concentrations versus *rangia* abundance. Except for the scarcity of *rangia* in very highly organic sediments (over 10%) there seem to be no correlations. These highly organic sediments are predominately broken down plant detritus. In these areas *rangia* may have difficulty in becoming stabilized. *Rangia* does occur in sediments high in plant detritus where small pockets of detritus collect in swales in hard packed clay. In this clay the clam burrows with difficulty. Therefore, the correlation may only represent problems of maintaining stability where loose plant detritus exceeds the normal burying depth of the clam.

#### SIZE

The majority of clams collected exceeded 34 mm. At only 19 of 55 shallow water stations were smaller clams found and at 6 of these no clam was under 30 mm. Only at Grand Lake was there an abundance of small clams, over 10/m<sup>2</sup> (Fig. 4),

and the majority of these were between 20 and 30 mm. However, numerous clams over 35 mm were also present at some stations in the Lake and at one station in the northeastern part they averaged 48 mm, or about as large as that found anywhere in the study area.

At deep stations the majority of clams were within the 30-42 mm range although some smaller clams were often found. The mean size for clams at the deep water stations ranged from 30-52 mm which was closely comparable to those found in shallow water. The majority of clams over 50 mm were taken in numbers under 10/m<sup>2</sup>, the only exceptions being at one station in Grand Lake and two in Atchafalaya Bay. The largest clam taken was in a tidal creek off Vermilion Bay; it measured 75 mm. Gooch (1971)<sup>1</sup> reported a record 86 mm clam from the area and the average size of some populations was over 75 mm. While large *rangia* seem most common in tidal creeks where the water remains practically fresh, there is no obvious correlation of size with environmental factors.

An example of the most common length-frequencies are shown in Figure 4. Means of *rangia* populations in excess of 10/m<sup>2</sup> (outside of Grand Lake) ranged from 38-52 mm. Only 14% of the clams were over 48 mm and 40% of them were

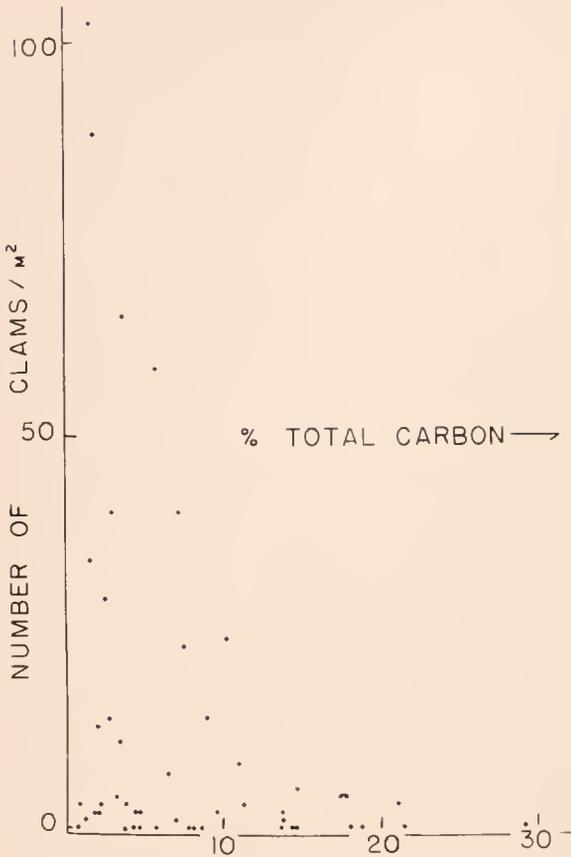


FIG. 3. Comparison of number of clams/m<sup>2</sup> in shallow water with amount of sediment carbon.

between 40 and 44 mm. In contrast, at Grand Lake only 2 populations were above 37 mm (both at 48 mm) and 84% of the clams were between 22 and 31 mm.

Samples containing juveniles below 10 mm were rare. Collections were made at all times of the year, and occasionally coincided with the time that veligers were metamorphosing. For example, collections made between 24 March and 21 April 1970 in Vermilion and West Cote Blanche Bays coincide with the time of setting previously reported by Fairbanks (1963). A total of 27 small juveniles was taken, mostly from areas where less than 5 adults were taken per drag. While this may suggest that *rangia* larvae only settle in areas where clams are scarce, large populations of small clams are often found very close to, although not intermixed, with adults.

One accidental capture of very small clams may provide some insight into settlement of larvae. A large uncounted group of young less than 1 mm

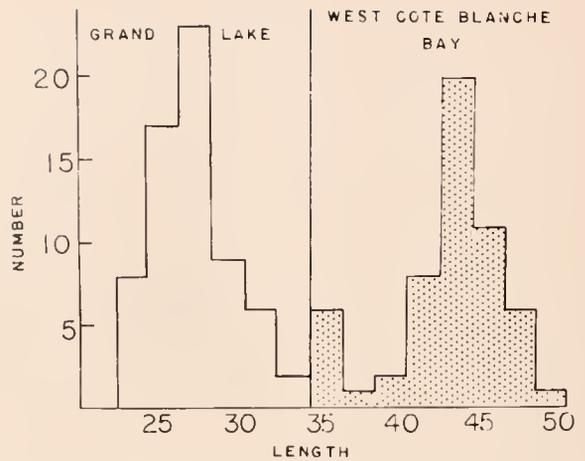


FIG. 4. Comparison of size distribution (in 2 mm intervals) of *rangia* from one station on north shore of Grand Lake and one on north shore of West Cote Blanche Bay.

in length was accidentally snagged with a small hydroid colony caught on the end of the oxygen probe in West Cote Blanche Bay. The clams had apparently clamped onto the colony by the shell margins.

#### ASSOCIATED SPECIES

*Rangia* apparently has no infaunal competitors in southwestern Louisiana estuaries. Occasionally we found the marsh clam, *Polymesoda caroliniana*, the small low salinity tellinid, *Macoma mitchelli* and unidentified unionids among the *rangia* populations.

*P. caroliniana* lives in the intertidal zone buried in mud in *Spartina patens*-*Sagittaria lancifolia* type marshes and sometimes reaches fair abundance there. Young clams (1-4 mm) were also found intertidally on Mud Point above mean sea level. Live *P. caroliniana* were common in intertidal burrows and loose clams were found scattered all the way to adjacent subtidal areas, where they are undoubtedly inadvertently transported. In an adjacent tidal creek *rangia* were abundant; however, none were found above mean low water. It appears, therefore, that these two species do not mix. Harry (1942) reported *P. caroliniana* among roots of marsh grasses in Barataria Bay, Louisiana, and Andrews and Cook (1951) describe their range and habitat in Virginia.

*Macoma mitchelli* was found only at the saltier and deep water stations in southern Vermilion Bay close to southwest Pass. Here they barely overlap *rangia* populations in the western part of the bay.

Closely associated forms seem largely limited to the two tiny gastropods, *Littordina sphinctostoma* and *Vioscalba louisianae* (Gooch, 1971)<sup>1</sup>. These two species live among the rangia, but their mode of life is unknown.

Oysters, *Crassostrea virginica*, and hooked mussels, *Brachidontes recurvus*, occur predominately seaward of rangia, although both occasionally set and survive for a short period of time in areas where rangia are found. Other than these animals and several demersal fishes and crustaceans (Norden, 1966; Perret, 1967) only two other mollusks were found. The gastropod *Nereitina relicivata*, is common in the lower intertidal zone and on some of the higher oyster reefs. They feed on green and blue-green algae, and occasionally overlap with rangia. The mussel, *Modiolus demissus*, occurs rarely in the marshes; only two records are known. Other than a rare chironomid larva or polychaete, there was no other macroscopic animal associated infaunally with rangia.

Only *R. cuneata* (Gray) was found; living specimens of *R. flexuosa* (Conrad) seem to be very rare, and have been reported in Louisiana by Harry (1942), Behre (1950), and Gooch (1971)<sup>1</sup>. Although recent *R. flexuosa* seem rare, many shells were found in old assemblages.

#### DISCUSSION

The distribution of rangia in Louisiana clearly follows the lower salinity waters that range from 0.5 - 9.0 ‰. This zone is perhaps best called oligohaline, although the term does not fit the salinity limits given by other authors. However, rangia clearly occupies this lower zone where there is some salt water intrusions. Other infaunal pelecypods, both fresh-water and marine, are absent. The absence of rangia along the eastern Atchafalaya Bay shoreline may be explained by the possible lack of salt water intrusion. This came about in the past two decades with increasing river flow. However, we were unable to sample the open waters of the Bay because of recent shoaling in the central and eastern part (Shlemon, 1971). It is possible that rangia would be in these waters.

The same may be true of White Lake which, like Grand Lake, has been isolated since 1951 by control structures to prevent seasonal salt water intrusion. Rangia were abundant in White Lake in 1952 (Gunter and Shell, 1958), but very few were taken there by Gooch (1970) as late as 1969. Howe, Russel and McGuirt (1935) reported that in 1934 Grand Lake was too saline for rangia. Today Grand Lake has considerable numbers of rangia in-

cluding populations of small individuals (below 30mm). These must have set after the control structure was built. Penaeid shrimp are still found in White Lake indicating that there may be some salt water intrusion, especially since the opening of Freshwater Bayou to the Gulf of Mexico. However, no rangia were found in this lake.

The lack of rangia in Calcasieu Lake can be explained by the higher salinities, probably increased by the ship channel. Instead, *Tagelus plebius* is a common infaunal mollusk. Large numbers of recent rangia shells on the bay bottom attest to its presence within historic times. Rangia was reported to be extremely common in upper Calcasieu Lake by Kellogg (1905). We failed to find any although no samples were taken in the center of the bay, in the river above the bay or in Lake Charles.

Although rangia does not penetrate the moderate salinity area of estuaries, it is not clear what factors are limiting. It tolerates moderate salinities (O'Heeron, 1966)<sup>6</sup> and occurs in small numbers off Marsh Island in the Gulf of Mexico where salinities often reach 20‰. O'Heeron (1966)<sup>6</sup> suggested predation by *Thais*, but this is an epifaunal feeder. Two drilled rangia were found in lower Vermilion Bay, but based on the bevel of the hole they were apparently drilled by *Polynices*. *Polynices* is a common predator of infaunal pelecypods. Other possible predators discussed by Gooch (1971)<sup>1</sup> do not seem to be segregated by salinity. There is no evidence of competition with other pelecypods at the seaward edge or no changes in bottom types which might explain the lack of rangia.

One of the most intriguing findings made during this study was the uniform size of populations and the apparent slow growth (Gooch, 1971)<sup>1</sup>. Another interesting observation was the lack of clams, young or old, in many apparently suitable areas suggesting that recruitment is rare. One explanation is the possible need for degrading plant detritus on which rangia might first attach to before burying into the sediments. The lack of plant detritus or other suitable materials at time of setting may contribute to setting failure. However, other hypotheses need to be investigated and studies on spawning, larval abundance, settlement and recruitment should be done.

The great abundance of rangia in southwestern

<sup>6</sup>O'Heeron, M. K., Jr. 1966. Some ecological aspects of the distribution of *Rangia cuneata* Gray. M.S. thesis, Texas A & M Univ. 55 pp.

Louisiana is undoubtedly related to the great width of the upper part of the estuarine zone. Its width in the Vermilion-Cote Blanche area is about 12 miles and the length exceeds 100 miles. *Rangia* appears well adapted to the very organic, turbid waters and reduced sediments that typify this area. The importance of the clam to the area seems to be as follows:

(1) Important converter of detritus to animal matter and reservoir for many nutrients, especially  $\text{CaCO}_3$ ;

(2) Fills a niche in a habitat (infaunal, oligohaline) that no other similar animal tolerates;

(3) Provides shell for storm built marsh beaches;

(4) Provides a hard substrate in bay bottoms for attachment of epifaunal species; and

(5) Probably has many unknown effects on sedimentation and survival of burrowing species of other groups.

*Rangia* have commercial applications both potential and realized. While some of the more obvious applications, such as mudshell, receive the most attention, some unstudied aspects may be more important. *Rangia* maintains a productive, stable area, which produces one of the largest commercial catches of other animals in the world. Hopefully future utilization of this clam will be considered over a long term view since the data gathered in our studies suggests that *rangia* may be very susceptible to rapid depletion.

#### ACKNOWLEDGMENTS

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PATTERN OF DISTRIBUTION OF THE SURF CLAM  
(*SPISULA SOLIDISSIMA*) IN THE POINT JUDITH,  
RHODE ISLAND HARBOR OF REFUGE<sup>1</sup>

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ABSTRACT

The object of this study was to determine the nature of the distribution of patches of the surf clam, *Spisula solidissima solidissima*. Two areas were selected inside the breakwater at Point Judith, Rhode Island. A statistical method designed for plant populations was utilized and a method of solving for the unknown parameters was developed. The collection of data was carried out by scuba divers. From the analysis and observations it was concluded that the pattern of patches of *Spisula* were density dependent, with high density areas tending toward complete aggregation while medium and low density areas consisted of randomly distributed discrete patches.

INTRODUCTION

Surveys of commercially important shellfish are made frequently to determine numerical abundance for management purposes. Most survey methods involve collecting individuals within a specified quadrat cast in a statistically valid manner. From the number collected, inferences are made as to the distribution of individuals and their numerical abundance. All of these sampling methods are carried out from the surface using mechanical sampling devices. Hard clams exhibit various degrees of contagion. That is, they are not distributed randomly on the bottom but are in patches. Previous work by Sails, Flowers and Campbell (1966) indicated that the quahog, *Mercenaria mercenaria* is contagiously distributed and further work by Sails and Gaucher (1965) confirmed this contagious nature for other marine pelecypods. Little has been done to determine the nature of the distribution of these patches of hard clams. Surface sampling techniques are inadequate for this determination in that they do not yield

the required precision. In recent years underwater photography has been used in studying benthic organisms. This method is effective on an organism which is readily identifiable from a photograph. For the hard clam this method was found to be ineffective in that the only visible indication is the presence of a siphon hole. Identification of the clam is difficult even with a practiced eye. Variable environmental conditions confound the problem by controlling the clams' condition. Under the proper conditions the clams pumped and the siphon holes were visible. At other times the clams were not pumping and the siphon holes were not visible.

As a first attempt at evaluating the nature of the patch and gap patterns of pelecypod mollusks, the surf clam, *Spisula solidissima solidissima* was selected. *Spisula* was chosen for its large physical size and ease in identification. The sampling was conducted by scuba divers using hand rakes in a depth of water varying from 18-25 ft. Identification of the clam in hand is an important factor in this type of sampling.

Two areas inside the breakwater at Point Judith, Rhode Island were selected. This location was selected for several reasons: a) virtually no commercial fishing of the surf clam has been done

<sup>1</sup>This work is a result of research sponsored by NOAA, Office of Sea Grant, Department of Commerce, under Grant #2-35190.

in the areas selected for the past 8-10 years; b) the density, as well as the physical size of the surf clams in the areas chosen differed; and c) minimal disturbance of the natural distribution of the surf clam is afforded by the wave force reduction of the Point Judith breakwater.

METHODS

Statistical Methods

Before sampling was begun the degree of aggregation or patchiness of the *Spisula* was empirically determined. Several small areas (20 x 20 ft) were completely searched for *Spisula*. Each clam was extricated from the bottom and placed by its respective hole. By rising 6-10 ft off the bottom, patch shapes and dimensions were clearly visible. The shapes of the patches varied. Some formed clusters where each individual was separated from another by a nearest neighbor distance. Others were in single file forming irregular curved patterns. Two instances where a patch consisted of a circle with the individual clams making up the perimeter of the circle were observed. Although the area within the circles was much greater than the nearest neighbor distances, this area could not be regarded as belonging to a gap. Maximum distances across patches were measured and visual notes on patch distribution were taken. From this empirical data a rough estimate of the mean patch radius *r* was established.

The method of sampling was one adapted from Pielou (1964) who used paired circular quadrats for estimating the patch and gap patterns of vegetatively reproducing plants. Pielou's basic methods and assumptions have been included as an explanation of the technique. On the basis of the empirical data gathered the patches and gaps were defined as follows: any point on the bottom at a distance greater than *r* from the nearest clam is to be regarded as belonging to a gap; and any point whose distance from the nearest clam is not greater than *r* is in a patch.

Although the density of clams in the two areas sampled varied, the mean patch sizes in each area was approximately the same. Hence, *r* for both areas was set at 20 in. since the mean diameter of patches was approximately 40 in.

If paired circular quadrats, each of radius, *r* are set down in an area, where *d* ≥ 2*r* is the distance between centers we have a possibility of four events, HH, HM, MH and MM. H denotes a hit or the presence of a clam within a circle and M denotes a miss or the absence of a clam within a circle. The events HM and MH are to denote the order of occurrence which will be dropped. When

a quadrat pair is set down in an area it may be thought of as constituting two observations of a two state discrete Markov Process. The states are a hit (state 1) and a miss (state 2). If the circles are tangent (*d*<sub>1</sub> = 2*r*) a single transition is assumed to have occurred. When *d*<sub>2</sub> = 4*r* two transitions have occurred and so on.

The matrix of transition probabilities may be written as:

$$\begin{matrix}
 & & \text{Second Quadrat} \\
 & & \begin{matrix} \text{H} & \text{M} \end{matrix} \\
 \text{p = First} & \text{H} & \begin{bmatrix} p_{11} & p_{12} \\ p_{21} & p_{22} \end{bmatrix} \\
 \text{Quadrat} & \text{M} & \\
 & & 
 \end{matrix} \tag{1}$$

where *p*<sub>11</sub> + *p*<sub>12</sub> = 1 and *p*<sub>21</sub> + *p*<sub>22</sub> = 1. Here *p*<sub>11</sub> is the probability that the second quadrat scores a hit given that the first one did, and the other three probabilities are similarly defined. To determine the four possible events HH, HM, MH, MM, it is necessary to know the probability *a*, that the first quadrat scores a hit, and the probability (*b* = 1-*a*) that it scores a miss. The vector (*a*, *b*) is the limiting probability vector of the Markov chain with transition matrix *P*. Thus *a* and *b* may be expressed in terms of the transition probabilities *P*<sub>ij</sub> by solving the matrix equation (*a*,*b*) *P* = (*a*,*b*). Whence

$$a = p_{21}/(p_{12} + p_{21}) \text{ and } b = p_{12}/(p_{12} + p_{21}) \tag{2}$$

The probability that both quadrats score hits is then

$$\text{Pr(HH)}_1 = ap_{11} = p_{11} p_{21}/(p_{12} + p_{21}) \tag{3}$$

where the suffix 1 in the first member denotes the single transition pair (*d*<sub>1</sub> = 2*r*) is being used. Also

$$\text{Pr(MM)}_1 = bp_{22} = p_{12} p_{22}/(p_{12} + p_{21}) \tag{4}$$

and

$$\text{Pr(HM)}_1 = \text{Pr(HM)}_1 + \text{pr(MH)}_1 = ap_{12} + bp_{21} \tag{5}$$

$$\text{Pr(HM)}_1 = 2p_{12} p_{21}/(p_{12} + p_{21}) \tag{6}$$

The absence of an arrow from the first member of the above equation signifies that the order in which the two quadrats are observed is disregarded.

When a pair of quadrats of length *d*<sub>n</sub> = 2*nr* is used *n* transitions are assumed to have occurred between each quadrat of the pair. The *n* step transition probabilities are given by the elements of *P*<sup>*n*</sup>. Putting 1 - *p*<sub>12</sub> - *p*<sub>21</sub> = *k*, we have

$$P^n = \frac{1}{p_{12} + p_{21}} \begin{bmatrix} p_{21} + k^n p_{12} & p_{12} (1-k^n) \\ p_{21} (1-k^n) & p_{12} + k^n p_{21} \end{bmatrix} = \begin{bmatrix} \binom{n}{1} & \binom{n}{2} \\ p_{11} & p_{12} \\ \binom{n}{2} & \binom{n}{1} \\ p_{21} & p_{22} \end{bmatrix} \tag{7}$$

Then  

$$\Pr(\text{HH})_n = ap_{11}^{(n)} = \frac{p_{21}(p_{21} + k^n p_{12})}{(p_{12} + p_{21})^2} \quad (8)$$

$$\Pr(\text{MM})_n = bp_{22}^{(n)} = \frac{p_{12}(p_{12} + k^n p_{21})}{(p_{12} + p_{21})^2} \quad (9)$$

$$\Pr(\text{HM})_n = ap_{12}^{(n)} + bp_{21}^{(n)} = \frac{2p_{12}p_{21}(1-k^n)}{(p_{12} + p_{21})^2} \quad (10)$$

Once the two parameters,  $p_{12}$  and  $p_{21}$ , are known it is possible to calculate the probabilities of the events, HH, HM and MM for any quadrat pair whose length is an integral multiple of the shortest pair.

*Estimation of  $p_{12}$  and  $p_{21}$*

Using the method of maximum likelihood, Pielou developed a set of equations for the estimation of  $p_{12}$  and  $p_{21}$ . To solve these equations for  $p_{12}$  and  $p_{21}$  a computer program was written in Fortran IV using Newton's method of solving systems of non-linear equations (McCalla, 1967).

Let  

$$f(p_{12}, p_{21}) = \frac{\partial \log L}{\partial p_{12}} \quad (11)$$

$$g(p_{12}, p_{21}) = \frac{\partial \log L}{\partial p_{21}} \quad (12)$$

then  

$$f'_{12} = \frac{\partial f(p_{12}, p_{21})}{\partial p_{12}} = \frac{\partial^2 \log L}{\partial p_{12}^2} \quad (13)$$

$$g'_{21} = \frac{\partial g(p_{12}, p_{21})}{\partial p_{21}} = \frac{\partial^2 \log L}{\partial p_{21}^2} \quad (14)$$

and  

$$f'_{21} = g'_{12} = \frac{\partial^2 f(p_{12}, p_{21})}{\partial p_{21} \partial p_{12}} = \frac{\partial^2 g(p_{12}, p_{21})}{\partial p_{12} \partial p_{21}} \quad (15)$$

$$\frac{\partial^2 \log L}{\partial p_{12} \partial p_{21}}$$

as first approximations of  $p_{12}$  and  $p_{21}$  let

$$p_{12,0} = \frac{n_2}{(2n_1 + n_2)} \text{ and } p_{21,0} = \frac{n_2}{(2n_3 + n_2)} \quad (16)$$

These are the estimates obtained by equating to expectation the observed frequencies using the shortest quadrat pair ( $d_1 = 2r$ ).

Expanding  $f$  and  $g$  in a Taylor series about  $(p_{12,0}, p_{21,0})$  we have

$$f(p_{12}, p_{21}) = f(p_{12,0}, p_{21,0}) + f'_{12}(p_{12,0}, p_{21,0})$$

$$(p_{12} - p_{12,0}) + f'_{21}(p_{12,0}, p_{21,0})(p_{21} - p_{21,0}) + \dots \quad (17)$$

$$g(p_{12}, p_{21}) = g(p_{12,0}, p_{21,0}) + g'_{12}(p_{12,0}, p_{21,0})(p_{12} - p_{12,0}) + g'_{21}(p_{12,0}, p_{21,0})(p_{21} - p_{21,0}) + \dots \quad (18)$$

We want to find  $(p_{12}, p_{21})$  such that  $f(p_{12}, p_{21}) = g(p_{12}, p_{21}) = 0$ . Using only linear terms of the above series expansion and setting them to zero will give us an approximate solution.

Let  $\Delta p_{12} = (p_{12} - p_{12,0})$  and  $\Delta p_{21} = (p_{21} - p_{21,0})$  (19)

and  

$$0 = f(p_{12,0}, p_{21,0}) + f'_{12}(p_{12,0}, p_{21,0})\Delta p_{12} + f'_{21}(p_{12,0}, p_{21,0})\Delta p_{21} \quad (20)$$

$$0 = g(p_{12,0}, p_{21,0}) + g'_{12}(p_{12,0}, p_{21,0})\Delta p_{12} + g'_{21}(p_{12,0}, p_{21,0})\Delta p_{21} \quad (21)$$

The solution to this system of linear equations, using Cramer's rule is found to be:

$$\Delta p_{12} = \frac{gf'_{21} - fg'_{21}}{f'_{12}g'_{21} - f'_{21}g'_{12}} (p_{12,0}, p_{21,0}) \quad (22)$$

$$\Delta p_{21} = \frac{fg'_{12} - gf'_{12}}{f'_{12}g'_{21} - f'_{21}g'_{12}} (p_{21,0}, p_{21,0}) \quad (23)$$

If  $p_{12,1} = p_{12,0} + \Delta p_{12}$  and  $p_{21,1} = p_{21,0} + \Delta p_{21}$  (24)

then  $(p_{12,1}, p_{21,1})$  is an approximation of  $(p_{12}, p_{21})$ . Using  $(p_{12,1}, p_{21,1})$  as the new approximation the procedure was repeated. Four iterations were necessary to obtain six place accuracy.

To obtain the variances of  $\hat{p}_{12}$  and  $\hat{p}_{21}$  letting  $p_{12} = \hat{p}_{12}$  and  $p_{21} = \hat{p}_{21}$

$$\text{Var}(\hat{p}_{12}) = \left[ \frac{\partial^2 \log L}{\partial p_{12}^2} \right]^{-1} \quad (25)$$

and similarly for  $\text{Var}(\hat{p}_{21})$  (Kendall and Stewart, 1967).

Pielou's assumptions in developing this two state discrete Markov Process were:

1) The probability of a hit or miss with one quadrat depends only on the result with the other quadrat.

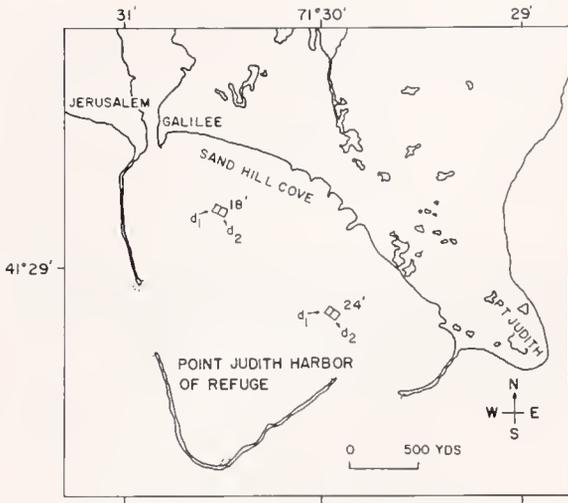


FIG. 1. Harbor of Refuge, Point Judith, Rhode Island.

Area 1, 18' depth at mean low water

$d_1 = 2r$  area sampled with circles tangent

$d_2 = 4r$  area sampled with circles separated by one diameter

Area 2, 24' depth at mean low water

$d_1 = 2r$  area sampled with circles tangent

$d_2 = 4r$  area sampled with circles separated by one diameter

2) These probabilities are constant throughout the area sampled.

If the observed frequencies of hits and misses show these assumptions to be justified, the pattern may be regarded as being random.

Sampling Methods

In the first area sampled the depth at mean low water was approximately 18 ft (Fig. 1) and the bottom was composed of fine sand. *Spisula* size in this area ranged from 5.5 - 6.8 in., this measurement being the maximum diameter across the shell. Nearest neighbor distances within a patch varied from 10-14 in. Using the radius  $r$  established from empirical observations as a basis, circular quadrats were constructed of heavy gauge iron wire each having radius  $r$ . One pair of quadrats was attached tangent to each other and a second pair separated by one diameter. Grid patterns 150 x 100 ft were constructed on the bottom using two foot sections of iron reinforcing rod and heavy fishing twine. The grid interval was set at 10 ft, the maximum distance across the largest quadrat pair. A total of 300 samples were taken, 150 with  $d_1 = 2r$  and 150 with  $d_2 = 4r$ . The individual

samples were independent of each other. Each grid was sampled by dropping a quadrat pair at random within the 10 x 10 ft area and digging within each circle with hand rakes to determine the presence (H = Hit) or absence (M = Miss) of a clam.

Complete rather than random sampling was used to minimize the basic problems encountered by the scuba divers during the sampling. Visibility was never more than 20 ft and a great deal of the time was less than 10 ft. The poor visibility and dulled mental state of the divers made the problem of random sampling extremely time consuming. Complete sampling actually consumed less time than random sampling would have.

The second area chosen was in a depth of 24 ft at mean low water. The bottom was made up of a silt-sand mixture. *Spisula* in this area varied from 4.5-6 in. in largest diameter. Nearest neighbor distances varied from 4-10 in. Grid patterns were set up and the sampling was carried out in the same manner as in the first area.

RESULTS AND CONCLUSIONS

The following estimates for  $p_{1,2}$  and  $p_{2,1}$  and their variances were computed for each area.

Estimates for the first area were calculated such that

$$\begin{aligned} \hat{p}_{1,2} &= 0.498579 & \text{var}(\hat{p}_{1,2}) &= 0.0014182 \\ \hat{p}_{2,1} &= 0.382237 & \text{var}(\hat{p}_{2,1}) &= 0.0009293 \end{aligned}$$

which gives a matrix of transition probabilities

		Second Quadrat	
		H	M
P =	First	H	M
	Quadrat	0.50142	0.498579
		M	0.617763

Estimates for the second area were calculated such that

$$\begin{aligned} \hat{p}_{1,2} &= 0.324245 & \text{var}(\hat{p}_{1,2}) &= 0.0006641 \\ \hat{p}_{2,1} &= 0.652231 & \text{var}(\hat{p}_{2,1}) &= 0.0021517 \end{aligned}$$

giving us a matrix of transition probabilities

		Second Quadrat	
		H	M
P =	First	H	M
	Quadrat	0.675755	0.324245
		M	0.347769

Using the maximum likelihood estimate

$$a = \frac{1}{N} \sum (F_{HH} + (1/2) F_{HM}) \tag{26}$$

TABLE 1. Fit of the theoretical distribution to observations.

		AREA 1	
Length of Quadrat		Frequency	
Pair $d_1 = 2ir$	Event	Observed	Expected
$d_1$	HH	35	32.639
	HM	67	64.909
	MM	48	52.452

$d_2$	HH	29	28.771
	HM	66	72.645
	MM	55	48.584

$x^2 = 2.07297 < F(x^2, .70, 2) = 2.41$

		AREA 2	
Length of Quadrat		Frequency	
Pair $d_1 = 2ir$	Event	Observed	Expected
$d_1$	HH	75	67.705
	HM	61	64.973
	MM	14	17.322

$d_2$	HH	54	66.941
	HM	82	66.502
	MM	14	16.502

$x^2 = 8.1747 < f(x^2, .99, 2) = 9.21$

where  $F_{HH}$  and  $F_{HM}$  are the observed frequencies of the events HH and HM and N is the size of the total sample, the proportion of the total area a, occupied by patches may be calculated.

For the first area sampled, again using the computer program developed for this problem

$\hat{a} = .43500$

and for the second area

$\hat{a} = .66833$

A (Pearson's  $X^2$ ) goodness of fit test (Fisz, 1963) was used to test the validity of assuming randomness for the patches of surf clams in each area (Table 1).

A good fit of the observed to expected frequencies was obtained for Area 1. For the second area the hypothesis of randomness is acceptable at the  $\phi = .01$  level.

Pielou (1964) described a method for determining the mean patch and gap size for a pattern which is

assumed to be random.

Denoting the matrix of transition rates by R where

$$R = \begin{bmatrix} -\lambda_p & \lambda_p \\ \lambda_g & -\lambda_g \end{bmatrix} \tag{27}$$

we have

$R = \ln P$  (28)

whence

$$\lambda_p = \frac{-p_{12}}{p_{12} + p_{21}} \ln(p_{11} \cdot p_{21}) \tag{29}$$

and

$$\lambda_g = \frac{-p_{21}}{p_{12} + p_{21}} \ln(p_{11} \cdot p_{21}) \tag{30}$$

The length of the intervals along a random line transect that lie in patches or gaps are exponentially distributed with mean  $1/\lambda_p$  or  $1/\lambda_g$  where the unit is the length of the shortest quadrat pair used in estimating the transition probabilities of P.

For the first area a patch size  $1/\lambda_p = 33.222$  in. and a gap size  $1/\lambda_g = 43.334$  in. were computed. Computations for Area 2 yielded a patch size of  $1/\lambda_p = 32.126$  in. which is very close to patch size for Area 1 and a gap size  $1/\lambda_g = 15.971$  in. Noticing that the computed mean gap size for the second area is not much larger than the nearest neighbor distance between individual clams indicating a tendency toward complete aggregation.

From the assumptions and methods used in this experiment it may be inferred that the distribution of patches of the surf clam, *S. solidissima solidissima*, is density dependent. That is, areas of high density tend toward complete aggregation and areas of medium to low density are composed of discrete randomly distributed patches. The observed differences in the size range of the clams in each area apparently had little effect on patch size. This implies that the second area having smaller clams and less distance between nearest neighbors would have more clams per patch. The determination of this implication would involve within patch distributions.

The techniques developed in this experiment coupled with a dredging technique to assess density in an area could be used effectively in making population assessments. It could also be used in determining the effectiveness of dredges and dredging techniques by sampling before and after an area has been dredged.

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## ASSOCIATION AFFAIRS

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### ANNUAL CONVENTION

The 64th annual meeting of the National Shellfisheries Association and the Shellfish Institute of North America was held jointly 25-29 June 1972 at the Williamsburg Logde, Williamsburg, Virginia.

Officers and Executive Committee members elected for 1972-1973 were:

President . . . . .	R. Winston Menzel
President-Elect . . . . .	Ronald Westley
Vice-President . . . . .	Dexter Haven
Secretary-Treasurer . . . . .	Michael Castagna
Member-at-large . . . . .	Herbert Hidu
Editors of the Proceedings . . . .	William N. Shaw Sara V. Otto

Mr. Darryl J. Christensen, National Marine Fisheries Service, Oxford, Maryland 21654, is custodian of back issues of the Proceedings, and John Ropes is archivist.

A change in dues from six to eight dollars per year was passed effective January 1973.

An amendment to the constitution was passed to include a President-Elect instead of two Vice-Presidents. It was moved that Vol. 63 be dedicated to Dr. Imai (deceased) in recognition of his contribution to shellfish biology. A resolution was passed in recognition of Mrs. Haynie's work as Secretary-Treasurer of the organization.

Twenty-nine new members were accepted making a total of 349 general members, 7 honorary members and 3 life members as of May 1st.

The Pacific Coast Section of NSA and the Pacific Coast Oyster Growers Association met August 18-19 at the Evergreen Inn, Olympia, Washington. New officers are:

Chairman . . . . .	Herb Tegelberg
Vice-Chairman . . . . .	Robert Herrmann
Secretary-Treasurer . . . . .	Gerald Lukas

Section dues were reduced from \$2.00 to \$1.00 per year.







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