1965 PROCEEDINGS

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HONORED GUESTS

Honored guests, Dr. and Mrs. Paul Galtsoff, in conversation with keynote speaker, Mr. James Engle, at right.

Dr. and Mrs. Paul S. Galtsoff were the honored guests of the National Shellfisheries Association and the Oyster Growers and Dealers Association throughout the entire Convention. Under Secretary of the Interior John A. Carver, Jr. prefaced his luncheon speech before the 57th joint convention (see article, p. 9) with these remarks:

> A distinguished American scientist honors all of us with his presence today. For the last ten years of his active professional service with the Department of the Interior, Dr. Paul Galtsoff was a member of a small corps of senior scientists in the Bureau of Commercial Fisheries. These men are permitted to relinquish their administrative responsibilities as laboratory directors, or administrators, and concentrate their attention upon the compilation and publication of the results of their research and experience, to advise and consult within the Bureau and with States and universities, and generally to do the kind of thing represented by Dr. Galtsoff's recent book.

Mr. James B. Engle, chief of the Shellfish Advisory Service, U. S. Bureau of Commercial Fisheries, delivered a major address at the opening of the 57th joint session (see article, p. 13) -ed.

PROCEEDINGS OF THE NATIONAL SHELLFISHERIES ASSOCIATION

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ABSTRACTS OF TECHNICAL PAPERS PRESENTED AT THE 1965 NSA CONVENTION

DERMOCYSTIDIUM IN TRAY POPULATIONS OF OYSTERS IN DELAWARE BAY

W. J. Canzonier

Department of Oyster Culture
Rutgers University, New Jersey

The incidence of *Dermocystidium* and mortalities attributable to this agent have been closely followed in experimental tray populations of oysters on the Cape Shore of Delaware Bay over the past six years.

The remnants of old stocks (1958-1960 imports) that had suffered heavy *Dermocystidium* mortalities, though maintaining a high incidence, exhibited reduced mortality in recent years. Two stocks, laboratory spawned progeny of previously selected parents, appear to have inherited some degree of resistance. They have relatively low mortalities during periods of *Dermocystidium* kill in other stocks — and incidence, at least in the 1961 year class, was still negligible as of October 1964.

The effects of proximity in the infection of newly introduced stocks have been most striking. Isolation of recent years' imports has been somewhat effective in reducing *Dermocystidium* mortalities, at least for the first year in trays.

SALINITY TOLERANCE LIMITS OF SOME SPECIES OF PELECYPODS FROM VIRGINIA

Michael Castagna and Paul Chanley

Virginia Institute of Marine Science
Wachapreague, Virginia

Salinity tolerance limits have been determined experimentally for 24 species of adult pelecypods. Survival and activities such as burrowing, feeding and byssal attachment were used as criteria of adaptation. In two species larval development and nest building were also used. It was demonstrated that most pelecypods studied are more euryhaline than their natural distribution would indicate.

LARVAL DEVELOPMENT OF *RANGIA CUNEATA* AND *LYONSIA HYALINA*

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Larvae of *Rangia cuneata* and *Lyonsia hyalina* were reared from eggs in laboratory cultures. *Rangia cuneata* increased from 75 to 175 μ in length during a 7-day larval life. Height varied from 5 to 20 μ less than length and thickness 45 to 65 μ less than length. The "straight-hinge" line was 55 to 60 μ long. A round inconspicuous umbo appeared when larvae were about 120 to 130 μ long. Both anterior and posterior shoulders were rounded. Swimming larvae had a conspicuous apical flagellum. Neither eye spots nor hinge teeth were observed in larvae.

Lyonsia hyalina larvae measured from 155 to 175 μ in length and from 120 to 130 μ in height. They resembled the elongated "straight-hinge" larval stage of other pelecypods except for an indentation in the hinge line and a dark gray or black opaque appearance. The larval period was brief and larvae metamorphosed without developing an umbo in three days. Adults are functionally hermaphroditic and autofertilized eggs developed into apparently normal larvae.

ON THE STRUCTURE, MODE OF INFECTION, AND FATE OF *TYLOCEPHALUM* IN THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA*

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During a survey for zooparasites of *Crassostrea virginica* collected from West Loch, Pearl Harbor, Honolulu, Hawaii, larvae of the lecanicephaloid cestode *Tylocephalum*, among other parasites, were identified. Whole, living larvae were recovered by subjecting the oysters to digestion for

one hour at 37°C in an aqueous solution containing 1 per cent pepsin and 1 per cent HCL followed by sedimentation. The cestode larvae were resistant to digestion. Histological sections of additional infected oysters revealed encysted and unencysted *Tylocephalum* larvae in several regions of the mollusks' bodies. The largest number was found in the zone immediately beneath the epithelial lining of the stomach and intestine followed by the gill matrices. Occasional larvae were also found in Leydig tissue in the digestive gland where they were situated between the digestive diverticula, and in between the diverticular cells.

In addition to finding larvae in oyster tissues, the ciliated larva (coracidium) of *Tylocephalum* was found intimately associated with gill surfaces and in the stomach. The ciliated larva, which is the precursor of the tissue form, possesses penetration glands, a complete ciliated epithelial surface, and several types of cells comprising the parenchyma. These larvae are the infective form and enter the oyster by penetration.

When ciliated larvae shed their surface epithelia and penetrate through the oyster's alimentary wall, the majority become encapsulated by connective tissue fibers underlying the lining epithelium; however, some do succeed in infiltrating deeper. When histologically discrete, recently established larvae were found in the deeper tissues; no appreciable host cellular and connective tissue reactions occurred. However, in time, heavy aggregates of leucocytes and a thick connective tissue capsule surround each larva followed by decomposition and resorption of the parasite.

It is concluded tentatively that *Crassostrea virginica* is not the natural molluscan host of *Tylocephalum* since destruction of the latter occurs. The complete life cycle of *Tylocephalum* remains unknown.

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PERIVASCULAR LEUCOCYTOSIS AND OTHER TYPES OF CELLULAR REACTION IN *CRASSOSTREA VIRGINICA* (GMELIN) EXPERIMENTALLY INFECTED WITH THE METASTRONGYLID NEMATODE *ANGIOSTRONGYLUS CANTONENSIS* (CHEN)

Thomas C. Cheng

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U.S. Public Health Service
Narragansett, Rhode Island [1]

In an earlier paper (Cheng and Burton, 1965. *J. Parasitol.* 51:296) it was reported that both the American oyster, *Crassostrea virginica,* and the quahaug, *Mercenaria mercenaria,* were suitable experimental intermediate hosts of the metastrongylid nematode, *Angiostrongylus cantonensis,* the causative agent of eosinophilic meningo-encephalitis in man in parts of Asia and the Pacific Basin. In experiments designed to study the effect of the larvae of *A. cantonensis* on the experimental oyster host and to study the reaction of the oyster to this parasite, it was found that a certain number of the first-stage larvae of *A. cantonensis* will readily invade oysters by penetrating the stomach wall and will eventually develop to the third-stage which is infective to susceptible mammals.

Within the oyster's body, the nematode larvae can be distributed via blood vessels. During the intravascular phase, a characteristic histopathological syndrome, termed perivascular leucocytosis, is evident. The condition is characterized by the aggregation of large numbers of the host's leucocytes around blood vessel walls. This condition suggests the attraction of the host's leucocytes to some substance elicited by the parasite. The molecular size of the "leucocyte attracting substance (LAS)" must permit it to permeate the blood vessel wall. LAS may be in the form of the nematode's molting fluid.

The larvae of *A. cantonensis* are not encapsulated in the oyster's tissues; rather, they are motile and, as a result, cause lesions, especially in the Leydig tissue. Leucocytic response to motile larvae is apparent although destruction of the parasite does not appear to occur.

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THE FATE OF A VIRUS, *STAPHYLOCOCCUS AUREUS* PHAGE 80, INJECTED INTO THE OYSTER

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A virus, *Staphylococcus aureus* phage 80, was intracardially injected into the oyster, *Crassostrea virginica,* held at various temperatures to study (1) the fate of the virus particles in the oyster, (2) the response of the oyster to virus particles, and (3) the effect of temperature on the shellfish-virus model. The phage population within the oyster decreased with time following injection and the decline was faster at higher temperatures than at lower temperatures. Leucocytes pinocytosed only a small number of the phage particles. A significant portion of the phage lost was recovered from shell liquor, rejecta, dejecta, and the sea

water surrounding the injected oysters.

The fate of phage particles was also studied *in vitro* in sea water and in oyster blood plasma. The plasma from both normal and previously phage-injected ("immune") oysters was tested. At 5° and 15°C, both normal and "immune" oyster plasma showed little or no effect on the phage. At 25°C, phage was inactivated in normal oyster plasma inoculated with a low concentration of phage (3.34 log PFU/ml) and also in "immune" oyster plasma.

BIOLOGICAL ASPECTS OF HARD CLAM PURIFICATION

S. Y. Feng

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Conditions under which hard clams are most active in the laboratory were studied as a key to conditions under which clams will purify themselves most effectively. Clam activity is affected by the ambient temperature and salinity and may be increased by presence of various organic materials; e.g., glucose. A definite diurnal cycle of clam activity appeared in the laboratory populations. It appears that by light manipulation a higher percentage of active clams may be obtained. In all studies some clams were inactive for several days. These recalcitrants, if polluted, could seriously affect a purification procedure. At lower temperatures (5°C), clams are more likely to retain viable viral particles due to their inactivity and protective environment. Viruses are not completely eliminated nor destroyed by clams when sea water is changed repeatedly and/or treated with ultraviolet light.

SOME STUDIES ON PHYSIOLOGICAL VARIATION AMONG POPULATIONS OF THE OYSTER DRILL, *UROSALPINX CINEREA*

David R. Franz

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During the past two years, laboratory investigations have been in progress to determine the nature and extent of physiological variations among populations of *Urosalpinx cinerea* from Long Island Sound, Delaware Bay, the Eastern Shore of Virginia and Bogue Sound, North Carolina.

When acclimated for long periods of time to cold water (6-10°C) none of the populations shows sustained activity below 6°. At 3°C, the populations do not differ significantly in their mobility or ability to attach to the substrate. At 6°, Eastern Shore and L.I. drills are more mobile than the N. J. and N. C. drills. However, only the Eastern Shore population differs significantly from the others with regard to its rate of activity at this temperature.

All populations begin drilling at temperatures between 10 and 11°C. At 16.5°, the feeding rates of all of the populations are similar although the Eastern Shore drills show indications of a higher rate which becomes quite pronounced at higher temperatures (20 and 24°C). At these temperatures, the differences between the N. C., N. J. and L. I. populations are probably not significant.

At 16.5°C, the Eastern Shore drills fail to lay eggs and the L. I. drills show a considerably greater fecundity than either the N. J. or N. C. populations. At the higher temperatures, however, the fecundity of the Eastern Shore population approaches that of the L. I. snails. Although the Eastern Shore and L. I. drills are quite similar in fecundity at higher temperatures, they differ in that the Eastern Shore snails produce more egg cases per clutch.

DISPERSION IN A SUBTIDAL *MYA ARENARIA* (LINNAEUS) POPULATION

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GENERAL DYNAMICS/ELECTRIC BOAT Groton, Connecticut

Areal dispersion patterns are described on the basis of three separate surveys of the soft-shell clam population in a shallow salt pond in Rhode Island.

A census of juvenile clams in Green Hill Pond, Rhode Island, yielded members from the whole range of sediment types and from all depths. A general dispersal of clams throughout the pond is indicated from the few zero counts obtained (12 per cent of sample) and the value of the Moyle and Lound index of dispersion (0.19). The density of juveniles in deeper areas is appreciably greater than in the shallow regions. This condition may result from hydrologic processes which concentrate veliger larvae in the deeper zone.

A census of the adult clam population showed a marked difference in distribution from that of juveniles. The adults are contiguously distributed as indicated by the goodness-of-fit of the negative binomial distribution and the high zero count (50 per cent of sample). Also, an inverse relationship was noted in the depth distribution of the two groups. The deeper portions of the pond, representing about one-half the total area, contained high concentrations of juveniles but were barren of adults.

The scarcity of adults in deeper areas was attributed to unsuitable substrates. Adults are not present in sediments for which the levels of organic carbon, total nitrogen, and median diameter of particle sizes are, respectively, >1.0 per cent, >0.1 per cent, and <0.1 mm. Furthermore, clams are almost always present when these threshold levels are not violated. (Only 4 barren stations from a total of 47 stations with suitable substrates.) The harmful effects from this sediment type may be due to the suspension of silt and sedimentary organic matter during autumn storms. A condition of low oxygen concentration and high turbidity would ensue which may be sufficiently adverse to cause mass mortality.

The three significant variables, median diameter, organic carbon, and total nitrogen, may serve the need for simple tests to determine the suitability of bottom areas for use in clam transplantation programs. The high correlation noted between organic carbon and total nitrogen for all sediment types means only one variable need be measured and the other estimated from the regression equation.

The distribution of adult soft-shell clams in densely populated sub-areas of the pond is in aggregations. The pattern was found to consist of clusters which decrease in areal expanse and, consequently, in numbers of individuals, as the size of clams increases. The distribution of members within clusters is random indicating biological suitability of the substrates. Consequently, the gradual diminution in size of clusters may indicate a disturbance of outside origin. A stochastic process involving active predation on the clam population is formulated to account for the patterns noted.

The dispersion in an intertidal population was found to be similar to that of the subtidal population. The influence of hydrology, substrate properties, and predation, may have a marked influence on this result.

A management program of transplantation and suspension culture is recommended for the local fishery. The intention is to overcome the natural limitations of the environment for propagation of the species as noted from this study of distribution and pattern.

RECENT OBSERVATIONS ON "MSX" IN DELAWARE BAY

Harold H. Haskin

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In 1964, after a three-year period of reduced intensity of "MSX" kills in Delaware Bay, experimentally introduced susceptible stocks followed the patterns and the high intensity of deaths characteristic of "MSX" in earlier years. Native Delaware Bay stocks, though carrying high levels of "MSX" infection, are showing very low mortality rates in comparison with imported susceptibles. This is interpreted as further evidence for the existence of a relatively "MSX"-resistant oyster stock in Delaware Bay.

SPAWNING AND REARING OF DELAWARE BAY STOCKS OF *CRASSOSTREA VIRGINICA*

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An artificial-rearing program was conducted during the summer of 1964 with the objectives of obtaining progeny of "MSX" resistant stocks and of developing a hatchery-rearing procedure most applicable to Delaware Bay conditions.

Oysters were spawned successfully after several weeks of conditioning at 28-30°C. Also, the summer spawning period was prolonged by holding oysters below 24°C throughout June and July. Spawning reactions of Delaware Bay oysters appeared to be somewhat different from those of Long Island Sound oysters. Several hours of running unfiltered 30°C water plus addition of stripped spawn were necessary to produce successful spawning. Larvae were reared successfully using both unialgal cultures and natural food in unmodified Delaware Bay water. All spat were reared on flowing unmodified Delaware Bay water. Spatfall of four Delaware Bay stocks and a Long Island Sound control stock were obtained on approximately 2,000 *Spisula* shells during the summer.

INFESTATION OF THE HARD CLAM, *MERCENARIA MERCENARIA*, BY THE BORING POLYCHAETE WORM, *POLYDORA CILIATA*

Warren S. Landers

Bureau of Commercial Fisheries Biological Laboratory Milford, Connecticut

The accidental infestation of juvenile *M. mercenaria* by *P. ciliata* in the absence of a sand substrate in laboratory trays is described. The extent of boring by this worm and the resulting damage to the shells suggest that this activity could contribute to mortalities of clams.

Experimental infestation of clams out of the

substrate was successful using larvae from adult worms of the original infestation. At sea water temperatures of 20°-22°C, newly hatched larvae introduced into trays containing clams continued their pelagic development and metamorphosed in 14 to 20 days. At metamorphosis many worms settled on the clams and constructed tubes. In clams ranging from 5-8 mm long, perforation of the shell occurred within 18 to 20 days. In clams 30-35 mm long, perforation took approximately twice as long. Although all parts of the shell were perforated, there was a tendency for the umbone region to be especially vulnerable, probably because the hinge joint provides a convenient depression in which the worm can attach. The internal mud blisters, typical of *Polydora* infestation, were present within 30 days after perforation of the shell.

Despite an indication that mortality was slightly higher in the experimentally infested clams of each size range than in the controls, chi square analysis failed to show a significantly higher death rate.

Clams in a sand substrate were not invaded by larvae of *P. ciliata* introduced into the water above the substrate but several clams that failed to bury themselves for some time after the introduction of the larvae became infested. The worms disappeared and further shell damage ceased when the clams eventually burrowed in.

Under natural conditions *P. ciliata* is apparently not an enemy of clams because of the clams' subsurface location. However, under hatchery conditions where post-set clams may be reared for a time in the absence of a substrate into which they can dig, *P. ciliata* may become a serious pest.

PROBLEMS IN IDENTIFICATION OF BIVALVE LARVAE

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Criteria used in recognition of bivalve larvae are considered and evaluated. The relative merits of identification and description by culture of spawned and plankton-collected larvae are discussed. Problems of identification that arise when larvae of several species of the same genus occur simultaneously in the water are considered. The importance of accurate identification of larvae in plankton samples and the practical value of this capability are stressed.

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STUDIES ON OYSTER SERUM

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Delaware Bay oysters were bled either from the heart or the muscle sinus. The blood was pooled, freed of leucocytes, and analysed for protein and amino acids. Protein levels were determined by Kjeldahl analysis. The mean serum protein concentration for 158 oysters was 17.67 mg/ml.

Two uncommon amino compounds were found free in oyster serum in the highest quantities: taurine (0.226 μM/ml) and beta-alanine (0.120 μM/ml). Most unexpected was the appearance of taurine at a level of 0.025 μM/g on the chromatogram of an acid hydrolysate of OSP (oyster serum protein). To our knowledge, there is but one published account of taurine as a protein substituent (*Chem. Abst.* **51**, 1957, 8876a). OSP is rich in histidine, asparatic acid and glycine; also present are phenylalanine and tyrosine, responsible for the marked UV absorption of OSP noticed in this laboratory.

Ultracentrifugation of serum dialysed against phosphate buffer revealed one rapidly sedimenting component present in high concentration, another slower component of considerably lower concentration, and a possible third component of intermediate velocity. The sedimentation constant (s) at 20°C and 44,770 rpm of the major component was 31.40 S (Svedbergs); for the minor component, 19.55 S. From these high *s* values, it is apparent that OSP consists of some very large molecules.

SOME HISTOCHEMICAL OBSERVATIONS ON "MSX"

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After fixation in 10 per cent formalin sea water, "MSX" shows a consistent pattern of Feulgen-positive structures. Two types of Feulgen-positive material were noted — each type undergoing a regular series of changes that are associated with the development of plasmodia.

One type of Feulgen-positive material is located in nuclei. The structure, size, and arrangement of this material goes through a regular series of changes. This series of changes apparently can go through more than one cycle without an accompanying cytoplasmic division.

The second type of Feulgen-positive material

found in "MSX" is one or more irregular shaped masses in the cytoplasm. This material also undergoes a regular series of changes, but the function, if any, of this material is not at all clear.

When fixed in 10 per cent formalin sea water, the "MSX" nuclei appear to be localized into a central compact mass. This is indicated from two separate histochemical techniques: (1) After staining with Giemsa the nuclei of many plasmodia appear to be surrounded by a strongly eosinophilic granular network. It is suggested that this eosinophilic network is protamine. (2) The only PA/S positive material found in "MSX" is a granular material that surrounds the central nuclear complex.

Both of these techniques indicate that "MSX" nuclei, in many plasmodia, are not distributed in a random fashion in the cytoplasm but are massed in the center of the plasmodium.

THE DISTRIBUTION OF "MSX" IN MIDDLE CHESAPEAKE BAY

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Within the past two years the oyster microparasite "MSX" has penetrated farther into Chesapeake Bay, at least as far north as Broad Creek on the eastern shore and Cedar Point Hollow on the western shore. Furthermore, within the past year an increase in the incidence of the parasite has been observed in areas where it has previously been found. Although mortalities as determined by fresh box-gaper counts have not been observed to be unusually high in the areas sampled, mass mortalities have been reported in other areas.

GILL TISSUE RESPIRATION AS A FUNCTION OF SALINITY AND TEMPERATURE IN FOUR SPECIES OF PELECYPODS

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Measurements have been made of oxygen consumption of excised gill tissue from four species of bivalves. The organisms used were *Mercenaria mercenaria, Modiolus demissus, Mytilus edulis,* and *Crassostrea virginica.* These four were chosen because of the differences in distribution of the adults with respect to salinity.

Results to date using winter acclimated animals indicate that the rate of oxygen consumption (Qo_2) is independent of salinity over the range 5-30 ppt for gill tissue from *Mytilus, Modiolus,* and *Crassostrea.* This is true at 10, 18, and 26°C. The Qo_2 of *Mercenaria* gill tissue, on the other hand, is significantly higher at the lower salinities. In addition this increase appears to be temperature dependent in that it is greater at lower temperatures.

NSA PACIFIC COAST SECTION

THE CULTURING OF *GONYAULAX* SP. AND THE ACCOMPANYING PROBLEMS INHERENT WITH THIS ORGANISM

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Kenneth K. Chew and Benny C. C. Hsu

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Washington

Samples of *Gonyaulax* sp. were collected from Sequim Bay, Washington during the summer of 1965 and were cultured in the laboratory. Unialgal cultures of *Gonyaulax* were obtained by serial washing of these cells with the use of a micromanipulator. The *Gonyaulax* found in the field samples were generally in two, three, and four cell chains. However, in the laboratory two, three, four, six and eight cell chains were common at the beginning of the growth phase. Ten and 16 cell chains were observed, but not commonly.

Although an increase in the number of cells in chains was generally thought to indicate reproduction, it was observed that two cell chains joined to make four cell chains and four cell chains joined to make eight cell chains. Dimension of these cells varied (15-25 μ; 25-45 μ). The smaller cells were observed to be those of recent divisions. Cultures past the logarithmic growth phase were larger with a deeper coloration.

Five separate groups containing 20-50 cells each were put in depression slides with enriched sea water containing soil extract, $NaNO_3$ and $NaNO_4$. These cells were then later transferred to tubes containing 10 ml of medium. *Gonyaulax* was found to be extremely sensitive to mechanical shocks of any type.

Of the five groups originally isolated, when using the same batch of medium, only two groups

showed rapid growth suggesting clonal differences within the population in Sequim Bay. Concentrations of 1,500 cells were observed in 21 days when starting with 50 cells.

One tube containing approximately 15,000 cells of *Gonyaulax* sp. per 10 ml of medium was processed using the standard method for extraction of paralytic shellfish toxin. Using the standard method for mouse bioassay of this toxin, the extract was observed to be definitely toxic.

THE PACIFIC OYSTER AND CERTAIN ENVIRONMENTAL CONDITIONS IN GRAYS HARBOR

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Weyerhaeuser Company, Longview, Washington

A study has been conducted by the Weyerhaeuser Company since 1963 in Grays Harbor to define the environmental factors responsible for the condition, growth, and mortality of Pacific oysters on two fattening areas in North Bay. Oysters are sampled off the grounds to determine meat composition at bi-weekly intervals; shell growth and animal deaths are determined from special groups of oysters on trays. The estuarine waters at the stations are sampled at weekly intervals through a 6 to 8 hour period. Samples are analyzed for routine quality measures, phytoplankton, nutrients, and related materials. Features irregularly studied at the stations include water velocity and the composition of the substrate. Continuous records of certain climatological and hydrological factors are also maintained.

Findings thus far indicate that food supply may be the primary limiting factor to the condition and growth of the oysters at the stations. Nutrient supply (as formerly measured) was less precisely related to the oyster condition. Extreme reductions in salinity and temperature during the winter and excessive amounts of suspended materials at various times throughout the year were found to have a negative effect on oyster condition and, in extreme cases, survival.

ACCUMULATION AND ELIMINATION OF A BACTERIOPHAGE BY THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*

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PHS Shellfish Sanitation Laboratory, Washington

A bacteriophage is being used as a model virus in further studies of the accumulation and elimination of particles smaller than bacteria by shellfish. Examination of feces of oysters exposed to phage pollution showed that the phage is taken in by the oysters and survives passage through the digestive tract.

By the use of high pollution levels and a more sensitive method of phage enumeration than previously used, the elimination of phage was studied in detail. Usually 99 per cent reduction of phage concentration in oyster meats occurred within one day. Very low concentration continued to be detectable for 10 to 15 days in some instances.

SHELLFISH TOXICITY STUDIES IN SOUTHEASTERN ALASKA

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College of Fisheries, University of Washington Washington

Samples of butter clams, *Saxidomus giganteus* (Deshayes), little-neck clams, *Protothaca staminea* (Conrad), and bay mussels, *Mytilus edulis* Linnaeus, were taken at regular intervals from five locations near Ketchikan, Alaska between May, 1963 and February, 1965. Tests conducted to determine levels of toxicity in the shellfish indicated considerable differences between stations. Levels of toxicity of little-neck clams and mussels were used as indices of the presence of the toxin producing organism since these shellfish concentrated and released the toxin rapidly. Butter clams released the toxin much more slowly. In general the shellfish concentrated the toxin during May and June, and during August and September, although in specific instances it was concentrated in the months of October through February.

Large samples of butter clams dug at the same time on the same beach were divided into size groups. The larger sizes contained a higher concentration of toxin than the smaller sizes. To compensate for the size differences in the regular samples, the levels of toxicity were adjusted to a standard size for each of the three most toxic beaches.

A group of live butter clams were moved from an area of high toxicity to an area of low toxicity and a similar group was moved from the area of low toxicity to the area of high toxicity. During a period of eight months the clams of high toxicity lost approximately 50 per cent of their toxin while the clams of low toxicity increased slightly in toxicity.

With each periodic sample, a sample of butter clams was taken on which separate tests of toxicity were run for the siphons and the bodies. A study of the percentage of the total toxin in the clams which was located in the siphon indicated that some of the toxin moves from the body of the

clam, where it is first concentrated, into the siphon. An average of 63 per cent of the toxin present in a butter clam is stored in the siphon.

INFECTION IN PACIFIC COAST MOLLUSKS BY THIGMOTRICHID CILIATES

Gilbert B. Pauley [1], Albert K. Sparks
Kenneth K. Chew and Evelyn J. Robbins

College of Fisheries, University of Washington Washington

Numerous mussels, *Mytilus edulis* Linnaeus, in Humboldt Bay, California, have been found infected with large numbers of ciliates, which are found both free in the branchial chambers of the gills and attached to the ciliated epithelium of this organ. Although exact identification of these protozoans has not been made, it appears that there are at least two distinct species of thigmotrichid ciliates infecting *M. edulis*. Both species are characterized by having longer cilia on one side than the other, one or more contractile vacuoles, and a cytoplasm of even-staining texture. The two species observed closely resemble *Ancistrum mytili* (Quennerstedt) and *Ancistrum caudatum* Fenchel, neither of which has been previously recorded from Pacific Coast mollusks.

It appears that ciliates are potential pathogens, capable of infecting a large number of individuals within a shellfish population and causing pathological changes in the hosts, under certain adverse conditions when the bivalves are weakened.

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OYSTER BED CULTIVATION IN WILLAPA BAY

Clyde Sayce

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The standard English harrow and a larger version of it have been used by the Willapa Bay oyster industry to break and scatter clustered oysters during recent years. The effects of these harrows upon oysters have not been investigated. Two experiments were conducted to evaluate their actions on oysters and Experiment No. 1 was completed.

An area having a natural oyster bed was divided into four lanes 60 feet by 200 feet each. These were designated Control and Lanes 1, 2 and 3. A ten weeks harrowing experiment was planned so that one lane, the Control, would not be dragged; one lane would be dragged once; another dragged 3 times at the beginning, middle and end of dragging. The third lane would be dragged once each week for ten weeks. Results of this first experiment are of interest.

1. Oysters were induced to spawn although not all at once. Their condition declined rapidly to a low point and gradually recovered.
2. Undragged Control oysters spawned all at once and their condition dropped to a very low value before a long gradual recovery.
3. Mortality of oysters by dragging was quite low and may be disregarded.
4. Cluster breaking by using a harrow is good and scattering is adequate.
5. Additional benefit is gained because dragged oysters catch more spat than undragged ones.

SOME RESULTS OF A STUDY ON MASS MORTALITIES OF PACIFIC OYSTERS

C. F. Woelke

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An 18 month study of mortality, growth and fatness of four stocks of Pacific oysters cross transplanted between four bays of Puget Sound is described. The measurements made on the oysters and discussed in relation to: temperature, salinity, nutrient salts, chlorophyll and carbon 14; total organic carbon, per cent ash and particle size of the bottom sediments; and growth, mortality and fatness of adjacent commercial oyster plantings. Shortcomings of the experiment and its design are discussed and suggestions for avoiding these problems in the future are made.

Proceedings of the National Shellfisheries Association
Volume 56 — May 1966

SOME CRITICAL PROBLEMS OF THE SHELLFISH INDUSTRY

John A. Carver, Jr. [1]
UNDER SECRETARY OF THE INTERIOR

The Department of the Interior's conservation program is a source of pride. It is also a challenge. It emboldens me to express a few thoughts today which have been distilled from the exciting years of service in the Department under the leadership of its greatest Secretary, Stewart Udall.

For it is the genius of the Secretary's leadership which has brought a common theme of conservation-consciousness to the diverse tasks assigned to our kaleidoscopic department.

Indeed, Stewart Udall has had much to do with a nationally heightened consciousness of conservation.

I am not fully confident of my ability to relate my general theme to your industry. My theme is a double one: one part is that technological inefficienty is not a good tool, for conservation efforts cannot be compartmentalized. The other has to do with man's destruction of the natural environment, primarily through pollution.

I will confess at the outset that some of the statistics shown to me to illustrate your problems set me to thinking about this: U. S. per capita consumption of domestically produced fish is only half what it was 50 years ago; molluscan shellfish are becoming less important, as population since 1880 triples, and oyster harvest declines from 175 million pounds to 50 million.

And then there is the interrelationship between our national concern for clean rivers, and our instruction from the President to clean up the Potomac and keep it clean, to make it a model of conservation.

Harvest of shellfish is directly related to the quality of the water, whether in Puget Sound or Chesapeake Bay or the Potomac Estuary.

For several months now we have had a large group of hydrologists, biologists, engineers, and others, representing the Federal Government, the States, and the local communities, looking at the problems of the Potomac Basin very broadly and deeply. Within this single river system exist all the major problems that affect river basins and estuaries all around our country: supply, water flow, and water pollution; conflicts between those who need water for domestic use, for power, for industrial uses, for fish and wildlife production, and for recreation; and navigational requirements.

With particular reference to the fisheries, the Potomac offers examples of most of our domestic fishery problems. We have important commercial and sport fisheries on the River, both of which have great undeveloped potential. The annual catch of shad was once at least 20 times as great as it is today. The annual oyster harvest has dropped more than 80 per cent in the last hundred years. We think we know how to reverse these trends and to establish once again prosperous fisheries for these valuable resources. The key to rehabilitation of the shad resource is pollution abatement, restoration of historic characteristics of river flow, and passage over dams. The secret of oyster production lies principally in developing informed public opinion and more liberal laws and public policies and establishing adequate administrative machinery to put these enlightened views into action.

Other fishery resources in the Potomac River appear to have resisted man's encroachment more successfully. The relatively new commercial fishery for soft-shell clams is still growing and we do not yet know its full potential. Striped bass have been yielding a fluctuating but increasing catch since the middle 1930's. Blue crab catches have been rising since 1880, also with wide fluctuations from time to time. We do not really know why these resources have continued to yield increasing catches in the face of a steadily increasing fishery. This is not to say that the catch of striped bass and blue crabs will continue to increase. We may be overfishing these resources at the present time. Research and management over an area much broader than the Potomac River itself is indicated,

[1] Remarks of the Honorable John A. Carver, Jr., Under Secretary of the Interior, before a luncheon meeting of the 57th joint annual convention of the Oyster Institute of North America and the National Shellfisheries Association at Baltimore, Maryland, on June 28, 1965.

for these are migratory animals, which move seasonally into Chesapeake Bay and even into the Atlantic Ocean.

Each kind of fish or shellfish has its own optimum yield. If the resource is to be managed for maximum yield, the sport catch and the commercial catch must both be controlled.

How is this done?

Here we enter the jungle. Throughout our country, sport and commercial interests are competitive, and bringing order to the system defies our best efforts. Fish pay no attention to State or national boundaries; regulatory schemes within States are frequently entirely separate, and often at war with each other; time and effort is consumed in internal struggles, when the real enemy is outside. And in the midst of this, we live with the legacy that conservation can be efficiently served by regulating the means of the taking.

As I look over the efforts to conserve shellfish resources, I am struck by the fact that legislation covering this field deals almost exclusively with means and not ends. A law which limits oyster harvesting to admittedly inefficient hand tongs channels the inventive genius of an industry away from concern with the resource to concern with the tools.

These laws stay on the books for many reasons. Some have a vested interest in the status quo. In industries where the competition for innovation is particularly fierce, the small firm is often at a serious handicap. Your industry is largely made up of small independent operators for whom expensive technological change could work substantial hardship.

But do the techniques for oyster growing and harvesting developed in the 19th century serve the conservation objective? The unfavorable competitive position of many American fisheries enterprises in relation to the fishing fleets of other nations is dramatic evidence that outdated technology cannot help the resource *per se*.

We have no laws requiring foresters to cut down trees with axes instead of power saws and move logs with horses instead of crawler tractors — but we require hand tonging and sail power for oyster boats.

Isn't this really a kind of backwards approach to conservation — legislating tools instead of legislating conservation goals? If the problem now is one of over-harvesting shouldn't the harvest be limited directly rather than forcing the oysterman to confine his efforts to a primitive technology?

And to extend the conservation argument, wouldn't it be better to legislate major reseeding and shellfish bed rehabilitation projects instead of continuing to see more and more of the industry go out of business because of resource failures and the competition from foreign suppliers? Is it not time now to press hard for changing the laws and technology of shellfish conservation into the proven principles of sustained yield? It is certainly unlikely that new laws will come into being unless you seek them.

The problem is general. In the wildlife field, for example, the deep-seated psychological and sometimes legal prohibition against hunting does flies squarely in the face of the biological facts of life. Wildlife managers generally agree prohibitions against harvesting by sex begins with a major handicap.

In mining, old laws and tradition call for the digging of prospect holes and the performance of annual assessment work — forcing people to go out and dig useless holes in the ground simply because the law says so. Modern geophysical and geochemical prospecting techniques negate the need for adherence to this century-old ritual.

Irrational reaction to bad conservation practices reaches its height in anadromous fisheries. The rape of the salmon resource in Alaska prior to statehood was a national scandal, from a conservation standpoint quite equivalent to the rape of the virgin forests of the Lake States before the concept of sustained yield took hold.

As to forest management, the reaction was against the operators; as to fisheries management, the reaction was against their tools. In the forests, the operators were required to reforest, to repair damage, to burn slash carefully, to protect the watershed and to yield to recreation requirement. In the fisheries industry, the reaction was against the tools. So the fishtrap, which could be opened or closed, and efficiently patrolled to assure escapement, was replaced with gill nets; and the job of watching to see that no boat, legislatively limited to 50 feet or less in size, moved into a stream to interrupt the spawning fish, is virtually insuperable.

Frustrated by the political power of the fisherman, the law took on the inanimate boats.

Some will say it worked; that the resource is returning. To which I suggest that it is just possible that the industry itself saw the necessity of sustained yield management, and good conservation practices, and that part of the reason lies in this evidence of maturity, not wholly in the wisdom of legislated inefficiency.

As some of you know, the United States and the Japanese are now engaged in a major dispute over fishing practices in the North Pacific. Though the issues are complicated by disagreement over many of the biological facts, its is also a fact that the Japanese fishing fleet is substantially more efficient than ours. One way we obviously cannot

solve this problem is by asking the Japanese to reduce their efficency to the equal of ours. The answer will come when we can agree on conservation objectives and conservation methods which are directed at the preservation of the resource.

We are no less aware of the other side of the conservation issue in which you and your industry are involved.

In the shellfish industry you work with bottoms you do not own. and which you often cannot even lease.

You work in an environment of water whose quality and quantity you cannot control and over which your influence is often very slight.

You and your product are at the mercy of upstream soil practices which may silt over your producing grounds or so pollute the environment as to prevent or destroy a shellfish crop.

Pesticides used in the saltwater marshes to control mosquitoes may so contaminate your product as to make it unmarketable. Chemicals used hundreds of miles away may later also prevent or spoil your crop. The significance of the relationship between pesticides and shellfish has been dramatically underscored by the results of recent research which indicate the almost fantastic degree to which shellfish are susceptible to the action of certain pesticides.

. . . . DDT concentrations of 1 part-per-billion will kill blue crabs in 8 days.

. . . . Commercial brown and pink shrimp exposed to 0.3 to 0.4 parts-per-billion of a widely used pesticide were paralyzed in 48-hour laboratory tests (in the lab, paralyzed fish or shellfish may live for days, even weeks, but in the sea, where only the fittest survive, death may come immediately.)

. . . . A concentration of 5 ten-thousandths of 1 part-per-million of DDT proved toxic to shrimp after 72 hours.

. . . . Oysters — your product — have a mysterious capability to soak up DDT from water containing fantastically small concentrations. Oysters exposed to DDT at 0.5 parts-per-million in small aquaria removed over 50 per cent of the pesticide from the water within 6 hours and 96 per cent in 2 days. Under experimental conditions the oyster detects and stores pesticides present in the water at concentrations as low as 10 parts-per-trillion.

Dredging projects may pre-empt good bottoms and effluent from cities and factories may so load up the water that shellfish can no longer survive.

I know of no natural resource activity in which the producers are so dependent on others for the environment in which they work. I suspect that the facts of this dependence often seem insurmountable to individual shellfish producers.

In a sense you are fighting the same conservation battle that every city dweller in the Nation is involved in. The resident of Baltimore whose health may be affected by polluted air and whose recreation opportunities are related to the quality of land and water management in a park hundreds of miles away has the same relationship to the conservation issues confronting the Nation. But unlike many people who find it difficult to define more than an arm's length relationship to parks and wildlife and water quality and pesticides these issues are your economic bread and butter. They are issues to which you should commit your individual and collective energies.

Acid mine drainage from the hills of Appalachia can be a critical economic issue to a Chesapeake Bay oysterman. Pesticides used on cotton farms in the Mississippi Valley may very well be one of the most important issues for a Gulf Coast shrimpman. The control of forest pests in the high watersheds of the Pacific Northwest may be an issue of economic life or death to a Pacific Coast oyster grower.

We in the Department of the Interior have assigned responsibilities to assist American fisheries in a variety of ways. The backbone of these programs is a research effort covering the fields of biology, economics, industrial technology, and marketing. We are pleased that recent development of the Shellfish Advisory Service has been so sympathetically received by your industry because we have long felt there was a major communications gap between the work of our scientists and those of you in the industry who could benefit from the results of this research. We hope to do even better in the future.

As I have tried to suggest today, however, the shellfish industry has a major war to fight on two fronts — one to bring the framework of laws and techniques of operation up to date and the second, to jump into the larger battles of concern for the total environment in which we live and work.

This concern for the whole of man's relationship with the natural world around him has new dimen sions and new impetus under President Johnson's leadership.

The President has made this issue his own and has articulated a concern long felt by many people but long muted by other forces which tended to splinter and divide both the issues and the individuals and groups concerned about them.

There is a new kind of unity in President Johnson's New Conservation in which your industry would feel very comfortable because it is a concern that begins from the assumption that conservation has dimensions much broader than the classic preservation of parks and native wildlife habitat —

a new dimension which must concern itself with the total relationship of man to his environment — all of it.

President Johnson called this "a creative conservation and restoration and innovation" concerned "not with nature alone, but with the total relation between man and the world around him. Its object is not just man's welfare but the dignity of man's spirit."

Your role in this is a challenge I commend to you. Your Government will welcome and support you in it.

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THE MOLLUSCAN SHELLFISH INDUSTRY CURRENT STATUS AND TRENDS

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The fishery resources of the world's seas and estuaries are being increasingly utilized for domestic consumption, and for export to bolster the economies of coastal nations. The greatest use of fishery products is to provide protein to people in areas dangerously short of this vital nutritional requirement. Consequently, well-managed use of these resources should be the aim of nations that depend on the sea. In international meetings to discuss the equitable use of fisheries resources, delegates are depending to an ever-greater degree on research information mostly concerning population dynamics to design programs and assign responsibilities for managing common fishing grounds. The bounties of the sea that were incompletely inventoried until recently are now being carefully evaluated in many areas of the world. If the newly inventoried resources are used to supplement the world's animal and agricultural products, we may yet meet the nutritional demands of our exploding population.

In a review of the world fisheries which appeared in a Bureau of Commercial Fisheries statistical publication, Lyles (1965) made the following statement:

"The world seafood harvest increased from 71.9 billion pounds in 1958 to 102.3 billion pounds in 1963. The 1964 harvest will doubtless be even larger. The *use* of fishery products in the United States expanded steadily from 7.6 billion pounds in 1958 to 12.0 billion pounds in 1964 but domestic fishermen benefited little from the population explosion."

[1] Mr. Engle, Chief of the Shellfish Advisory Service and Senior Scientist of the U. S. Bureau of Commercial Fisheries, sums up current status and trends in the shellfish industry, basing his thoughts on a background of over 30 years experience with shellfish problems. Mr. Engle delivered this keynote address at the opening session of the 57th Joint Session of the Oyster Institute of North America, Baltimore, Maryland, June 28, 1965.

The review also pointed out that in 1964 about 62 per cent of our national supply of fishery products was of foreign origin. Despite the increase in population and consequent increase in food consumption, our fish harvest declined and the United States continued to be the world's largest importer of fishery products (including molluscan shellfish).

We cannot ignore the significance of attractively packaged imported frozen fresh scallops, canned oysters, and clams in competition with domestic products on retail shelves. Some, such as sea scallops, are caught in waters adjacent to our shores, and later shipped into this country (O'Brian, 1961; Bourne, 1964).

This serious problem of foreign competition must be solved by meeting the various facets of competitive harvesting, processing, quality control, and other details of merchandising. Our task here today, however, is to evaluate the internal status of our local shellfish industry.

It must be emphasized that this is a period of change during which we must use accumulated knowledge to control environment and to increase production. First, let us examine some of the conditions that exist today in the oyster industry. In a like manner we will discuss the other commercial mollusks.

THE OYSTER

The oyster is the commercial mollusk that has been and continues to be the most vexing to the biologist and to the processor in the industry. It has been an exotic food and a most plebian food. It has been the food of kings and emperors and the food of aborigines of this and other continents, as evidenced by kitchen middens unearthed by archaeologists. It has been plentiful and scarce. It existed before recorded history and has persisted through many generations. Unless, however, we now mend our conservation habits, clean our waterways, and learn to domesticate this im-

portant marine animal, it may become extinct in some parts of our country.

The oyster resource; for the most part, has not been adequately farmed and managed in the waters of its greatest potential production, Chesapeake Bay. Here 50 per cent of the national oyster harvest has been made during most of this century (Fig. 1). Production today, however, is only about 10 per cent of that in the early 1900's. Commercial shellfish are in most of our coastal bays and estuaries, but where oyster production is concerned, "As Chesapeake Bay goes — so goes the nation." The same has also been true in recent years for soft-shell clams.

Preliminary data on the U. S. oyster harvest for 1964 present a slightly more optimistic picture (Fig. 2). Production was 60.2 million pounds, an increase over 1962 of 3 per cent in weight, and 1.1 million dollars in value (Lyles, 1965). It would be more gratifying generally if this improvement were more equally distributed. Because it was not, we must look at the harvest in different regions to get a true perspective (Fig. 3). When production since the turn of the century (Figs. 1, 2, 3) is compared with production during the past decade (Galtsoff, 1956; Lyles, 1965), interesting changes are apparent. Production has decreased somewhat in Chesapeake Bay and the south Atlantic States, and has decreased considerably more in the middle Atlantic States and New England, but has increased many-fold along the coast of the Gulf of Mexico and the Pacific Ocean (McHugh, 1963). The understanding of the reasons for these changes and the lessons we may draw from them are basic elements to consider in facing the complex problems we are now trying to solve.

As the figures show, diminishing returns have been a feature of the oyster production since the heyday of the late 1800's. Until recently we could say that the picture illustrated the usual story of over-fishing the "unlimited" natural supply. At certain locations and during various periods, non-management or poor management of natural oyster beds has caused serious reductions in the available commercial supply (Glude, 1951). The problem is complex, and involves local traditions, political differences, pressures and expediencies, individual selfishness, ignorance of biological facts, and factors. Many elements only indirectly involved with shellfish management and production have complicated the problem in the following ways: (1) The increase in population and lagging sanitation improvements which have made our rivers and estuaries too polluted for shellfish production; (2) the so-called improvements of our water fronts for navigational, industrial, and domestic purposes, and the use of headwater impoundments for power, flood-control, and increased water requirements; and (3) the related but extremely vexing difficulties of controlling predators and disease organisms, which have had their effect on molluscan shellfish production (Engle, 1963; Engle & Rosenfield, 1962).

One other significant factor is the sales competition from other food products presented to a consumer conditioned to the accelerated pace of modern living and the convenience of prepared-food packaging. Those of us who have lived a few more decades than the present generation of American housewives say *"The oyster ain't what*

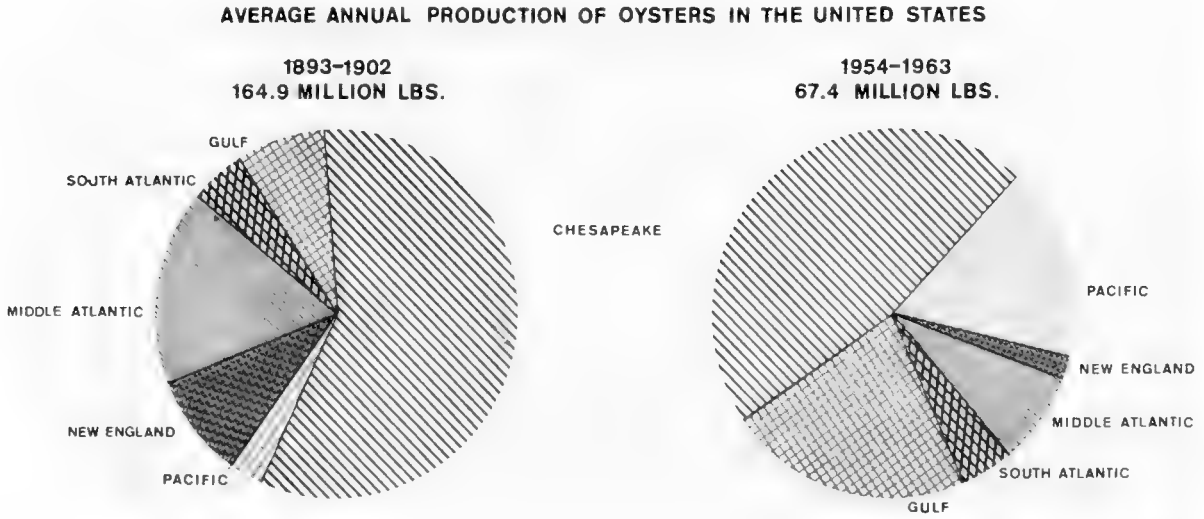

FIG. 1. *Average production of oysters by regions at the decade including the beginning of the 20th century and the decade including the present period (Modified after Galtsoff, 1956).*

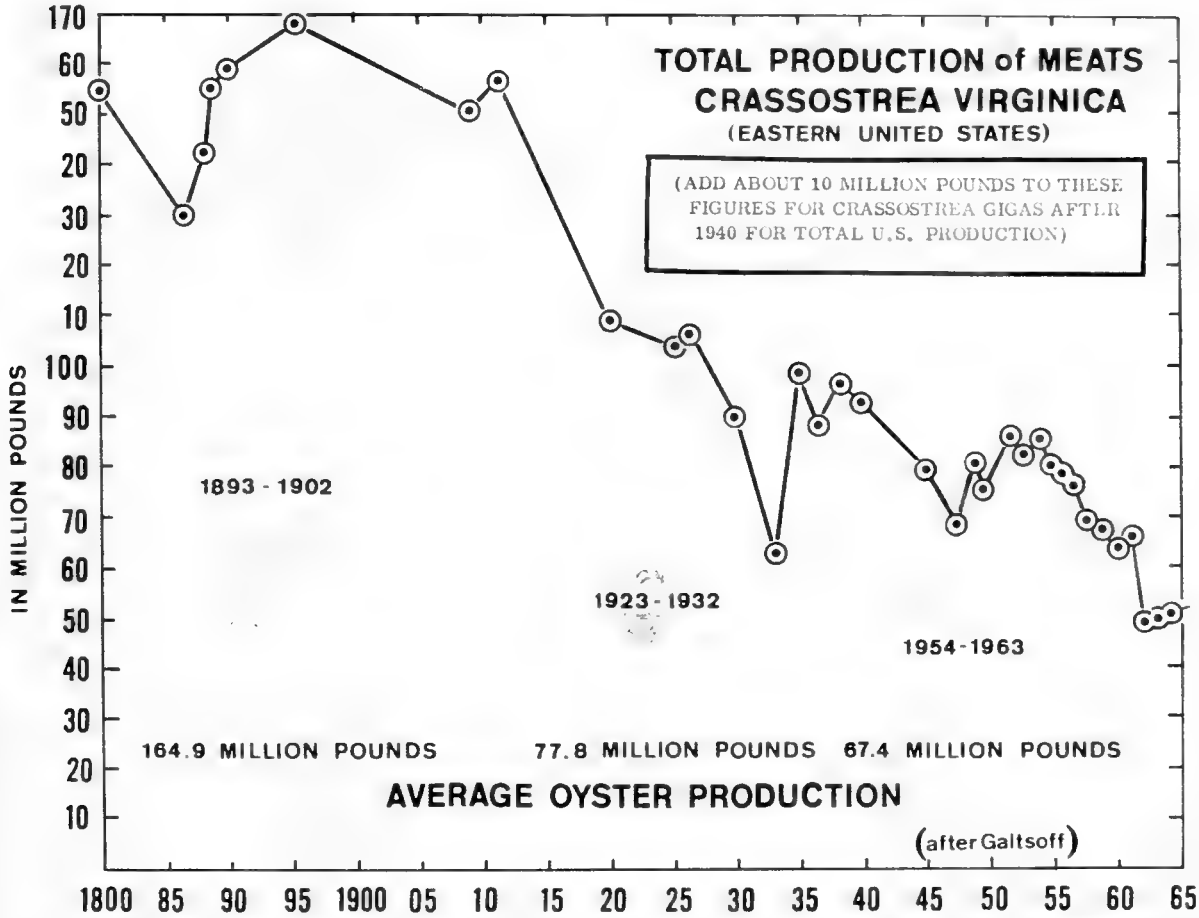

FIG. 2. *Total production of oyster meats, 1880 to 1964 with average production for three separate decades during this period (Modified after Galtsoff, 1956).*

it used to be." It is a far better food product from the point of view of sanitation, but something has been lost in processing that at one time made oysters the king of seafoods. If we go back into the history of oyster eating in this country, we can see the subtle changes in consumer attitudes. George Washington and other landowners of the Chesapeake Bay area bought or gathered oysters to supply a high-protein diet for their hard-working field hands. Then came the Epicurean period of Delmonico's, raw oyster bars, and a widespread appreciation of this marine animal in every coastal and many an inland household. If you will forgive a personal reference, I can recall oysters on our family table at least once a week; traditionally in the dressing in the holiday fowl and in an oyster stew on Easter morning. Perhaps these food habits still exist in many places, but the general trend in production of molluscan shellfish has been downward while the human population has surged upward — an indication that we are not maintaining the custom of eating oysters at home or as an aperitif when dining out.

Earlier, I pointed out the striking increases in oyster production of the Pacific Coast and the Gulf of Mexico. In the middle and late 1800's and the early 1900's, oystering on the Pacific Coast was a casual industry. The relatively small production depended on the excellently flavored but delicate native Olympia oyster and on imports of the eastern American oyster, replanted in the bays and tributaries of the Pacific Ocean. The Olympia oyster has not been able to compete with progress, for industry has produced changes in the water conditions which were once suitable for growth and reproduction. Nor has it been able to compete with the more progressive and vigorous Pacific oyster industry. The eastern oyster, unlike the human population that came from the east, was not able to thrive, prosper, and reproduce in the

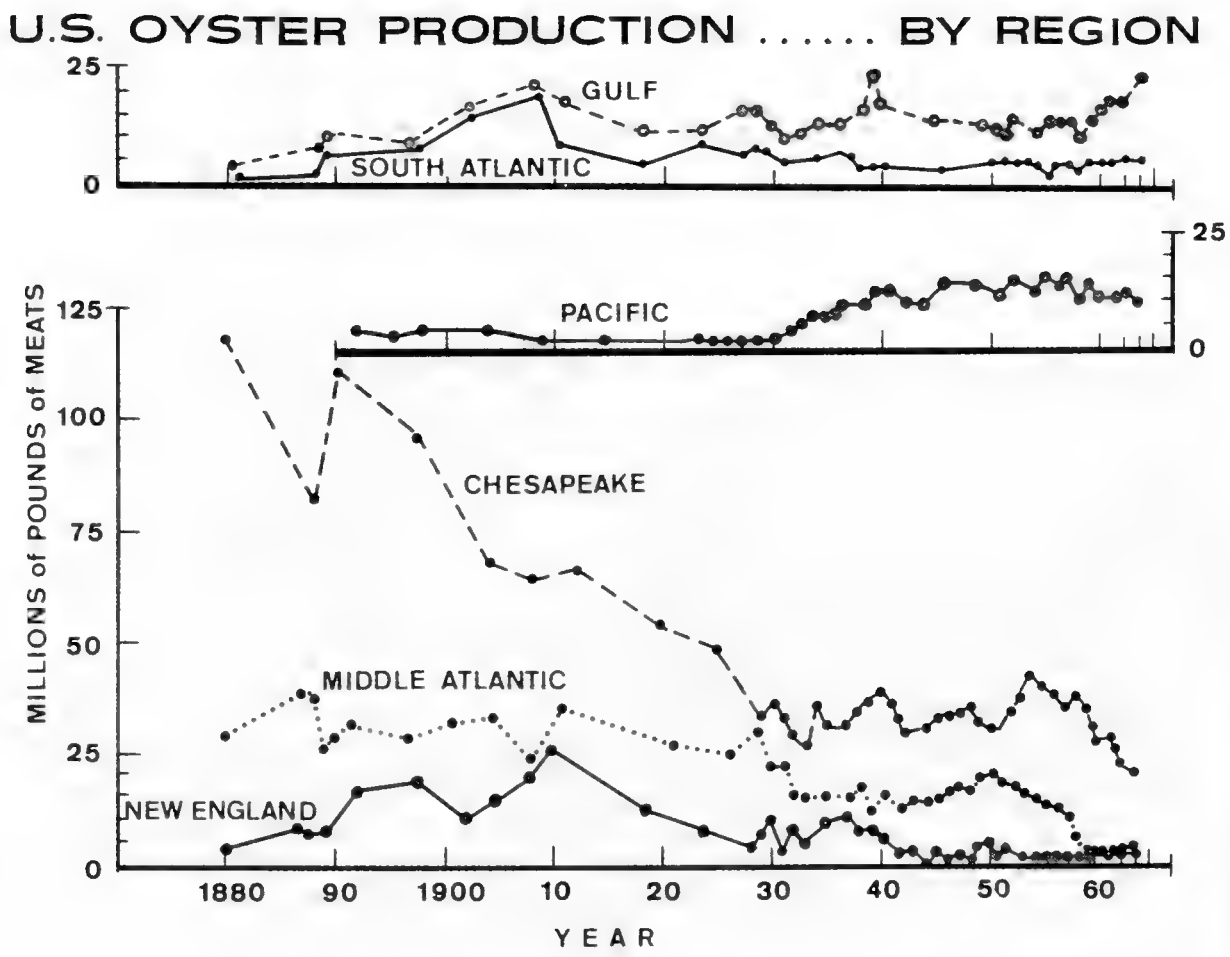

FIG. 3. *United States oyster production by regions: Gulf of Mexico, south Atlantic, Pacific coast, Chesapeake Bay, Middle Atlantic, and New England from 1880 to 1964 (From McHugh, 1963).*

west. Consequently, West Coast oyster production of those days failed to keep up with the increasing demands of an expanding population. It was here that ingenuity, business adventure, and luck changed the story of declining oyster production to one of success and stability by the introduction of a species new to the area. The Japanese oyster, now called the Pacific oyster, imported as seed from areas in Japan devoted entirely to seed production, has been able to grow and keep the West Coast industry supplied with a stable source of oysters for more than the past quarter century. The present oyster industry is strictly one of private enterprise, vigorously pursued by energetic business interests.

The increased harvest of oysters from the Gulf States is another story. Here the catch has been stimulated by a drop in production in northern oyster-producing states. In 1964 oyster production of the Gulf States exceeded that of Chesapeake Bay by about 1 million pounds (Anonymous, 1965). Much of the southern production is transported by truck to be shucked and distributed by northern processors. Danger in this harvesting program is its demands on natural production, that are not always offset by an increased effort to expand the planting of cultch or the employment of other cultivation procedures. The increased harvesting in the south has, however, temporarily stemmed the decline in the total production of oysters in the United States.

Molluscan shellfish other than oysters are involved in our national shellfish economy. I started the discussion about this animal in deference to the great organization sponsoring these meetings and in whose name *oyster* is so prominent, the Oyster Institute of North America. I am not sure we can spare the time to examine the fishery for

each of the economic mollusks in as great detail so, I have selected those of most economic importance. Importance is often a matter of personal interest so you will have to accept my interpretation, some of which is backed up by statistical evidence.

CLAMS

Clams — razor, soft-shell, hard, surf, and others — altogether yielded 63.5 million pounds of meat in 1964. This was an increase of about 1 million pounds over 1963. The increase represents an improvement in production of hard and soft-shell clams, and no change in the production of surf clams. These three clams constitute the bulk and principal value of the clam industry (Fig. 4).

The surf or sea clam, harvested mostly from the waters of the middle Atlantic Coast, is by far the leading meat producer among the clams. As its name indicates, it is a product of the open sea and abundant enough to be exploited in ever-increasing numbers, as the catch records over the past 15 years show. The annual harvest since 1950 has increased five-fold and now stands at more than 38 million pounds of meat. The industry, aware of the fallacy in the idea of an inexhaustible supply, initiated a government supported exploration and research program through the Oyster Institute and the U. S. Bureau of Commercial Fisheries, to take surf clam fishing out of the truly "wild-hunting" category. As you are fully aware, this has been the past history of the surf clam industry and, for that matter, it is still the way most mollusks are exploited. It is unlikely that the surf clam can be farmed and cultured in the same manner as many of the estuarine mollusks. We can, however, apply the techniques of conservation that evolve from accumulated knowledge of life history, natural recruitment and mortality, seasonal variations in growth, distribution, and the effect of fishing on a restricted age class of organisms. Again, this is the general formula for population studies upon which a management program must be built. Furthermore, this is not static information and the factors involved must be re-examined and adjusted periodically.

At this time the U. S. Bureau of Commercial Fisheries, through its Gloucester, Massachusetts,

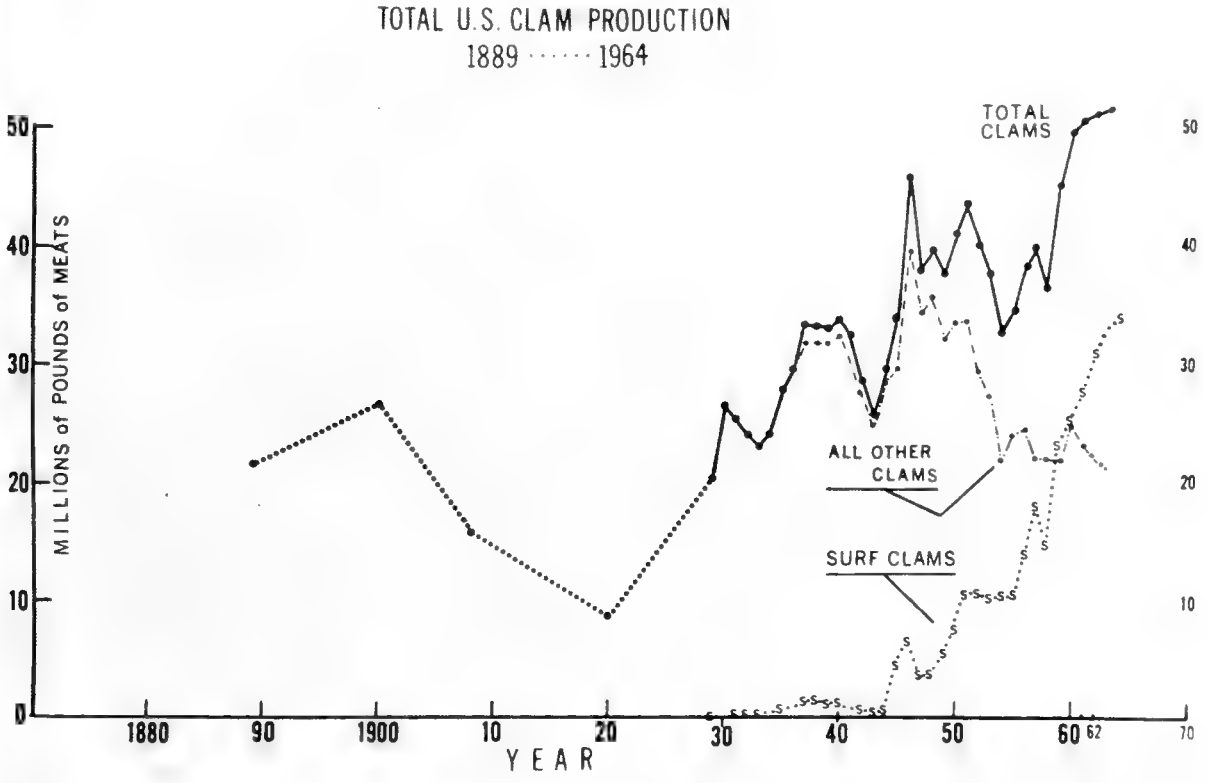

FIG. 4. *Total U. S. Clam production 1889-1964. Note rapid rise of surf clam harvest from 1945 to 1964 (From McHugh, 1963).*

exploration and gear research facility and the Oxford, Maryland, Biological Laboratory, is engaged in just this kind of information-gathering. The surf clam program of the Oxford Biological Laboratory discovered, collected, measured, and marked a large group of 1-year-old clams and put them back in the sea. Information on survival and growth rate has been garnered from periodic examinations of this population. Studies of abundance and distribution, in addition to the biological factors now being observed, are part of a program leading to good management of the resource.

Next in importance, based on pounds of meats produced, is the hard clam. This animal has filled a very important role in the economy of the Middle Atlantic and lower New England area. Many of the molluscan shellfish producers who formerly depended on the declining oyster resource, have converted to hard clam fishing as a significant adjunct to their business. Long Island Sound, Narragansett Bay, the coastal bays of Long Island and those of Maryland and Virginia and Lower Chesapeake Bay account for 95 per cent of hard clam production; about 50 per cent comes from the Middle Atlantic area. We might elaborate on this production even more by saying that a large part of it comes from the bays of Long Island where a great natural expansion of the clam population has occurred, and where there is efficient harvesting with modern methods and equipment, efficient management, and aggressive marketing. In New York State, a minimum size law and the use of selective methods of harvesting with efficient hydraulic dredges have greatly assisted in the cultivation, recruitment, and conservation of this important resource.

What is the status of the soft-shell clam industry? The statistics indicate a moderate upswing in production (Fig. 5). Maryland increased its production in 1964 by 19 per cent, to a record catch. Soft-shell clam production in 1963 in New England was 5 per cent more than in 1962. A controversy exists in this industry, however, over the manner of fishing for the animal. In New England most areas restrict the harvesting of soft-shell clams to hand digging in the intertidal zone and prohibit the use of any hydraulic device. The pros and cons of New England's objections to a more efficient method involve traditional, biological, and economic factors that need not be argued here. Maryland, as a relative newcomer in the soft-shell clam industry, is in it because of a new hydraulic device which can harvest clams to moderate depths of water with little or no dam-

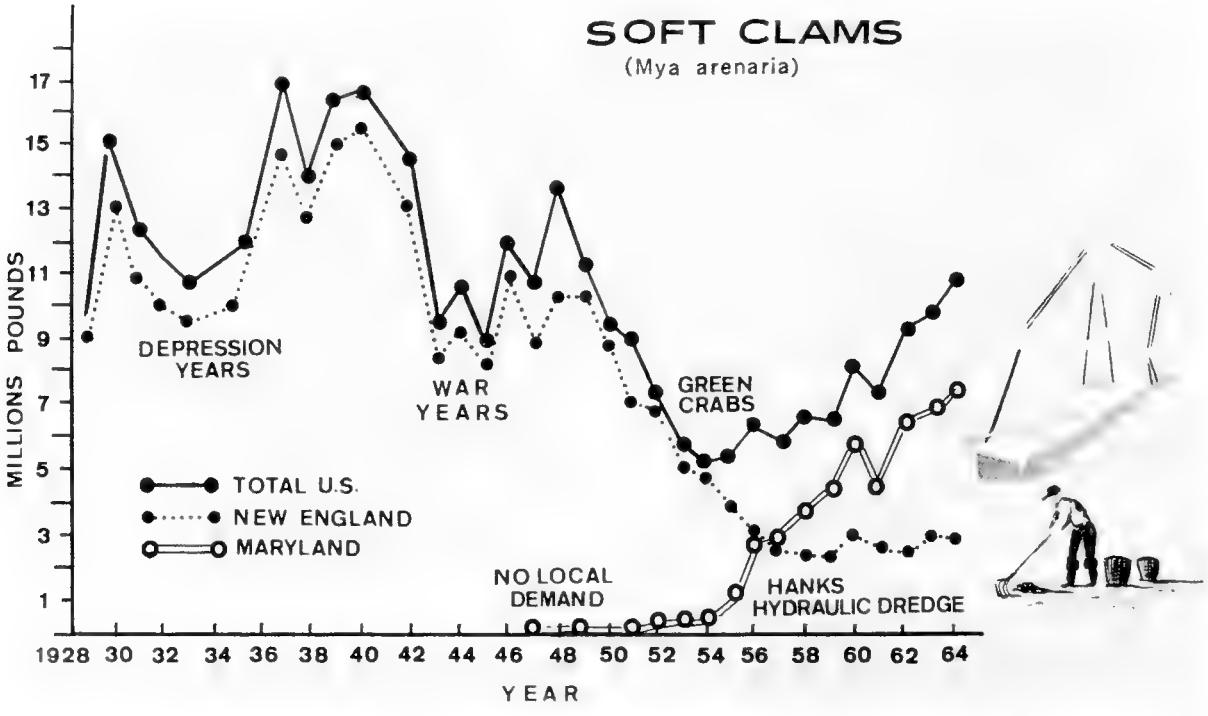

FIG. 5. *Soft Clam,* Mya arenaria, *total production United States, New England and Maryland with suggestions for the cause of variations in supply.*

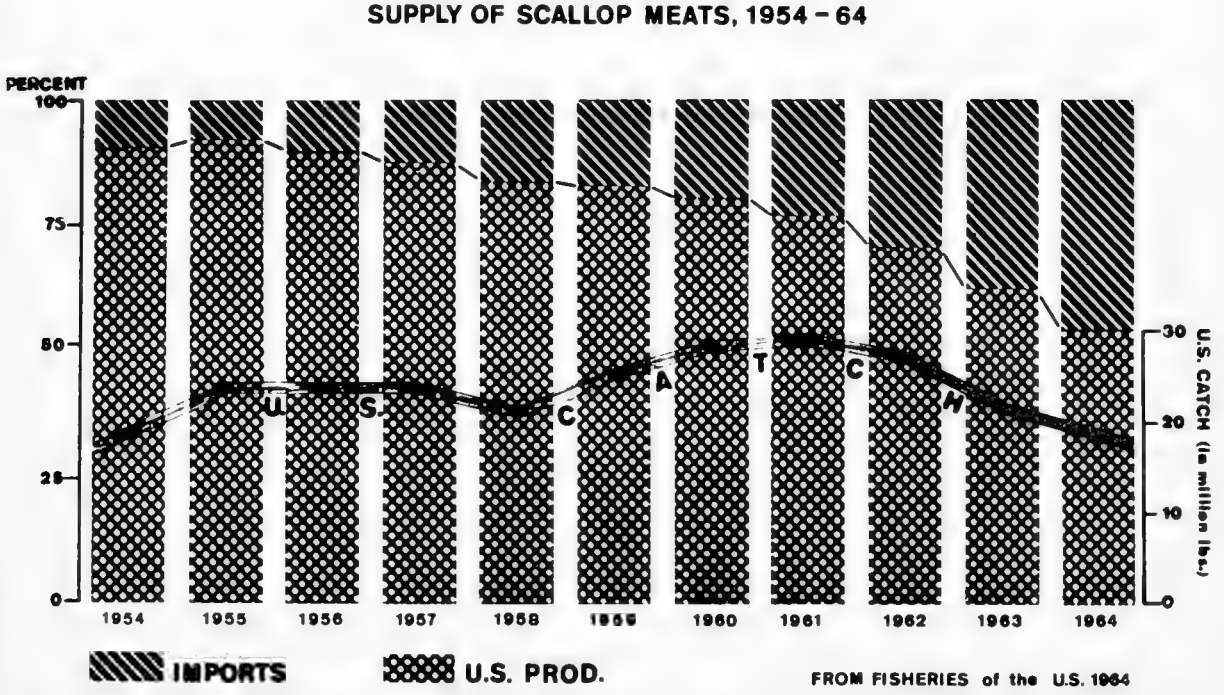

FIG. 6. *The per cent of the scallop supply from import and domestic sources consumed in the United States and the United States catch 1954 to 1964 (From Fisheries of the United States 1964).*

age to the clam. The device is efficient, labor-saving, and can be selective. It automatically cleans mud from the outside of the clam. Clams are adundant here; present indications are that these fast growing animals reproduce sufficiently to withstand the current method of exploitation.

I stand in the middle of another controversy built around regional pride in quality. In the cold light of competitive marketing, the Chesapeake soft-shell clam has found a place in the trade which makes it an important item in the whole economy of the molluscan shellfish industry. Though the epicure may be able to tell the difference between a New England clam and a Maryland clam, a great many people enjoy eating clams without regard to their regional origin.

The Pacific Coast produces a diversity of clams which provide quarry for clam-digging sportsmen. Of course there is a substantial commercial harvesting of some clams, but it is small as compared with the East Coast production, and makes up only about 1.6 per cent of the U. S. total. The principal clam of the West Coast is the razor clam, caught by hand-digging at very low tides on the ocean front. The sport has become so popular on some parts of the coast that bag and size limits had to be set to manage the resource for the sportsman.

SCALLOPS

Scallop production has varied seasonally, but over the years since 1950 annual production has fluctuated between 17 and 27 million pounds (Fig. 6). In 1964 the catch was near the lower amount. The U. S. Bureau of Commercial Fisheries, in its statistical digests, lists three scallops: bay, calico, and sea. The first two are of small consequence in the total production, although they supply a select and local seashore trade; sea scallops form the bulk of the catch. The sea scallop, like the surf clam, inhabits the off-shore waters of the ocean and is found in great abundance on Georges Bank off the New England Coast. The distribution of the sea scallop in the coastal waters extends from Newfoundland to Cape Hatteras, although commercial fishing ceases approximately at the Virginia Capes.

In my opening remarks the importation of sea scallop meats was mentioned. Herein exists a complex problem involving labor, economics, quality, price control through catch limits, and foreign competition. Within the last few years critical analyses of these factors have been made (O'Brien, 1961; Anonymous, 1964; Bourne, 1964). Marketing

campaigns have brought the sea scallop to the dinner tables of the consumer frequently, but the public is often eating an imported product fished from what we consider our waters.

THE EXOTICS

A denizen of the underwater rocks of the Pacific Coast is the abalone, a gastropod mollusk, which produces almost a million pounds of meat classed as a delicious gourmet food. California, where most abalones are taken, exercises strict control of the fishery, which is both commercial and recreational.

Nor should mollusks such as squid, octopuses, periwinkles, conchs, cockles, and mussels be overlooked. They are taken in small amounts for some of our citizens whose specialized tastes for seafood include these sea animals. In a country such as ours, where all except the Indians are foreigners, seafood consumption takes on an international flavor.

THE FUTURE

I have tried to sketch briefly the current status of the molluscan shellfish industry, with some historical information as background. To forecast events and developments is more difficult; still, I am inclined to feel optimistic about the future. The molluscan industry and science are now cooperating with nature to save shellfish crops from disease and predation, to artificially produce the seed we need, and to control the type, quality, and other special features of the crop through selective breeding.

The Ultimate or at least a most efficient concept of the future development of shellfish culture has been proposed by Donald L. McKernan, Director, U. S. Bureau of Commercial Fisheries. Mr. McKernan, in an address to the shellfish industry, assembled in Convention in Washington, D. C., July 22, 1963, described a shellfish factory of the future. He projected for the year of 2000 the completely controlled culture of molluscan shellfish freed of the vicissitudes and impediments of nature by the application of science and industrial ingenuity. Parts of the concept are presently being tested in the laboratory. Other parts have passed this stage and are in the hands of industry being incorporated in the practical program of shellfish production.

Shellfish hatcheries are in operation on the Atlantic and Pacific coasts. Not all of these are test-tube operations; some are plants capable of producing commercial supplies of molluscan seed. Federal, State, and industry efforts are combined to accelerate the achievement of this necessary step in answering the number one problem — insufficient seed production (Loosanoff and Davis, 1963).

Control of predators remains in most areas a principal problem, although a measure of success has been made through chemical and physical controls. The Ocean Pond seed project on Fishers Island, New York, is experimenting with collection of seed oysters on suspended cultch in a salt pond where the seed is kept away from predatory snails. Other ventures of this nature by representatives of both science and industry are in progress on Martha's Vineyard and Cape Cod, Massachusetts; Milford, Connecticut; Oxford, Maryland; Gloucester Point, Virginia; and possibly elsewhere (Shaw, 1962, 1965).

As efforts to control genetic characteristics proceed, more and more dependence will be placed on pond culture; at least this will be necessary for clams and oysters to maintain hybrid purity. They must be treated very much like hybrid corn which can only be raised from protected and selected parent stock. I can picture many advantages through breeding; as we improve the stock, methods of culture will become more precise — and shellfish caught by the "catch as catch can" method will be less acceptable. Shellfish farming will be a necessity and will further supplement the "wild-hunting" methods now used to catch about half of today's crop. Those concerned with molluscan shellfish are making a more critical evaluation of current conditions and are realizing that the talents of industry leaders and the waterman, the scientist and the administrator, and the lawmaker and his constituents must be brought together and applied to common problems. Above all, when we have made the product available, we must encourage the public to eat it.

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MORPHOLOGICAL AND CULTURAL STUDIES OF A MOTILE STAGE IN THE LIFE CYCLE OF *DERMOCYSTIDIUM MARINUM* [1, 2]

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ABSTRACT

Dermocystidium marinum *hypnospores, obtained by exposure to thioglycollate (the Ray technique), were isolated from oyster tissue and placed in sea water under aerobic conditions. The cells differentiated into sporangia, and sporulation occurred by successive bipartition of the protoplast, resulting in the formation of motile cells within the hypnospore wall. The planonts escaped through one or two preformed discharge pores and tubes. All motile cells were biflagellated with both flagella attached laterally and subapically. The anterior flagellum had* Flimmern *and the posterior flagellum was a whiplash. The planont cell body was intermediate between reniform and pyriform. Sporulation morphology is discussed from light microscope studies. Infection of oyster organ and tissue explants was accomplished with motile cells from single sporangia and from populations of sporangia. Living oysters were infected with the hypnospore isolates, but it was not determined which cell type gave rise to the infections — hypnospores, prehypnospores, or planonts.*

INTRODUCTION

The literature on *Dermocystidium marinum* has been voluminous since the initial description by Mackin, Owen, and Collier (1950). Excellent reviews by Johnson and Sparrow (1961) and by Mackin (1962) summarize most of the literature.

The assay method of Ray (1952) has been of great value in determining the incidence and intensity of *D. marinum* infections, but, in addition, has raised the question of the role of the hypnospores in the life cycle. Hypnospores were found in heavily infected oysters and oyster explants in sea water (Ray, 1954a, 1954b; Mackin, 1962); therefore, it was suggested that the response to thioglycollate medium was normal but exaggerated (Mackin, 1962). Mackin and Boswell (1956) suggested that the hypnospores develop and release aplanospores which could act as infective elements if ingested by oysters and/or phagocytized by oyster amoebocytes. However, the hypnospores were never observed to produce aplanospores.

This study demonstrates that hypnospores develop into sporangia which, in turn, develop planonts. The term sporangium is used in its general sense to include asexual structures (Starr, 1955), because it was not demonstrated whether the planonts were isogametes or zoospores or either. A morphological description of the sporulation process is given and the motile cells are shown to be capable of infecting oyster explants.

METHODS

Dermocystidium marinum cells were induced to differentiate into hypnospores by the Ray technique (Ray, 1952). Infected oyster tissues were held for 48 hours in fluid thioglycollate medium (F. T. M.) then digested for 6-8 hours in 0.25 per cent trypsin (1:250; NBCo), prepared in Cameron's

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[4] A portion of a dissertation submitted in partial fulfillment of the requirements for the Ph.D degree from Florida State University, Tallahassee, Florida.

sea water (Cameron, 1950). The artifical sea water was equivalent to 32 ppt salinity and was adjusted to a pH of 7.8 to 7.9.

A magnetic stirrer was used to agitate the trypsin preparation. During digestion, temperatures rose no higher than 32°C. This procedure separated all tissues into individual cells or into small clumps of cells around 0.5 mm^3 or less (with the exception of gills and associated gill bars).

After trypsinization, the suspension was poured through six to eight thicknesses of cheesecloth to remove the larger clumps of cells and tissue debris. The suspension was then centrifuged at 340 x *g* for two minutes. Four washings were made in each of three conical, 15 ml centrifuge tubes. The fungus cells were resuspended each time with Cameron's sea water.

Following the washing procedure, the cells were placed in flasks with Cameron's sea water or natural sea water (28 to 32 ppt). Five-tenths of a mg/ml of penicillin "G" potassium and of streptomycin sulfate were added to retard bacterial growth. The incubation temperature was 30 ± 1°C and a shaker table was used to agitate the culture. Cell densities from several hundred to 50,000/ml were used.

The morphology of sporulating sporangia and of planonts is described from light microscope studies. Periodic observations of single sporangia undergoing sporulation and of planonts were made with an inverted microscope. The cells were contained in a chamber perfused with Cameron's sea water.

Whole and sectioned sporangia were stained in Heidenhain's haematoxylin, using the method outlined by Starr (1955), in acetocarmine (Humason, 1962), in Schiff's reagent, using the Feulgen method (Jensen, 1962), and in Weigert's iron haematoxylin (Humason, 1962). Whole planonts were stained, using the methods of Couch (1941). Lipid was detected, using a Sudan IV method (Humason, 1962).

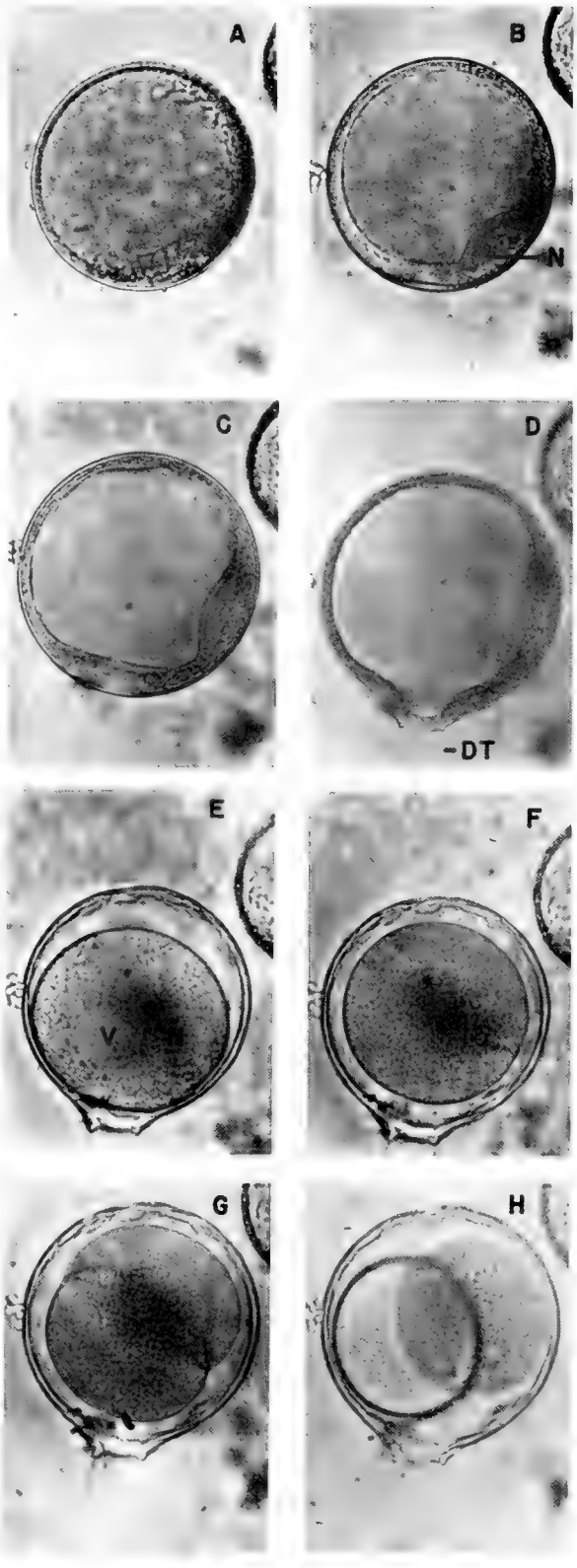

FIG. 1 *(A-D). Sporulation of a single sporangium in Cameron's sea water. All micrographs; 730X. Note enlargement of sporangium between* 1*B and* 1*C.* FIG. 1*A — plus 13 hours;* FIG. 1*B — plus 30 hours; FIG.* 1*C — plus 39 hours;* FIG. 1*D — plus 42 hours. Nucleus (N); discharge tube (DT). Light micrographs.*

FIG. 1 *(E-H). Sporulation of a single sporangium in Cameron's sea water. All micrographs; 730X.* FIG. 1*E — plus 42 hours, 40 minutes;* FIG. 1*F — 43 hours, 10 minutes;* FIG. 1*G — 43 hours, 20 minutes;* FIG. 1*H — 43 hours, 41 minutes. Nucleus (N); vacuole (V). Light micrographs.*

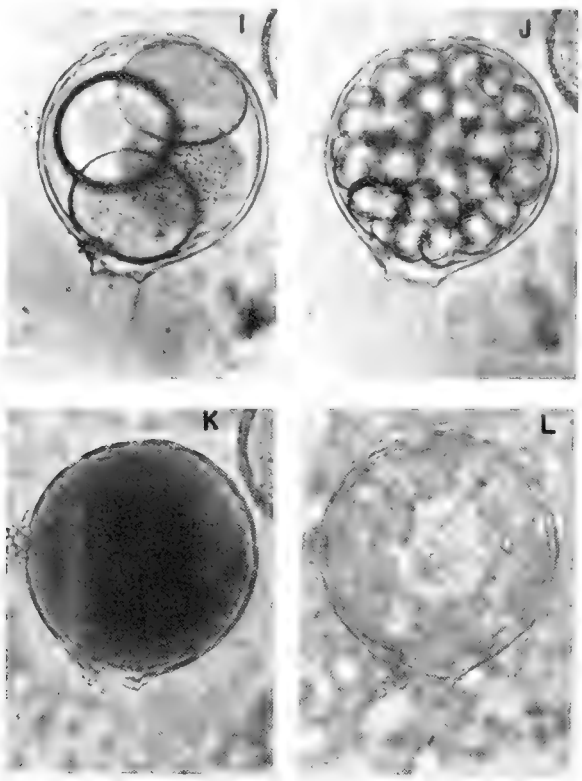

FIG. 1 *(I-L). Sporulation of a single sporangium in Cameron's sea water. All micrographs; 730X.* FIG. 1*I* — *plus 44 hours, 22 minutes;* FIG. 1*J* — *plus 46 hours, 35 minutes;* FIG. 1*K* — *plus 60 hours, 30 minutes;* FIG. 1*L* — *plus 92 hours. Light micrographs.*

Attempts were made to infect oyster explants and whole organs with planonts. Two methods were used. In the first method, explants and single sporangia were sealed in perfusion chambers and observed under an inverted microscope. Each chamber consisted of a glass ring, 3.5 mm high and 10 mm inside diameter, a microscope slide, a coverslip (22 mm square), and two, 0.8 mm (I.D.) glass capillary tubes, tapered at one end to 0.1 to 0.2 mm (I.D.). Two notches were scored in each glass ring so that the capillary tubes could be slipped between the ring and slide. The tubes were positioned across the ring from each other. All components, except the coverslip, were sealed together with epoxy resin (Durcopan ACM). The explant and sporangium were placed in a drop of Cameron's sea water on the coverslip and a small fragment of coverslip was used to sandwich the preparation against the main coverslip. The system was then inverted over the glass ring and sealed to the ring with paraffin. A reservoir of Cameron's sea water was used to perfuse the chamber at about 1 to 3 ml per hour.

In the other method, organ explants were placed in an experimental apparatus designed to allow only motile infective elements to reach the organs. Adductor muscle and heart explants were placed in a flask, containing Cameron's sea water, which was connected to another flask by means of side arms. The side arms were 5 to 10 mm above the bottom of the containers, and were 15 mm long and 4 mm inside diameter. A thick suspension of about 2×10^6 hypnospores was gently layered on the bottom of the flask not occupied by the explants. The preparations were maintained at room temperatures (22-25°C).

Individual living oysters were exposed to planonts in experimental systems, each of which consisted of a beaker, connected to a flask by means of side arms, 5 to 10 mm above the bottom of each container. An oyster was placed in each beaker and a suspension of hypnospores was added to each flask. Cameron's sea water was the medium and incubation temperatures were 22-25°C.

In this study observations were made using *D. marinum* cells from oysters of Apalachicola Bay, Florida, and Galveston Bay, Texas. No differences in morphology or infectivity were detected. Uninfected control oysters were obtained from upper Delaware Bay.

RESULTS

Morphology of Sporulation

Hypnospores were observed in sea water perfusion chambers as single cell isolates and in populations of hypnospores. The hypnospores used in this study stained blue, blue-black, or green in Lugol's iodine solution as reported by Ray (1954b).

The observed pattern of development is pictured in Figure 1 (A-L). The elapsed times for the series are given with each figure; but in the following discussion, elapsed times will not be given, because of the extreme variations in elapsed times which occur. In a population where 97 per cent sporulation occurs, cells may demonstrate first cleavage as soon as 24 hours after being placed in sea water, and others may not initiate cleavage until plus five days.

Prior to first cleavage of the protoplast large lipid droplets are either dissolved and utilized by the cell or are subdivided, because the cytoplasm assumes a fine granular appearance (Fig. 1A) rather than being masked by the droplets as in hypnospores recently isolated from F. T. M. (Fig. 2). Further subdivision and/or utilization of the lipid deposits allows one to clearly see the large, characteristic vacuole and the nucleus (Fig. 1B).

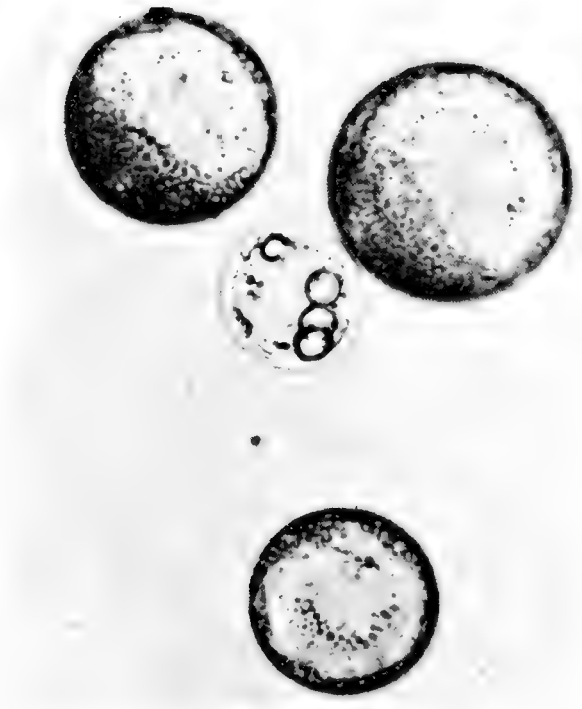

FIG. 2. *Hypnospores in sea water, showing concentrations of lipid droplets within the cytoplasm. The smallest cell has lysed and most of the lipid has coalesced into several large droplets. Light micrograph; 1,600X.*

A clear cytoplasmic area, immediately under the cell wall, appears early in development (Fig. 1B). The area becomes progressively larger (Figs. 1C; 3), a bulge appears in the wall overlying the area (Fig. 4A) and rupture occurs, thereby forming a discharge pore (Fig. 1D). One, or rarely two, pores may be formed. Sections indicate that the clear area represents a plug of material which gives rise to a discharge tube and also serves to block the pore area until planonts have differentiated. The plug is quite strong. Until the last few hours prior to planont discharge, the sporangia can be crushed without being ruptured.

The wall, surrounding the pore, is flared out and ragged in appearance (Fig. 4B), indicating that pore formation results from a forceful pressure from within. The bulge which forms prior to rupture also supports this idea (Fig. 4A). As can be seen in Figure 4B, discharge tubes do not stain blue in Lugol's iodine.

Sporulation stages, prior to first cleavage, show a startling change in the nucleus. Hypnospores, as isolated from fluid thioglycollate medium, have peripherally located, compact nuclei which are spherical or oval (Fig. 5, A-B). The longest axis is usually 3 to 6μ. A nucleus may have one or two lightly staining areas (Fig. 5A) or it may stain homogeneously dark in haematoxylin (Fig. 5B). It is not known what the light areas represent. If they represented endosomes or nucleoli, one would expect them to be more, rather than less, basophilic than the rest of the nucleus. Acetocarmine-stained preparations gave a similar picture. Feulgen preparations did not yield reproducible or interpretable results when used on whole cells. Sectioned cells were not subjected to the Feulgen procedure.

When hypnospores are placed in sea water and differentiate into sporangia, the nuclei enlarge greatly prior to first cleavage (Fig. 1A-E), and the chromatin is either dispersed or chromatin duplication occurs (Fig. 6). The striking increase in size indicates that the latter possibility exists. One would expect that nuclear division would be preceded by condensation rather than dispersal of chromatin.

Prior to the first bipartition, the protoplast contracts and forces much of the vacuolar fluid into the area between the cell wall and plasma membrane (Fig. 1E). The vacuole becomes irregular in outline and is subdivided. Upon completion of the contraction process the cytoplasm becomes dense, and the nucleus and vacuoles can no longer

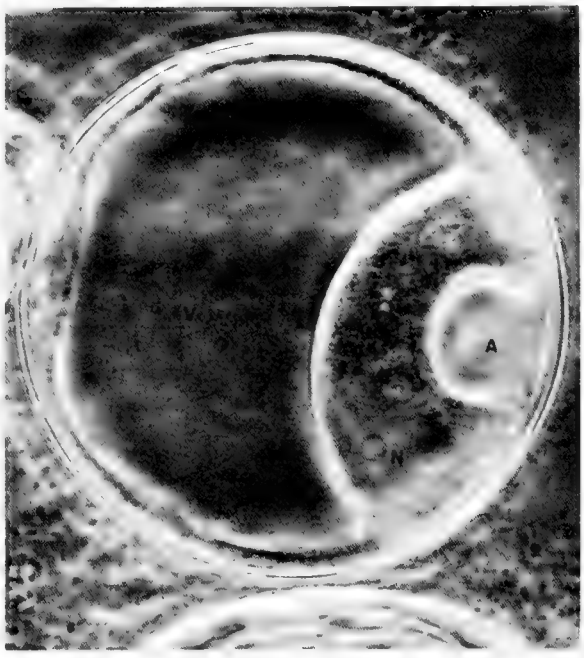

FIG. 3. *A living precleavage sporangium which had been in sea water 36 hours. Vacuole (V); nucleus (N); area responsible for discharge pore and tube formation (A). Light micrograph; 3,600X.*

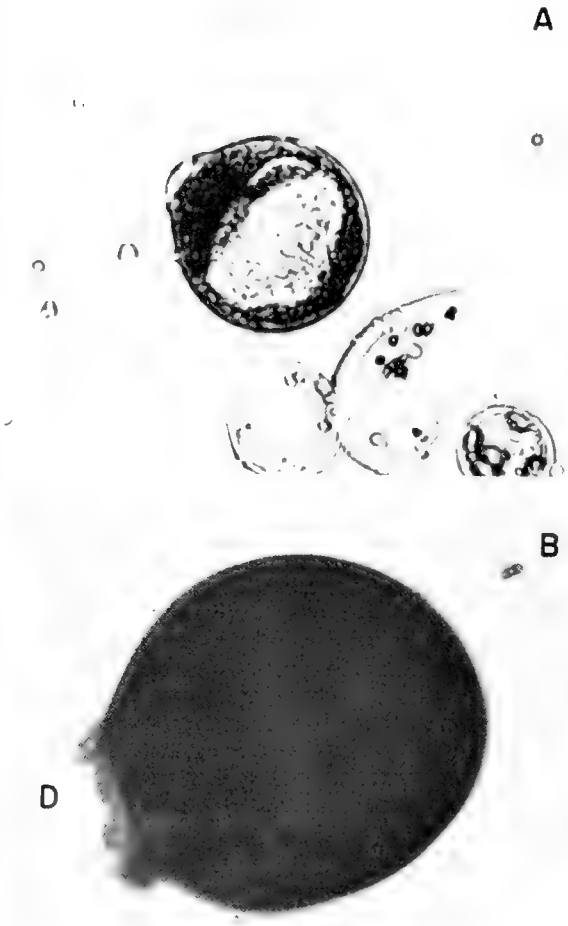

FIG. 4 *(A-B). Sporangia before and after discharge pore formation.* FIG. 4*A is a light micrograph of a living sporangium prior to pore formation.* FIG. 4*B is a light micrograph of a sporangium stained in Lugol's iodine. The ragged edge of the pore and the nonstaining discharge tube (D) can be seen.* FIG. 4*A — 1,200X; 4B — 1,650X.*

be seen (Fig. 1F). At this time the protoplast has drifted to the center of the region encompassed by the cell wall.

Cleavage of the protoplast is similar to the process observed in the first few divisions of some invertebrate eggs, such as in sea urchins. With each division, cleavage furrows divide the protoplast into equal halves (Fig. 1F-J). The first two bipartitions are synchronous. Subsequent divisions are slightly asynchronous; however, no two cleavages are more than about one division out of phase.

Mitotic figures were found in cells resulting from the first five cleavages (Fig. 7). Apparently spindle fibers are formed, but it was not ascertained whether the nuclear membrane disappeared during nuclear division, nor was it possible to count the number of chromosomes. The chromatin material appears to be subdivided with each division. In some early cleavage stages the nucleus can be seen in living cells which have just completed a cleavage (Fig. 8), thereby suggesting that the nuclear membrane is intact at that time.

As bipartition continues, the extracellular fluid within the cell wall is apparently reabsorbed by the cells (Fig. 1 I-K). About 1.5 to 2 days prior to release of planonts from the sporangia, the cells become motile. They move almost imperceptibly

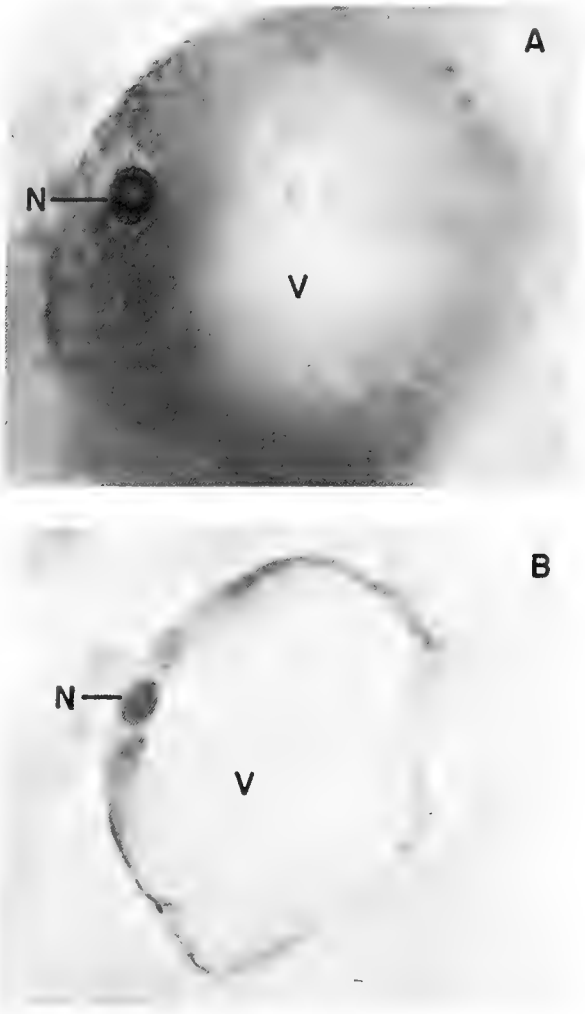

FIG. 5 *(A-B). Sectioned hypnospores, stained in Weigert's iron haematoxylin. Nucleus (N); vacuole (V). Light micrographs; 1,900X.*

at first, but the frequency of gyrations slowly increases over a period of about two days until the mass of planonts is undergoing rapid, random oscillations. At the peak of activity the plug apparently parts in the center and the planonts swim out singly and doubly. It is not unusual for a sporangium to be emptied of material, leaving no cell particulates or lysed planonts.

The mature planont is a biflagellated uninucleated cell with subapically and laterally attached flagella (Fig.9). The cell body is 2-3 μ by 4-6 μ and is intermediate between pyriform and reniform with a slightly invaginated area at the point of insertion of the flagella. The anterior flagellum propels the cell without direct aid from the posterior flagellum; therefore, on the recovery stroke the cell slows down, resulting in a jerky swimming motion. The posterior flagellum is about two-thirds as long as the anterior one (6-10 μ and 9-16 μ, respectively), and appears to act mainly as a rudder trailing behind the cell. The cell body does not change shape as do many fungal planonts.

The anterior flagellum has tinsels *(Flimmern)* along one side as shown by stained preparations (Fig. 10). Unless a mordant and stain are used to increase the apparent diameter of the tinsels, they are invisible in bright field or phase microscopy. The posterior flagellum is naked; i.e., a whiplash flagellum.

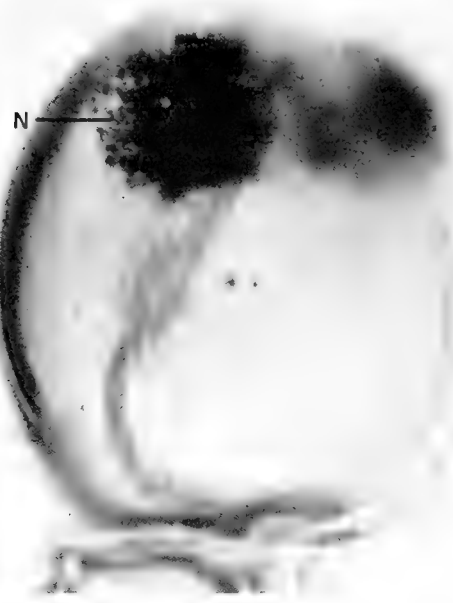

FIG. 6. *Precleavage sporangium in sea water 35 hours. Fixed in Schaudinn's fixative and stained in Heidenhain's haematoxylin. Nucleus (N). Light micrograph; 4,800X.*

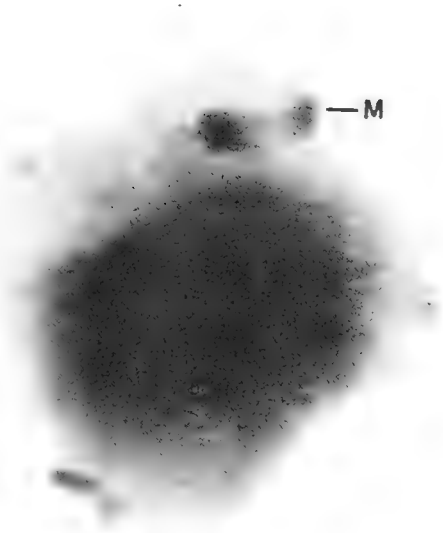

FIG. 7. *Sporangium in third cleavage. Mitotic figure (M) represents late anaphase. Stained in Heidenhain's haematoxylin. Light micrograph; 3,500X.*

Koch's methods (Koch, 1958) for revealing rhizoplasts, blepharoplasts, and nuclear caps were used, but none of these structures was detected. Little intracellular detail was seen. A large refringent body in the posterior end of the cell body, the nucleus, and two vacuoles in the anterior end were the only evident structures.

In about 0.1 per cent or less of the sporangia, a secondary sporangium with a normal cell wall was found within the primary cell. Both cells have the potential to sporulate. Several times motile planonts were released from both sporangia simultaneously, although it was more normal for the primary sporangium to release its planonts prior to first cleavage of the secondary cell. This double sporulation is probably the result of endogenous budding of the immature thallus which forms a cell within a cell. Endogenous budding has been previously reported by Mackin *et al.* (1950).

Infection Experiments

Oyster explants were successfully infected with the planonts using the methods described above. After 4 to 14 days of maintenance the explants in perfusion chambers were demonstrated to contain

infective cells by perfusing F. T. M. into the chamber and observing hypnospore formation *in situ*. Six out of 11 explants were successfully infected. The maximum number of hypnospores demonstrated in a single explant was four and they were always found separately, never in clusters; therefore, no reproduction occurred after the planont differentiated into a nonmotile cell.

Intermediate stages between the motile form and the hypnospore were not observed in a developmental sequence. Presumably the planont becomes an immature thallus and, when placed in thioglycollate, differentiates into a mature thallus (prehypnospore), then into a hypnospore.

The explants contained in the flask systems were infected in five of eight attempts, but in only two explants out of two experiments was there any indication that multiplication of the fungus had occurred. One cluster of 21 hypnospores and another cluster of four were found after culturing the explants in F. T. M.

Ray (1954b) demonstrated that after culturing infected oyster tissue in F. T. M., the tissue could be used to infect oysters by injecting the mass into the mantle cavity. He suggested that those cells which did not differentiate into hypnospores were responsible for the infections. In the present study, the remote possibility exists that nonmotile cells caused the infections; however, it is extremely unlikely that cells flowed passively through the connecting tubes to the explants — a distance of 90 mm. The fact that no hypnospores with discharge pores were found in the flasks with the explants is interpreted as strong evidence that the experimental procedures were effective in preventing passive flow of cells through the flask side arms.

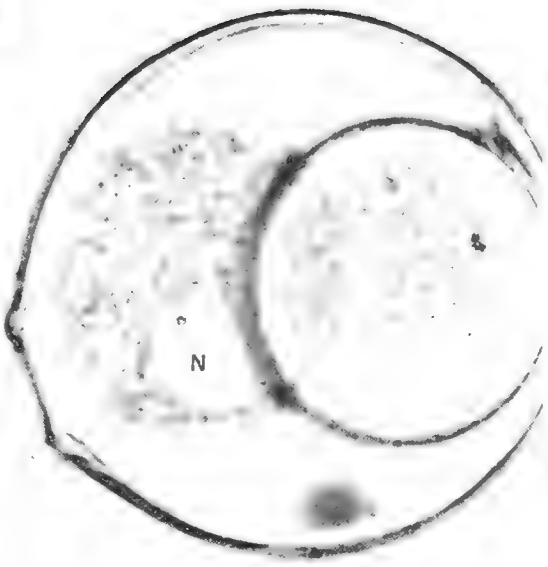

FIG. 8. *Living sporangium after completion of first cleavage. Nucleus (N). Light micrograph; 2,300X.*

Three oysters out of five were infected by using the beaker and flask method. One oyster was infected heavily enough to isolate the hypnospores after exposure to F. T. M. and to induce sporulation. Planonts which were morphologically typical *D. marinum* biflagellates were obtained; there-

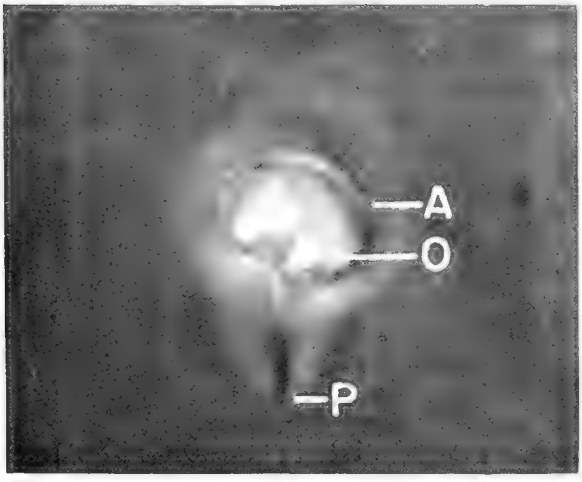

FIG. 9. *Planont fixed in 1 per cent Os04 fumes. Anterior flagellum (A); posterior flagellum (P); osmophilic body (O). Phase micrograph; 3,400X.*

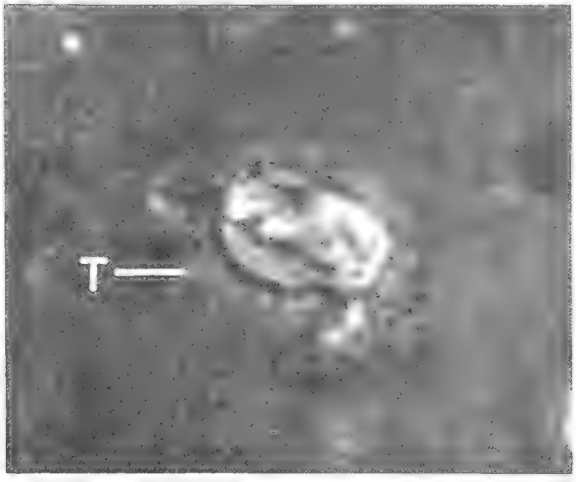

FIG. 10. *Planont, stained with mordant and crystal violet. Tinsels (T). Light micrograph; 4,000X.*

fore, the life cycle was completed under laboratory conditions, if one assumes that some of the prehypnospores, which differentiated into hypnospores in F. T. M., arose from planonts and not from prehypnospores. The authors know that prehypnospores were present in the experimentally infected oyster, because individual hypnospores were observed to form in F. T. M. slide preparations.

As was anticipated, hypnospores were drawn across the bridge between the flask and beaker in each experiment, probably by the ciliary action of the oysters. Empty hypnospores with discharge pores were found on the mantle surface of the infected oysters; therefore, it cannot be definitely stated that planonts were responsible for the infection. Nonmotile elements probably caused at least some of the infections.

DISCUSSION

The developmental stages which follow hypnospore formation have been demonstrated. Whether the planonts represent zoospores or isogametes has not been shown. Attempts to induce copulation of the planonts were inconclusive. Some *D. marinum* biflagellates were observed to be linked in rare instances, but the point of fusion was randomly located on the cell bodies. If copulation occurred, one would expect the copulation point to be a constant feature. It is believed that the "doublet" cells were examples of incomplete cell division.

The planonts can definitely infect oyster explants; therefore, it is reasonable to assume that living oysters are also infected by the biflagellates. The relative importance of the motile cells in transmission of infections under natural conditions is not certain. Ray (1954b) has demonstrated by injection of nonmotile cells into oysters that planonts are not the only infective elements. Further evidence concerning the role of planonts in transmission of infections will be discussed in a later paper.

ACKNOWLEDGEMENTS

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CYCLOHEXIMIDE: INHIBITION OF *DERMOCYSTIDIUM MARINUM* IN LABORATORY STOCKS OF OYSTERS [1]

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ABSTRACT

Cycloheximide (acti-dione), an antifungal antibiotic, prolonged the life of oysters that were naturally infected with Dermocystidium marinum, *a lethal fungus parasite. In closed aquaria, oysters treated with four concentrations (1, 5, 10 and 50 μg/ml/wk) of this antibiotic lived for several weeks to months longer than untreated control oysters. Presumably suppression of* D. marinum *infection by cycloheximide accounted for extended oyster longevity. The cycloheximide concentrations employed were chiefly inhibitory for lethal infections developed several weeks to months following cessation of treatmnt in all but one of the treated oyster groups. None of the oysters receiving 50 μg/ml/wk for 164 days showed* D. marinum *infection, by cultures, at death. The survival period of oysters treated for 45 days appeared directly related to antibiotic concentration. However, the survival period of those treated for 164 days was somewhat similar for all concentrations employed. Generally, oysters treated for 164 days lived longer and showed much fewer heavy infections at death than those treated for only 45 days. The feasibility of controlling* D. marinum *infections of oysters used for experimentation in closed systems by continuous treatment with a low level (1 μg/ml/wk) of cycloheximide is suggested by this study.*

INTRODUCTION

Cycloheximide (acti-dione), an antibiotic produced by *Streptomyces griseus,* inhibits the growth of many fungi and yeasts, but has little activity against bacteria (Whiffen, 1948 and 1950). Studies with 12 antifungal antibiotics showed that cycloheximide was the most effective in retarding *Dermocystidium marinum* enlargement and development in excised oyster tissues in thioglycollate and sea water cultures (Ray (a), in press).

This fungus, *D. marinum,* described by Mackin *et al.* (1950), is a common parasite of oysters, *Crassostrea virginica,* on South Atlantic and Gulf of Mexico coasts. A preponderance of field and laboratory evidence has incriminated *D. marinum* as a cause of massive oyster mortality during the warm season in relatively high-salinity estuarine waters (Andrews and Hewatt, 1957; Mackin, 1962; Ray, 1954). Furthermore, *D. marinum* is so widespread in the Gulf region that it is practically impossible to consistently obtain parasite-free oysters for physiological and pathological studies, thus severely impairing the conduct of such studies.

Moreover, even the use of very lightly infected stocks may prove unsatisfactory for some studies, especially in closed aquaria, at water temperatures of 25°C or more. There is a marked tendency in the course of laboratory studies for *D. marinum* infections to produce either debilitating or lethal effects within a few weeks. Interpretations derived from experimental results obtained with oysters infected with an organism displaying such pathogenicity as *D. marinum* may therefore be questionable. Data obtained with parasitized oysters are often misleading because control animals survive poorly. The need for *D. marinum*-free oysters, especially for disease studies, stimulated a search for a method to control this fungus for laboratory experiments.

This report presents results of two *in vivo*

[1] This research was supported by Public Health Service Research Grant EF-00338 from the Division of Environmental Engineering and Food Protection and by Organized Research Fund, Texas A&M University.

studies designed to test the effects of cycloheximide on the development of *D. marinum* in naturally-infected oysters.

MATERIALS AND METHODS

Oysters used in these experiments, designated as *in vivo* studies No. 1 and No. 2, were obtained from upper Galveston Bay, Texas. The oysters were vigorously scrubbed and animals such as attached barnacles, bryozoa and hydroids were scraped from the shells. Each experimental oyster population was graded into size groups of 10-mm increments (e.g., 51-60, 61-70) and an equal number of animals of each size category was used as experimental groups. In order to eliminate cross-infection of oysters, each animal in each group was held separtely in a 1-quart specimen jar (covered with a Bakelite cap) containing 500 ml of filtered (Whatman No. 12 paper) sea water that received continuous aeration. The sea water, salinity about 25 ppt, and cycloheximide were renewed at weekly intervals. Water temperatures varied between 25° and 26°C during all but five weeks of the 379-day experimental period. The lowest temperatures, averaging 20°C, occurred during one week (November 18-24, 1964); and during the other four weeks (December 2-15, 1964, and February 24 to March 8, 1965) water temperatures of 22° to 23°C prevailed.

All oysters were checked twice daily for "gapers" (dead or dying oysters), and diagnosed for *D. marinum* by the original (P+S)[2] (Ray 1952a and 1952b) and modified (M+C) (Ray (b) in press) thioglycollate culture technique. A portion of gill and mantle was cultured by both methods and the rectum was additionally cultured

[2] The modified method differs from the original in that nystatin (mycostatin) and chloramphenicol (chloromycetin) serve as antimicrobial agents instead of penicillin G and dihydrostreptomycin. In this paper, the original method is indicated as P+S and the modified as M+C.

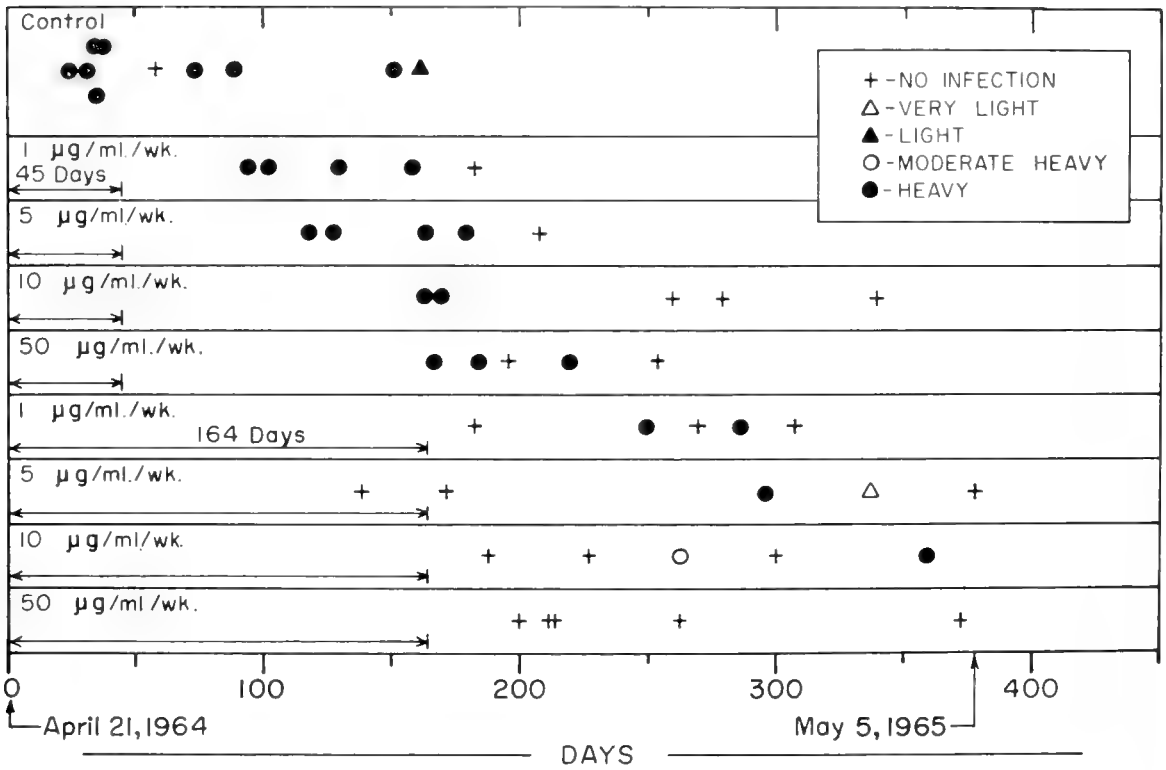

FIG.1. *Effect of cycloheximide on* Dermocystidium marinum *infection and oyster survival (*in vivo *study No. 1). Cycloheximide concentration indicated as µg/ml/wk and treatment period, by length of arrow. Position of each symbol, representing infection intensity according to legend, indicates time required for each oyster to become a "gaper".*

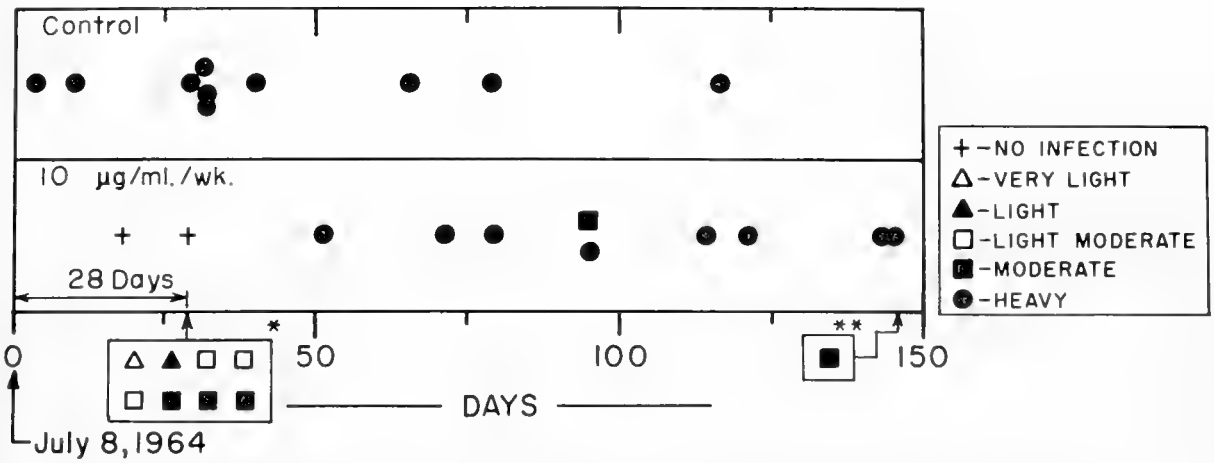

FIG. 2. *Effect of cycloheximide on* Dermocystidium marinum *infection and oyster survival (*in vivo *study No. 2). Cycloheximide concentration indicated as µg/ml/wk and treatment period, by length of arrow. The position of each symbol, representing infection intensity according to legend, indicates time required for each oyster to become a "gaper".*

**Infection intensity of eight oysters sacrificed immediately after cycloheximide treatment was discontinued.*

***Infection intensity of an oyster sacrificed after 146 days.*

by the M+C method. The infection intensity was estimated for "gapers" and live oysters by the procedure of Mackin (1962) and Ray (1954). The weighted incidence (Mackin, 1962) was also determined for live oyster samples. All "gapers", except badly decomposed ones, were fixed in Zenker's fluid and stored for future histological study. Tissues from oysters diagnosed as *D. marinum*-free by culture techniques were sectioned and stained by the Giemsa method. These slides were examined to corroborate results obtained with cultures.

IN VIVO STUDY NO. 1

The first study, *in vivo* No. 1, was conducted with a population of oysters having a mean length of about 70 mm. The extremes of length varied from about 60 to 75 mm. A base sample of 19 oysters was checked for *D. marinum* on April 16, 1964. The incidence of infections was about 70 per cent (13 of 19) and the weighted incidence was 0.90. This weighted incidence indicated an average infection level of "light" for this oyster population. However, the incidence and weighted incidence may have been a little higher than indicated since the diagnosis was made almost a week before the study was begun (data presented in Figure 1 suggest that the incidence in this population may have been as high as 80 or 90 per cent by April 21, 1964). Ten oysters of each group received each of the following cycloheximide concentrations: 1, 5, 10 and 50 µg/ml/wk for 45 days. Another group of 10 oysters, used as controls, was maintained in filtered sea water. After 45 days the cycloheximide treatment was discontinued for one-half (5) of the oysters in each of the four experimental groups. The other half (5) of each group continued to receive the indicated concentrations of cycloheximide for an additional 119 days (total exposure period, 164 days).

The results of *in vivo* study No. 1 (Fig. 1) show that oysters treated with cycloheximide lived much longer than the untreated control animals. One-half (5) of the control oysters, all of which were heavily infected with *D. marinum*, died within 41 days, whereas not one of the 40 treated oysters died during the same period. Furthermore, 94 days elapsed before the first experimental oyster died. During this period, however, 80 per cent (8) of the control oysters died.

These data show further that oysters treated for 45 days lived from 94 up to 339 days. Sixty-five per cent (13 of 20) of the oysters treated for 45 days showed heavy *D. marinum* infection at death and the remaining 35 per cent (7 of 20) showed no *D. marinum* by thioglycollate tests. In the groups treated for 164 days, the extremes of survival varied from 138 days to 379 days. Moreover, only one of 40 experimental oysters died (after 138 days) during either treatment period. Only 30 per cent (6 of 20) of the "gapers" receiving the 164-day

treatment showed *D. marinum* infections, and only 20 per cent (4 of 20) of these oysters displayed heavy infections at death.

Collectively for all experimental groups approximately one-half (19 of 40) of the treated oysters were positive for *D. marinum* when diagnosed by thioglycollate tests. Seventeen of 21 thioglycollate-negative "gapers" were suitable for histological preparation. Upon examination of stained tissue sections, all but two of the "gapers" revealed cells that were recognizable as *D. marinum*. The infection intensity of the 15 positively diagnosed oysters was rated as follows: light, 4; moderate, 4; moderate heavy, 3; and heavy, 4.

IN VIVO STUDY NO. 2

The mean length of the experimental oyster population in the second study was 65 mm. Extremes of length ranged from about 50 to 80 mm. A base sample of 10 oysters from this population checked on the first day (July 8, 1964) of the study had a *D. marinum* incidence of 100 per cent; and the incidence and weighted incidence with the M+C method was 100 per cent and 3.85, respectively; the values were slightly lower with the P+S method: incidence of 90 per cent and weighted incidence of 3.00. The average infection level (between moderate and moderate-to-heavy) of the oysters used for *in vivo* study No. 2 was considerably higher than that of the first experiment.

Thirty oysters were subjected to a cycloheximide concentration of 10 μg/ml/wk for 28 days and 10 others were used as untreated controls. After this period 28 of the treated oysters survived. Eight of them were sacrificed to determine the *D. marinum* infection level at this time and 10 of them were transferred to jars containing filtered sea water. The remaining 10 treated oysters were used for other studies and are not considered in this report.

The second *in vivo* experiment (Fig. 2) indicates that mortalities of moderate-to-heavily infected oysters are arrested by exposure to cycloheximide. During the 28-day treatment only two (about 7 per cent) of the 30 treated oysters died (after 17 and 28 days), both of which were negative for *D. marinum* by the culture tests. During the same period, however, three (30 per cent) of the 10 control oysters died and each was heavily infected with *D. marinum*. Survival periods for the 10 control oysters varied from 3 to 117 days, whereas 11 of 12 treated oysters lived for 17 to 145 days. One treated oyster, which was moderately infected, was sacrificed after 146 days to conclude the study. All but one of the oysters, including both treated and untreated, that survived beyond 28 days were heavily infected with *D. marinum* at death. The exception was a moderately-infected treated oyster that lived for 90 days.

Among those oysters surviving beyond 28 days it was found that four of seven control oysters died within 40 days, whereas 52 days elapsed before a cycloheximide-treated oyster died. Moreover, only one control oyster survived beyond 78 days (lived for 117 days); in contrast, seven of 10 treated animals lived beyond this period.

All eight of the oysters sacrificed after the 28-day treatment period were infected; however, the average infection with both culture methods was considerably lower than that of the sample checked initially (28 days earlier). The weighted incidence with the M+C method (2.06) was higher than with the P+S method (1.25). Both of these values are about 50 per cent less than those obtained 28 days before.

DISCUSSION

Although these data are limited, results indicate that cycloheximide may be employed to arrest the development of lethal levels of *D. marinum* infections in lightly infected oysters maintained in closed laboratory systems. These data also show that prolonged treatment will extend survival. For example, oysters treated for 45 days with 1 mcg/ml/wk lived for 94 to 181 days in contrast to survival periods of 181 to 307 days for those receiving the same concentration for 164 days.

Similarly, an increased survival period was noted with longer exposures to the three other concentrations employed. It also appears that survival may be prolonged by increasing the concentration of cycloheximide. Animals receiving 1 μg/ml/wk of cycloheximide for 45 days survived 94 to 181 days. Those treated for the same period with 5 μg/ml/wk lived for 118 to 212 days, and those treated with 10 μg/ml/wk lived 163 to 339 days. The difference in survival periods at various concentrations of the drug was less uniform for the 164-day period than for the 45-day period. The lack of uniformity may be due to the influence of other adverse conditions, such as the lack of food, during the prolonged exposure period.

The results of *in vivo* study No. 1 permit some comments regarding influence of starvation on oyster survival. These data suggest that oysters survive for surprisingly long periods and are difficult to starve to death if lethal agents, such as *D. marinum*, are suppressed or excluded. For example, 28 of 40 treated oysters survived for long periods, between 6 to 12 months. Therefore, the failure of at least one of the 10 control (untreated) oysters to live as long as 6 months is in striking contrast. Other workers (Gillespie *et al.*, 1964 and Mackin, unpublished data) previously have observed prolonged survival of starved oysters. Gillespie and co-workers found that about 75

per cent (17 of 23) of the unfed oysters maintained in filtered artificial sea water (about 15 liters per oyster, changed at monthly intervals) lived at least 150 days. One oyster survived for 390 days. (The report contained no data on *D. marinum* incidence.) Consequently, prolonged survival of oysters subjected to marked starvation weakens the tenability of the belief that *starvation* (insufficient food supply) constitutes a significant cause of natural oyster mortalities. The unlikelihood of oysters starving to death has been suggested earlier (Gillespie *et al.*, 1964 and Mackin, 1961).

During investigations of adult oyster mortalities, absence of obvious causes has led some workers to speculatively blame paucity of food (Mackin, 1961). Because it is doubtful that there are occurrences of starvation conditions as severe as those in the present studies, it seems unreasonable to attribute oyster mortality to temporary periods of inadequate food supply. The above comments are not intended to minimize the role of food and proper nutrition as a requirement for the general well-being and propagation of natural oyster populations. They apply only to my belief that *starvation* is of little consequence as a direct and major cause of adult oyster mortality.

This extended survival of treated oysters probably resulted from the effects of cycloheximide on *D. marinum*. About one-half (21 of 40) of the treated oysters were free of the parasite according to thioglycollate tests. Nevertheless, 15 of 17 thioglycollate-negative "gapers" which were examined histologically showed infections with intensities ranging from light to heavy. Compared with *D. marinum* in sections of either treated or untreated thioglycollate-positive "gapers", the parasites in treated thioglycollate-negative "gapers" stained very lightly and appeared abnormally distorted. Moreover, multinucleate (rosette) forms were rarely observed in thioglycollate-negative "gapers". Since *D. marinum* failed to develop in thioglycollate cultures of several "gapers" obtained months after treatment was ended, it appears that cycloheximide permanently altered or killed the parasites in some oysters.

Cycloheximide showed some effectiveness in retarding death and inhibiting *D. marinum* in an oyster population that had a high level of infection. A 28-day treatment with 10 μg/ml/wk (*in vivo* study No. 2) appeared to reduce significantly the infection intensity in live oysters. This reduction was temporary, however, since lethal infections began to develop about 25 days after discontinuation of treatment. All oysters that survived beyond the treatment period showed lethal *D. marinum* infections at death.

Although cycloheximide treatment did not have the desired result of completely destroying *D. marinum* in an oyster population, it shows promise for use in suppressing development of lethal infections. Information obtained from the present study suggests the feasibility of using low dosages continuously throughout the experimental period to inhibit *D. marinum* in oysters held in closed systems. Oysters receiving a low level (1 μg/ml/wk) of cycloheximide for 164 days lived almost as long as those subjected to higher dosages (5, 10 and 50 μg/ml/wk) for the same period.

Mention should be made of the properties of cycloheximide that make its use in aquaria and hatchery operations more practicable than many other antifungal antibiotics. It is commercially and readily available in technical grade since it has agricultural uses (Ford *et al.*, 1958). Also, unlike most antifungal antibiotics, it is stable at room temperature and is not inactivated by light (Ford and Klomparens, 1960). Its stability, however, in alkaline solutions is poor.

The Upjohn Company has conducted some studies to determine the stability of cycloheximide in a sample of sea water from the supply used in the present study (salinity about 25 ppt). The test results (personal communication from Dr. Gerald A. Boyzack, Upjohn Co., Kalamazoo, Michigan) show, at concentrations of 12, 42 and 100 μg/ml, a loss of about 25 to 40 per cent of the activity after one week's storage at room temperature; after two week's storage, about 40 to 50 per cent of the activity remained. Despite this activity loss, the results obtained in this study show that weekly applications at low levels do inhibit *D. marinum*.

Additional studies, employing much higher concentrations of cycloheximide than those used in the present studies, are now being conducted. Also, since *D. marinum* development is markedly inhibited at water temperatures of 15°C and lower (Andrews and Hewatt, 1957), oysters are being treated with cycloheximide at 16° to 17°C. The combination of low temperature and cycloheximide-treatment may completely destroy *D. marinum* in naturally infected oysters held in closed laboratory systems and may be a useful method for controlling mortalities in aquaria.

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SOME OBSERVATIONS ON THE SEASONAL DISTRIBUTION OF SELECTED ENZYMES IN THE AMERICAN OYSTER AS REVEALED BY ENZYME HISTOCHEMISTRY [1]

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ABSTRACT

The seasonal distribution of some hydrolytic and mitochondrial enzymes was observed in normal and diseased oysters. During the active feeding months it was noted that the digestive gland was especially rich in enzymes; their cellular localization and distribution are discussed. As the epithelium of the digestive gland regresses during the winter hibernation period, activities of both classes of enzymes fall to low levels. Also, enzyme fluctuations in the developing and regressing gonad are marked for several of the enzymes surveyed. Epithelial tissues in the oyster appear to be quite reactive for many of the enzymes reported here; ciliated epithelial tissues, especially, show a concentration of mitochondrial enzymes in a broad zone immediately under the ciliary border. As a consequence of a heavy infection of an unnamed haplosporidian parasite called "MSX", normal digestive physiology appears deranged as enzyme levels drop to near hibernation levels in the digestive gland.

INTRODUCTION

Much time and effort has been expended in studying the normal and abnormal physiology of the American oyster, the latter particularly since 1957 with the discovery of the parasite know as "MSX". To date, very little work on enzymes, particularly enzyme histochemistry has been published on the oyster. It seemed appropriate, therefore, that studies along these lines could profitably be undertaken with special emphasis on abnormal distributions of enzymes due to the diseased state. Since the normal histochemical pattern of enzymes is not well understood, it was imperative to work on this phase before describing the diseased and therefore the aberrant state. Also, since oyster tissues vary with the seasons, it was decided to follow and describe the seasonal fluctations, if any, of the various enzyme systems under consideration.

MATERIALS AND METHODS

Thin slices of tissue from the visceral mass, systemic heart and the region of the adductor muscle were fixed in either cold (O-2°C) acetone or alcohol-acetone (1:1) for 18 hours, then embedded in low-melting point (52-54°C) paraffin and sectioned at 8μ. Alkaline phosphatase was determined by the method of Gomori (1952); esterase by the method of Burstone (Pearse, 1960); and acid phosphatase according to Burstone (1958). All sides were appropriately counterstained to reveal nuclear detail; control sections were incubated either without substrates or were inactivated by hot water (80°C for 15 min) prior to incubation.

To demonstrate the mitochondrial enzymes, malic dehydrogenase, cytochrome oxidase and NAD [2] diaphorase, thin slices of tissue were removed from the visceral mass and immediately frozen in a cryostat maintained at — 20°C. After sectioning at 20 μ , sections were fixed for 30 seconds in cold (4°C) acetone and air dried before incubation. Malic dehydrogenase was determined by the method of Hess, Scarpelli and Pearse (Pearse, 1960); cytochrome oxidase by the method of Burstone (1961); and NAD diaphorase accord-

[1] The work reported here was supported by contracts No. 14-17-0003-88 and No. 14-17-0003 111 with the U. S. Bureau of Commercial Fisheries.

[2] The following abbreviations are used in this report: NAD (Nicotinamide Adenine Dinucleotide) ATP (Adenosine Triphosphate).

ing to Novikoff (1963). Slides were counterstained for nuclear detail and control sections were run as previously described.

RESULTS

A. *Alkaline Phosphatase*

During the spring, summer and fall heavy concentrations of this enzyme are found throughout the tubules of the digestive diverticula and in the apical border of the gut epithelia (Fig. 1). Leucocytes in the tissues are also very reactive. The gametogonia but not the mature sex cells, show marked concentrations of alkaline phosphatase. The winter hibernation period shows the enzyme limited to the apical border of the flattened epithelia of the digestive diverticula tubules; the regressed gonad still exhibits low to moderate amounts of the enzyme. The gut epithelial levels of the enzyme also drop significantly during this period.

B. *Esterase*

The tubules of the digestive diverticula in the spring, summer and fall feeding months show high concentrations of non-specific esterase in the secretory-absorptive cells but not in the generative cells (Fig. 2). The apical portions of the gut epithelia exhibit moderate amounts of the enzyme in this period. The winter condition of the digestive gland shows the enzyme still associated with the secretory-absorptive cells but present in reduced amounts consistent with the general regression of the gland. High levels of esterase are seen in the mantle, gill epithelia, kidney and ceroid cells throughout the year (Figs. 3, 4).

C. *Acid Phosphatase*

Marked concentrations of this enzyme are located in the secretory-absorptive cells of the digestive gland tubules during the spring, summer and fall feeding periods (Fig. 5). A striking contrast is seen in enzyme levels in the small ducts of the digestive gland: cell type I (Battle and Shaw, 1958) is completely devoid of the enzyme while a copious supply is evident in cell type II (Fig. 5). During the winter non-feeding condition, enzyme levels are much reduced consonant with the regressed gland. High levels of this enzyme are also found in the apical portions of stomach and intestinal epithelia throughout the year (Fig. 6). The mantle and gill epithelia also show moderate to intense levels of acid phosphatase. Leucocytes exhibit heavy concentrations of the enzyme visible as discrete particles which have been labeled tentatively as lysosomes.

D. *Malate Dehyrdogenase*

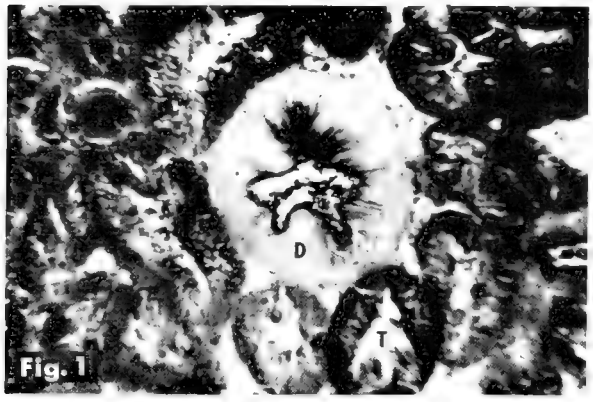
The distribution of this enzyme is typical of the many mitochondrial enzymes presently under investigation. Heavy concentrations are observed in the apices of the gut epithelia during the spring, summer and fall (Fig. 7); marked amounts are also observed in the basal portions of the secretory-absorptive cells as well as in the generative cells of the tubules of the digestive diverticula. Mature egg cells also show heavy concentrations of this enzyme. The winter hibernation period shows a marked regression of the enzyme in the gut epithelia and digestive gland. Relatively strong amounts can be detected, however, in the winter gonad. Moderate amounts are found in the mantle, gill epithelia, leucocytes, kidney and heart muscle throughout the year.

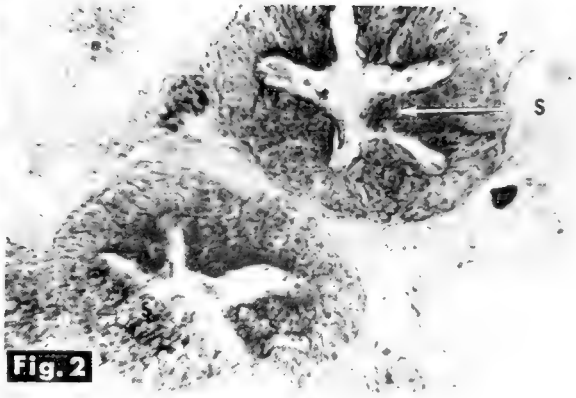
E. *NAD Diaphorase*

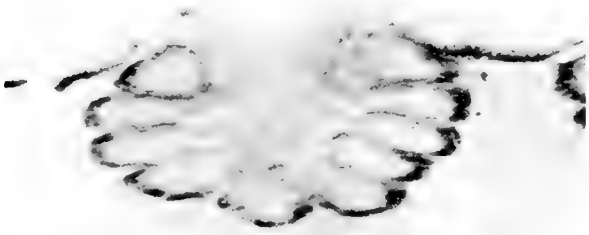
High concentrations of NAD diaphorase are found in all ciliary epithelia, especially just under the layer of basal granules. The tubules of the spring, summer and fall digestive gland also show heavy concentrations of the enzyme, particularly in the basal portions of the secretory-absorptive cells. The distribution of this mitochondrial enzyme in the small ducts of the digestive diverticula is just the reverse of that described for acid phosphatase; i.e., marked concentrations are observed in cell type I but practically none in cell type II. The winter non-feeding digestive gland has much reduced levels of enzyme but the distribution is very similar to the summer gland. The cytoplasm of the Leydig cells exhibits high levels during the spring and fall and heart muscle, especially the ventricle, has very high concentrations of this enzyme throughout the year. Copious amounts of the enzyme are found in the apical, vacuolated ends of the cells of the kidney and urinary bladder, particularly during the seasons of active feeding. Developing and mature egg cells exhibit high concentrations of this enzyme; a marked activity is also noted in the vicinity of the spermatozoa relative to the rest of the male gonadal follicle. The winter, dormant follicles show weak to moderate enzyme activities.

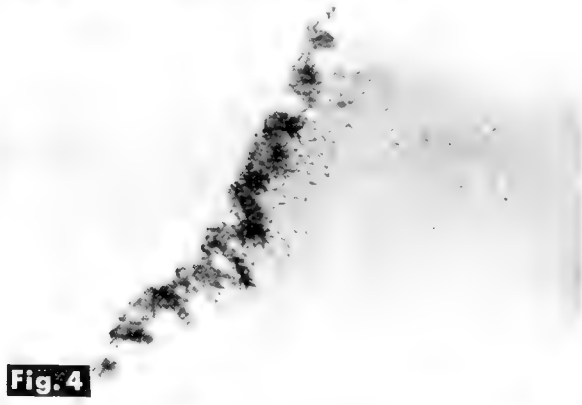
F. *Cytochrome Oxidase*

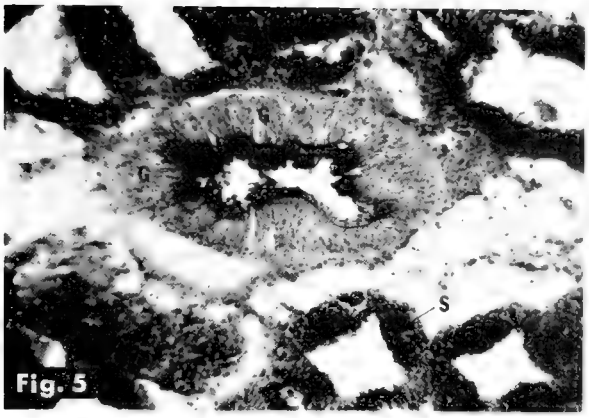
The apical border of gut epithelia (stomach, intestine, style sac, mid-gut) shows very heavy concentrations of cytochrome oxidase, with somewhat lesser amounts distributed throughout the middle and basal portions of these epithelial layers. The distribution of the enzyme in the spring, summer and fall digestive gland follows a pattern similar to the other mitochondrial enzymes; i.e., heavy concentrations in the generative cells and basal layers of the secretory-absorptive cells of the tubules; heavy concentrations in the apical borders of cell type I of the small ducts, while cell type II shows only moderate amounts. Leucocytes, whether in the lumen of the gut or in tissues contiguous











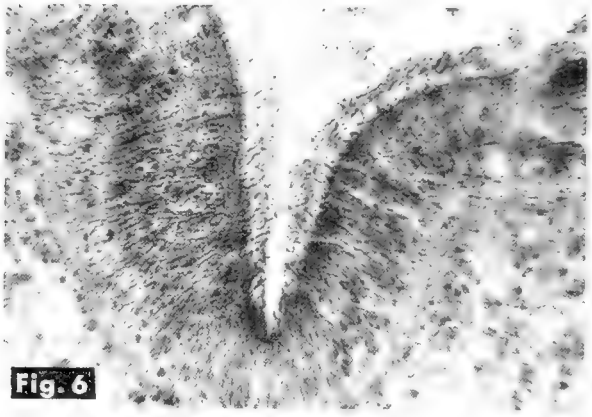


FIG. 1. *Distribution of alkaline phosphatase in the ducts and tubules of the active digestive gland. D, duct; T, tubule. X 200.*

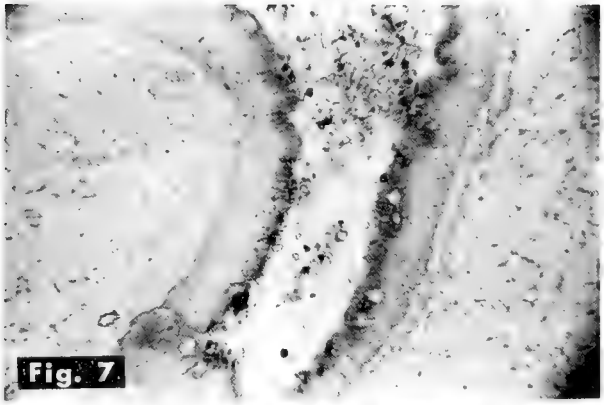
FIG. 2. *Distribution of non-specific esterase in the tubules of the active digestive gland. Note the presence of the enzyme in the secretory-absorptive cells(S). X 430.*

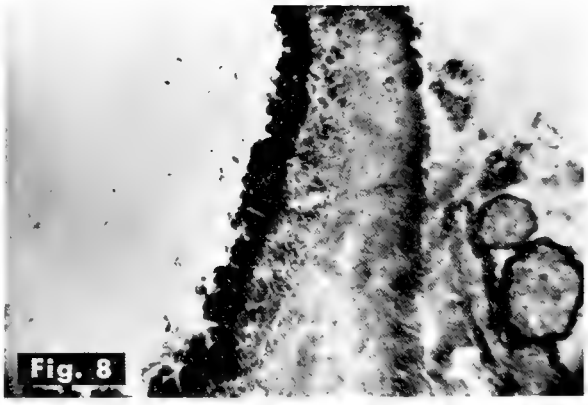
FIG. 3. *Localization of non-specific esterase in the epithelium of gill filaments. X 200.*

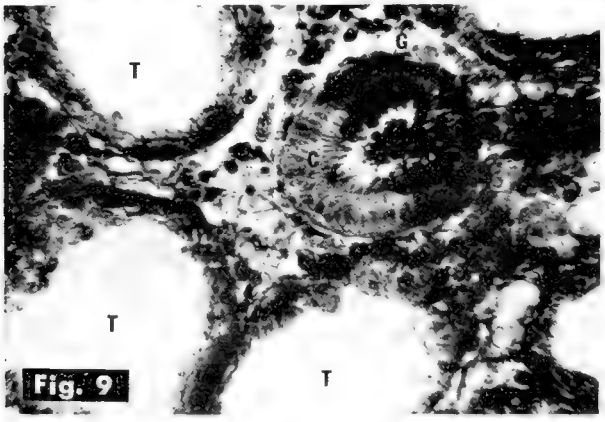
FIG. 4. *Distribution of non-specific esterase in mantle. Note high activity in apical portions of epithelium. X 430.*

FIG. 5. *Distribution of acid phosphatase in active digestive gland. Note intense reactions in secretory-absorptive cells (S) of the tubules and cell type II (G) of the ducts. X 200.*

FIG. 6. *Localization of acid phosphatase in a large duct of the active digestive gland. Note the concentration of the enzyme just under the ciliary border. X 430.*







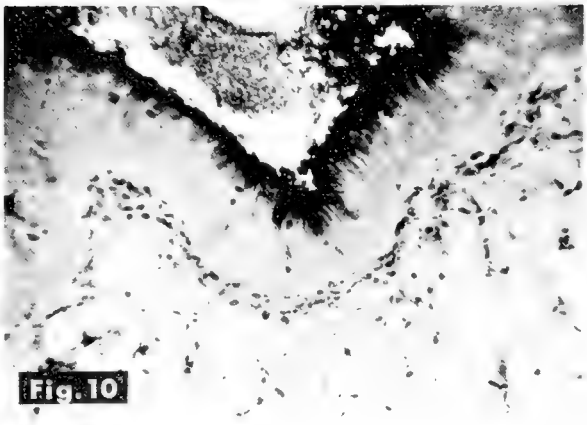


FIG. 7. *Presence of malate dehydrogenase in the intestine. Note the concentration just under the ciliary border. X 100.*

FIG. 8. *Localization of cytochrome oxidase in the mantle. Note the heavy concentration of the enzyme on the shell-side relative to the gill-side. X 100.*

FIG. 9. *Distribution of alkaline phosphatase in the hibernating digestive gland. Note the paucity of the enzyme in the regressed epithelium of the tubules (T) and the sharp distinction in enzyme localization between cell type I (C) and cell type II (G) of the small ducts. X 430.*

FIG. 10. *Distribution of alkaline phosphatase in the stomach. Note the concentration of the enzyme just under and extending into the ciliary border. Numerous enzyme-rich leucocytes may be seen in the peri-gastric blood sinus as well as wandering across the gastric epithelium. X 200.*

with the gut, usually exhibit marked activities for cytochrome oxidase. Enzyme levels in the digestive system drop sharply during the winter non-feeding condition.

The mantle and gill epithelia also show heavy concentrations of this enzyme, especially during the spring, summer and fall (Fig. 8). Heart muscle exhibits a marked enzyme activity at all seasons as does the kidney and urinary bladder. The distribution of cytochrome oxidase in the gonad is not unlike that for NAD diaphorase.

DISCUSSION

The hydrolytic enzymes studied to date, alkaline and acid phosphatase and non-specific esterase, have essentially similar distributions. The heavy concentrations present in the apical portions of the secretory-absorptive cells agree very well with the known digestive functions of these cells (Fig. 2). No trace of these enzymes is found in the generative cells of the digestive gland and the intensity of the enzyme reaction is seen to diminish sharply during the winter non-feeding condition. During this time the epithelium of the tubules of the digestive gland undergoes a marked regression as the cells become quiescent during the hibernation period. The distribution of the hydrolytic enzymes in the small ducts of the digestive gland is also of considerable interest. Heavy concentrations are

seen in cell type II which is a large, glandular type cell that apparently secretes mucous among its other functions (Fig. 9). The presence of hydrolytic enzymes in these cells may aid in the formation of the mucous material or initiate the digestive process as the food material travels toward the more distal tubules. The heavy concentrations of the enzymes near the ciliary border of the stomach and intestine (Fig. 10) suggest a more active role in digestion and absorption for these organs than has previously been indicated.

The fact that entire stretches of the mantle may be quite active for alkaline and acid phosphatase while other regions of this organ exhibit very slight activities, suggests that the shell-building role of the mantle is probably a discontinuous process, especially in the adult. Local areas of the mantle may be actively engaged in the construction of new shell or "chalky deposits" (Korringa, 1952) in order to maintain proper contact between shell and mantle while other portions may be quiescent. The heaviest concentrations of alkaline phosphatase are usually found on the shell-side of the outer or shell lobe and in the periostracal groove. This appears to agree well with the work of Galtsoff (1964) who pictures a portion of the mantle "stained" for this enzyme. This area of the mantle is instrumental in the initiation of shell growth, hence high concentrations of enzyme here are not surprising. Galtsoff (1964) and others have shown and suggested that alkaline phosphatase is instrumental in shell-building in the oyster and other mollusks.

The high concentrations of hydrolytic enzymes in leucocytes also tends to support their important roles as digestive, scavanger and defensive cells.

Strong activities of mitochondrial enzymes are seen in ciliated epithelium — gut, mantle, gonoducts (Fig. 7). Apparently the mitochondria are concentrated in a layer immediately under the ciliary border, producing ATP necessary for ciliary movement. As ATPase has been located on the cilia of many animals, including man (Cress, 1965), it would be interesting to determine the distribution of this enzyme in the oyster. The concentrations of mitochondrial enzymes in the basal regions of the secretory-absorptive cells of the tubules of the digestive gland suggest their energy-producing support, possibly even in the synthesis of the hydrolytic enzymes located in the apical portions of these same cells. The heavy concentrations of cytochrome oxidase in the mantle, especially the mantle margin, lends support for the active role of this complex organ.

The annual cycle of the gonad shows marked fluctuations in levels for alkaline phosphatase and the mitochondrial enzymes. Developing and mature egg cells show intense activities for malate dehydrogenase, cytochrome oxidase and NAD diaphorase. Oyster egg cells apparently are generously endowed with mitochondria, so necessary for supplying the required energy for the active embryos and larvae. Strong activities of these enzymes are also noted in the male maturing gonadal follicles with a distinct concentration about the mature spermatozoa. Oyster sperm (Galtsoff and Philpott, 1960) like that of higher animals, have many mitochondria in the mid-piece to supply the energy for locomotion.

Diseased animals show abnormal enzyme distributions usually in the digestive gland and alimentary canal. A heavy parasite burden of "MSX", for example, frequently manifests itself in the gross appearance of the digestive gland which appears quite pale. Histochemically, this organ shows a significant decrease in enzyme levels especially in acid and alkaline phosphatase and esterase. The plasmodium of the parasite appears to be rich in mitochondria and especially the enzyme, malate dehydrogenase. This agrees well with some recent work on the electron microscopy of the parasite[3]. More information is needed on abnormal enzyme levels in various tissues and leucocytes, especially in the terminal stages of the disease.

ACKNOWLEDGEMENTS

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EXPERIMENTS WITH OYSTER PURIFICATION IN FLORIDA[1]

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ABSTRACT

Twelve tests on purifying oysters, using ultraviolet light as the sterilizing agent, were conducted. The depuration unit employed was capable of purifying oysters using a low circulation factor at water temperatures of 24 to 26°C. No special cleaning procedure of the bivalve was necessary. In all tests except one, meats were at acceptable bacterial levels within 12 hours. In seven tests the bacterial counts of the water dropped to or remained at zero within four hours and in all tests, within 24 hours. Removal of fecal material did not appear to alter results.

INTRODUCTION

Florida, with approximately 8,000 miles of shoreline, has many acres of actual or potential water bottoms suitable for shellfish culture. In the past, most of Florida's leading estuaries supported thriving shellfish industries. Many of these areas have been rendered unsafe for production due to sewage pollution. Mollusks growing in these waters may carry many diseases such as typhoid, dysentery, and hepatitis. An effective control of pollution is desirable but not always possible. Depuration, or cleansing of shellfish from marginal or polluted waters, could be a solution to this dilemma.

The objectives of this study were to check factors which influence purification and to develop a shellfish purification system suitable for commercial use in Florida.

Depuration is not a new process and has been successfully used for many years to cleanse mussels and clams. In England, Dodson (1928) developed a system using chlorine as the active sterilizing agent and Wood (1957) modified this method for subsequent use on oysters. Several clam depuration plants using chlorine are or have been in operation in the United States, including one at Newburyport, Massachusetts, since 1930.

Use of chlorine in shellfish purification has a serious drawback. Kelly *et al.* (1960) established that chlorine had an adverse effect on the feeding activity of the American oyster, not removed by dechlorination.

Kelly *et al.* (1960) and Wood (1961) tested ultraviolet light as a sterilizing agent and found no adverse effects on feeding activity of oysters. Wood further believes that depuration plants capable of treating 10,000 oysters per operation could be constructed.

The practice of depuration is based on the physiology of shellfish. Briefly, these animals are filter feeders capable of passing several gallons of water per day through their gills. Nutrients are obtained from the water by the ciliary activity and mucus collection which strains off microorganisms and suspended organic materials. Pollutants, including sewage bacteria, can be discarded as pseudofeces or eliminated with the feces. According to ZoBell and London (1936), some of the microorganisms are probably utilized as food by these animals. Shellfish will purify themselves when placed in clean water. The purpose of ultraviolet treatment in a purification system is to keep the water free of harmful bacteria.

MATERIALS AND METHODS

Tests were conducted in a small pilot system with recirculated salt water. The physical structure of the plant (Fig. 1) consisted basically of five parts: sterilizing unit, aeration baffles, holding tank, circulating pump and plumbing, and filter. Most of the component parts were made of

[1] Contribution No. 98, Division of Saltwater Fisheries, Florida Board of Conservation.

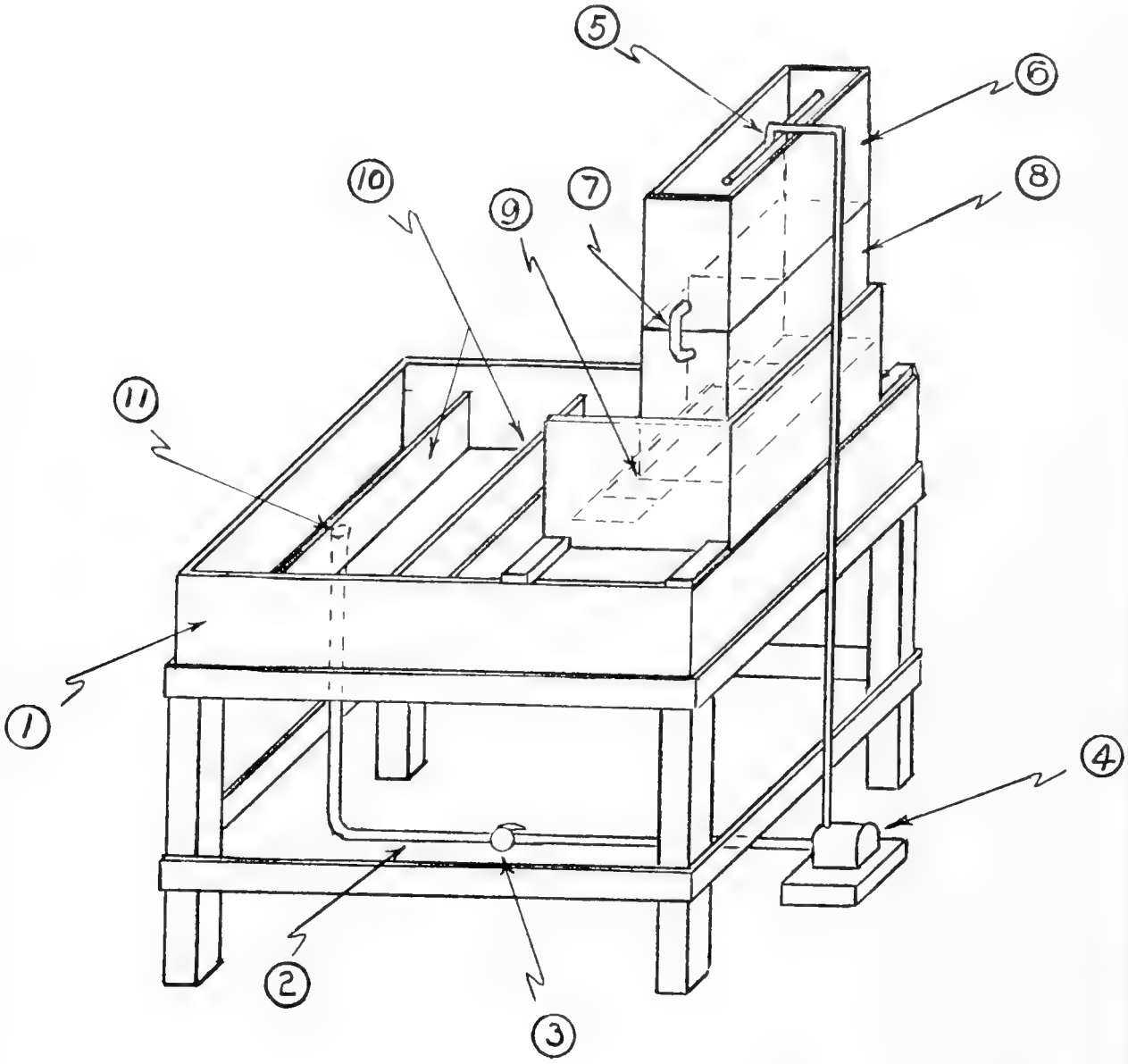

FIG. 1. *Illustration of oyster purification unit: (1) holding tank, (2) plumbing, (3) valve, (4) pump, (5) inlet nozzle, (6) filter unit, (7) connecting pipe, (8) ultraviolet sterilizer unit, (9) aeration baffles, (10) weirs, (11) outlet.*

epoxy-covered plywood and 3/4-inch PVC plumbing fixtures. A polyethylene pump and valve were also used.

The sterilizing unit was 10 inches wide, 24 inches deep, and 22 inches long. It housed three 15-watt ultraviolet germicidal lamps located six inches above the water line. Water from the sterilizer flowed down the aeration baffles into the holding tanks.

The holding tank measured five feet long, three feet wide, and one foot deep. Good circulation was accomplished in this tank by dividing it with three weirs into four compartments. The first three compartments were 18 inches wide and the last com-

partment at the outlet end was 6 inches wide. The first section served as a splash area for incoming water, the middle two held the shellfish, and the last served as an outlet and settling basin. Water flowed from the aeration baffles into the first compartment, over the first weir, under the second weir and over the third, into the last compartment. A slight drop in the water level was maintained at the first and third weirs. Water flowed through a 1/16-inch space under the middle weir. Epoxy-covered plywood racks, one inch from the bottom of the tanks, held the oysters stacked in two or three layers, depending on their size. Two hundred liters of water were maintained in the system.

Water was pumped from the last compartment into the filter box located above the sterilizing unit. The pump was rated at nine gallons per minute head and the rate of flow was controlled by a polyethylene ball and socket valve placed in the line at the inlet of the pump. The filter box was 24 inches by 10 inches by 12 inches and contained a 1-inch layer of replaceable glass wool. Water was allowed to flow by gravity from this box back into the sterilizer to complete the cycle.

Water for these tests was drawn directly from Bayboro Harbor, filtered with a nylon fiber filter, adjusted with deionized water to 17-21 ppt, and tested for coliform bacteria. The filter removed all suspended material larger than 30 μ and most of the smaller particles. No direct control of the temperature was attempted; however, the system was located inside a building and the water temperatures varied from 24 to 26°C. The pH varied from 7.7 to 7.9.

EXPERIMENTAL PROCEDURES

Twelve experiments were conducted in this study. The following factors were checked: (1) the ratio of oyster numbers to water volume; (2) the circulation factor (the number of times the whole water volume passed through the sterilizer in one hour); (3) the effect on bacterial counts of the removal of feces and pseudofeces from the water. Observations were also made to determine whether cleaning the animals and sanitizing the tanks and equipment influenced coliform counts.

The experiments were evaluated by the rate of removal of coliform bacteria from the oyster meats and the concentration of coliform bacteria in the water. The lactose presumptive and bile green confirmation tests used in these experiments were conducted under rigid laboratory control and the methods are described in "Recommended Procedures for the Bacterial Examination of Shellfish and Shellfish Waters" (1947) and "Standard Methods for the Examination of Water and Waste Water" (1960). A series of standard agar plate counts, as described in "Standard Methods for the Examination of Dairy Products" (1963), were made on the oyster meats for each experiment in an effort to correlate the progressive decline of the coliform with the non-coliform bacteria.

Three 15-watt ultraviolet lights and 200 liters of water were used in all experiments. The first depuration study involved 195 oysters while 400 were used in all other experiments to gain a better insight of a commercial operation. With the exception of the first experiment, the oyster-to-water ration was two.

Because polluted oysters were scarce, the shellfish used in experiments five through 12 were treated with sewage effluents. A safe level of coliform content for shucked commercial oysters was considered by health authorities to be below the MPN (most probable number) of 16,000 per 100 ml of sample, and for our paper this standard will be used to classify acceptable coliform counts. Pollution was accomplished by adding raw sewage effluent to a circulating tank system for four days. Slightly more than 400 animals were treated each time by adding one quart of effluent to 400 liters of water daily in the tank. Ample aeration was provided.

During the first two experiments a high circulation factor of 4.6 was used and the feces and pseudofeces were siphoned off once a day. The third experiment involved a reduced circulation factor of 2.23. No fecal materials were removed from the tank in this or any of the remaining tests.

In Experiments 4 through 8, the circulation factor was varied from 1.00 to 2.97. In Experiments 4 and 5, before the bivalves were placed in the tanks, the water was treated with ultraviolet light and the coliform content tested zero; also, all tank surfaces, pipes, and valves were sanitized with ethyl alcohol and thoroughly rinsed with tap water. In other experimental conditions, tests 4 through 8 were nearly identical.

In the first eight experiments the bivalves were separated into singles, cleaned of fouling organisms, and scrubbed thoroughly with a stiff fiber brush. In Experiments 9 through 12, the oysters were separated into small clumps only and simply hosed off. The circulation factor was varied from 1.00 to 2.70.

RESULTS AND DISCUSSION

Values for some of the experimental variables and a summary of results are given in Table 1.

Initial MPN of the meats in Experiment 1 was 9,500 per 100 ml, a figure indicating insufficient pollution. The water was free of coliform bacteria after four hours and the count in the meat had dropped to 330 per 100 ml in 24 hours. For the

TABLE 1. *Values for some experimental variables and summary of results.*

Exp. no.	Date	Temp. °C.	Circ. Factor	Sal. ppt		Bacterial Analysis: Initial MPN/100 ml	4-hour test MPN/100 ml	24-hour test MPN/100 ml	48-hour test MPN/100 ml
1	10-15-64	24	4.60	20.0	Meats	9,500	2,400	330	58
					Water	4.5	0	0	0
2[1]	10-26 64	26	4.60	18.7	Meats	240,000	1,300	490	79
					Water	5.6	7.8	0	0
3	11-19-64	25	2.23	17.1	Meats	3,300	490	0	0
					Water	170	0	0	0
4	11-23-64	25	2.23	18.5	Meats	11,000	700	9,200	1,400
					Water	0	0	0	0
5[2]	12- 7-64	25	2.97	18.5	Meats	95,000	330	3,300	130,000
					Water	0	0	0	0
6	1- 4-65	24	2.97	20.7	Meats	18,000	700	490	70
					Water	0	0	0	0
7	1-18-65	20	2.00	20.9	Meats	22,000	1,100	230	79
					Water	0	0	0	0
8	2-21 65	26	1.00	18.5	Meats	18,000	790	330	230
					Water	34	22	0	0
9	3-22-65	26	1.00	21.0	Meats	18,000	330	240,000	490
					Water	0	46	0	0
10	4- 5-65	26	2.97	20.5	Meats	17,000	230	330	330
					Water	0	6.1	0	0
11	4-26-65	24	2.00	20.7	Meats	22,000	790	230	0
					Water	2	2	0	0
12	5-18-65	26	1.00	21.0	Meats	18,000	330	230	79
					Water	0	0	0	0

[1] Incidental test at 12 hours: water recorded 0 MPN.

[2] Incidental test at 36 hours: meats recorded 17,000 MPN.

second experiment, the initial meat MPN was 240,000, dropping to acceptable levels after four hours and the water was coliform free after 12 hours. In the third experiment, results showed the water count was zero and the bivalve flesh was 330 MPN after four hours. Tests on water and oyster meats showed no increases in coliform count throughout the experiment. This indicated that fecal material did not cause a build-up of coliform bacteria in the meats or water. However, these bivalves were not sufficiently polluted initially.

In Experiments 4 through 8, with the exception of Experiment 5, the coliform counts were reduced within four hours to less than 16,000 MPN. Although in some cases slight increases were noted within the 48-hour test period, the MPN did not exceed the accepted standard. The bivalves tested in Experiment 5 were those used in the preceding experiment. They were re-treated with sewage effluent before use. Coliform counts on the meats fluctuated erratically in Experiment 5 and the final two tests were high. After 36 hours, the coliform count increased to 17,000 MPN and the 48-hour test showed a MPN of 130,000. When compared with the other experiments, no physiological difference was noted in these oysters. However, since they were reused from the prior experiment, the possibility existed that the oysters were induced into inactivity by this treatment. The high coliform count could be attributed to inactivity.

For the last four experiments, the counts made at the end of four hours showed a significant decrease in the coliform MPN count. No appreciable subsequent increases were recorded in Experiments 10, 11, and 12. However, in Experiment 9, a build-up of coliform in bivalve flesh occurred at the end of 12 hours. The MPN ranged from 240,000 at the end of 12 hours to 4.9 million at the end of 18 hours and back to 240,000 at the end of

24 hours. The final 48-hour count had dropped to 490 MPN. During this experiment the oysters spawned extensively and rapid multiplication of coliform bacteria could have been engendered by the spawn. Since the coliform counts in the water for the same period were low, it seems likely that the bacterial growth occurred within the oysters.

Coliform level of the water was low throughout each experiment. In seven tests the bacterial counts of the water dropped to or remained at zero within four hours. No coliform were observed within 24 hours.

Total plate counts showed a progressive decline in noncoliform bacteria that in most cases could be correlated with the decline in coliform bacteria. This indicates that the depuration system is effective against microorganisms other than the coliform groups.

General characteristics common to all the experiments should be mentioned. They include: (1) a loss of water volume from the tanks due to evaporation; (2) the presence of physiological by-products evidenced by numerous frothy bubbles; (3) a mortality of less than one oyster per trial.

Although our operation did not involve more than two bushels of oysters, it would appear that any problems that might be anticipated in a commercial operation could be overcome. More detailed investigations on a commercial scale are needed to ascertain the full potential of depuration utilizing ultraviolet light as the sterilizing agent.

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THE LABORATORY REARING OF THE SHIPWORM, *BANKIA SETACEA* (TRYON)

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ABSTRACT

The laboratory rearing of the marine borer, Bankia setacea *(Tryon), is described from the fertilized egg to metamorphosis. The conditions for egg and sperm release and the difficulties encountered in rearing this mollusk are discussed.*

INTRODUCTION

The culture of the marine borer larvae of *Bankia setacea,* the only species of the Teredinidae commonly found in the waters off the northwest coast of North America, is a preliminary goal of a research program designed to elucidate possible methods of control. Up to the present time, this species of marine borer has never been cultured from the fertilized egg to metamorphosis. However, it is needless to state that without the pertinent contributions of such investigators as Loosanoff and Davis (1) and Quayle (2), progress as reported herein would have been much more difficult.

Loosanoff and Davis (1) have described the conditions and equipment required for rearing a number of mollusks, among these the marine borer, *Teredo navalis* (Linne). *Teredo navalis* is larviparous, raising the fertilized egg to the young larval stage within its gill system before release. On the other hand, *Bankia setacea* is oviparous, releasing eggs and sperm for fertilization and development in the surrounding sea. Adult *Teredo navalis,* when maintained at 15° to 20°C, spawned at 14°C or higher, and released larvae in the temperature range of 16° to 20°C. Immature larvae or fertilized eggs removed from the gill system developed normally to metamorphosis in a period of four weeks at 20°C (1). Quayle (2) studied the early development of the pelagic larvae of *Bankia setacea* from plankton samples. From the dimensions of the larvae obtained from successive samples, the pelagic period was estimated to be in the range of four weeks at a temperature of 12° to 15°C.

The purpose of this investigation was to define the conditions for the laboratory culture of the marine borer, *Bankia setacea.*

METHODS

Living adult *Bankia setacea* were obtained from catcher sticks, 1/2 x 1/2 x 6-inch or 1 x 1 x 12-inch fir sticks which were completely protected with neoprene except for one end to permit the entry of larvae. Infested sticks, when cleaned of debris, could be maintained in aerated sea water (25 ppt) for at least one year, provided sufficient wood remained for the borers. The sticks, which were renewed periodically, were the source of adult *Bankia setacea* from which the eggs and sperm were obtained. The algae food cultures, *Monochrysis lutheri* and *Isochrysis galbana,* were supplied by Dr. R. Ukeles, Bureau of Commercial Fisheries, Biological Laboratory, Milford, Connecticut. These pure cultures were grown in aseptic conditions in a sterile "Universal" medium as described by Loosanoff and Davis (1).

RESULTS AND DISCUSSION

The inhalant and exhalant siphons of male and female *Bankia setacea* exhibit morphological sex characteristics (3). The male exhalant siphon has four rows of papillae extending down the full length of the siphon and attempts to enter into the end of the female siphon during copulation. The siphons of the female do not have papillae and are very passive during copulation. If the male does not succeed in releasing sperm into the siphon of the female, both eggs and sperm are

released into the water where fertilization takes place (Fig. 1). A similar observation on copulation in *Bankia gouldi* was noted by Clapp (4).

FIG. 1. *Photograph of* Bankia setacea *showing the release of sperm by the male exhalant siphon after an unsuccessful attempt to fertilize the female.*

The conditions under which the eggs and sperm are released are difficult to define. If wood sticks containing mature adults are removed from the sea and placed in the laboratory tanks, they may or may not immediately release their eggs and sperm. From many observations, the release of eggs and sperm appears to depend on the degree of maturity of the adults. If *Bankia setacea* are brought into the laboratory before their gonads are fully mature they require approximately one to two weeks at 15°C before they will release their products. Specimens with ripe gonads may be induced to spawn by introducing fresh sea water at temperatures ranging from 7° to 25°C.

Bankia setacea were incubated at 15°C because this temperature approximates that of the sea in this area during the summer months when the food algae supply is greatest. Adults which had been maintained in the laboratory at 15°C consistently released eggs and sperm when the borers were exposed to fresh sea water at the slightly lower temperature of 8°C. In general, when the temperature of the fresh sea water varied from 8°C, either higher or lower, there was little induced sexual activity. Thus, two conditions were necessary for the release of eggs and sperm from adults held in aquaria at 15°C. First, fresh sea water was necessary. For some unknown reason the adults do not release their eggs and sperm into water in which they have been living in captivity for more than several hours. Secondly, the fresh sea water had to be at or near 8°C. When both these conditions were met, the release of eggs and sperm was generally assured.

Bankia setacea larvae below 0.068 mm would not survive in the presence of microorganisms. The septic cultural conditions successful with *Teredo navalis* and other mollusks did not apply to *Bankia setacea* (1). For example, fertilized eggs, washed free of debris and sperm, never grew beyond 0.080 mm, due to infection by bacteria or fungi. For survival they had to be removed from the adults, under completely aseptic conditions, by alcohol swabbing and isolation by organ culture techniques (5), and then kept under aseptic conditions with added penicillin and streptomycin for three days for the development of the early veliger stage. If the larvae were then placed either in large containers of fresh-running sea water or in five-gallon carboys containing aerated non-sterile sea water they would die of infection.

In one experiment, four-day old, healthy-appearing larvae were placed in 2-gallon containers fitted with screened openings of 0.060 mm mesh, lowered into the ocean 25 feet below low-tide level and about two feet above the ocean floor. Even though the sea around these containers was heavily infested with *Bankia setacea* larvae, the artificially-born, captive larvae survived only two weeks and grew to a maximum size of only 0.080 mm.

Artificial propagation of *Bankia setacea* to the stage of metamorphosis could be achieved only by culturing under completely aseptic conditions with algae added as food (Fig. 2). The absolute demand for aseptic conditions may be only a local problem and not one to be faced in laboratories in other areas. The authors have been notified recently that the larvae of the *Bankia setacea* can be reared to metamorphosis with relative ease in the clean marine water used by the Friday Harbour Laboratory, University of Washington.

The following procedure for obtaining eggs and sperm aseptically must be followed carefully. The catcher sticks containing adult *Bankia setacea* are placed in 95 per cent alcohol for five minutes. The adults are then removed from the sticks and placed in a fresh solution of 95 per cent alcohol for approximately one minute. While still submerged

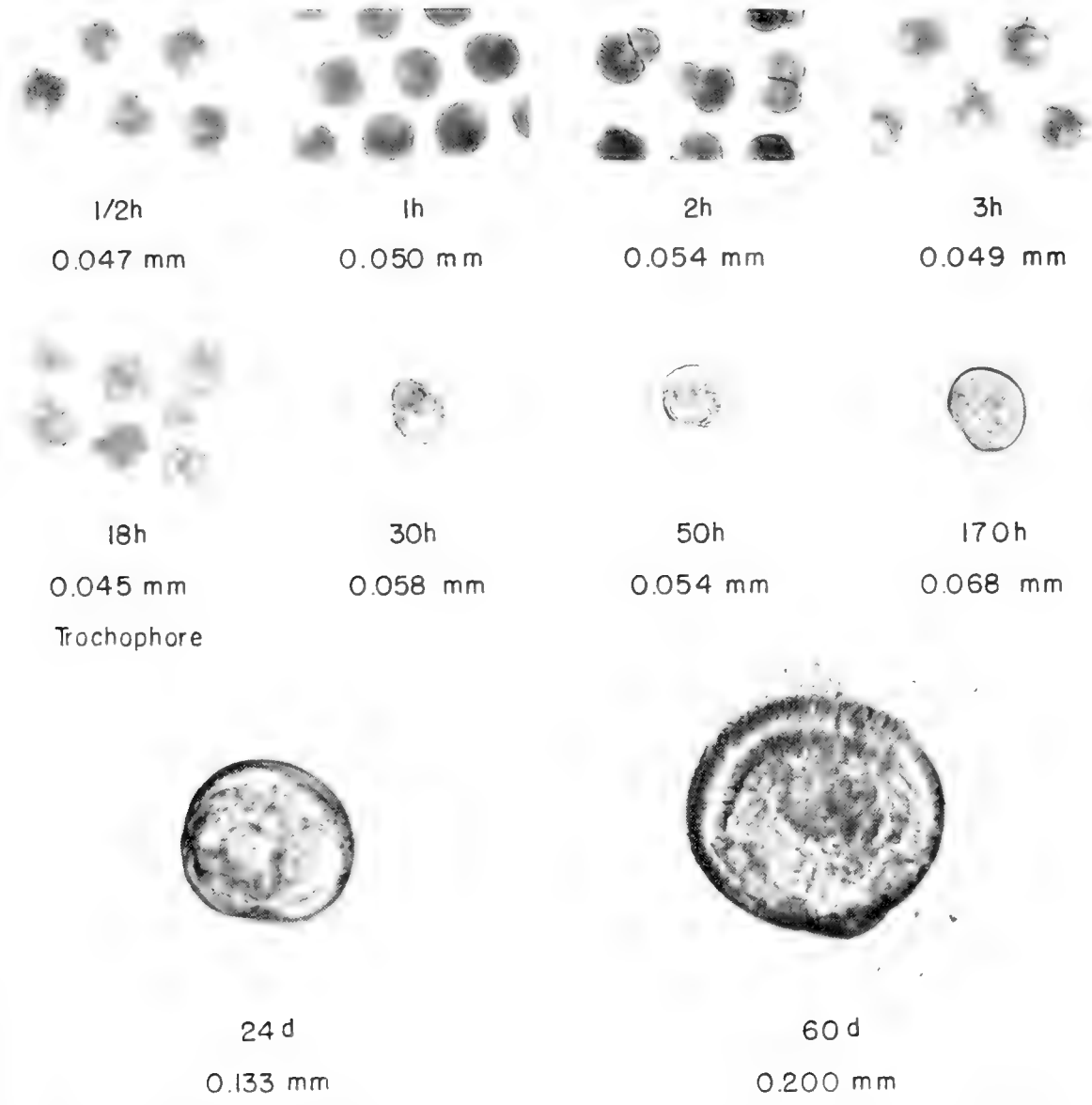

FIG. 2. *Photographs of* Bankia setacea *showing the development of the fertilized egg to the larvae at metamorphosis. All photographs are at the same magnification.*

in alcohol the mantle tissue is quickly removed and sections of the male and female gonadal tissue are placed immediately together into sterile sea water at 15°C. After a period of approximately 15 minutes, the fertilized eggs are washed by several cycles of slow-speed centrifugation and resuspended in sterile sea water. The fertilized eggs are then dispersed into 25 ml volumes of sterile sea water containing 50 units per ml each of penicillin and of streptomycin and incubated at 15°C for three days or until the early veliger stage is present. The larvae are placed then into suitable flasks which contain steam-sterilized sea water; e.g., 50 ml sea water in a 250 ml Erlenmeyer flask. One ml of concentrated growing culture of either *Monochrysis lutheri* or *Isochrysis galbana* is added to the flasks. Under present conditions, metamorphosis requires a minimum of two months and occurs when the larvae measures 0.186 mm in width. Only approximately 10 per cent of the

larvae reached metamorphosis.

This rate of development was double the time taken for the metamorphosis of laboratory-cultured *Teredo navalis* larvae and of *Bankia setacea* larvae growing naturally as estimated from plankton samples (2). As more knowledge of the optimum conditions for the culture of the mollusk becomes available, the total time for complete development of the *Bankia setacea* to the metamorphic stage may be decreased.

Larvae were found to develop to the veliger stage in salinity values from 40.4 ppt to 16 ppt. At 14 ppt salinity and below, normal cell development did not occur and the larvae failed to develop. The sudden osmotic shock to the 3-hour fertilized eggs used in this experiment was decreased by placing the eggs inside a dialysis bag and permitting the salinity to change from 25 per cent to the desired salinity over an estimated 4-hour period. The cultures were not followed beyond the veliger stage.

ACKNOWLEDGEMENTS

The authors are grateful for the support given by the Biology Branch of the Biological Science Division, Office of Naval Research, Washington, D. C. (NR104-760) and by the National Research Council Consolidated Grant No. CG-9. The authors are indebted also to Dr. C. C. Walden for his interest and encouragement.

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LARVAL DEVELOPMENT OF THE LARGE BLOOD CLAM, *NOETIA PONDEROSA* (SAY) [1]

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ABSTRACT

Larvae of Noetia ponderosa *were raised from eggs in laboratory cultures. Lengths increased from 80 to 210 μ over a 4-week free-swimming period. Height is 15 μ less than length in small larvae but as much as 55 μ less than length when larvae are ready to metamorphose. Depth varies from 25 to 70 μ less than length over the same period. The straight-hinge line is 65 to 80 μ long. The umbo is first formed at a length of 150 to 160 μ and becomes long and broad. Umboned larvae are brown with the shell becoming reddish-brown at the anterior end. However, these typically arcid colors are not as dark as in other species of this family. The anterior end is more pointed than the posterior in umbo larvae. The hinge consists of a central undifferentiated area with a series of small taxodont teeth at both the anterior and the posterior end of the hinge line in both valves.*

INTRODUCTION

Noetia ponderosa (Fig. 1), one of the common larger members of the family Arcidae, occurs in estuaries from Virginia to Florida and in the Gulf of Mexico (Abbott 1954). Its distribution is usually limited to high-salinity (above 25 ppt) subtidal areas where it is frequently attached to shells or other objects in a soft mud or sand substrate. This report describes the larval development of this species.

Adult *N. ponderosa*, collected periodically from the channels between the high-salinity marshes near Wachapreague, Virginia, contained gametes throughout most of the year, though they spawned in the laboratory on but three occasions during the fall and winter. Spawning was induced and the larvae were raised in the laboratory by the techniques described by Loosanoff and Davis (1963). Although some larvae were fed a variety of algae, only those receiving *Monochrysis lutheri* survived to metamorphosis. Water temperature ranged from 19 to 26°C in larval cultures.

Both male and female clams released a steady stream of gametes during spawning. Spermatozoan heads were triangular and 3.5 to 4.5 μ long. They were bluntly pointed anteriorly and 2.5 μ wide at the base of the tail which was about 60 μ long. Normal eggs were released either singly or in clumps. They were coral-red and about 65 μ in diameter. The yolk appeared coarse and granular under the microscope. Sample counts indicated that two isolated females had released 11,314,000 and 11,034,000 eggs respectively within a few minutes during a single spawning.

LARVAL DEVELOPMENT

Larvae of *N. ponderosa* are similar in appearance to those of other Arcidae. They grew slowly in our cultures. Straight-hinge larvae have a minimum length (maximum anterior-posterior dimension) of 80 to 85 μ. The apical flagellum becomes inconspicuous and disappears while larvae are still less than 100 μ in length. The hinge line is 65 to 70 μ long in one-day-old larvae (80 to 90 μ in length) but increases to 75 to 80 μ long in two-day-old larvae (95 to 105 μ in length). There is no further increase in the length of the hinge line during larval development. In early stages length is usually 15 to 20 μ greater than height (the dimension perpendicular to length) but the difference increases to 25 to 30 μ by the time the umbo stage begins at 150 to 160 μ. The difference continues to increase until length usually exceeds height by 45 to 55 μ at a length of 200 μ. Depth (the maximum left-right dimension) is about 25 μ less than length in the smallest larvae. In larger straight-hinge larvae depth is 45 to 55 μ less than length but may be as much as 70 μ less in larvae at metamorphosis.

At a length of 130 μ the anterior end begins to

[1] Contribution No. 217 from Virginia Institute of Marine Science.

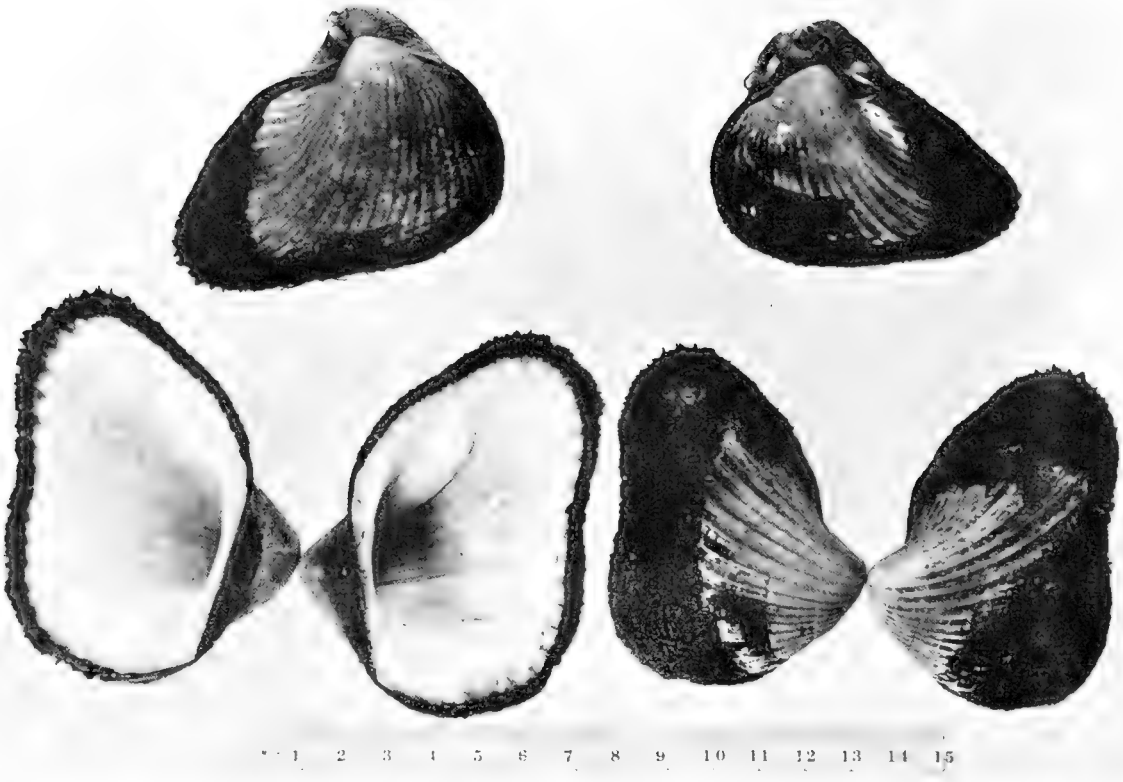

FIG. 1. *Above, living* Noetia ponderosa; *lower left, internal view of opened valves; lower right, external view of opened valves.*

develop a more pointed appearance than the posterior end. This difference becomes more noticeable as size increases. Nevertheless, the anterior and posterior ends are of nearly equal length. Shoulders are short and form a continuous line obscured by the umbo. They slope equally and gradually to the differently shaped ends.

A broadly rounded umbo begins to project above the hinge line when larvae reach a length of 150 to 160 μ (2 to 4 weeks under our culture conditions). The umbo becomes increasingly conspicuous and somewhat flat. Eventually it appears as a long, broadly-rounded knob with a silhouette that is discontinuous with the shoulders (Fig. 2).

Shortly after the development of the umbo, the anterior end of the shell becomes a darker reddish-brown. This shell coloring may extend into the umbo region in larger larvae. Larvae are darker than most pelecypods, but are not as dark as larval *Anadara transversa*. The adductor muscles become prominent shortly after the formation of the umbo, and are more conspicuous than in most species of pelecypods. An indistinct eye spot forms in larvae about 180 to 185 μ in length. It rapidly becomes conspicuous and is typically dark as metamorphosis proceeds. Larvae metamorphose at lengths ranging from 185 to 210 μ.

The foot is short and blunt. It is heavily ciliated at the distal end and has a large blunt heel that is used to apply the strong byssus threads with which this clam is capable of attachment at metamorphosis. The prominent features of the internal anatomy are illustrated in Figure 3.

The distinctive hinge structure of the Arcidae is evident in larvae as small as 80 μ in length (Fig. 4A). At both the anterior and posterior ends of the hinge line of each valve are a series of small taxodont teeth. The number of readily identifiable teeth in each series increases from four to six during larval development. The teeth on one valve mesh with those on the opposite valve to form a toothed area about 25 μ long. The anterior toothed area is separated from the posterior area by an undifferentiated central area of the hinge 35 μ long. Frequently at least part of the hinge structure can be seen through the umbo when larvae are lying quietly. The development of the hinge can be seen in the photomicrographs in Figure 4.

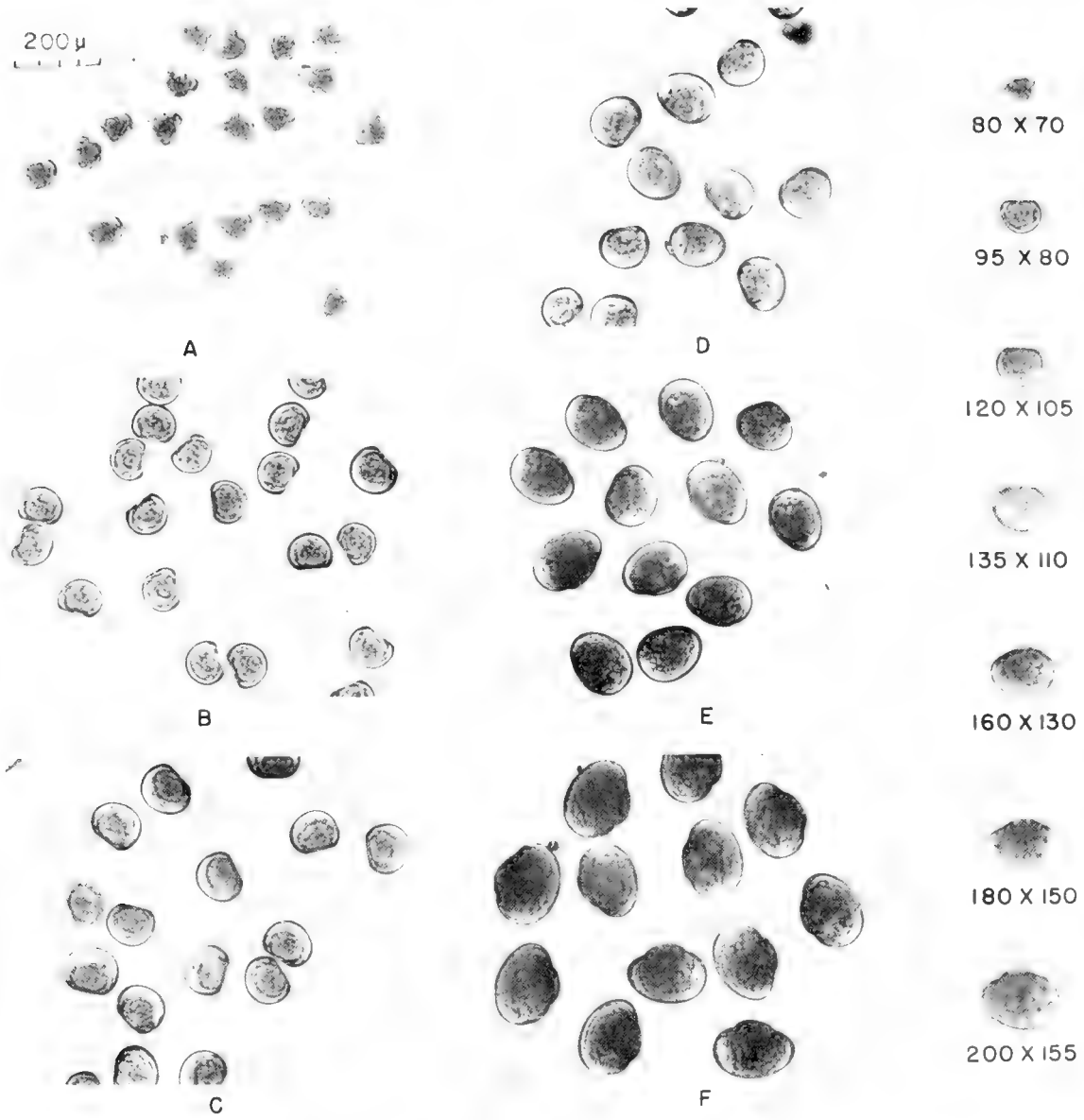

FIG. 2. *A. One-day-old larvae. Many with shell still incompletely formed. B. 10-day-old larvae. C. 12-day-old larvae. D. 20-day-old larvae. E. 26-day-old larvae. F. 30-day-old larvae.*

DISCUSSION

The Arcidae are common, widely-distributed clams. Some species are of commercial importance (Kan-no and Kikuchi, 1962; Pathansali, 1964), yet larval development of only five species has previously been described (Table 1). From descriptions and illustrations accompanying them, several characteristics appear to be common to larvae of this family. Growth is relatively slow, and the larval period comparatively long (4 to 6 weeks). Length exceeds height by up to 70 μ; more than in most pelecypod larvae. Larvae have a distinct brown to reddish-brown color and become eyed and metamorphose at lengths between 200 and 300 μ.

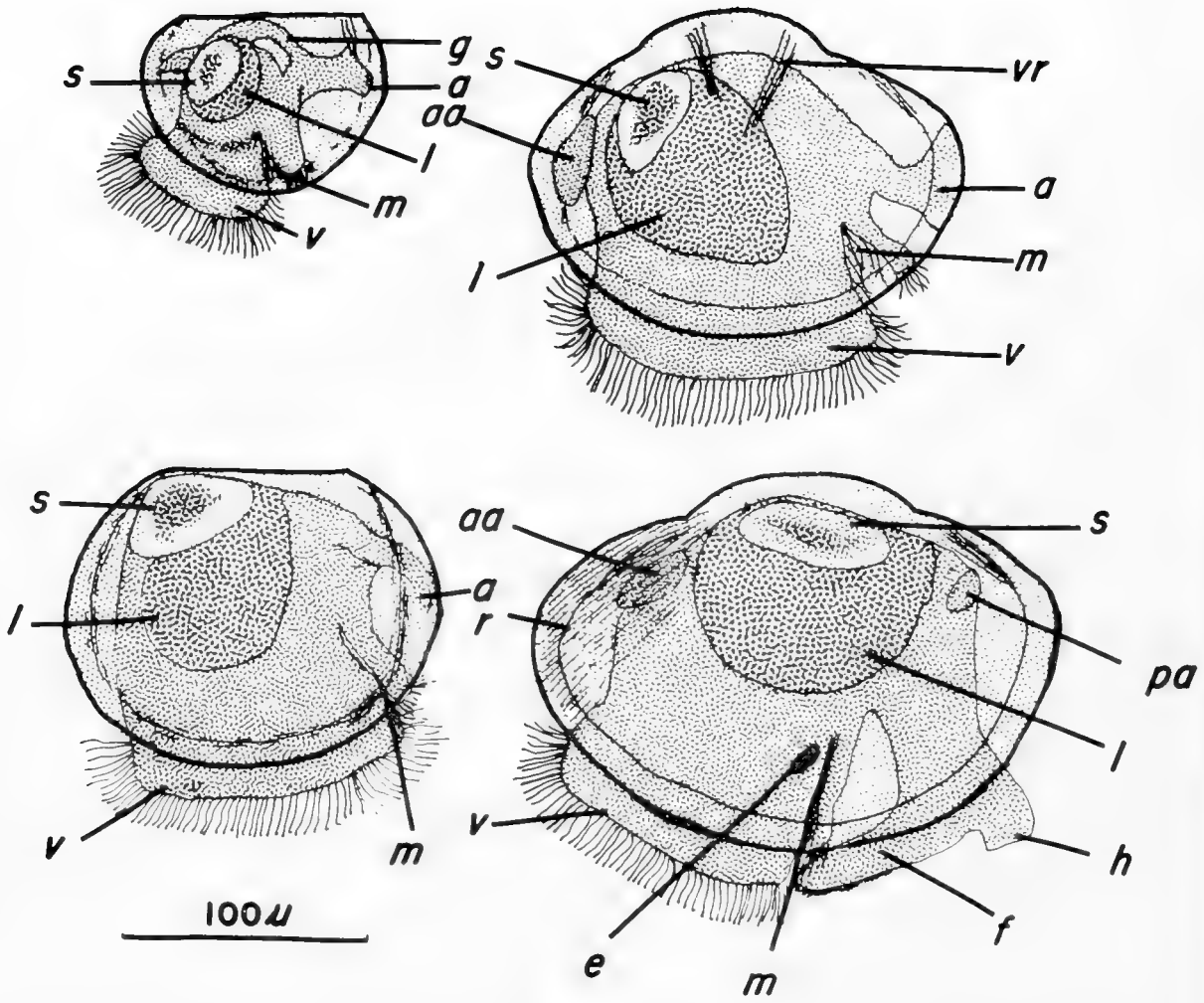

FIG. 3. *Schematic drawing of development of the internal anatomy of larval* Noetia ponderosa. *aa — anterior adductor muscle, a — anus, e — pigmented eye spot, f — foot, g — gut, h — heel of foot, l — liver or digestive diverticula, m — mouth, pa — posterior adductor muscle, r — reddish-brown color, s — stomach, v — velum, and vr — velar retractor muscles.*

Larvae of *N. ponderosa* have the shape, color, comparatively short height, and dentition typical of arcid larvae. In Virginia only larvae of the closely related blood clams, *Anadara transversa* and *Anadara ovalis*, could be confused with larval *N. ponderosa*. The umbo of *N. ponderosa* is broader, longer, and flatter than the umbo of *A. transversa* and the shoulders of the latter slope more steeply. In addition, the ventral margin of *N. ponderosa* slopes more steeply toward the anterior end than in larval *A. transversa*. Although the larval stages of *A. ovalis* have not been described, the similarity of other arcid larvae suggests that larval *A. ovalis* would resemble those of *N. ponderosa*.

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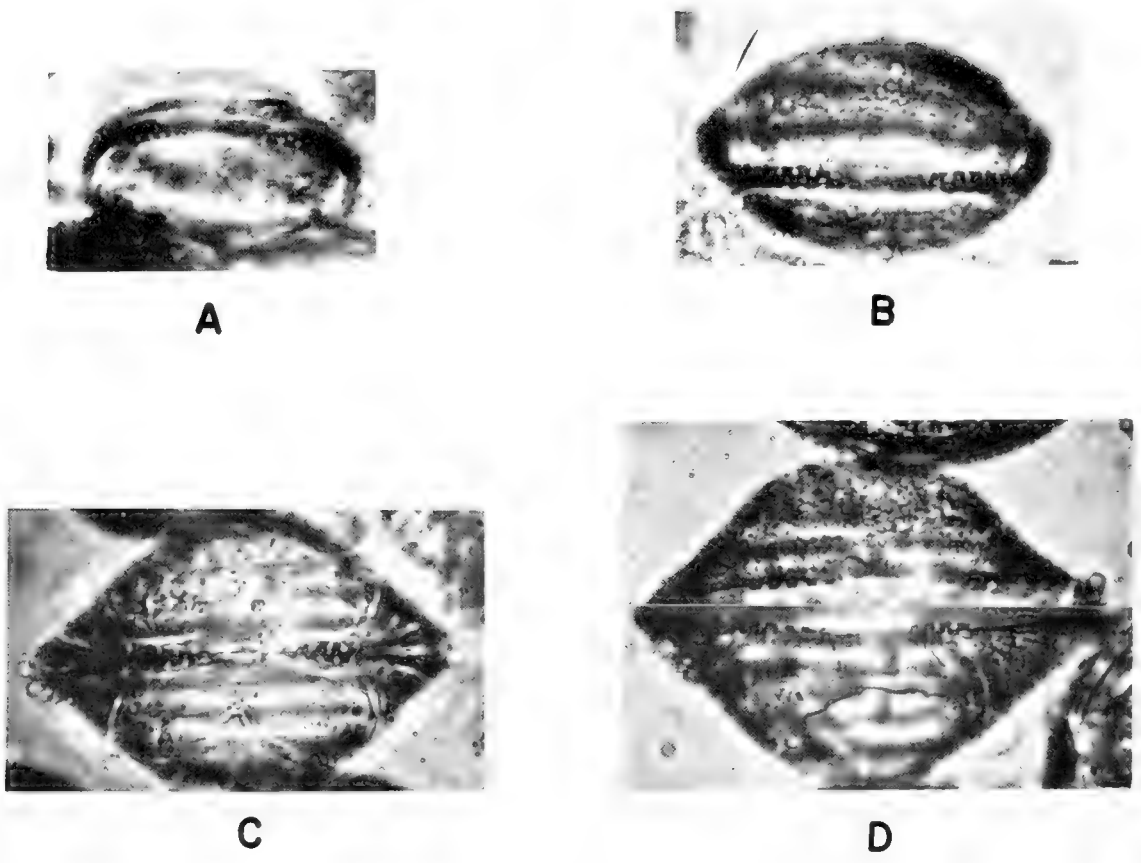

FIG. 4. *Development of the hinge structure in* Noetia ponderosa *larvae. A. Larval shell 80 μ long. B. Larval shell 120 μ long. C. Larval shell 155 μ long. D. Separate valves of larval shell 175 μ long. Dorsal view with left valve on top. Anterior end to the right.*

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TABLE 1. *Description of Larval Arcidae*

Species	Author	Description
Arca noae Linnaeus	Odhner (1914)	Reddish-yellow, metamorphose at about 200 μ. Hinge line with 8 anterior and 8 posterior taxodont teeth. (Drawings)
Anadara broughtonii (Schrenk)	Yoshida (1953)	Recognizable at length of 150 μ. Metamorphose at about 250 μ. Height 120 to 190 μ. Both ends rounded. (Drawings)
Anadara broughtonii (Schrenk)	Kan-no (1963)	Minimum length 83 μ. Height 67-180 μ. Metamorphose at 230 μ after about 40 days. (Photomicrographs)
Anadara broughtonii (Schrenk)	Kan-no and Kikuchi (1962)	Minimum length 90 μ. Metamorphose at 190-230 μ in 4 weeks. (Photomicrographs)
Anadara granosa Linnaeus	Pathansali (1964)	Metamorphose at lengths from 187 to 208 μ. Height 21 to 28 μ less than length. Pale yellow; 16 large taxodont teeth with small gap in mid-provinculum. Concentric lines in shell. Narrow anterior end more darkly colored than more rounded posterior.
Anadara granosa bienensis (Schrenk and Reinhart)	Yoshida (1957)	Metamorphose at 231 μ. Elongated. Height 44-60 μ less than length. About 7 concentric lines on shell.
Anadara transversa (Say)	Loosanoff and Davis (1963)	Minimum length 70 μ. Metamorphose in 27 to 37 days at lengths of 215 and 310 μ but usually 240-260 μ. Umbo at 130-140 μ. Eye at 205 μ. (Photomicrograph)
Anadara subcrenata (Lischke)	Yoshida (1937; 1953)	Minimum length 110 μ. Metamorphose at about 280 μ. Height 90-200 μ. Knobby umbo. Yellow. (Drawings)

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THE GROWTH AND MORTALITY OF SEED OYSTERS, *CRASSOSTREA VIRGINICA*, FROM BROAD CREEK, CHESAPEAKE BAY, MARYLAND, IN HIGH-AND LOW-SALINITY WATERS

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ABSTRACT

Seed oysters from low-salinity water of Broad Creek were transferred and suspended off bottom in the low-salinity water of Tred Avon River and high-salinity water of Chincoteague Bay. Growth and mortality were measured and compared for two years. The rate of shell growth was similar in both areas. A high second-year mortality was observed in Chincoteague Bay which apparently was not caused by high salinity.

INTRODUCTION

Certain natural bars in Chesapeake Bay are set aside by the State of Maryland for production of seed oysters, *Crassostrea virginica*. Clean shells, either from shucking houses or dredged off the bottom, are placed on these preserves each spring just before the oyster-setting season. In the fall or early the following spring, the shells with newly attached oysters, called spat, are moved to natural oyster bars. Approximately three years later, after the oysters have reached the 3-inch legal size, they are dredged or tonged and sold by oystermen.

One of the most important seed-producing bars in the Maryland portion of Chesapeake Bay is on the Eastern Shore, at Mulberry Point in Broad Creek. (In 1963 and 1964, for example, 297,420 and 205,460 bushels of shells were planted on this bar to catch seed oysters.) Little is known about the growth and mortality of seed oysters from Broad Creek after they are moved, although Beaven (1952) reported on the growth of seed oysters from several other bars in Chesapeake Bay. Annual surveys by the Maryland Department of Chesapeake Bay Affairs provide information on the density of oysters on many natural bars, but it is difficult to evaluate the growth or survival rate of recently planted seed oysters, since they are usually mixed with other year classes already on the bar.

This paper reports on the growth and mortality of Broad Creek seed oysters of the 1960 year class after their transfer to both the relatively low-salinity water of the Tred Avon River and the high-salinity water of Chincoteague Bay. These areas represent two distinctly different types of environment within the region where seed oysters from Broad Creek could be planted. The salinity is similar at Broad Creek and Tred Avon River; it fluctuates from 8 to 16 ppt (average about 12 ppt). In Chincoteague Bay salinities range from 17 to 35 ppt (average 30 ppt). Seasonal temperatures in both areas vary from 0° to 30° C.

MATERIALS AND METHODS

Samples of seed oysters of the 1960 year class were collected in March 1961 from Mulberry Point, Broad Creek, Maryland (76° 15′ W, 38° 45′ N). On 28 March a portion of the sample was strung on stainless steel wires (50 oysters per wire) and suspended above the bottom from a stationary platform in the Tred Avon River, Oxford, Maryland (76° 10′ W, 38° 41′ N). On 7 April a second portion of the sample was divided into two groups. One group of approximately 250 oysters was placed in a tray 40 inches long, 20 inches wide, and 4 inches deep; a second group of 100 was divided equally and strung on two stainless steel wires. Both the tray and strings were suspended from a tray rack (Castagna, 1964) in Chincoteague Bay at Franklin City, Virginia (75° 23′ W, 38° 0′ N).

Growth of oysters in both localities was measured monthly (except during winter). Growth was determined by an increase in shell height (greatest dorsoventral distance) and length (greatest anteroposterior distance). Initially at Chincoteague Bay, only the oysters on the strings were measured. On 4 August 1961 one string was missing, how-

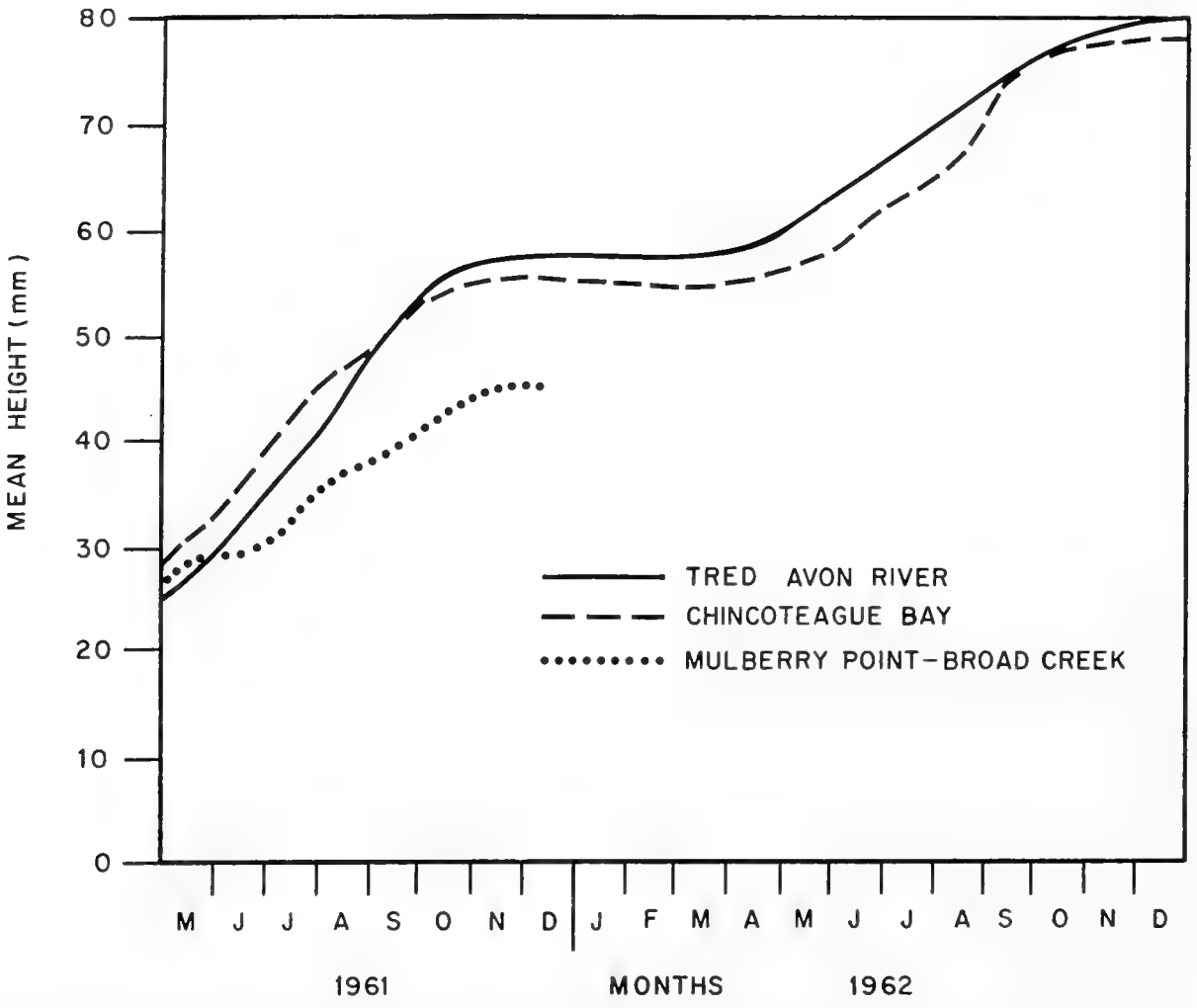

FIG. 1. *Growth curves for Broad Creek oysters suspended off the bottom in the Tred Avon River, Maryland, and Chincoteague Bay, Virginia, and on the bottom at Mulberry Point, Broad Creek, Maryland.*

ever, and on 6 October 1961 the second string was lost; it then became necessary to measure the growth of oysters in the experimental tray. During 1961, samples of seed oysters were also collected periodically from Broad Creek at Mulberry Point and their growth was compared with the growth of oysters suspended off the bottom in the other two areas.

Total counts of live oysters in each stock were made monthly to determine the number of oysters surviving. At the end of the study, on 20 and 28 December 1962, 20 oysters from each group were sacrificed and their per cent solids (Engle, 1950) and index of condition (Grave, 1912; Higgins, 1938) were measured.

GROWTH, SURVIVAL, AND CONDITION OF OYSTERS AFTER TRANSFER

Several investigators have studied the effects of transferring oysters into a new environment. Shaw and McCann (1963) observed that oysters moved from Long Island Sound, Connecticut, to Cape Cod, Massachusetts, grew as well as native populations. Beaven (1952) found that, in general, seed oysters grew best when transferred to nearby waters.

Extensive studies have been carried out on the growth and survival of South Carolina oysters which have been planted in Chesapeake Bay.

TABLE 1. *Mean height, length, and percentage mortality on different dates after transfer from Broad Creek to the Tred Avon River.*

Date	Number Measured	Height (mm) Mean	Height (mm) Standard Error	Length (mm) Mean	Length (mm) Standard Error	Cumulative Mortality (%)
1961						
May 1	102	25.5	0.60	19.9	0.48	0
June 2	101	30.2	0.67	24.2	0.53	0
July 5	101	36.6	0.67	29.3	0.57	0
July 31	100	41.3	0.75	33.7	0.59	3
Sept. 1	100	47.3	0.79	37.8	0.60	7
Oct. 3	100	54.0	0.89	42.1	0.70	14
Nov. 3	100	57.3	0.89	44.6	0.70	14
Dec. 1	100	55.2	0.86	43.3	0.74	14
1962						
April 18	100	57.8	0.57	45.4	0.77	34
May 15	100	61.2	0.94	50.2	0.87	38
June 19	101	64.7	0.97	53.9	0.87	38
July 19	100	67.4	0.99	54.7	0.90	38
Aug. 16	100	71.5	1.03	56.3	0.88	38
Sept. 13	101	73.1	1.04	56.8	0.90	38
Oct. 16	101	77.8	1.14	57.9	0.86	44
Nov. 14	101	78.7	1.13	57.8	0.90	44
Dec. 27	100	80.1	1.12	57.8	0.85	44

Beaven (1953) found that the survival of these oysters was higher in the southern than in the northern portion of Chesapeake Bay. Andrews and McHugh (1957) observed that the growth of South Carolina oysters was almost equal to the growth of native Virginia stocks, but the rate of survival of the southern oysters was lower during the colder seasons from November to May.

In Figure 1 the growth curves are shown for Broad Creek oysters in the Tred Avon River and Chincoteague Bay from May 1961 through December 1962, and at Mulberry Point from May to December 1961. The growth of suspended oysters was approximately the same in the low-and high- salinity waters. By the end of 1961 the oysters averaged 55.2 mm in height and 43.3 mm in length in the Tred Avon River (Table 1), and 56.1 mm and 39.1 mm in Chincoteague Bay (Table 2). At the end of the experiment in December 1962, the average heights and lengths were 80.1 mm and 57.8 mm in the Tred Avon River, and 77.9 mm and 54.8 mm in Chincoteague Bay. Statistical analyses based on methods given in Hubbs and Hubbs (1953) revealed that the small difference in growth between the two areas was not significant.

The growth of oysters on the bottom at Mulberry Point was considerably slower than growth of stocks which were suspended off the bottom (Fig. 1). The average shell height in December 1961 was 11 mm less for oysters at Mulberry Point than for the two suspended stocks (Table 3). Similar results were reported by Shaw (1962) for Cape Cod, Massachusetts. (Since seed oysters are moved by the State from Mulberry Point to other areas, only one year of comparison was possible.)

Little mortality was observed during 1961 at Chincoteague Bay. The survival rate continued high until May 1962, when 34 per cent of the transferred oysters were found dead; by December, when the study was terminated, 74 per cent of the original population had died. Total mortality for the two years was about 44 per cent for oysters in the Tred Avon River; one-half of the deaths in the river were caused by a winter kill which occurred when some of the oysters on the strings were exposed to freezing air temperatures at low tide. It can be assumed that the other half died from natural causes.

On 20 and 28 December 1962, index of condition and per cent solids were determined for 20 oysters from each group. The index of condition was 13.3 and per cent solids 16.4 in Chincoteague Bay; comparable values for oysters in the Tred Avon River were 13.4 and 18.0.

POSSIBLE CAUSES OF MORTALITY IN CHINCOTEAGUE BAY

The effects of moving oysters from low- to high-salinity water was the first factor considered as

TABLE 2. *Mean height, length, and percentage mortality on different dates after transfer from Broad Creek to Chincoteague Bay.*

Date	Number Measured	Height (mm) Mean	Height (mm) Standard Error	Length (mm) Mean	Length (mm) Standard Error	Cumulative Mortality (%)
1961						
May 2	100	28.6	0.65	21.2	0.49	—
June 1	100	32.8	0.57	24.9	0.49	—
July 3	100	39.4	0.72	29.6	0.62	—
Aug. 4	49	46.3	1.32	35.9	1.08	—
Sept. 5	36	48.6	1.60	36.7	1.33	—
Oct. 6	100	53.4	0.95	38.9	0.59	—
Oct. 31	100	55.3	0.99	40.0	0.64	2.8
Dec. 5	100	56.1	1.03	39.1	0.59	9.3
1962						
May 2	100	56.2	0.94	40.6	0.66	34.0
June 13	100	59.2	0.99	42.4	0.60	50.0
July 12	90	62.8	0.97	44.9	0.65	58.1
Aug. 15	81	67.1	1.04	48.1	0.74	62.3
Sept. 19	76	75.3	1.11	53.3	0.74	64.6
Oct. 18	74	77.4	1.12	55.5	0.77	65.1
Nov. 16	75	77.4	1.12	54.8	0.78	65.1
Dec. 18	55	77.9	1.48	54.8	0.96	74.4

1/ One string of oysters lost.

2/ Second string of oysters lost; measured in tray stock.

a possible cause of death in Chincoteague Bay, but since the greatest mortalities in the Bay occurred during the second year, it does not appear that a sudden salinity change was the cause of mortality. Loosanoff (1950) transferred oysters from low salinities (between 3.0 and 10.0 ppt) directly to water of high salinity (about 27.0 ppt). In general, he observed that oysters withstood sharp changes from low to high salinity without serious physiological effects. Galtsoff (1964) observed total mortality of oysters 3 to 4 weeks after they were transplanted in September from the low sa'inity (10 to 12 ppt) of upper Chesapeake Bay to the high salinity (32 to 33 ppt) of Sinepuxent Bay at the northern end of Chincoteague Bay. Similar transfers succeeded in October and November, when the air and water temperatures were much lower. He believed that the sudden change in salinity during hot weather was the cause of mortality.

In recent studies at the Oxford Laboratory, little mortality was observed when oysters were transferred from low to high salinity during either cool or hot weather. The oysters were moved from Harris Creek (an area adjacent to Broad Creek) into Chincoteague Bay during March, June, September, and December.

Chincoteague Bay is heavily infested with two serious oyster predators — the Atlantic oyster drill, *Urosalpinx cinerea*, and the thick-lipped drill, *Eupleura caudata*. In the present study, however, the oysters were suspended off the bottom, out of reach of these enemies. Though an occasional drill has been found in adjoining trays, no drills nor drilled oysters were found in the tray holding the Broad Creek stock; thus predation apparently was not the cause for the higher mortality in Chincoteague Bay.

The haplosporidians, "MSX" (multinucleate-sphere x) and *Haplosporidium (=Minchinia) costalis* have been found in the high-salinity waters of Chincoteague Bay (Wood and Andrews, 1962). It is possible that some of the mortality was due to these parasites. To date, neither of these forms has been found in the lower salinity waters of the Tred Avon River.

Until the cause of the high mortality is known and controlled, it appears unwise to transplant stocks of seed oysters from Upper Chesapeake Bay into Chincoteague Bay.

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TABLE 3. *Mean height and length of oysters living on the bottom at Mulberry Point, Broad Creek, on different dates in 1961.*

Date	Number Measured	Height (mm) Mean	Height (mm) Standard Error	Length (mm) Mean	Length (mm) Standard Error
May 10	100	27.6	0.50	20.2	0.39
June 6	100	29.2	0.76	21.1	0.52
July 6	100	31.2	0.61	23.5	0.51
Aug. 1	100	35.6	0.73	25.6	0.51
Aug. 29	100	39.0	0.69	26.8	0.50
Sept. 26	98	40.7	0.67	28.1	0.46
Nov. 7	100	44.4	0.71	31.7	0.50
Dec. 7	100	44.8	0.71	31.1	0.49

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REVIEW OF OYSTER CULTURE IN ALASKA, 1910-61

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ABSTRACT

There has been limited success with culture of the Pacific oyster, Crassostrea gigas *in the Coon Cove-Carroll Inlet area near Ketchikan since 1910. Since 1938 from 110 to 227 acres of tidelands have been leased yearly for oyster culture in this area. Several companies have participated at different times but the annual harvest has never exceeded 550 gallons of shucked meats.*

Culture of the Pacific oyster, *Crassostrea gigas* (Thunberg), may be practical under certain conditions in regions that are commonly regarded as unsatisfactory by oyster culturists in the continental United States (records available in the files of the U. S. Bureau of Commercial Fisheries Biological Laboratory, Auke Bay, Alaska).

Seed oysters from Japan (obtained through the State of Washington Department of Fisheries or the Pacific Oyster Growers Association) were planted in areas extending from southeastern Alaska to Kachemak Bay on Cook Inlet at various intervals in 1910-61, but success was limited except in the Coon Cove-Carroll Inlet area near Ketchikan in southeastern Alaska. This area has proved moderately productive and has been a site of oyster culture for the past 50 years. Before 1937, tidelands were held by right of occupancy; in 1937-60 they were leased from the Federal Government under the Oyster Bottom Leasing Act; and beginning in 1960 the State of Alaska assumed responsibility for tideland leases. Acreages of tidelands leased are not available for all years, but it appears that from 110 to 227 acres have been used for oyster culture since 1938. Oyster production and acreage used for culture in the Coon Cove-Carroll Inlet area for 1940-61 are given in Table 1.

Celia Fairbanks and Judy Conkle were responsible for the first Alaskan plantings of Pacific oysters in George Inlet near Ketchikan in 1910. Oyster culture continued there and at Coon Cove and Carroll Inlet until 1920. Unfortunately, no details of this operation are available and records are lacking for the period 1920-38.

The Alaska Oyster Company leased tidelands at Coon Cove in 1938, and a year later marketed an unknown quantity of oysters. Because it takes as long as three years from seed to market size in this area, oysters planted prior to the 1938 lease date must have been cropped. About 300 acres were under lease in southeastern Alaska from 1938 to 1947, but oysters were produced on only 40 acres in Coon Cove and 100 acres at Shoal Cove, Carroll Inlet (also leased to the Alaska Oyster Company). They were sold on the local (Ketchikan) market. As no seed was imported from Japan from 1941 to 1947, the beds were nearly exhausted by 1945. Some seed oysters were planted in 1948, but none between 1949 and 1955. The Alaska Oyster Company went out of business in 1953.

The North Gem Oyster Company of Ketchikan leased 10 acres on the east shore of George Inlet in 1955 and added to their holdings each year until they held 247 acres in 1957. The company planted at the rate of 7,000 spat per acre in 1955, 5,500 per acre in 1956, and 28,600 per acre in 1957. In this 3-year period they harvested 100 gallons of shucked oysters, which probably represented a clean-up of some of the beds of the defunct Alaska Oyster Company.

In April 1955 the North Gem Oyster Company began an experiment with raft culture on the east shore of George Inlet opposite Beaver Falls — the only known trial of raft culture in Alaska. The experimental raft was made of eight cross-bars, each supporting 14 strings. The strings were

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TABLE 1. *Oyster Production, Coon Cove-Carroll Inlet Area near Ketchikan, Southeastern Alaska, 1940-61. (Asterisk indicates no data available; years with no data are omitted. Figures are from annual reports of area management agents.)*

Year	Company	Acres in production	Gallons (shucked)	Price per gallon
1940	Alaska Oyster Co.	140	*	$4.00
1941	-do-	-do-	82	3.50
1943	-do-	-do-	550	4.00
1944	-do-	-do-	500	4.00
1947	-do-	-do-	299	4.00
1948	-do-	-do-	114	6.00
1949	-do-	-do-	177	5.29
1950	-do-	-do-	0	—
1951	-do-	-do-	12	*
1956	North Gem Oyster Co.	110	100	*
1957	-do-	227	0	—
1960	Alaska Oyster Co.	227	100	*
1961	-do-	227	100	*

about 6 feet long and each held 11 or 12 seed shells. Buoyancy was provided by six empty 50-gallon oil drums. The 7,500 spat from Japan on the strings had attained an average length of 55 mm by September 11. Japanese seed usually is about 10 mm long when received for planting. Water temperatures ranged from 39° to 62°F during this period.

The same shipment of spat included 28,000 that were planted April 18 on 10 acres of nearby bottom. By September, losses were 3 per cent for the raft culture and 63 per cent for the bottom culture. The causes of mortality are unknown. Growth rates of two groups were reported by the company to differ considerably; the raft groups showed a much faster rate of growth. Oysters suspended from the raft attained lengths up to 160 mm (6.5 inches) in two years. No measurements are available for oysters grown on the bottom.

The North Gem Oyster Company went into receivership in 1958 and their holdings were taken over in 1960 by the newly formed Alaska Oyster Company, which immediately began to import seed and harvest oysters. During the winter of 1960-61 about 200 gallons of shucked oysters were sold locally. This company planted 700,000 spat on part of their holdings in 1960 and 2 million spat on 227 acres in 1961.

Two natural spawnings have been recorded in or near George Inlet. In 1939 a set was produced from natural spawning in George Inlet and a set occurred in Coon Cove in 1943.

Oyster culture was in progress in Alaska in 1961, but in a limited area near Ketchikan in southeast Alaska. Past attempts at oyster culture have been undertaken by undercapitalized companies with little experience. Well-planned and well-financed trials of Pacific oysters and introduction of the European oyster might expand the area of culture and increase oyster production.

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SETTING AND GROWTH OF THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA*, ON NAVIGATION BUOYS IN THE LOWER CHESAPEAKE BAY

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ABSTRACT

Samples of oysters were collected for 7 years from selected navigation buoys, moored in the lower Chesapeake Bay from the mouth of the Potomac River to Cape Charles, to obtain data on oyster growth and mortality. The shells of the live oysters were measured, year classes separated, and an average rate of growth determined. Indexes of mortality were estimated by counting dead oyster valves attached to the buoys.

Average ranges of shell height were 25 to 35 mm at the end of the setting year; 70 to 80 mm by the end of the first year; and 80 to 95 mm by June of the second year (buoys are generally on station a maximum of 2 years). This growth is extremely rapid, apparently the result of being off-bottom. Not only was growth rapid, mortalities were unusually low on the buoys.

The extensive setting of oysters on navigational buoys, followed by rapid growth and low mortalities, suggests the commercial possibilities of off-bottom culture of oysters in certain areas of the lower Chesapeake Bay.

INTRODUCTION

Buoys used as navigational aids are placed at vantage points along practically all coastal and large inland waterways. They are brought ashore to Coast Guard bases periodically for cleaning and servicing (Fig. 1). Any biological material attached to the buoys while they are "on station" is available for collection and study (Merrill, 1965). Not only is material from buoys easily attainable but each buoy has its own community structure. Because buoys are on station for known periods of time, growth of the animals collected from them can be interpreted.

Growth of various bivalves from buoys has been studied over the past few years. In the sea scallop, *Placopecten magellanicus*, for instance, only occasional very young specimens are taken in their natural habitat (Merrill, 1965). Most of what is known of this scallop's early rate of growth has been learned from collections taken from many buoys in the New England area. Galtsoff and Merrill (1962), who studied *Ostrea equestris* using material collected from an ocean buoy off Georgia, determined, among other things, a rate of growth for this species in surface waters. Merrill (1959) reported an unusually fast growth of the soft-shell clam, *Mya arenaria*, which had settled and nestled in a thick hydroid growth far up in the

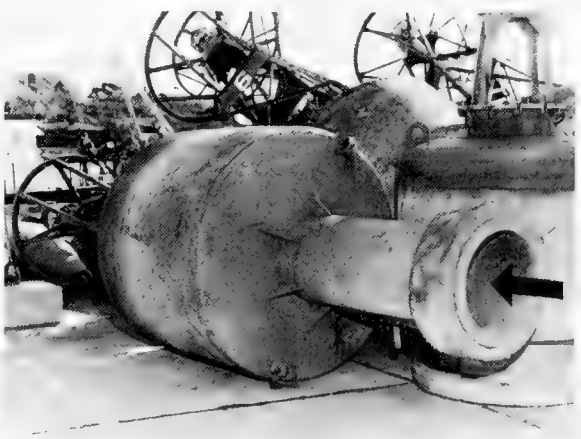

FIG. 1. *Navigation buoy; arrow indicates the sheltered inner portion of the stabilizer tube from which oysters were taken.*

stabilizer of a navigation buoy at Buzzards bay, Massachusetts.

Study of the growth of bivalves from surface buoys is tantamount to the study of growth from raft culture. Reports have shown that growth is faster nearer the surface than on the bottom. Belding (1916) reported faster growth for soft-shell clams suspended from rafts than for those in boxes buried to the level of the substrate; in Europe, where farming of the mussel, *Mytilus edulis*, is practiced extensively, growth is faster in those cultivated on wooden frames above the sea bottom. Studies by Shaw (1962, 1964, 1965) have shown that the American oyster, *Crassostrea virginica*, grows almost twice as fast when suspended from rafts as on the bottom. Bonnot (1935) and Quayle (1956) stated that the culture of Pacific oysters, *Crassostrea gigas*, from rafts greatly increased the growth of the suspended oysters. Quayle (1956) further determined that oysters cultured by this method show lower mortality because of elimination of silting and reduction of predation. Belding (1930) considered the most important factor in the growth of clams to be a good current which carries food, oxygen, and salts; this current also acts as a sanitary agent.

The purpose of this paper is to describe the results of a study of the growth and mortality of *Crassostrea virginica*, which had settled and grown on navigation buoys near the water surface. An evaluation is made on the possibilities of developing off-bottom culture of oysters in these waters.

PROCEDURE

The Coast Guard keeps accurate records for all buoys — when and where they are placed on station, and when they are brought ashore for cleaning (Table 1). It was from these buoys that we collected our samples.

Oysters were taken from selected navigation buoys for 7 years at the Portsmouth, Virginia, Coast Guard Base. These buoys had been moored in the lower Chesapeake Bay from near the mouth of the Potamac River to Cape Charles (Fig. 2). All live oysters were collected from at least 50 square feet of the inner tube surface; we also removed the oysters over 30 mm (which could be seen easily) that had died but whose valves or under-valves remained attached to the buoy. These dead oysters were counted to determine indexes of mortality. Height (greatest dorsoventral distance) of 1,536 oysters from 20 buoys (an average of 79 specimens from each buoy) was measured. Size frequencies were established and then, with the aid of probability paper, year classes were separated and the mean height for each year class determined (see Harding, 1949). Comparison of mean heights of oysters from most northern and most southern

TABLE 1. *Location of buoys, date of placement and retrieval, and number of live oysters collected from each buoy. Location numbers correspond to those in Figure 2.*

Location					Number of oysters
Number	Latitude N.	Longitude W.	Date In	Date Out	
1	36°51.5′	75°30.0′	5-22-58	7-25-60	77
2	36°53.6′	76°20.0′	1-14-60	5- 2-62	55
3	36°53.6′	76°20.0′	5- 2-62	4-27-64	36
4	36°58.2′	76°20.0′	8- 9-60	8-16-62	85
5	36°59.2′	76°18.8′	12-19-61	8-27-63	70
6	36°58.7′	76°07.5′	11-20-59	11-21-62	45
7	37°04.3′	76°04.8′	4- 7-58	5-19-60	45
8	37°12.0′	76°08.5′	1- 2-60	10-31-61	18
9	37°07.0′	76°07.8′	4-19-61	8-18-62	7
10	37°07.4′	76°09.2′	11- 4-57	10-10-59	92
11	37°09.4′	76°08.5′	8-21-59	12-15-61	115
12	37°08.7′	76°11.5′	6-28-56	5-28-58	104
13	37°13.5′	76°08.4′	2-28-62	11- 1-62	17
14	37°15.0′	76°22.6′	5-28-58	5-19-60	190
15	37°15.0′	76°22.6′	5-19-60	5-22-62	181
16	37°15.0′	76°07.0′	10- 6-59	10-19-61	77
17	37°23.5′	76°11.5′	10-10-60	10- 9-62	25
18	37°34.5′	76°10.4′	10- 1-62	6- 8-64	94
19	37°38.8′	76°07.5′	12-10-61	6- 8-64	50
20	37°59.4′	76°10.9′	6- 5-58	9- 5-60	58

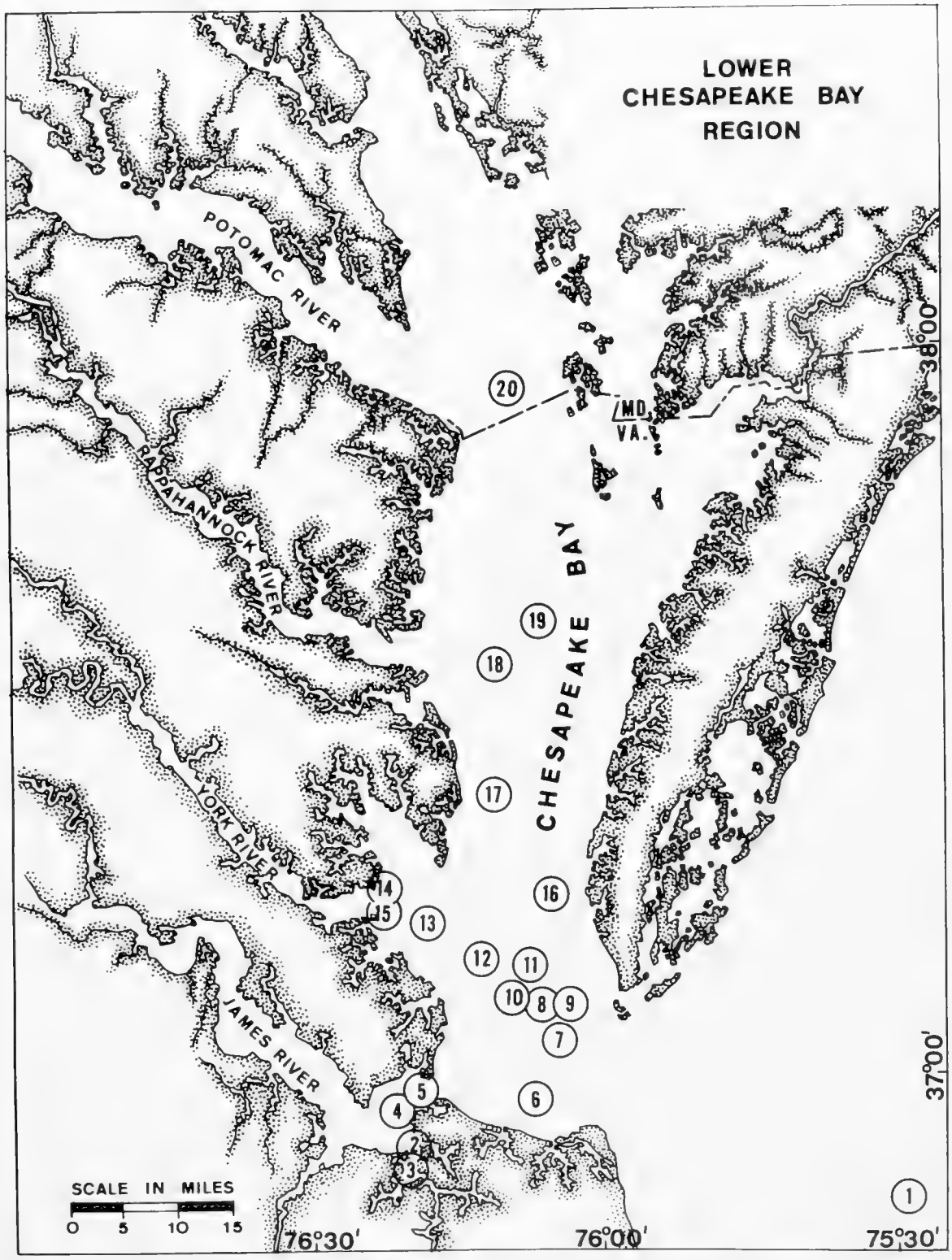

FIG. 2. *Lower Chesapeake Bay region showing locations 1-20 of navigation buoys from which samples were collected.*

stations proved the rates of growth to be closely similar. Thus we were able to combine all data and establish an average growth curve for the entire area of lower Chesapeake Bay.

RATE OF GROWTH

Oysters set in lower Chesapeake Bay from June through October (Loosanoff, 1932; Andrews, 1951, 1954). Shell growth takes place from April to November in the Bay, but little or no growth is laid down from December through March (Mc Hugh, unpublished manuscript[1]). The average height of buoy oysters ranged from 25 to 35 mm by the end of the setting year (time of setting to January 1), from 70 to 80 mm by the end of the next year, and 80 to 95 mm by June of the following year. Since buoys are generally in the water a maximum of 2 years, data on later growth were not available.

Comparison of the growth of buoy oysters with that of other experimental stocks grown off the bottom in Chesapeake Bay makes it obvious that the oysters attached to buoys grow faster. As an illustration, the comparison of the growth of oysters collected from buoys with that of Broad Creek seed suspended on ropes in the Tred Avon River, Maryland, can be seen in Figure 3. Comparative measurements for South Carolina and York River seed suspended in trays at Gloucester Point, Virginia, are also indicated. At the end of the setting year a difference in growth rate is already apparent; the buoy oysters averaged 8 mm larger than the Broad Creek stock suspended in the Tred Avon River. By the end of the next year the buoy oysters averaged 15-23 mm larger than the York River, South Carolina, and Broad Creek stocks. In fact, many of the oysters from buoys were 75 mm or greater at the end of 1 1/2 years; some suspended stocks did not attain this height until almost the end of the following year, or 2 1/2 years after setting.

MORTALITY OF BUOY OYSTERS

As mentioned earlier, a count was made of all "boxes" and "scars" for those oysters 30 mm or greater. The number of dead oysters was extremely low. Only 32 scars and boxes were observed; the indicated mortality is about 2 per cent (computed from the total number of oysters examined).

INTENSITY OF SETTING

The number of oysters collected from individual buoys varied from 7 to 190 (Table 1), and average 78.6 per buoy. Among the areas monitored in the lower bay, setting was the heaviest at the mouth of the York River on buoys 14 and 15 (Fig. 2). At this station 190 and 181 oysters were collected from the two buoys. Counts also exceeded 100 oysters on buoy No. 12, 5 miles ENE of Plumtree Point, and on buoy No. 11 in the middle of the Bay off the town of Cape Charles (Fig. 2).

POSSIBILITIES FOR OFF-BOTTOM CULTURE IN LOWER CHESAPEAKE BAY

The rate of growth of buoy oysters was greater to our best knowledge than that of any stock north of Cape Hatteras. This rapid growth is probably the result of currents that bring plentiful food materials (Merrill, 1965). In more protected waters, the reduction of currents lessens the amount of food available to the oysters. The slower growth rate of the suspended stocks in York River and Tred Avon River as compared with those attached to buoys are more than likely related, in part, to the strength of water current.

Currents over the bottom are further reduced, and it is in this zone that oysters grow the slowest. Beaven (1952) observed that oysters growing on the bottom in Holland Straights, Chesapeake Bay, Maryland, grew to only 48 mm in height after 2 years. Buoy oysters reached this size during the first year of growth (Fig. 3).

It is possible that the oysters are not in the water long enough (a maximum of 2 years or less) to be killed by diseases known to be prevalent in these areas (Andrews, 1964). It would be worth a study to check the incidence of the common oyster disease organisms in large oysters from buoys.

The fact that the height of buoy oysters averaged 80 to 95 mm after only 2 years of growth, and the apparently low mortality among these oysters warrants, in our opinion, further investigations into the possibilities of developing off-bottom culture in certain areas of the lower Bay.

One site worth considering is the mouth of the York River, where setting was the heaviest on the buoys. Shells could be suspended from rafts moored in this locality. Following setting, the rafts could remain at the site until November when growth for the season has terminated. They could then be towed to more protected waters for the duration of winter to shield the oysters from floating ice and storms. The following spring the rafts could be moved to more open waters in order to take advantage of faster currents and increased amount of food materials which contribute to oysters' faster growth. This method of culture is similar to that now being conducted so successfully in Japan.

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[1] McHugh, J. L. (unpublished manuscript), "Growth of oysters at Gloucester Point, Virginia."

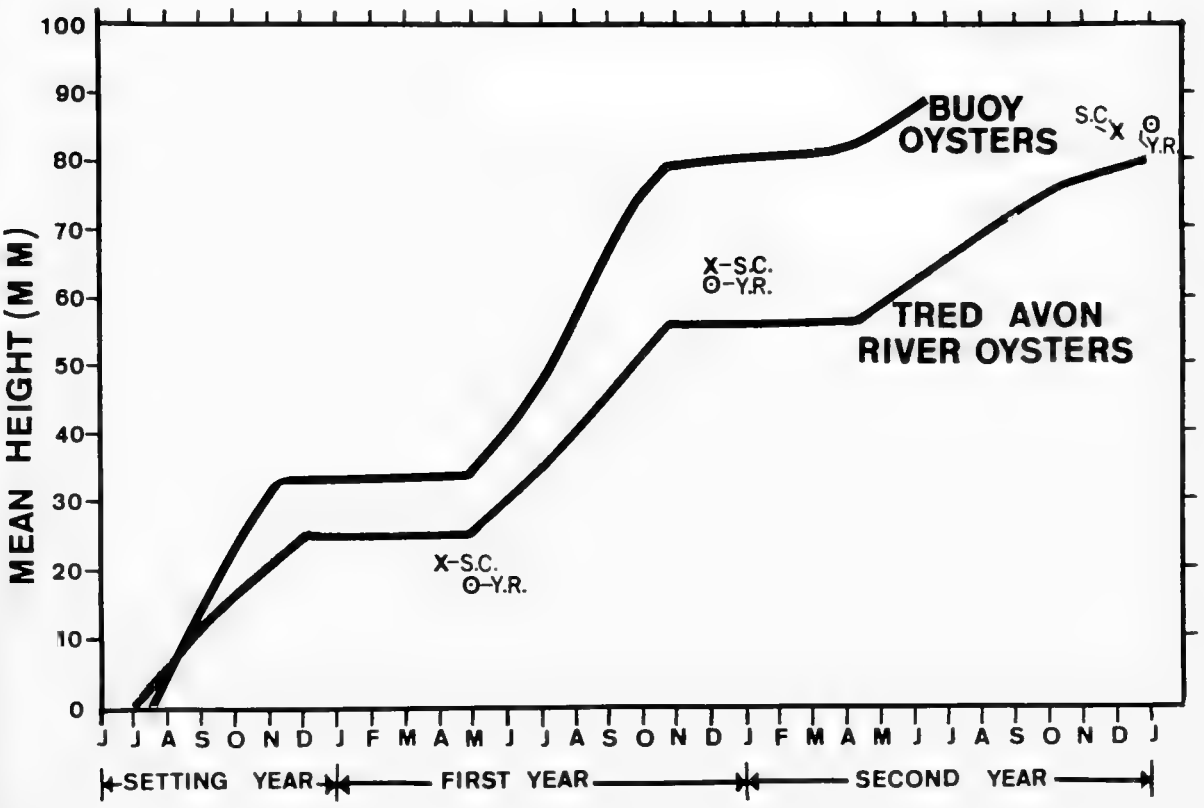

FIG. 3. *Growth of buoy oysters and Tred Avon River oysters. Measurements of tray-held York River and South Carolina oysters are indicated by ⊙-Y.R. and X-S.C.*

CONCLUSIONS

The continuous decline of oysters in Chesapeake Bay, and more recently, the rapid loss of oysters in the lower Bay from diseases and predation, necessitate a close look at present-day culture techniques. The collections from buoys suggest that suspended culture may be one means of supplementing production of a declining industry. Although this method of culture increases costs, the present scarcity of oysters in turn increases the value of the product. In areas where off-bottom culture has been studied, faster growth and greater survival has been found in comparison with oysters grown on the bottom. Oysters grown off-bottom can be marketed earlier than similar stocks growing on the bottom. Also, the lower mortality among suspended oysters means the marketing of greater volumes.

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ESTIMATION OF THE SAMPLING DISTRIBUTION AND NUMERICAL ABUNDANCE OF SOME MOLLUSKS IN A RHODE ISLAND SALT POND

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ABSTRACT

An approach for refining population density estimates based on census surveys is presented. Frequency distributions of five molluscan species enumerated from a sampling survey of a Rhode Island salt pond were fitted by the negative binomial frequency distribution. Approximate normality was achieved by an inverse hyperbolic sine transform of the data, permitting estimations of abundance with relatively narrow confidence limits. Stratification of the sample by depth contours increased the precision of the estimates.

INTRODUCTION

Resource assessments of shellfish populations are customarily based on census surveys. However, such numerical abundance estimates often give wide interval estimates because of irregularities in the areal dispersion of members of the population. This study was made to demonstrate an approach for refining population density estimates based on a census survey of the common molluscan fauna in a brackish embayment.

Individuals of many aquatic species are not uniformly nor randomly distributed in space, but rather are contagiously distributed (Barnes and Marshall, 1951; Taylor, 1953; Holmes and Widrig, 1956; Kutkuhn, 1958; Cassie, 1959; and Moyle and Lound, 1960). Such aggregations were also noted in this survey. To satisfy our objective it was therefore necessary to find a suitable mathematical function to describe the observed distribution of organisms by species. As Skellam (1952) has pointed out, knowledge of frequency distributions can suggest suitable transformations for purposes of applying standard statistical tests of significance. A normalizing transformation was desirable for purposes of estimating numerical abundance with relatively narrow confidence limits.

This survey was conducted during June and July of 1962 in Green Hill Pond, Charlestown, Rhode Island (Fig. 1), a shallow brackish-water pond of approximately 170-hectares surface area with a maximum depth of about 2 meters. The physiography and recent geological history have been described by Conover (1961).

METHODS AND RESULTS

A single large random sample consisting of 138 stations was obtained for the survey. A map of the entire pond was partitioned into 50 x 100 foot (15.24 x 30.48 m) grid intervals. Sampling stations were selected by means of a table of random numbers. The grid interval was chosen by a consideration of the accuracy by which stations could be located in the field by triangulation procedures. The total number of samples was restricted by time and cost factors.

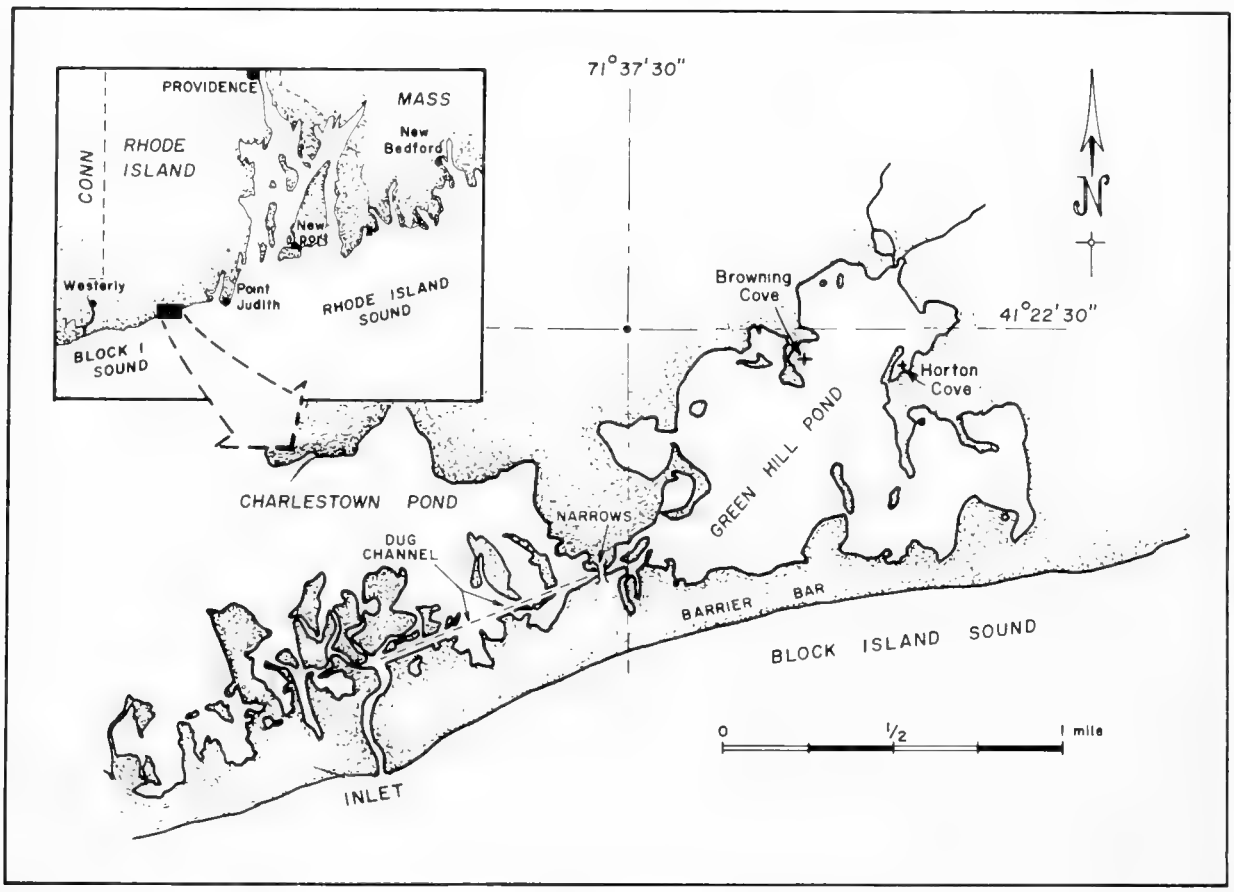

FIG. 1. *Geographic location and general features of study area.*

A 1/2-cubic yard (.38 cu m) capacity clam-shell construction bucket was used to recover the bottom samples from each station. The bucket was operated from a flat-bottomed barge by means of an A-frame and hand winch. The samples measured 4 1/2-square feet (.42 sq/m) in surface area and the sampling device penetrated the bottom to a depth of 1/2 to 2 feet (.15-.61 m), depending on the compaction of the sediments and the depth of water. The sediments taken were washed through a screened box consisting of 1/4-square inch (.64 cm) mesh hardware cloth from which the retained mollusks were collected, identified, and counted. Five species were taken in sufficient abundance for the purposes at hand. They were: *Crassostrea virginica* (Gmelin), *Macoma balthica* (Linnaeus), *Modiolus demissus* (Dillwyn), *Mya arenaria* (Linnaeus), and *Macoma phenax* (Dall).

A preliminary analysis of the frequency distributions of catches of these species indicated varying degrees of superdispersion. This was manifested by a large number of samples with low counts and a smaller number with high counts. (A typical frequency distribution obtained is shown in Figure 2.) The problem of superdispersion (a significantly larger variance than mean) has been considered by several workers and has been summarized by Bliss and Fisher (1953). Particular attention has been given to the negative binomial distribution as a reasonable approximation to observed sampling distributions.

In this instance, the choice of a negative binomial frequency function to describe the observed distributions was suggested by the shapes of the frequency distribution curves and by its general application in biological studies, and it was therefore fitted to available data. The negative binomial $(q-p)^{-k}$ is completely described by two parameters, the mean m and the index k. In this expression k is a positive constant which decreases for any particular distribution with increasing heterogeneity; and $q=1+p$ (k positive) where

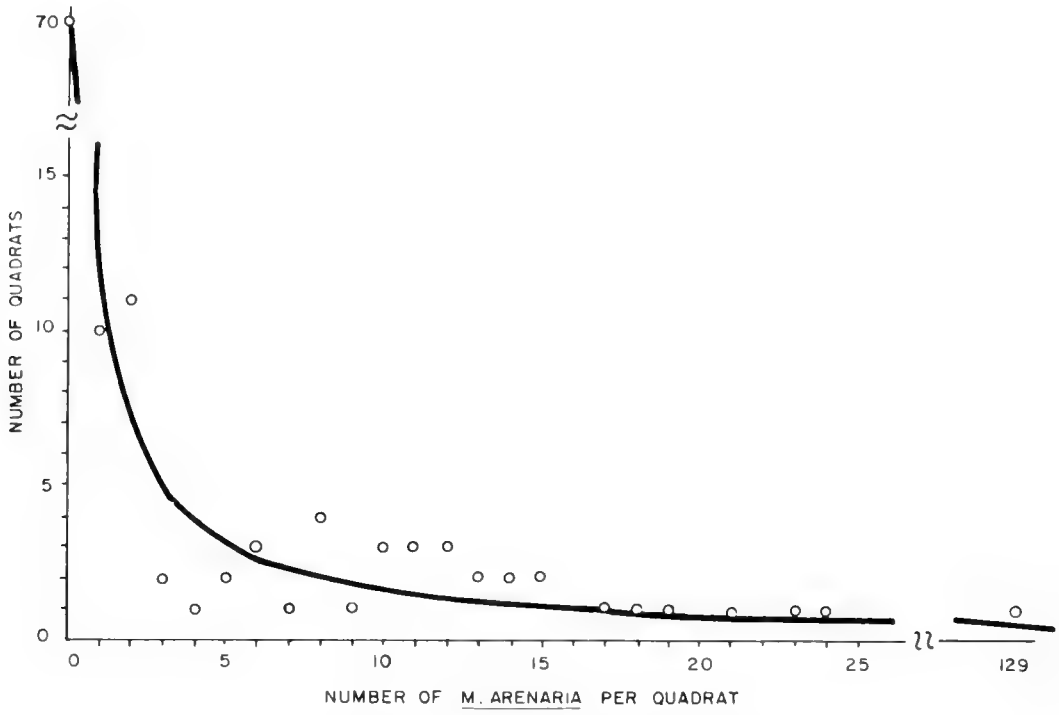

FIG. 2. *Numerical frequency distribution of adult clams and fitted negative binomial curve.*

— p=m/k. The fully efficient maximum likelihood method given by Haldane (1941) was used for the estimation of k.

The parameters of these distributions with chi-square goodness-of-fit probabilities are indicated in Table 1. The degrees of freedom are equal to the number of classes after grouping less three, since, in addition to the total frequency, two parameters have been efficiently fitted (Fisher, 1941).

The inverse hyperbolic sine transformation was used for all species since it has been shown to be a normalizing transformation for the negative binomial as well as for other contagious distributions (Beall, 1954). Specifically, the $1/\beta \sinh^{-1} \beta\sqrt{x}$ transformation described by Quenouille (1950) was used, where x is the value of the observed datum and β is the slope of the line giving the dependence of the standard deviation on the mean. The coefficient β was obtained by partitioning the several frequency distributions into equal intervals for purposes of calculating means and standard deviations to which a least-square line was then fitted. The β coefficient was estimated

TABLE 1. *Parameters of the negative binomial and chi-square goodness-of-fit probabilities by species for Green Hill Pond data.*

Species	Mean	Var.	Expected Var.	k	1/k*	df	χ^2_p
Modiolus	4.60	303.58	461.35	0.046	21.55	3	0.025
Crassostrea	3.97	217.06	239.18	0.067	14.90	4	0.90
Mya	8.76	417.26	451.11	0.174	5.76	8	0.05
Macoma phenax	3.86	28.44	38.52	0.425	2.35	8	0.10
Macoma balthica	81.59	10956.76	11893.72	0.564	1.77	21	0.10

*Dispersion index suggested by Moyle and Lound, 1960.

TABLE 2. *Tests of normality in the transformed frequency distributions of Green Hill mollusks.*

Species	Skewness	Kurtosis	Values of t	
	g_1	g_2	g_1	g_2
Modiolus demissus	0.5259	-.5918	1.154	0.668
Crassostrea virginica	0.3950	-.7748	0.967	0.970
Mya arenaria	0.4233	-.6256	1.456	1.090
Macoma phenax	0.5420	-.5427	2.039	1.032
Macoma balthica	0.1414	-.9363	0.663	2.212

t_∞, 0.025 — 2.241; t_∞, 0.10 — 1.645; t_∞, 0.20 — 1.282.

as the slope of the regression line.

The effectiveness of a transformation may be judged to some extent by the normality of sets of transformed data. Both skewness and kurtosis were tested by the method of Snedecor (1956) to determine how the distributions of the transformed observations might depart from normality. Skewness is measured by g_1, and complete symmetry is indicated in the sample when its value is zero. Kurtosis (g_2) is also zero if there is no departure from normality. The results of tests for normality in the transformed data for the five species are shown in Table 2.

The transformed values of the mean number of individuals per sampling unit for 2-foot depth contour intervals are given in Table 3. The number of units actually taken and the total number of possible sampling units for each stratum are also given in Table 3.

Since neither proportional nor optimal allocation were provided for in the sampling scheme, a weighting factor based on the number of units taken in relation to the size of the stratum was used to compute the means and variances. The method with notations taken from Cochran (1953) is described. Let

h — stratum,

N_h — total number of possible sampling units in stratum h,

n_h — number of units sampled in stratum h,

i — unit within the stratum,

y_{hi} — value obtained for the i th unit in stratum h,

N — total number of possible sampling units in all the strata,

L — total number of strata, and

$$\bar{Y}_h = \frac{1}{N_h} \sum_{i=1}^{N_h} y_{hi}$$

The stratified population mean per unit is

$$\bar{y}_{st} = \frac{1}{N} \sum_{h=1}^{L} N_h \bar{y}_h \qquad (1)$$

and the variance of y_{st} is

$$s^2_{(y_{st})} = \frac{1}{N^2} \sum_{h=1}^{L} N_h (N_h - n_h) \frac{S_h^2}{n_h} \qquad (2)$$

TABLE 3. *Transformed means of individuals by species within 2-foot depth contour intervals.*

Species	Depth Interval in Feet		
	0.00-1.99	2.00-3.99	4.00-5.99
Modiolus demissus	0.60	0.85	0.00
Crassostrea virginica	0.49	0.69	0.05
Mya arenaria	1.70	1.19	0.24
Macoma phenax	0.70	1.30	2.40
Macoma balthica	5.26	5.21	6.52
No. units sampled*	45	57	19
No. units in stratum	1,009,789	1,089,968	1,988,272

*Depth data not available for 17 stations sampled.

where $$y_h = \frac{1}{n_h} \sum_{i=1}^{n_h} y_{hi}$$

is the sample mean for stratum h, and

$$S_h^2 = \frac{1}{N_h - 1} \sum_{i=1}^{N_h} (y_{hi} - Y_h)^2$$

is the sample variance for stratum h.

The confidence interval is then

$$y_{st} \pm ts\ (y_{st})$$

for the population mean, and

$$N\ y_{st} \pm t\ N_s\ (y_{st})$$

for the population total. The multiplier is read from tables of the normal distribution.

Transformed data were used to calculate the estimates of the stratified population means and standard deviations. The values obtained were then converted back to the original scale to give derived values upon which the population size estimates were based. The derived values with their respective interval estimates for the five species are given in Table 4.

DISCUSSION AND CONCLUSIONS

The probabilities associated with the chi-square goodness-of-fit values of Table 1 give the probability of obtaining a worse fit by chance. The value obtained for the distribution of oysters *(Crassostrea virginica)* shows an unusually good fit. The values for the other species are sufficiently good with random sampling to retain the hypothesis of a negative binomial distribution with the possible exception of *Modiolus demissus*. The large value for the dispersion index obtained for this species indicates a high degree of heterogeneity. This distribution might be more closely represented by some other contagious distribution. These data suggest that the assumption of a negative binomial distribution as a description of the occurrence of all observed species of mollusks may not be entirely valid. However, the course of action with regard to a transformation is not greatly affected by the apparent deviation of *Modiolus* from a negative binomial distribution. As stated previously, a major concern was selecting a transformation to yield a variable whose distribution is approximately normal. On this basis, the five species of mollusks are sufficiently well fitted by negative binomial distributions to warrant use of a $\sinh^{-1}$ transformation. In the formal test for normality of the transformed data (Table 2), it is clear that all but three values of g_1 and g_2 were small and non-significant at the 20 per cent level of t. The exceptions, g_1 for *Mya arenaria* is non-significant at the 10 per cent level, whereas g_1 for *Macoma phenax* and g_2 for *Macoma balthica* are non-significant at the .025 level of t. The consistently negative values of g_2 suggest that all of the distribution curves are somewhat plateau-like near the center, but there is little evidence for departure from normality.

The range in the means for the depth intervals indicated that a more precise estimate of abundance could be obtained by stratifying the sample. The variability noted permitted pooling the data into the three strata shown in Table 3. Weighted means and variances (equations 1 and 2) were obtained to offset unequal sampling intensity between the various depth strata, and the converted values were used to estimate the size of the populations at the time of sampling. The relatively narrow confidence intervals obtained for the five species at the 95 per cent confidence level can be taken to indicate successful normalization of the data by means of the $\sinh^{-1}$ transformation.

ACKNOWLEDGEMENTS

The authors are indebted to Messrs. Donald B. Horton and Ramish Nayak of the Narragansett Marine Laboratory for assistance in the field sampling and enumeration of the data. This study was financed in part by the Marine Resources Program of the U. R. I. Graduate School of Oceanography, and the General Dynamics, Electric Boat Division graduate education program.

TABLE 4. *Derived means and standard deviations for quadrat samples and population numerical abundance estimates with 95% confidence limits.*

Species	Mean	Std. Dev.	Estimate	Interval
Modiolus demissus	0.142	0.0052	580,000	± 41,000
Crassostrea virginica	0.111	0.0030	450,000	± 25,000
Mya arenaria	0.792	0.0096	3,240,000	± 78,000
Macoma phenax	2.958	0.0172	12,090,000	±141,000
Macoma balthica	53.166	0.0420	217,340,000	±343,000

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APPENDIX [2]

Further Description of the Sampling Problem

The area of Green Hill Pond (420 acres) contains over 4,000,000 units equal in area to that taken by the clam-shell bucket used in the survey. In this, as well as in most marine surveys, the number of samples is often limited by economics, time, and other factors. A total of 138 samples was taken in the study. It seems as if this is a very small sample at first glance. However, it should be appreciated that pollsters are quite successful in predicting national election results on the basis of sample sizes of a few thousand voters out of a population of tens of millions. For both these cases, as well as for many others, mathematicians have devised procedures for making inferences about population parameters from a small set of observations *provided* these observations constitute a random sample of independent, identically distributed random variables. To the extent that we are able to specify a sampling process which should produce such a random sample, there is reason to expect that the set of observations actually obtained can be used to make inferences about the populations. Our random sample was obtained by partitioning the pond into a grid and then assigning the stations to be sampled in a random fashion. That is, each unit had an equal chance of being chosen.

A basic problem encountered when sampling natural populations is to distinguish between variability due to the distribution of the population in the environment from that inherent in the sampling method. In this instance, special effort was made to keep the size of the sampling unit (quadrat) constant. The variability in numbers of mollusks subsequently noted among stations was therefore ascribed to irregularities in the areal distribution of members of the species sampled.

The three fundamental distributions which are characteristic of natural populations are random, infradispersed, and superdispersed (Hutchinson,

[2] This appendix has been added by the authors at the request of the editor so that some of us not closely concerned with mathematics might have a better understanding of the thinking processes involved in setting up a population model of the kind presented here.

1953). The individuals of a species are randomly distributed if the position of each in space is independent of that of all the others. An observed distribution giving rise to such a stochastic pattern is the Poisson series. The variance of this distribution, in the statistical sense, is equal to the mean. Where the variance is much less than the mean, the members are more uniformly spaced (infradispersed) than would be expected if they were distributed according to chance. Aggregated populations (superdispersed) are those in which individuals of the species occur in groups or clusters. In such a case, the variance will be much greater than the mean. In our example, the observed frequency distribution clearly indicated aggregation of individuals. This is apparently the usual case for counts of plants or animals per unit area.

The conventional approach to the assessment of superdispersion is to compare the observed frequencies of quadrats occupied by 0, 1, 2, 3, etc. members against the expected frequencies for theoretical distributions with known characteristics. A satisfactory fit to a theoretical model implies that the spatial relationship of members under study must be similarly distributed (Ghent and Grinstead, 1965). A variety of nonrandom distributions have been employed for this purpose; the most favored being the negative binomial (Bliss and Fisher, 1953), Neyman's Type A (Neyman, 1939), the double Poisson (Thomas, 1949), the Poisson binomial (Skellam, 1952; McGuire, Brindley and Bancroft, 1957), the logarithmic series (Fisher, Corbet and Williams, 1943), and the lognormal distribution (Cassie, 1962). The choice of which mathematical model to use is generally based upon theoretical considerations of the variable or from previous emperical evidence. Our choice of the negative binomial distribution was based on the similarities in the shape of the frequency distribution curve obtained (Fig. 1) and by the general applicability of the negative binomial in biological studies.

The goodness-of-fit of the various species to the negative binomial distribution demonstrates the existence of superdispersion. Thus the variability noted is due to heterogeneity in the populations sampled. With heterogeneity, there is an increased probability that a second organism of the same type will be found in the immediate vicinity of the first.

The chi-square test was used to determine the goodness-of-fit of the theoretical distribution to the emperical data. This is an appropriate test of the fit of discrete distributions and evaluates the overall fit of the curve, not simply a few parameters (Whitney, 1959).

After the nature of the distributions of the series of counts has been determined it is necessary to transform the data because applications of most statistical tests depend on various assumptions concerning the nature of the observations. Probably the most common transformation is to equalize variances which also serves to make the distribution more nearly normal so that standard statistical tests can be applied without difficulty.

An inverse hyperbolic sine transformation is recommended to normalize a variable which is graduated by the negative binomial distribution (Anscombe, 1948; Beall, 1954). Successful normalization by use of an inverse hyperbolic sine transformation was verified as shown in Table II in the body of the paper.

The transformed data were used to compute means and variances within 2-foot depth contour intervals. The values obtained were deemed sufficiently variable to warrant retention of the stratification for purposes of estimating population densities of the various species in the whole pond. A gain in precision of the estimates could thereby be realized. The unequal sampling intensities among the strata (Table III) necessitated use of a weighting factor for purposes of estimating the density of the entire population of each species.

To briefly summarize, we have attempted to avoid bias by random sampling, to demonstrate the nature of the observed distribution of mollusk counts, to make an appropriate transformation, to increase precision by stratification, and to make inferences concerning the populations.

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BENTHIC ECOLOGY AND FAUNAL CHANGE RELATING TO OYSTERS FROM A DEEP BASIN IN THE LOWER PATUXENT RIVER, MARYLAND

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ABSTRACT

Living oysters from deep water (130 feet) were taken in an area of the lower Patuxent River. These oysters were studied to determine their well-being at this unusual depth. This was done by comparing the benthic ecology, including faunal changes, physical and chemical parameters and oyster growth rate at the deep-water station with shallower water stations nearby. Faunal change was more variable at the deep-water station, but physical and chemical characteristics were similar at all stations. The oysters from 130 feet grew more slowly; however, they were successful in maintaining a population and surviving well under rather unusual conditions.

INTRODUCTION

The main distribution and abundance of the American oyster, *Crassostrea virginica* (Gmelin), is in shallow estuarine waters. Galtsoff (1964) gave 100 feet as the greatest depth in which *C. virginica* lives. Merrill, Emery, and Rubin (1965), from scattered data relative to present bathymetric distribution of natural oyster populations in Chesapeake Bay, estimated 6 meters (about 18 feet) as the average depth.

Since oysters usually live in shallow water, the following observation in a Report by the Board of Shellfish Commissioners of Maryland (1912, p. 120) of living oysters at an unusually great depth in an area of the lower Patuxent River, Maryland, is of interest and significance:

> In the vicinity of Point Patience, oysters were taken at depths of 120 and 130 feet, and the results of the examination in this deep water showed the bottom to be densely stocked and the oysters to be in a most thriving condition. While the oysters may originally have been carried to these deep bottoms from adjoining oyster bars by currents (as many oyster men claim), no evidence was found to indicate that the conditions of the deep lying bottoms are not just as favorable for the growth of oysters as those which prevail on the bottoms in shallower water.

Stimulated by the Maryland Report, we investigated the current status of the oyster population in this deep basin. Upon confirmation of the existence of the oysters in depths of 130 feet off Point Patience (Boss and Merrill, 1965), we felt that a comparison of this population with populations from shallower depths in the same area would be valuable. Therefore, at several stations in June and December 1964, chemical and physical data were taken, growth rates of shells of the oyster and "condition" indices of the oyster meats were determined, and changes in faunal composition recorded. This paper reports the results of the study.

DESCRIPTION OF THE AREA

The inset in Figure 1 shows the geographic position of the Patuxent River as it enters the western part of Chesapeake Bay at Solomons, Maryland, about 25 miles north of the mouth of the Potomac River. The Patuxent River is approximately 80 miles long and drains an area of about 960 square miles (Bonsteel and Burke, 1907). The lower por-

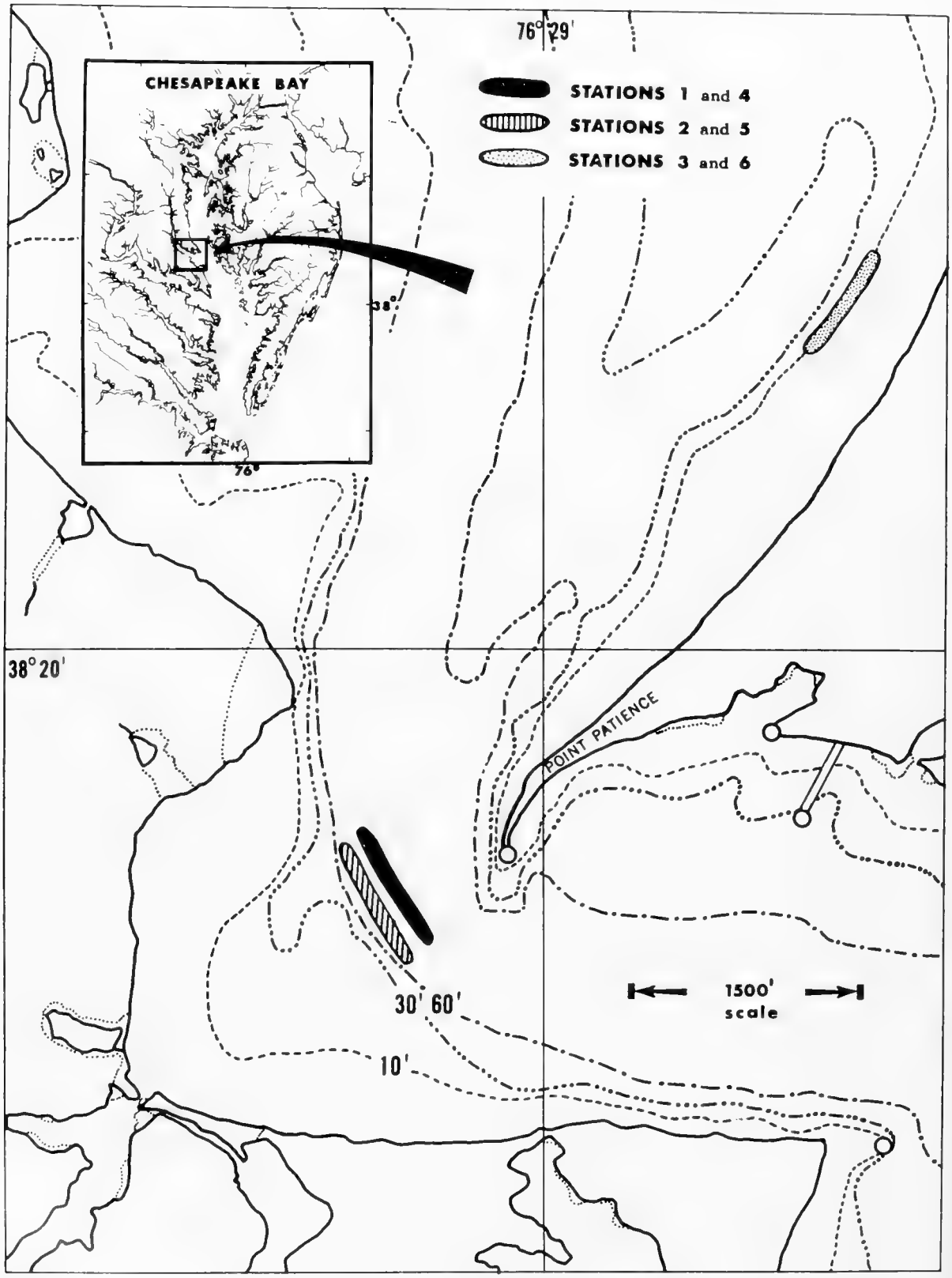
76° 29'
CHESAPEAKE BAY
STATIONS 1 and 4
STATIONS 2 and 5
STATIONS 3 and 6
38°
76°
38° 20'
POINT PATIENCE
30'
60'
10'
1500'
scale

TABLE 1. *Plotted positions of dredging stations in the lower Patuxent River. Stations 1-3 (June); repeat Stations 4-6 (December). Locations are taken from Coast and Geodetic Survey Chart 553.*

Station	Date	Lat. (N)	Long. (W)	Depth (Feet)
1	12 June 1964	38°19′44″	76°29′15″	130
2	12 June 1964	38°19′41″	76°29′18″	65
3	12 June 1964	38°20′30″	76°28′27″	10
4	3 Dec. 1964	38°19′44″	76°29′15″	130
5	3 Dec. 1964	38°19′41″	76°29′18″	65
6	3 Dec. 1964	38°20′30″	76°28′27″	10

tion of the river is estuarine and has a tidal amplitude of 1.4 feet at the mouth of the estuary and 2.9 feet near its upper limit (Nash, 1947).

Stations were established off Point Patience at positions and depths indicated in Table 1 and Figure 1. The area sampled is about 1 mile upstream from Solomons in tidal zone V as classed ecologically by Mansueti (1961). This portion of the river has a steeply walled channel or basin which at places exceeds a depth of 130 feet. Pritchard (1951) noted that the lower Patuxent River has a subsurface upstream movement of saline water and a surface downstream current of fresh water with each tidal cycle.

Station positions were established on board ship by triangular alignment with salient land features and depth readings on the fathometer. As in the study by McErlean (1964), the exact positions are subject to some degree of error, in the range of 30 feet.

PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE AREA

Physical and chemical data were taken at each station at the surface and bottom. The salinity and temperature were recorded simultaneously with a salinometer. Oxygen determinations were made by the Winkler method and chlorophyll "a" measurements by spectrophotometric analysis. Table 2 summarizes these measurements.

Temperatures in June were nearly isothermal (22.1-22.4°C), except for the bottom temperature at the deep-water station which was 21.0°C. Likewise, water temperatures were closely similar in December (8.0-8.7°C); the shallow-water surface waters were slightly cooler than the bottom waters.

Salinities in June were also similar (11.29-12.23 ppt), but the bottom waters were slightly more saline (by less than 1 ppt). The situation was the same in December, when salinity averaged 18.5 ppt.

Oxygen levels were low at all stations in June. Values ranged from 7.46 to 7.99 ppm except at the bottom at the deep-water station where the concentration was 5.99 ppm. Oxygen values were higher in December; they averaged about 11 ppm but were somewhat lower at all bottom stations and lowest at the deep-water station (10.66 ppm). These levels indicate fair circulation the year around.

Chlorophyll "a" was generally low at all stations in both June and December. In each season, however, the amount was lowest on the bottom at the deep-water station.

FIG. 1. *Geographic location of the lower Patuxent River in the area of Pt. Patience (inset) and location of stations at which oysters were collected. The 10-, 30- and 40-foot depth contours are shown by broken lines.*

"CONDITION" OF OYSTERS

A "condition" index was established for the summer samples by visual examination of the oyster meats, and for the winter samples by determination of the percentage dry weights of the meats (Table 3). Each sample contained 10 adult oysters. The condition of the oysters in shallow water was better in both seasons than in either the deep or medium water. No samples contained oysters that would classify higher than average and no oysters would classify as good.

GAMETOGENESIS OF OYSTERS

Sex of 10 oysters from each station in June and December was determined and the conditions of the gonads recorded. In June, oysters from all stations appeared ready to spawn (gonads classified as "ripe"). In December, gonads of the oysters from shallow depths were flaccid, but some of those from deep and medium-deep stations were only partially spawned. At the medium-deep station the gonads of 3 of the 10 oysters contained some eggs or sperm; at the deep-water station, 4 of the 10 had only partially spawned. The sexual products were still viable. Fertilization took place when eggs and sperm were placed together in the laboratory. Larvae developed through to the "straight-hinge" veliger stage.

TABLE 2. *Physical and chemical data at dredging stations in the lower Patuxent River. Stations 1-3 occupied June 12, 1964; stations 4-6, December 3, 1964.*

Station	Depth (feet)	Temperature (°C)		Salinity (ppt)		Oxygen (ppm)		Chlorophyll "a" (μg/l)	
		Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
1	130	22.10	21.00	11.67	12.23	7.92	5.99	1.38	1.17
2	65	22.40	22.30	11.29	11.73	7.96	7.46	1.62	1.42
3	10	22.30	22.30	11.38	11.46	7.99	7.96	1.78	2.70
4	130	8.42	8.68	18.46	18.92	11.51	10.66	3.34	1.87
5	65	8.42	8.55	18.57	18.67	11.25	10.90	2.35	4.31
6	10	8.00	8.49	18.20	18.57	11.26	10.71	2.17	2.07

To our best knowledge, these populations of oysters are the only natural oysters in the Chesapeake area that could be utilized out of season for spawning experiments.

THE BENTHIC FAUNA AND CHANGES IN THE FAUNAL COMPOSITION

A 5-minute tow was made at each station with a standard 30-inch oyster dredge with a 3-inch-mesh bag. At no station was the bag completely filled. Biological materials were sorted after each tow and all, or an aliquot, of the material was preserved. Table 4 lists the various species of animals found at each station and their abundance. The data for the stations show a seasonal fluctuation in the population of certain species.

The most noticeable changes in the faunal composition were at deep-water stations 1 and 4. Both the oysters, *Crassostrea virginica*, and the mussels, *Brachidontes recurvus*, showed a nearly 10-fold decline in numbers in the December dredging as compared with the June dredging. The numbers of the sea grape, *Molgula manhattensis*, increased sharply in December and the anemone, *Aiptasia eruptaurantia*, declined. Both *Molgula* and *Aiptasia* are sedentary organisms which often attach to the shells of oysters or mussels.

The increase in *Molgula* in deep water was phenomenal — from 200 per 5-minute tow in June to an estimated 30,000 in December. Of the 2-bushel volume of material in the deep-water dredge haul in December, *Molgula* made up about 1 1/2 bushels.

The dominant epizoic organism of the oyster at the medium-depth station in December was the red-finger sponge, *Microciona prolifera*. This species fouled oyster shells heavily, but did not appear to affect the oyster population adversely. Although the sponge was dominant, it was highly localized; it was found only in December and only at this station.

GROWTH OF OYSTERS AND MUSSELS

Shapes of the oysters ranged from long and narrow to ovate. To obtain a comparable shell size for all shapes, measurements of height and length of the oysters were taken, totalled, and the sum divided by 2. After size frequencies were established an attempt was made to separate the populations into age groups with the aid of probability paper as described by Harding (1949). This separation was not possible because the great variation in yearly growth masked the modes of the age groups. The mode of first-year oysters was easily distinguished, however, in all collections. A mean, calculated to represent that group, provided an index of growth for the first-year oysters at each station. This figure is the "low" mean in Table 5. The remaining oysters in each collection were given a "high" mean which represents the remaining range of ages.

Each oyster population showed growth during the 7 months as indicated in both "low" and "high" means in Table 5. The mean size of the small oysters in deep water was less than at the other stations on both collecting dates. Furthermore, the mean growth for the juveniles of the deep-water population was less, only 11 mm in 7 months com-

TABLE 3. *"Condition" of Oyster Meats.*

Stations	Depths (feet)	12 June 1964 (by observation)	2 December 1964 (by percentage solids)
1 and 4	130	Medium minus	10.20
2 and 5	65	Medium	9.81
3 and 6	10	Medium plus	12.77

TABLE 4. *Benthic fauna, in numbers of individuals per 5-minute tow, taken at stations off Point Patience in the lower Patuxent River, Maryland (Stations 1-3, June 1964; Stations 4-6, December 1964).*

	Station numbers, and depths in feet (in parenthesis)					
	1	2	3	4	5	6
Organism	(130)	(65)	(10)	(130)	(65)	(10)
Porifera						
Microciona prolifera (Ellis & Solander)	—	—	—	—	abundant	—
Coelenterata						
Aiptasia eruptaurantia (Field)	400	—	—	52	308	—
Aiptasiomorpha luciae (Verrill)	—	—	—	—	4	—
Diadumene leucolena (Verrill)	—	—	2	—	—	66
Thuiaria argentea (Linnaeus)	—	—	—	some	—	—
Annelida						
Nereis (Neanthes) succinea (Frey & Leuckart)	—	5	54	32	114	122
Polydora ligni Webster	—	1	1	—	—	—
Phyllodoce (Anaitides) maculata (Linnaeus)	—	—	—	6	11	—
Glycera dibranchiata Ehlers	—	—	—	2	—	—
Polyclad worms	—	—	—	—	3	3
Crustacea						
Balanus improvisus Darwin	many	many	—	—	common	common
Balanus eburneus (Gould)	common	common	—	—	rare	rare
Callinectes sapidus Rathbun	—	—	4	—	—	—
Eurypanopeus depressus (Smith)	8	101	1	23	66	52
Rithropanopeus harrisii (Gould)	36	12	—	12	2	—
Crangon septemspinosus (Say)	2	—	—	7	—	—
Palaemonetes pugio Holthuis	—	6	—	—	—	—
Palaemonetes vulgaris (Say)	—	—	—	1	10	—
Palaemonetes intermedius Holthuis	—	—	—	—	6	—
Mollusca						
Nassarius vibex (Say)	6	—	1	18	—	—
Epitonium rupicola (Kurtz)	16	—	—	2	—	—
Odostomia impressa (Say)	—	—	2	—	—	—
Odostomia bisuturalis (Say)	—	—	—	—	4	—
Haminoea solitaria (Say)	—	—	34	—	—	—
Crassostrea virginica (Gmelin)	258	1677	227	49	1058	162
Brachidontes recurvus (Rafinesque)	1004	1356	47	51	546	62
Mulinia lateralis (Say)	—	—	—	—	—	5
Gemma gemma (Totten)	—	—	12	—	—	—
Mya arenaria (Linnaeus)	3	18	4	—	—	—
Tagelus plebius (Solander)	—	—	41	—	—	—
Macoma balthica (Linnaeus)	5	8	1	—	—	1
Laevicardium mortoni (Conrad)	—	1	—	—	—	—
Tunicata						
Molgula manhattensis (DeKay)	200	120	5	30,000	648	293
Pisces						
Gobiosoma bosci (Lacepede)	—	6	4	3	2	1
Gobiesox strumosus Cope	—	—	2	1	2	—
Chasmodes bosquianus (Lacepede)	—	—	1	1	6	1
Opsanus tau (Linnaeus)	—	—	3	3	2	—
Syngnathus fuscus Storer	—	—	—	3	2	—

TABLE 5. *Comparison of oyster populations per 5-minute tow at three depths in the lower Patuxent River, Maryland in the summer and winter of 1964. Stations 1-3 sampled June 12, 1964; stations 4-6, December 3, 1964. (See text for explanation of low and high means.)*

Station	Depth (feet)	Number in Sample	Low Mean (mm)	High Mean (mm)
1	130	258	22	67
2	65	1677	27	69
3	10	227	25	93
4	130	49	33	69
5	65	1058	43	76
6	10	162	47	97

TABLE 6. *Size range (10-mm intervals), and average sizes of a sample of* Brachidontes recurvus *from Stations 1-6, lower Patuxent River.*

Size	Stations					
(mm)	1	2	3	4	5	6
1-10	7	13	1	3	7	2
11-20	78	44	8	14	28	12
21-30	146	50	12	17	41	14
31-40	182	71	13	13	96	15
41-50	83	44	10	3	82	11
51-60	11	4	2	1	17	8
61-70	—	—	1	—	1	—
Total	502	226	47	51	273	62
Average	31	30	30	27	35	33

pared with 16 mm at the 65-foot station and 22 mm at the 10-foot station. The change through 7 months in the "high" mean reveals a similar situation in that the growth for the entire population of the oysters at the deep station was lower.

While the oysters from 130 feet grew more slowly than those at either of the shallower stations, they were maintaining a population and surviving well under rather unusual conditions.

Although the oysters from deep water, as a population, exhibited growth for the period of study, the population of mussels showed "negative" growth. An attempt to separate the modes for age groups of the mussel, *Brachidontes recurvus*, was unsuccessful. In every collection the total size frequency of the population blended into an indistinguishable single mode (Table 6). The lack of a distinctive "low" mean can be explained by the long spawning season of *B. recurvus*. Allen (1962) and Shaw (1965) have shown that this mussel spawns over a long period, possibly from April to December. An almost continual setting of post-larvae during the growing season would make it impossible to establish a mean for young-of-the-year.

The mean size for mussels increased at the medium-deep and shallow-water stations over the 7 months, but decreased sharply at the deep-water station. The decrease of size at the deep-water station is attributable to the lower ratio of larger to smaller animals (Table 6, compare stations 1 and 4). Greater mortality of larger mussels would account for the decrease in mean size.

Molgula and *Brachidontes* are of a similar size and both foul the oyster, which is much larger. *Molgula*, like the oyster and mussel, is an extremely efficient suspension feeder, and has a surprisingly high dry-weight of about 5 per cent (Haven [1], in litt.). Galtsoff (1964) found that oysters suspended on rafts off Cape Cod, Massachusetts, showed no ill effects when they were totally covered with fouling organisms, including *Molgula*. The great numerical increase of *Molgula*, however, may have adversely affected the growth of mussels through direct competition.

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ASSOCIATION AFFAIRS

ANNUAL CONVENTION

The National Shellfisheries Association 1965 Convention was held jointly with the Oyster Institute of North America and the Oyster Growers and Dealers Association of North America, Inc., on June 27 — July 1, 1965, at the Lord Baltimore Hotel, Baltimore, Maryland.

The Pacific Coast Section of the NSA met on August 19-20, 1965, at Olympia, Washington. Officers of the Section were Charles Woelke, chairman, William Beck, vice-chairman, and Kenneth Chew, secretary-treasurer.

The NSA secretary-treasurer reported that the Association had 211 members in good standing, including 33 new members accepted since the last Convention. Three members resigned but two of these are no longer in shellfisheries work. Membership dues were raised from four to six dollars; library dues were held at four dollars to insure wide distribution of the Proceedings.

Dr. and Mrs. Paul S. Galtsoff were the Honored Guests of the joint Association throughout the entire Convention.

Mr. P. R. Walne of the Conway Laboratory, Ministry of Agriculture, Fisheries and Food, Wales, U. K., was an invited guest of NSA. He gave three papers on shellfish investigations including the popular evening lecture.

A symposium on hatchery and pond culture technique was held with invited speakers.

John L. Myhre was awarded the T. C. Nelson Award for meritorious research in shellfisheries by a junior scientist. The award included a scroll recognizing this achievement and five years free membership in NSA.

Officers elected for the term 1965-1966 were as follows:

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