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ABSTRACTS OF TECHNICAL PAPERS PRESENTED AT THE 1966 NSA CONVENTION

STATISTICS OF THE NEW JERSEY SURF CLAM FISHERY — 1965

Allan M. Barker and Thomas M. Groutage

U. S. Bureau of Commercial Fisheries
Biological Laboratory
Oxford, Maryland

New Jersey landings of surf clams (*Spisula solidissima*) have increased greatly since 1943, while the contributions of other middle Atlantic states have declined and are now of minor significance. The marked increase in landings from 1957 (15 million pounds of meat) to 1965 (43.7 million pounds) can be attributed to the New Jersey fishery. Greater demand for the product and increased gear efficiency have both played their part in this rise.

Since 1959, surf clam fishing effort has stabilized and is concentrated in the area between Point Pleasant and Barnegat Lightship. A fleet of about 50 boats is based at Point Pleasant, with a few (7-9) boats at Cape May-Wildwood and Barnegat Inlet. In 1965 considerable effort was expended near Barnegat Lightship. In the spring a dense bed of small clams inshore at Cape May was heavily exploited. Record landings in 1965 were due in part to large catches of these small clams. Landings at Cape May exceeded those at Point Pleasant in March. Mean lengths of total samples by month at Point Pleasant were consistent at 6 inches throughout 1965. Mean lengths of Cape May samples were 5 to 6 inches.

Average catch and effort at Point Pleasant in 1965 reached a low of 607 pounds per hour in February and a high of 744 pounds per hour in September. Observations on commercial vessels at sea revealed that the amount of clams discarded is usually insignificant and rarely exceeds one bushel per trip.

Length-meat weight ratios indicated that meats from inshore Cape May clams were similar in weight to meats from offshore Point Pleasant clams of the same shell length. Monthly per cent solids of clams from Point Pleasant and Cape May were determined in 1965 and values were not significantly different between the two locations.

COMPARATIVE STUDY OF TWENTY-THREE SPECIES OF BIVALVE LARVAE

Paul Chanley

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Wachapreague, Virginia

Larvae of 23 species of bivalves have been reared in the laboratory and studied comparatively. Photomicrographs, keys, graphs, and tables are offered as aids to their identification. Characters of particular importance in identifying larvae include: hinge line length, size and shape of umbo, length-height relationship, length and shape of anterior and posterior ends as well as color or texture. In a few species hinge teeth are distinctive.

A SOURCE OF PARALYTIC SHELLFISH TOXIN IN SEQUIM BAY, WASHINGTON

John L. Dupuy, Albert K. Sparks,
Kenneth K. Chew, and Benny C. C. Hsu

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The seasonal rise in shellfish toxicity along the Strait of Juan de Fuca from 1961 to 1965, particularly in Sequim Bay, was coincident with the increased abundance of the dinoflagellate, *Gonyaulax* sp. (*catenella*?) and the appearance of toxin in plankton extracts. Experiments with unialgal mass cultures of *Gonyaulax* sp. isolated from Sequim Bay gave definite evidence that *Gonyaulax* is a primary source of toxin in this area and in the Strait of Juan de Fuca.

Final concentrations of *Gonyaulax* sp. varied when grown under similar conditions and in the

same batch of medium. Extracts of *Gonyaulax* sp. grown in similar and different culture media were found to yield variable amounts of paralytic shellfish poison.

FURTHER STUDIES IN CLAM DEPURATION [1]

S. Y. Feng [2]

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In a study of elimination of viral particles by hard clams, *Mercenaria mercenaria,* two bacteriophages, *Staphylococcus aureus* phage 80 and *Escherichia coli* phage S-13, were used as virus models. Hard clams experimentally contaminated with the two phages were treated in recirculating and flow-through seawater systems. The sea water, with salinities ranging from 20 to 22 ppt, was irradiated by UV light before reaching the clams. The experiments were carried out under temperatures ranging from 10° to 20°C and flow rates from 50 to 150 gallons per hour. The data reveal that the purification rates of the two bacteriophages, by hard clams, were independent of temperatures (10°, 12°, 15°, 18° and 20°C), and flow rates (50, 100 and 150 gallons per hour). The course of elimination is characteristically an initial rapid removal followed by a more gradual, 6-day attrition of the viral particles. The data also suggest that the clam eliminates the two bacteriophages at different rates: *S. aureus* phage 80, the less hardy of the two, is eliminated at a much faster rate than is the *E. coli* phage S-13 which resembles poliomyelitis virus in size, shape and nucleic acid content.

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[1] This investigation is supported in whole by Public Health Service Research Grant EF 00671.

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FURTHER STUDIES ON PHYSIOLOGICAL VARIATION AMONG POPULATIONS OF OYSTER DRILLS

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Physiological studies in the field and laboratory were carried out on oyster drills *(Urosalpinx cinerea* Say) from Long Island Sound, Delaware Bay, and Bogue Sound, N. C. and on oyster drills *(U. c. follyensis* Baker) from the Eastern Shore of Virginia. Short-term growth studies using drills reared from egg cases showed that the Eastern Shore drills grew significantly faster than the Long Island and North Carolina populations. However, the rate of oyster consumption by all three populations was the same.

Analysis of the cumulative feeding and fecundity curves of mature Long Island and Eastern Shore drills maintained in trays on the Cape Shore tide flats indicated that these drills consumed more oysters and produced more egg cases than similarly held New Jersey and North Carolina populations. Temperatures above 25°C did not appear to inhibit feeding or fecundity of drills in trays.

Laboratory studies on feeding rates of drills held at temperatures ranging from 10-30°C indicated that the Eastern Shore drills fed at higher rates than the other three populations, especially at low temperatures.

In the laboratory, the Long Island drills produced many more egg cases than the other populations at temperatures from 10 through 26°C. The New Jersey and North Carolina drills did not differ from each other in this regard and both produced fewer egg cases than the Eastern Shore drills at temperatures above 15°C.

These studies clearly show the higher fecundity of the Long Island drills relative to all other populations studied. Physiological differences in the Eastern Shore population have been demonstrated in growth rate, feeding rate and the number of egg cases produced per clutch.

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STUDIES OF THE F_1 and F_2 HYBRIDS OF THE NORTHERN AND SOUTHERN QUAHOG CLAMS

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Florida State University

During the first half of five years' observations of northern and southern parents and reciprocal hybrids, the southern species grew fastest and the northern slowest. Growth rates of the hybrids were intermediate, but closer to the southern parent. During the latter half of the observation period the growth rates of the hybrids surpassed both parents. The F_1 hybrid of the cross, female *Mercenaria campechiensis* X male *Mercenaria mercenaria,* grew larger than those of a reciprocal cross.

All the 4 possible F_2 crosses have been made. Although still too small for final characterization, they so far resemble the southern parent in shell morphology.

A PRELIMINARY REPORT ON MORTALITY AND MSX LEVELS IN OYSTER SPAT.

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Since 1958 there has been increasing evidence on the Delaware Bay Cape Shore for development of strains of oysters resistant to mortality due to *Minchinia nelsoni* (MSX) infection. As part of a detailed study of disease resistance, mortalities in two groups of spat, exposed in trays at the Cape Shore, have been monitored since July 1965. Both groups were laboratory reared; one from a stock of resistant parent oysters (old Delaware Bay stocks exposed to MSX for several years) and the other from a stock of very susceptible oysters (Navesink River, N. J.). From 29 July 1965 to 23 April 1966, 75 per cent of the Navesink spat and 80 per cent of the Delaware Bay spat died of all causes. Histological study of periodic samples of living oysters and "gapers" are incomplete but by 14 August 1965 more than 90 per cent of all spat sampled were lightly infected with MSX. By October the infections in the Navesink spat were generally heavy, while more than one third of the Delaware Bay spat had rare to light infections. Interpretation of these data must await completion of the study.

ELECTRON MICROSCOPE STUDIES OF *MINCHINIA NELSONI*

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Gill tissue from living oysters showing a moderate or heavy infection of *Minchinia nelsoni* (MSX) on examination of a wet mount was fixed in Palade's veronal-buffered osmic acid, embedded in methacrylate, and sectioned on an LKB Ultrotome. Sections were viewed and photographed on an RCA EMU-2 (Canalco modified) electron microscope.

Vegetative stages of MSX that have been recognized in these preparations to date have a morphology familiar to us, in an overall sense from studies with the light microscope. The cell membrane is distinct but ragged in appearance. No locomotive organelles such as cilia, flagella, or vestiges of a flagellar apparatus such as kinetoplasts have been observed in plasmodial stages. Only capped nuclei have been seen so far. The nucleoplasm is granular and homogeneous (i.e., without chromatin clumps). The nucleolar cap is distinguishable only by its greater electron density; no membrane separates it from the rest of the nucleoplasm. Other intranuclear structures such as spindles or "Kernstaebe" have not yet been encountered. The nuclear envelope, which consists of two membranes, is about 25 μ thick. In one collection of oysters, the outer membrane of the MSX nuclei exhibited small outpouchings or "blebs". The significance of the blebs is not known.

MSX mitochondria are approximately spherical and relatively large. The inner mitochondrial membrane is invaginated into microtubules rather than cristae; this microtubular condition is typically, though not exclusively protozoan. Mitochondria frequently appear in close association with nuclei. Many nuclei are surrounded by mitochondria and often appear to be indented by their mitochondrial "satellites". The large number and size of mitochondria, and their association with nuclei, suggest that MSX is a cell of great oxidative metabolic capacity, part of the energy from which may function in nuclear reproduction.

In addition to mitochondria, the cytoplasm contains a background of granules, saccules, and vacuoles which for descriptive purposes may be termed endoplasmic reticulum. Imposed upon this background have always been found numerous, small (about 150 μ), very dense (i.e., osmiophilic) particles. These particles bear a very strong resemblance to certain viruses. If these are viruses, one may now only speculate about their role: perhaps they are hyperparasites or symbionts (cf., the kappa particles of *Paramecium.)*

It is apparent that MSX as seen through the electron microscope is cytologically complex and unusual to an extent not hitherto imagined.

PROGRESS IN ARTIFICIAL CULTURE OF VIRGINIA OYSTERS

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For seven years the parasite, *Minchinia nelsoni* (MSX), has persistently killed oysters in Virginia and kept nearly half of our private oyster acreage out of production. The urgent need for MSX-resistant oysters has resulted in vigorous efforts to breed such stocks by artificial culture. Phase (1) was the collection of survivors from large planted beds, heavily selected by MSX, for five

to seven years. Phase (2) involved breeding these old survivors under controlled laboratory conditions. Numerous larval cultures were successfully reared in 1964 and 1965. All important stocks of selected oysters have been bred successfully. Phase (3) required nursery conditions for progeny which insured growth, survival and freedom from contamination by wild spatfalls. This was found in an acre-size pond with limited circulation. Phase (4) requires monitoring batches of progeny until evidence on resistance to MSX is available. This phase is the longest, most tedious and most demanding in terms of effort and time. All other phases have now been successfully completed. Monitoring requires a mimimum of two or three years. Each progeny group is held in large legged trays in York River. Oysters are counted, measured and sampled regularly to provide disease incidence and death rates. Some 25 lots of progeny and as many brood stocks are now under observation. Progeny set in 1964 are showing encouraging results in that those from MSX-selected oysters have lower disease incidences and less mortality than controls.

A PLANKTON SAMPLER FOR OYSTER LARVAE

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A plankton sampler has been designed primarily to sample oyster larvae uniformly in a vertical column of water. It may be used either as a spot or as a moving sampler. The apparatus requires a perforated pipe, water pump, water meter and plankton net.

THE LOCOMOTION AND BEHAVIOR OF SURF CLAMS, *SPISULA SOLIDISSIMA*

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The locomotion and behavior of surf clams, *Spisula solidissima,* were investigated to assess their possible migratory abilities. Both field and laboratory observations were included in the study.

Observations were made on beach and offshore populations of surf clams. On beaches juvenile clams 20 to 70 mm in shell length displayed four types of activity: (1) gliding through the water (2) exhuming themselves from the exposed beach at low tide, (3) rapidly burrowing into the substrate as the tide flooded in, and (4) crawling over the substrate. These observations suggested that small clams may shift location, especially if bottom currents are present to carry the clams once locomotion is initiated. In the laboratory, juvenile clams propelled themselves off the bottom by muscular movements of the foot and glided a distance of 8 to 12 inches.

Low water temperatures resulted in reduced activity. Clams in the laboratory acclimated to a 10°C increase from 8°C within 24 hours and burrowed more rapidly than clams under the influence of low seasonal temperatures. A 22°C rise from 8°C caused gaping, lethargy, and eventual death. Clams were frequently observed emerging from exposed sand at low tide, but this activity has not been duplicated in the laboratory either by draining the water from the aquaria sand, heating the sand surface, or applying a weight to the sand surface. Crawling, by alternately penetrating the tip of the extended foot in the sand and contracting it, has been observed only infrequently in either the field or laboratory.

MARKING SURF CLAMS FOR GROWTH STUDIES

John W. Ropes, Arthur S. Merrill, and Thomas M. Groutage

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Biological Laboratory
Oxford, Maryland

Various marking techniques were tested on surf clams, *Spisula solidissima,* as part of studies on population dynamics and growth. The methods of collecting large numbers of juvenile surf clams at Chincoteague Inlet, Virginia, in late 1964, applying colored liquids to the shells, attaching plastic discs and tapes to the shells with adhesives, and grinding notches in the shells are described.

The colored liquids were generally unsatisfactory marks on recovered clams. Notches clearly delimited increments of new shell growth. New adhesives appear to be promising as a means of attaching colorful individual markers and can be used in conjunction with shell notches for planting in the offshore fishery. The colorful marker improves the chance of recovering notched clams by attracting the attention of the fishermen.

THE GROWTH OF JUVENILE SURF CLAMS AT CHINCOTEAGUE INLET, VIRGINIA

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Juvenile surf clams, *Spisula solidissima,* were collected, marked, planted, and recovered over an 18 month period in Chincoteague Inlet, Virginia, to obtain measurements of growth. A notch ground into the shell margin was used as a reference mark of the clam's original shell length when planted.

Clams from the Chincoteague Point planting site grew an average of 42.1 mm in length after 18 months or about 2.4 mm per month. Slower growth (22.7 mm after 18 months or 1.3 mm per month), apparently due to unfavorable environmental conditions, was observed at the Assateague Cove planting site. Unmarked clams from Wallops Island increased in length from 21.1 mm to 66.2 mm in 18 months, an average of 2.5 mm per month. An analysis of variance showed no significant difference between the growth of marked clams from Chincoteague Point and unmarked clams from Wallops Island. Thus, notching the shell margin of surf clams appears to be a useful method of providing accurate measurements of growth.

A THREE-PLY REPRESENTATION OF THE MAJOR ORGAN SYSTEMS OF A QUAHAUG

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A series of semi-diagrammatic drawings have been made to outline and depict the relative positions of the major organ systems in the northern quahaug, *Mercenaria mercenaria* L. These drawings can be cut out, folded, slipped together, and stapled in the hinge area to form a sequence of visualizations from the exterior to the interior of this mollusk. Additional drawings provide orientation in sectional aspects.

Dissections of large quahaugs, about 12 cm in length, and sections of frozen specimens made with a diamond-blade, cut-off saw were the basis of the paper reconstruction.

These drawings were developed as a training aid in connection with the National Shellfish Sanitation Program.

THE PRESENT STATUS OF FLORIDA SPINY LOBSTER RESEARCH

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To find ways to increase production of the spiny lobster, large scale studies on its basic biology were begun in 1962. Nine cruises were made to the Yucatan Straits and northern Caribbean to study the possible Caribbean origin of Florida's spiny lobster population. Evidence is reviewed for the year-round production of larvae in the Caribbean. Spawning in Florida appears to be more extensive in spring and summer. Because of the long larval life and high natural larval mortality, rearing from eggs is not considered feasible as a farming practice.

CILIARY ACTIVITY AND OXYGEN CONSUMPTION IN PELECYPOD GILL TISSUE

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Measurements were made of ciliary activity and oxygen consumption of excised gill tissue from *Crassostrea virginica, Mercenaria mercenaria, Modiolus demissus,* and *Mytilus edulis.* The objective was to evaluate the influence of salinity, temperature, and season on these two parameters.

The cilia of gill tissue from low-salinity as compared to high-salinity acclimated animals beat faster at low experimental salinities. The four species are ranked as follows with respect to the ability of cilia to tolerate reduced salinities: *Modiolus* > *Crassostrea* > *Mytilus* > *Mercenaria.*

Of ecological interest is the relationship between gill tissue oxygen consumption and ciliary activity. At low salinities isolated gill tissue utilized as much or more oxygen than at high salinities. Yet, at these low salinities ciliary activity was greatly reduced. If this relationship holds true for gill tissue in the intact animal, it means that there is a constant or increased expen-

diture of energy under conditions where the food gathering machinery is working less efficiently.

A REVIEW OF PROGRESS IN SURF CLAM RESEARCH

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Studies of the biology and of the population dynamics of the surf clam, *Spisula solidissima* Dillwyn, are the two major divisions of the Surf Clam Program at the Bureau of Commercial Fisheries Biological Laboratory, Oxford, Maryland. Advances in our knowledge of the clam have been significant in recent years.

The annual reproductive cycle has been determined for New Jersey clams. Spawning in July is usually followed by a second but minor spawning in the Fall. Sexual maturity appears to be reached at a shell length of about 2-1/2 inches. Growth of juvenile surf clams has been followed at Chincoteague Bay, Virginia, for the past 18 months by sampling a beach population, and by planting and recovering marked clams.

In two 30-day surf clam cruises in 1965, a total of 591 stations were occupied in a 25,000-square-mile area extending from Montauk Point, Long Island, to Cape Hatteras, North Carolina. The number of clams taken, size frequencies, bottom type, associated organisms, temperatures, and salinities were recorded for each station.

Catch data were obtained during 1,400 interviews with boat captains, and 14,000 clams from 785 commercial landings were measured at Point Pleasant, Barnegat, and Cape May-Wildwood, New Jersey, in 1965. The amount of clams discarded at sea by fishermen was observed on individual trips and determined to be of minor importance.

The relation of length to meat-weight was established for each month of 1965 in samples from the fishery. Seasonal changes in clam meat condition, as measured by percentage solids, were also determined in 1965.

NSA PACIFIC COAST SECTION

METABOLISM OF ^{65}Zn IN CRUSTACEA[1]

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The metabolism of ^{65}Zn has been studied in the shore crab, *Hemigrapsus nudus*. Data for the uptake of the radionuclide from sea water, retention, and sites of concentration will be presented. Also the uptake, retention, and localization of ^{65}Zn from sea water by the benthic amphipod, *Anonyx* sp., and the pelagic euphausid, *Euphausia pacifica*, will be shown. The relationships between the ecology of these three species of Crustacea and their metabolism of this important radionuclide will be discussed.

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[1]This paper is based on work performed under United States Atomic Energy Commission Contract AT 45-1)-1830.

[2]A graduate student in the Department of Oceanography, Oregon State University, supported by the predoctoral AEC Richland Graduate Fellowship program.

GONYAULAX (CATENELLA?), ITS GROWTH, TOXIN PRODUCTION AND RELATIONSHIPS WITH BIVALVE MOLLUSKS

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Experiments with unialgal mass cultures of *Gonyaulax* isolated from Sequim Bay gave definite evidence that *Gonyaulax* is a primary source of toxin in this area and in the Strait of Juan de Fuca.

Under identical conditions of culture (with the exception of light intensity) production of paralytic shellfish toxin was found to be inversely proportional to regeneration time.

With an increase in the period of time after the

cells have reached the end of the log reproduction phase the amount of toxin present per unit number of cells decreased.

The uptake of paralytic shellfish toxin by shellfish has been demonstrated. Very low concentrations of *Gonyaulax* (about 20 cells/ml) had to be used without rejection becoming apparent.

The forms of *Gonyaulax* in culture have been described over a 96-day period.

STUDIES ON WET STORAGE OF OYSTER AND CLAM SHELLSTOCK

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Studies were initiated to determine the effect of wet storage on the sanitary quality of shellfish. A float capable of holding approximately 5 bushels of shellfish was anchored over a commercial oyster bed in Burley Lagoon. Shellfish and water samples were examined at the low, mid and high points of the tide for a 24-hour period and at the low and high tides for another 24 hours. Four experiments utilizing Pacific oysters *(Crassostrea gigas)* and Manila clams *(Venerupis japonica)* were completed during the winter and spring months of 1965-66 and one experiment was performed in July, 1966. The shellfish showed a rapid accumulation-elimination response to fluctuations in water bacterial density. In all experiments the coliform MPN's, fecal coliform MPN's and plate counts of shellfish and water samples varied inversely with the salinity. Salinity observations could not always be correlated with the stage of the tide. This may have been due to the presence of a large, strong eddy current causing intermittent influxes of fresh water into the float area. Further experiments employing constant recording salinity and temperature devices and an intensified sampling schedule will be conducted.

THE IDENTIFICATION AND METHODS OF REPRODUCTION OF THE CAUSATIVE ORGANISM OF SHELLFISH TOXICITY IN WASHINGTON STATE

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Paralytic shellfish toxicity studies in the State of Washington have been conducted for the past six years. The causative organism was found to be a chain-forming dinoflagellate. This organism is similar in appearance to *Gonyaulax catenella* Whedon and Kofoid and has the same plate formula of 4', Oa, 6", 6, 6"', 1p, 1''''. However, some morphological differences were noted. The organism studied in Washington contains extra left accessory sulcal plates and two antapical wings instead of two short antapical spines. The girdle curtain (curtain fin) is a delicate membrane attached to both ridges of the girdle but is not always present. The relative size, shape, and position of the plates are also slightly different.

The methods of reproduction of this organism are not yet fully understood. Examinations of pure cultures and field samples have thus far shown four methods of reproduction: (1) binary fission with theca, (2) division of protoplast into two daughter cells after escaping from theca, (3) cyst formation, and (4) development of autospores.

THE UPTAKE OF THE RADIOISOTOPE ^{65}Zn BY VARIOUS TISSUES OF THE FRESHWATER MUSSEL, *ANODONTA CALIFORNIENSIS* LEA[1]

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Marine mollusks are used extensively as indicators of radioisotopes in the environment. However, very little work has been done utilizing freshwater mollusks as radionuclide indicators.

Two separate experiments using the freshwater mussel, *Anodonta californiensis* Lea, were set up to determine (1) if this animal has an affinity for ^{65}Zn, (2) what effect the amount of isotope available in the environment has on the accumulation of it by the mussel and (3) which tissues of the mussel's body concentrate ^{65}Zn.

In the first experiment, utilizing ^{65}Zn concentrations of 1 μCi/l, 10 μCi/l, and 100 μCi/l, it was found that the total body burden accumulated by these mussels was approximately linear to the amount of radioisotopes available in the environment.

The second experiment ran for 36 days, using 100 μCi/l of ^{65}Zn. The tissues of the mussel's body accumulated ^{65}Zn both by μCi/g and total tissue burden in the following order: (1) gills, (2) man-

tle and palps, (3) body mass including the digestive gland, digestive tract and gonad, (4) adductor muscles and (5) foot. At the termination of the experiment, the mussels were still accumulating ^{65}Zn from the environment, as they had not come to equilibrium, and had an average total body burden (soft parts) of approximately 100 μCi.

Autoradiographs were made of four different areas of the mussels. The Leydig cells contained little or no ^{65}Zn. Moderate concentrations of the radioisotope were found in the gonads, gonadal ducts, outer mantle epithelium, intestinal and rectal epithelium, and the adductor muscles. Heavy concentrations of ^{65}Zn were observed in the kidney epithelium, the leucocytes, and the pericardial fluid.

The order of concentration of stable zinc and ^{65}Zn was the same within the various tissues of the mussel.

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[1]This paper is based on work performed under United States Atomic Energy Commission Contract AT(45-1)-1830.

TRANSPORT AND DISTRIBUTION OF RADIOACTIVE EFFLUENTS IN COASTAL AND ESTUARINE WATERS OF THE U. K.[1]

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The distribution and transport of radionuclides discharged to the Irish Sea from the chemical reprocessing plant at Windscale is discussed in terms of the hydrography and the composition of seabed material. Seaweed surveys around the U.K. coastline compare the background fallout levels with those in the Irish Sea and indicate the limit of Windscale contamination to less than 200 miles.

In the Blackwater Estuary, M.A.F.F. surveys of the distribution of ^{65}Zn are presented in relation to the contamination of the commercial oyster beds, and discussed in relation to the hydrographic conditions.

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[1]Work carried out while employed by the U.K.A.E.A. and previously presented, in part, at the I.A.E.A. Symposium on Disposal of Radioactive Wastes to the Oceans, Seas and Surface Water, 1966. Vienna (in press).

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PROBLEMS IN SHELLFISH PRODUCTION [1]

Jay D. Andrews

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Twenty years ago I attended my first oyster convention. A year later I gave a paper on setting in the James River. Now I am even more concerned about understanding the mechanisms of wild spatfall since setting has begun failing in the fabulous James River seedbeds.

I want to take as my topic *production*, which is the theme chosen for this convention, and as my guide I will use the program in your hands. Almost 90 years ago Prof. Wm. K. Brooks of Johns Hopkins University began marine studies in Chesapeake Bay on Fort Wool — the small island on the Chesapeake side of the Hampton Roads bridge-tunnel as you leave Norfolk. He noted the abundance of oyster drills around the island and you will find these predators still listed on our program in 1966. We are testing whether or not a chemical called Polystream can be used successfully in waters other than Long Island Sound.

Other familiar old subjects appear on the program. In the late forties Dr. Korringa of Holland was our guest and told us about shell disease of oysters in Europe. Since then we have discovered or acquired several diseases of our own — the most serious and famous of which is MSX. You will find this subject discussed together with an account of government programs to combat the disease. Studies of bivalve larvae were being made in the forties and are still in progress in laboratory and field. Our knowledge of larvae, especially artificial culture of them, has advanced considerably in two decades.

But I don't recall that production was a subject on our programs 20 years ago. When I came to Virginia, seed oysters sold for less than 50c a bushel and market oysters for $2 to $3. Now, from the southern states to New England, seed oysters range from $2 to $10 and market oysters from $4 or $5 to $20. Much has happened! First Mother Nature decided to demonstrate who controls the seas and sent a few hurricanes which ravaged all areas but particularly Long Island and New England where predators and failure of spatfalls were already pressing oyster farmers hard. The program this year offers an "explanation" of Mother Nature and the droughts she has recently inflicted upon us with disastrous results.

Then in the fifties as seed production failed in Delaware Bay, we were badly hurt by MSX which spread to Chesapeake and now has a large proportion of the high-salinity oyster grounds out of production in the middle Atlantic area. Production has declined precipitously.

Other subjects on our 1966 program, if not new, have become much more serious over the 20 years. The pesticides spread so liberally over our land have often ended up in our rivers. Hence the huge monitoring programs now in progress. Pollution has grown almost to the status of a national disaster, hence the public health scientists are busy with abatement programs in big new laboratories. Purification of shellfish is a growing concern and expense.

But back to production. Why can't we produce all the shellfish our people will eat? And why have our products changed from a poor man's food to almost luxury items? Any of you fortunate enough to have a reason to watch the stock market know that mergers of million dollar companies are almost daily occurrences in this country. What do they seek by merger — *control* of their products from raw materials until they are safely in the hands of consumers, and, size and diversification to protect against the unforeseen. What do we find in the shellfisheries industry — ancient methods despite modern technology, small units from towns in New England to county systems in the mid-Atlantic and over a hundred certified producers of shellfish in Virginia alone — each with a brand name but no coordination in production or marketing. Individual entrepreneurs number in the thousands if one includes tongers and small planters. Furthermore, few of these

[1] President's Annual Report, delivered June 6, 1966 at the 1966 NSA meeting, in Norfolk, Va.

small units have any control over seed production or marketing of their products. State and Federal governments have increased their efforts and subsidies to help stimulate production and in some measure, such as the use of buried shells for cultch, these have been successful. For a real success story in fisheries, read about the Maryland soft clam industry. This fishery has many of the features needed for today's market — quality, low price, high yields, efficient gear and lacks only aggressive marketing.

It would be easy to blame the 200 scientists who comprise NSA for not providing predator control, disease-resistant races, pollution abatement, and effective methods for regulating wild spatfall. These are not easy problems to solve in the open waters of our estuaries where shellfish are grown. Biological knowledge could help greatly if it could be applied without political and economic restrictions. For example, there are thousands of acres of barren public oyster grounds in Chesapeake Bay not being utilized. Most of these are in low-salinity areas where disease and predator problems are minimal. The Potomac River is a conspicuous example of an area with excellent growing grounds but no source of seed oysters.

Should we merge, enlarge and consolidate our shellfisheries industries in the interests of control and efficiency? There is no lack of interest in modernization. The several oyster hatcheries in the Long Island area and the three commercially-oriented hatcheries built or being built by England, Canada, and the United States at Milford attest to this. The attempts at pond culture from Martha's Vineyard to Florida also reflect the need and desire for control of shellfish production.

The accounts of attempts to move culture off the bottom — which has already occurred in most shellfish-producing countries — represent timely topics on our program.

We, shellfish producers and scientists, are a small group in this burgeoning country. In a society of expanding populations with rapidly increasing demands, in a country with a vigorous private economy and powerful government agencies, we had better have clear and unified purposes and objectives. Our shellfish are grown in natural resources — fresh and salt waters — which are coveted by many. Our increasing contacts with engineers and scientists who are trying to serve society's needs by storing water and manipulating waters and waterways are warning enough of things to come.

The program of this convention will only touch on the many facets of shellfish production and problems. Think earnestly on these matters during these few convention days and go home determined to seek this year a place for shellfisheries under the sun. The oyster growers and Dealers Association, the National Shellfisheries Association, and the Oyster Institute of North America should provide leadership and inspiration.

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EFFECTS OF "SOFT" DETERGENTS ON EMBRYOS AND LARVAE OF THE AMERICAN OYSTER (*CRASSOSTREA VIRGINICA*)

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ABSTRACT

Experiments were designed to determine the toxicity to oyster embryos and larvae of a standard linear alkylate sulfonate (LAS) detergent, a commercial liquid LAS detergent, and sewage effluent with and without degradation products of LAS. The percentage of fertilized eggs developing normally was reduced significantly at concentrations greater than 0.025 mg/l of the standard LAS and 0.25 mg/l of the commercial detergent. The percentage survival and growth of larvae decreased significantly at 1.00 and 0.50 mg/l, respectively, for LAS and at 2.50 mg/l for the liquid detergent. Although growth of oyster larvae was decreased by concentrations of 100 ml per liter of effluent with or without LAS, the reduction was not drastic except at concentrations of 200 ml per liter or higher. The toxicity, if any, of the degradation products of LAS was masked by the toxicity of the effluent itself.

INTRODUCTION

The quantities of synthetic detergents used in the United States and the effects of such detergents on freshwater animals were reviewed by Hidu (1965), who also reported the effects of some detergents on larvae of the marine bivalves, *Mercenaria mercenaria* and *Crassostrea virginica*. An industry-wide conversion was completed on June 30, 1965, from the manufacture of detergents of the alkyl benzene sulfonate (ABS) type, which are only very slowly degraded by bacterial action, to the new biodegradable linear alkylate sulfonate (LAS) type (Brenner, 1965). The effects of these new "soft" detergents on freshwater fish have been studied by Bardach, Fujiya and Holl (1965), Hokanson [1] (personal communication), and Thatcher and Santner [2] (personal communication). Since these detergents are almost completely degraded in an efficient sewage treatment plant, most of the detergent occurs in the effluent as degradation products rather than as the active detergent. Field tests have shown that LAS-based detergents break down rapidly when subjected to secondary (activated sludge) sewage treatment (Renn, 1965).

In the present study we determined the toxicity of a reference sample of undegraded LAS detergent, a commercial liquid biodegradable detergent, and of sewage effluents, both with and without degradation products of the detergent, to embryos and larvae of the American oyster, *Crassostrea virginica*.

METHODS AND MATERIALS

The methods of obtaining fertilized oyster eggs and oyster larvae and the standard method for rearing the larvae in the laboratory were described by Loosanoff and Davis (1963).

To determine the effect of various concentrations of pollutants on development of eggs into normal free-swimming larvae, we placed about

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13,000 recently fertilized eggs into each of a series of 1-liter beakers that contained filtered, ultraviolet-treated sea water. In all experiments, duplicate 1-liter cultures were maintained at each concentration of the detergent or effluent and two cultures were left untreated as controls. All cultures were kept in a constant temperature bath at 26.0° ± 1.0°C. After 48 hours, the free-swimming, straight-hinge larvae from each beaker were caught on a 325-mesh screen and resuspended in 250 ml of filtered sea water. A 4-ml sample of the resuspended larvae was then obtained from each beaker. The number of eggs developing normally in each culture was determined by counting the total number of straight-hinge larvae in each sample. The counts of samples from duplicate cultures were averaged and the results expressed as a percentage of the average number developing in control cultures. The values in the tables are the averages for three successive experiments except where noted.

To determine the effects of the detergents or effluents on survival and growth of larvae, we placed 10,000 to 12,000 larvae, which had been reared under normal culture conditions for 48 hours, into each of a series of 1-liter beakers containing filtered ultraviolet-treated sea water. The sea water was changed every second day to eliminate waste products of larval metabolism, and the experimental conditions were reestablished. Equal volumes of a mixture of live algal food (flagellates) were added to each culture daily, as described by Davis and Guillard (1958). A standard dose of Sulmet[3] (0.33 ppm) was added to each culture every second day, when the sea water was changed, to minimize mortality due to bacterial contamination.

To determine the percentage survival and increase in size of oyster larvae after 10 days under the experimental conditions, i.e., when the larvae were 12 days old, we sampled quantitatively in a manner similar to that at 48 hours. The number of surviving larvae in each 4-ml sample was counted and 100 larvae were measured. The average number surviving and the average length were determined for each pair of duplicate cultures. Survival is expressed as a percentage of survival in control cultures and increase in size is given as a percentage of the increase in mean length of larvae in untreated control cultures. The values in the tables are the averages for three successive experiments except where noted.

The sample of LAS was obtained from The Soap and Detergent Association, 295 Madison Avenue, New York, New York 10017, and was "a composite of a number of commercially available products, typical of the LAS presently being marketed" (letter from the Association, June 2, 1965). Analysis of the sample was 60.8 per cent LAS, 36.1 per cent sodium sulfate, 0.4 per cent free oil, and 2.7 per cent water. A sample of a commercial liquid biodegradable detergent of unknown composition was also used. A modified geometric series of test concentrations was used for both detergents: 0.0025, 0.005, 0.010, 0.025, 0.05, 0.10, 0.25, 0.50, 1.00, 2.50, 5.00, and 10.00 mg/l.

For tests of degradation products of LAS, the U.S. Testing Company, Hoboken, New Jersey 07030 (where biodegradability tests of LAS were in progress), furnished us with sewage effluents in which 81.25 per cent, 96.25 per cent, and 100 per cent of the 20 mg/l of added LAS detergent had been degraded. For the control effluent, i.e., effluent that contained no detergent degradation products, U.S. Testing Company took effluent just prior to adding detergent to the digestion process. Larval cultures were set up with effluent containing degradation products equivalent to original LAS concentrations of 0.1, 0.2, 2.0, 3.0, 4.0, and 5.0 mg/l, which meant that it was necessary to add 5, 10, 100, 150, 200, and 250 ml of effluent, respectively, per liter of larval culture. Another series of cultures was set up with equivalent volumes of effluent that contained no detergent degradation products to determine whether it was the degradation products or the effluent itself that affected the survival and growth of larvae.

EFFECTS OF LAS DETERGENTS AND THEIR DEGRADATION PRODUCTS ON OYSTER EMBRYOS AND LARVAE

Effects on Embryonic Development

The effect of active LAS detergents and sewage effluent, both with and without degraded LAS, on the development of fertilized oyster eggs into straight-hinge larvae is given in Table 1. Oyster eggs had a very low tolerance to active LAS detergent; concentrations of 0.05 and 0.10 mg/l permitted only 51 and 64 per cent, respectively, of the eggs to develop, and many of these were of abnormal shape or size, or both. No eggs developed at a concentration of 0.25 mg/l. Hidu (1965) reported that 47 per cent of oyster eggs developed normally at a concentration of 0.50 mg/l of ABS that was 54.8 per cent active, i.e., at an ABS concentration of 0.27 mg/l. It would appear that LAS is more toxic than ABS while in the active state.

[3] Registered Trademark — American Cyanamid Company's sodium sulfamethazine soluble powder.

TABLE 1. *Percentage of oyster eggs developing to straight-hinge larvae in various concentrations of LAS detergent and sewage effluent (see Methods and Materials for explanation of percentages).*

Concentration (mg/l)	LAS[1]	Liquid biodegradable[2] detergent	Effluent with degraded LAS	Effluent[3] without degraded LAS	Volume of effluent (ml/l)
0.0 (Controls)	100	100	100	100	0 (Controls)
0.0025	92	99	—	—	—
0.005	88	108	—	—	—
0.010	85	108	—	—	—
0.025	66	118	—	—	—
0.05	51[4]	115	—	—	—
0.10	64[4]	68	—	—	—
0.20	—	—	127	78	10
0.25	0	63	—	—	—
0.50	0	14	—	—	—
1.00	0	0	—	—	—
2.00	—	—	97	109	100
2.50	0	0	—	—	—
3.00	—	—	—	—	—
4.00	—	—	—	—	—
5.00	0	0	99[4]	66[4]	250
10.00	0	0	—	—	—

[1] Concentrations listed are of active LAS; gross product contained 60.8% active LAS

[2] Concentrations listed are of gross product; percentage of active LAS unknown

[3] Values listed based on single experiment

[4] Many larvae in these cultures abnormal in size or shape, or both

In experiments with the commercial liquid biodegradable detergent, oyster embryos tolerated reasonably well a concentration as great as 0.25 mg/l of the gross product, i.e., survival was equal to 63 per cent of that in the controls. The manufacturer will not divulge the percentage composition of this product, but its lower toxicity was undoubtedly the result of a lower concentration of active LAS. Hidu (1965) reported that oyster eggs developed to straight-hinge larvae at a concentration as high as 1.00 mg/l of the same brand of detergent with an ABS base, again indicating that LAS is more toxic than ABS while in the active state.

The toxicity of effluent from a sewage treatment process did not increase when it contained LAS (Table 1). Although a high percentage of eggs developed at a concentration of 5.00 mg/l, many of the resulting larvae were abnormal. The data clearly show that LAS had lost most of its toxicity to oyster eggs through degradation in treatment; any remaining toxicity of the LAS or its degradation products was masked by the toxicity of the effluent itself.

Effect on Survival and Growth of Larvae

Oyster larvae showed a somewhat higher tolerance to both standard LAS and the commercial liquid biodegradable detergent than did the embryos. Survival of larvae decreased significantly between concentrations of 0.50 mg/l and 1.00 mg/l for the LAS and between 1.00 mg/l and 2.50 mg/l for the commercial product (Table 2). The results again are similar to those obtained by Hidu (1965) with ABS detergents. Although the percentage survival of larvae in the control cultures was somewhat greater than in cultures receiving the effluent with or without degraded LAS, a degradation-product concentration equivalent to 4.00 mg/l of the undegraded LAS was required before survival was drastically reduced. (Our supply of control effluent without LAS was limited: therefore, fewer concentrations could be tested than for effluent with degraded LAS.) The

TABLE 2. *Percentage of oyster larvae surviving in various concentrations of LAS detergent and sewage effluent (see Methods and Materials for explanation of percentages).*

Concentration (mg/l)	LAS[1]	Liquid biodegradable[2] detergent	Effluent with degraded LAS	Effluent[3] without degraded LAS	Volume of effluent (ml/l)
0.0 (Controls)	100	100	100	100	0 (Controls)
0.0025	76	96	—	—	—
0.005	106	88	—	—	—
0.010	93	102	—	—	—
0.025	71	104	—	—	—
0.05	96	84	—	—	—
0.10	104	120	78	—	5
0.20	—	—	76	75	10
0.25	95	95	—	—	—
0.50	63	82	—	—	—
1.00	0	87	—	—	—
2.00	—	—	69	103	100
2.50	0	42	—	—	—
3.00	—	—	63	—	150
4.00	—	—	35	—	200
5.00	0	0	10	19	250
10.00	0	0	—	—	—

[1] Concentrations listed are of active LAS; gross product contained 60.8% active LAS

[2] Concentrations listed are of gross product; percentage of active LAS unknown

[3] Values listed based on single experiment

similarity of results for effluent with and without detergent degradation products suggests that any toxicity of the degradation products was masked by toxicity of the digested sewage effluent.

As indicated by the percentage increase in mean length (Table 3), growth of oyster larvae was normal at LAS concentrations of 0.0025 mg/l to 0.25 mg/l, but broke sharply between 0.25 mg/l and 0.50 mg/l of active LAS; all larvae died at concentrations of 1.00 mg/l or higher. The liquid detergent was somewhat less toxic but growth of larvae declined between detergent concentrations of 1.00 mg/l and 2.50 mg/l. The lesser toxicity was again probably due to a lower concentration of active LAS in the commercial detergent. These results are similar to those reported by Hidu (1965) for non-biodegradable detergents containing ABS.

Growth of larvae was approximately normal at concentrations of 100 ml/l (2.00 mg/l) or less in both the control sewage effluent and that containing degraded LAS. Slight growth occurred in the cultures receiving effluent containing degradation products at a concentration equivalent to 4.00 mg/l of undegraded LAS. Since one-fifth of the total volume of these cultures was effluent (200 ml/l) and the effluent without degradation products appeared to be equally toxic, we assume that LAS detergent in sewage, if passed through an effective sewage treatment plant, should have no more effect than an equal volume of effluent without LAS detergent degradation products.

SIGNIFICANCE OF DETERGENTS TO OYSTER FISHERIES

Although LAS detergents are known to be more readily biodegraded than ABS detergents, little has been published concerning the toxicity of LAS detergents or their degradation products. It has been generally assumed that the degradation products would be non-toxic.

Our results indicate that LAS detergents, in the active state, are at least as toxic to oyster larvae as the ABS compounds. Passage through an effective sewage treatment plant should, however, almost completely degrade LAS detergents,

TABLE 3. *Percentage increase in mean length of oyster larvae reared in various concentrations of LAS detergent and sewage effluent.*

Concentration (mg/l)	LAS[1]	Liquid biodegradable[2] detergent	Effluent with degraded LAS	Effluent[3] without degraded LAS	Volume of effluent (ml/l)
0.0 (Controls)	100	100	100	100	0 (Controls)
0.0025	96	100	—	—	—
0.005	97	101	—	—	—
0.010	98	101	—	—	—
0.025	106	100	—	—	—
0.05	94	102	—	—	—
0.10	96	103	111	—	5
0.20	—	—	105	92	10
0.25	88	101	—	—	—
0.50	31	103	—	—	—
1.00	Dead	100	—	—	—
2.00	—	—	85	71	100
2.50	Dead	44	—	—	—
3.00	—	—	55	—	150
4.00	—	—	32	—	200
5.00	Dead	Dead	—[4]	—[4]	250
10.00	Dead	Dead	—	—	—

[1] Concentrations listed are of active LAS; gross product contained 60.8% active LAS

[2] Concentrations[1] listed are of gross product; percentage of active LAS unknown

[3] Values listed based on single experiment

[4] Number surviving too small for accurate determination of mean length

thereby reducing their toxicity and rendering them safer to use than the ABS detergents. There is need, nevertheless, for data on the quantity of active LAS that may enter our streams and estuaries, comparable to data on ABS.

We found that effluent containing LAS degradation products equivalent to 20 mg/l of LAS is no more toxic to oyster eggs and larvae than an equal volume of effluent containing no degradation products. Thus, if sewage treatment plants are capable of degrading LAS compounds, it would require enough effluent to constitute 15 per cent or more of the total volume of streams and estuaries to affect seriously the development, survival, and growth of oyster larvae. In most areas a 15 per cent concentration of sewage effluent would not be expected, but in areas such as the immediate vicinity of sewage discharge where sewage effluent approaches or exceeds 15 per cent of the total volume of the body of water it enters, it could be expected to affect recruitment of oysters.

CONCLUSIONS

1. The percentage of fertilized oyster eggs developing normally was reduced significantly at concentrations greater than 0.025 and 0.25 mg/l of active LAS and a commercial liquid biodegradable detergent, respectively.

2. The percentage survival of larvae decreased significantly at 1.00 mg/l for LAS and at 2.50 mg/l of the liquid detergent.

3. Growth of oyster larvae was significantly reduced at 0.50 mg/l for LAS and at 2.50 mg/l of the liquid detergent.

4. Although growth of oyster larvae was decreased by concentrations of 100 ml per liter of effluent with or without LAS, the reduction was not drastic except at concentrations of 200 ml per liter or higher.

5. The toxicity, if any, of the degradation products of LAS was masked by the toxicity of the effluent itself.

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EFFECTS OF POLYSTREAM[1] AND DRILLEX[2] ON OYSTER SETTING IN CHESAPEAKE BAY AND CHINCOTEAGUE BAY

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ABSTRACT

The possibility of increasing the yield of oyster spat, Crassostrea virginica, *in Chesapeake Bay and Chincoteague Bay by treating shells with Polystream or Drillex was tested during 1963. Field studies were also conducted in Watts Bay, Virginia, to determine if the use of Drillex-treated sand in combination with Polystream-treated shells increased oyster setting.*

Significantly more oysters were caught on both Polystream-treated and Drillex-treated shells than on untreated shells when they were suspended either in the intertidal zone or placed on the bottom of Chincoteague Bay. Polystream-treated shells placed just off the bottom in the Tred Avon River, Chesapeake Bay, Maryland, also caught significantly more oysters than untreated shells. In Tangier Sound and Broad Creek in Chesapeake Bay, the difference in the amount of setting between chemically-treated and untreated shells was not significant.

Although in many cases more oysters were caught on chemically-treated shells, Polystream and Drillex apparently did not repel the principal fouling organism in Chincoteague and Chesapeake Bays.

In Watts Bay, setting was greater on plots with Polystream-treated shells and Drillex-treated sand than on either control plots (untreated shells only) or on plots with just Polystream-treated shells.

INTRODUCTION

In cooperation with the Bureau of Commercial Fisheries Biological Laboratory, Milford, Connecticut, the Biological Laboratory at Oxford, Maryland, initiated in 1963 the following studies: (1) measurement of the setting rates of oysters on cultch treated with Polystream or Drillex, and (2) evaluation of the use of Drillex-treated sand in combination with Polystream-treated shells as a method of increasing the yield of oyster spat.

Earlier experiments by the Milford Laboratory in Long Island Sound, Connecticut (Loosanoff, 1961a), indicated that shells dipped in Polystream (a mixture of chlorinated benzenes, of which 45 per cent is 1, 2, 3, 4-tetrachlorobenzene) had almost three times as many living spat as untreated shells, that spat were 20 to 25 per cent larger on treated shells than on undipped shells, that treated shells were much less fouled than untreated ones and that the number of drilled oysters on treated shells was only about one-eighth those on untreated shells. Loosanoff (1961b) also found that chemically-treated sand gave the oysters a considerable amount of protection from drill damage.

The purpose of this paper is to describe the re-

[1] A polychlorobenzene product produced by Hooker Chemical Corp., Niagara Falls, New York. Polystream is a registered trademark. Mention of commercial products does not imply endorsement by the Bureau of Commercial Fisheries.

[2] An experimental compound containing 98 per cent Polystream and 2 per cent Sevin (a product of Union Carbide). Drillex is no longer manufactured.

[3] Present address: Bureau of Commercial Fisheries Biological Laboratory, Galveston, Texas.

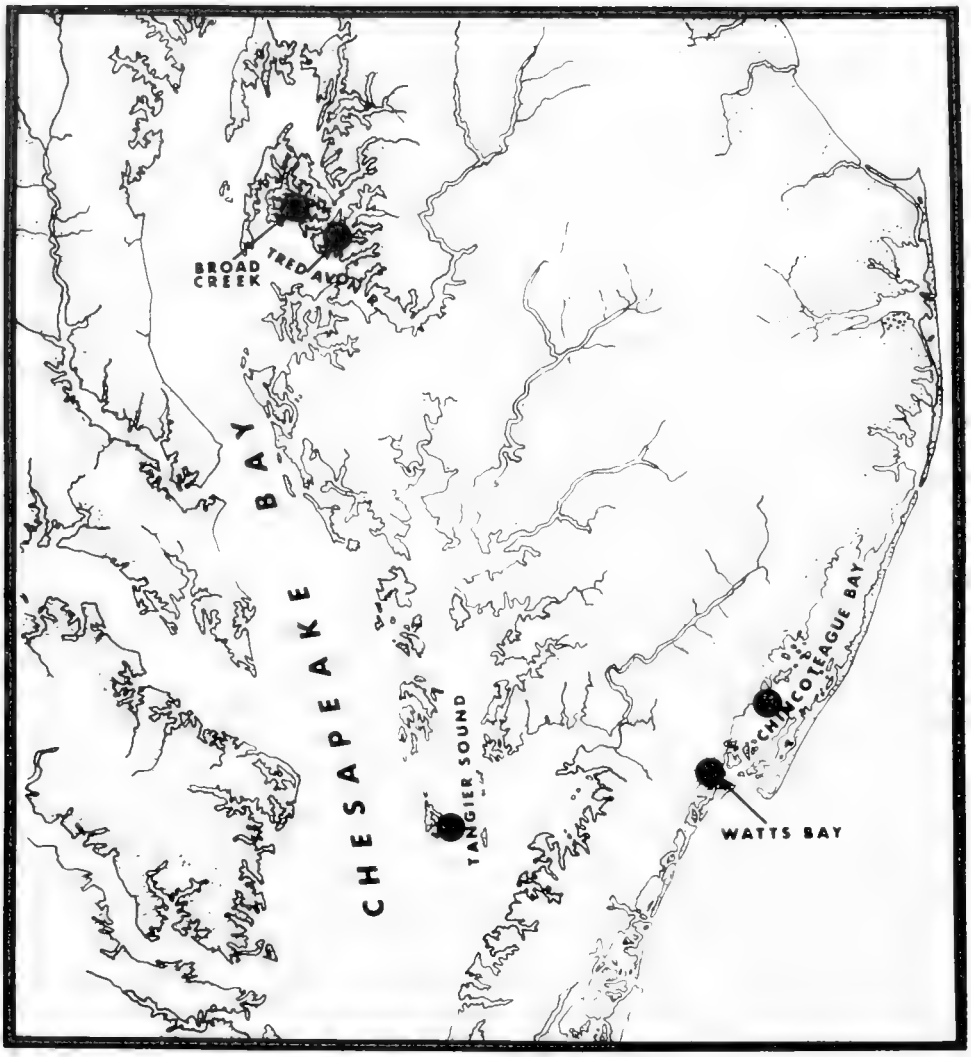

FIG. 1. *Location of stations, designated by solid black circles, in Chesapeake Bay, Chincoteague Bay, and Watts Bay where spat-collection studies were conducted.*

sults of a series of tests of shells, treated with Polystream and Drillex, to catch seed oysters and the results of attempts to increase oyster setting by using Polystream-treated shells in combination with Drillex-treated sand.

POLYSTREAM-AND DRILLEX-TREATED SHELLS AS CULTCH

Method

Following the procedure recommended by the Milford Laboratory (C. L. MacKenzie, Jr., Personal Communication), chicken-wire bags, each containing 24 clean, dry, oyster shells, were dipped into either Drillex or Polystream for 30 seconds and then allowed to drain for at least 2 to 3 hours. Chemically-treated and untreated shell bags were placed in four localities — three in Chesapeake Bay (Tangier Sound, Virginia, Tred Avon River and Broad Creek, Maryland) and one in Chincoteague Bay at Franklin City, Virginia (Fig. 1). The bags were left at all stations throughout the period of oyster setting.

Two ropes, each containing eight shell bags, 50 shells in each bag, were placed along the bottom (leased by a Virginia oyster grower) on the eastern side of Tangier Island. Each rope assembly had four bags of undipped control shells

TABLE 1. *Average number of spat on treated and untreated shells.*

Locality and treatment	Live spat per shell	Total spat per shell
Franklin City		
Intertidal — suspended		
Polystream	3.63	3.95
Drillex	2.48	2.75
Control	1.55	1.73
On bottom		
Polystream	0.20	0.57
Drillex	0.32	1.03
Control	0.04	0.25
Tangier Sound		
On bottom		
Polystream	0.63	1.91
Drillex	0.80	2.20
Control	0.48	1.66
Tred Avon River		
Off bottom		
Polystream	0.36	0.38
Drillex	0.20	0.21
Control	0.06	0.07
Broad Creek		
Off bottom		
Polystream	2.35	2.44
Drillex	2.47	2.59
Control	3.01	3.09

and four bags of chemically-treated shells — two dipped in Polystream and two in Drillex. These bags were in the water from 21 June to 25 September, 1963.

Six shell bags, two control and two dipped in each chemical, were suspended just off the bottom at four stations in Broad Creek and at five stations in the Tred Avon River. They were suspended on 13 and 14 June and retrieved on 30 and 31 October, 1963.

At Franklin City, 10 bags of shells treated with Drillex, 10 treated with Polystream, and 10 untreated bags were suspended in the intertidal zone, and a similar group were placed on the bottom. The bags at this station were in the water from 11 June to 14 October, 1963.

All shells at each locality were examined for the number of oysters, including live, drilled, boxes (dead oysters with both shell values still attached), and scars (only lower shell value present). In addition, all fouling organisms were counted either in per cent of shell covered, as with colonial calcarious bryozoans, or in actual number, as with barnacles. The shell heights (greatest dorsoventral distance) of all live spat were measured and their means compared statistically.

Setting on Shells

Oyster setting on the control and chemically-treated shells at each locality is shown in Table 1. In the intertidal zone of Chincoteague Bay, counts were 3.63 and 2.48 live spat per shell on those treated with Polystream and Drillex, respectively, as compared with 1.55 live spat per shell on undipped shells. The number of live spat on the Polystream-treated shells was significantly greater than on the control shells at the 5 per cent level; setting was also significantly greater on the Drillex-treated shells than on the control shells at the 5 per cent level. The difference between the setting on the Drillex-treated and Polystream-treated shells was not significant at the 5 per cent level.

Shells on the bottom in Chincoteague Bay caught considerably less spat than those in the intertidal zone. Shells treated with Drillex and Polystream had 0.32 and 0.20 live spat per shell

TABLE 2. *Average shell height of spat on treated and untreated shells.*

Locality and treatment	Mean height (mm)	Standard error of mean	Number
Franklin City			
Intertidal — suspended			
Polystream	35.5	0.33	879
Drillex	36.3	0.41	503
Control	34.9	0.53	375
On bottom			
Polystream	30.9	1.43	48
Drillex	26.3	1.00	75
Control	24.1	4.90	10
Tangier Sound			
On bottom			
Polystream	6.3	0.28	126
Drillex	6.6	0.32	156
Control	6.0	0.22	190
Tred Avon River			
Off bottom			
Polystream	33.5	1.15	47
Drillex	32.9	1.58	78
Control	33.0	2.88	14
Broad Creek			
Off bottom			
Polystream	30.9	0.50	447
Drillex	31.7	0.46	479
Control	32.5	0.39	573

whereas the control had 0.04 live spat per shell. The difference between the setting on chemically-treated and control shells was significant at the 5 per cent level. The difference between the two chemically-treated groups was also significant at the 5 per cent level.

Oyster setting in the Tred Avon River was light. Significantly more spat were caught on the Polystream-treated than on the control shells (at the 5 per cent level). The setting on the Drillex-treated shells and control shells did not differ significantly.

Untreated shells in Broad Creek had 3.01 live spat per shell compared with 2.35 and 2.47 per shell on the Polystream-and Drillex-treated shells, respectively. Differences among these were not significant at the 5 per cent level.

Setting was very light in Tangier Sound; numbers of live spat were only 0.63 and 0.80 per shell on shells treated with Polystream and Drillex respectively, and 0.48 on the untreated shells. As in Broad Creek, differences among the groups were not significant.

Degree of Fouling

At Franklin City, both the chemically-treated and untreated shells in the intertidal and bottom zones were heavily covered with fouling organisms. No differences were apparent between the two groups. Principal fouling forms were barnacles, calcareous tube worms and fleshy and calcareous bryozoans. MacKenzie, Loosanoff, and Gnewich (1961) found that Polystream was not effective against Bryozoa which are, unfortunately, among the principal fouling forms in Chincoteague Bay.

It is possible, since more spat were caught on chemically-treated shells, that both Polystream and Drillex inhibited or slowed down the growth of fouling organisms such as bryozoans when the shells were first placed in the water. This retardation would provide more clean surface for oysters to set. As the effects of the chemical become less, fouling would increase, and eventually reach a degree similar to that on the control shells. This hypothesis could be tested by examining chemically-treated and untreated shells weekly following their placement in the water.

TABLE 3. *Number of live oysters, scars, boxes, drilled boxes, and percentage of boxes drilled among shells placed on the bottom in Chincoteague Bay (see text for definition of scars and boxes).*

Shell treatment	Live spat	Total scars	Total boxes	Total set	Boxes drilled	
					number	percentage
Polystream	76	61	110	247	88	80
Drillex	48	41	48	137	39	81
Control (untreated)	10	36	15	61	13	87

It was difficult to evaluate the degree of prevention of fouling by the chemicals at Tangier Sound because of the accumulation of mud on the shells. Nevertheless, it appeared that the single-cell protozoan, *Folliculina* sp., was inhibited by Polystream and Drillex. Similar results for this species were found by MacKenzie, Loosanoff, and Gnewich (1961) in Long Island Sound.

In the Tred Avon River, difference in the degree of fouling between the chemically-treated and untreated shells was small, except for the number of barnacles. Setting of this species was 2½ to 3 times greater on the treated than on the control shells. In general, calcareous bryozoans were the principal fouling organism.

Chemically-treated shells placed in Broad Creek also caught 2½ to 3 times more barnacles than the control shells. As much as 50 per cent of the chemically-treated shells were covered with barnacles, which eliminated space on which oysters could attach.

Polystream and Drillex apparently do not repel the principal fouling organisms in Chincoteague Bay or Chesapeake Bay, i.e. barnacles, bryozoans, calcareous tube worms.

Growth of Spat

The shell height of all spat caught on both the chemically-treated and untreated shells was measured to the nearest millimeter. Mean heights at each locality of the oysters on treated or untreated shells were not significantly different (Table 2). The use of Polystream or Drillex neither improved nor hindered the shell growth of oysters.

Effectiveness of Chemicals in Repelling Oyster Drills

Among the shells placed on the bottom at Chincoteague Bay, 80 per cent of the boxes on Drillex-treated shells were drilled, whereas 81 and 87 per cent, respectively, of the total boxes on the Polystream-treated and control shells had been drilled (Table 3). It was not possible to determine if the scars had died from drill predation since the top shells were missing.

Several chemically-treated bags of shells in the intertidal zone fell to the bottom. One of them treated with Polystream had 17 drilled oysters and 48 live ones; a bag with Drillex-treated shells had 10 drilled oysters and 11 live spat. At Tangier Sound, as many oysters were killed by drills on the chemically-treated as on the control shells. It is apparent that the treatment of shells with Polystream or Drillex did not protect the spat from drill predation.

THE USE OF DRILLEX-TREATED SAND AND POLYSTREAM-TREATED SHELLS

Method

On 20 and 21 June 1963, three 1-acre plots, each measuring about 150 by 290 feet, were established on an intertidal seed oyster ground at Watts Bar in Watts Bay near Atlantic, Virginia. The distance between Plot I and II was 225 feet, whereas 750 feet separated Plot II from Plot III. Sixteen existing mounds of oyster shells, called rocks, were used on each plot. All rocks were covered with about 15 bushels of clean oyster shells, prior to the following treatment:

On Plot I, eight rocks received 15 bushels of shells dipped in Polystream and the other eight rocks were covered with an equal amount of untreated shells. Random ½-square meter samples of the plot indicated an oyster drill population of 5.5 snails per square meter.

On Plot II, eight rocks received Polystream-treated cultch and the others were covered with undipped shells. On 28 June, 1963, after the shells were planted, 5 cubic yards of washed, kiln-dried sand previously mixed with 55 gallons of Drillex were spread over the plot. A drill population of 6.6 snails per square meter was found on this plot.

On Plot III (the control), each of 16 rocks received an additioned 15 bushels of untreated shells.

Treated shells were dipped in Polystream 30 seconds prior to being distributed on the rocks.

The washed, kiln-dried sand and 55 gallons of Drillex were mixed in a concrete-mixing truck. The sand was then loaded on a barge, carried to Plot II, and distributed evenly over the whole area. Observations were made periodically to determine the effects of the sand on the animals in the area. Beginning in November, ½-to 1-bushel samples of shells were collected from each rock to determine the intensity of setting. Poor weather conditions and unpredictable tides made it necessary to continue sampling throughout the winter so that all rocks could be examined. A spring spat count was made to evaluate winter kill, and a final count was made in early July to determine spat survival before the new 1964 wave of oyster setting.

Effects of chemicals on invertebrates and planted shells

Many invertebrates were found to be dying immediately after the application of the Drillex-treated sand. Animals that showed distress incuded two species of shrimp, *Crangon* sp. and *Palaemonetes* sp., mud crabs (Xanthidae) and polychaetes, *Scoloplos* sp. Fresh oyster boxes appeared several days after the sand had been applied, none of which were drilled. These mortalities appeared to stop 2 weeks after application; only an occasional box was found thereafter.

Shells treated with Polystream collected a layer of sticky silt. Though the silt on untreated shells could be rinsed away, it was necessary to rub it off the treated shells.

Setting

Routine examination of the shells within the experimental area from July through October indicated a poor set. The set was light also in other areas of Chincoteague Bay.

Spat counts on each plot are shown in Table 4. Highest counts of live spat per bushel were on Plot II where Drillex-treated sand was used. Setting was greatest on those rocks that received both Drillex-treated sand and Polystream-shells. Lowest counts were on the control Plot III.

Resampling of Plot II in July 1964 showed a higher number of oyster spat per bushel on the treated shells than when the plot was originally sampled. in December-January. Possibly some small spat were missed in the earlier examinations. Fluctuations in the number of spat on the untreated shells were considerable in Plot I. For example, counts in July 1964 from each rock on this plot ranged from 0 to 133 spat per bushel of shells. No counts on Plot I or Plot III, however, approached those on the rocks in Plot II where treated sand and shells were combined.

Plots I and II were resampled in July 1964 to study for drills and drill predation. Plot II had one drilled spat and no drills, Plot I yielded 20 drilled oysters and 9 drills.

DISCUSSION

The success of oyster setting always depends primarily on the availability of clean surfaces (Galtsoff, 1964). The fouling of shells prior to oyster setting is a serious problem in oyster management. To reduce fouling, states attempt to time their plantings of shells as close as possible to the initiation of the setting season. This procedure is sometimes impossible when large quantities of shell must be distributed over a wide area. Shells planted too early become fouled and are inefficient as spat collectors.

One answer to the problem of early planting of cultch is treatment of shells with an anti-fouling chemical. Thus the shells, though in the water several months before oyster setting begins, are still clean. Some success in reducing fouling has been made in Long Island Sound by the use of Polystream (MacKenzie, Loosanoff, and Gnewich, 1961).

The biota differs in each geographic location; though chemically-treated shell may inhibit the

TABLE 4. *Average counts of spat per bushel of shells on experimental plots.*

Plot	Months of Sampling	Live spat per bushel on	
		Untreated shells	Treated shells
I	Nov. 1963	51.4	164.8
II	Dec.-Jan., 63-64	260.9	299.6
III	Mar. 64	13.1	—
I	Mar. 64	29.3[1]	—
I	July 64	92.7	74.7
II	July 64	255.3	586.0

[1] Sampled for winter kill,

setting of fouling organisms in one area, the same results may not be obtained at another location with a completely different group of animals. The results of our study offer an example of the importance of these local differences for in Chesapeake and Chincoteague Bays, the principal fouling organisms apparently were not repelled by Polystream or Drillex.

In Long Island Sound the setting of the barnacle, *Balanus balanoides,* was reduced considerably when shells were treated with Polystream; treated shells had only one-seventh as many barnacles as untreated controls (MacKenzie, Loosanoff, and Gnewich, 1961). In Broad Creek, Chesapeake Bay, the setting of a different species of barnacle, *Balanus improvisus,* was 2½ to 3 times heavier on chemically-treated shells than on untreated shells. Preliminary tests obviously should be made at each new location before commercial application is attempted.

Over seven times more spat were caught in this study on plots with Polystream-treated shells and Drillex-treated sand than on plots that had only Polystream-treated shells. Setting in Chincoteague Bay during the test year (1963) unfortunately was light. But, because of significant differences in setting between the chemically-treated and untreated plots, we believe further investigation on commercial use of chemical treatments should be made. Since Drillex is no longer manufactured, Polystream-treated sand could be substituted.

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Postscript

While this manuscript was in press, a paper entitled: "Effects of the Treatment of an Oyster Bed with Polystream and Sevin" by Dexter Haven, Michael Castagna, Paul Chanley, Marvin Wass, and James Whitcomb appeared in the December, 1966 issue of *Chesapeake Science,* **7**(4):179-188. The authors reported that Polystream-Sevin did not control drills on treated plots at Hog Island Bay, Virginia, and oyster production was not increased by treatment. As in our study, the addition of the chemically-treated sand initially killed the invertebrates in the area.

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PRELIMINARY STUDY ON THE USE OF BERGMAN-JEFFERTS CODED TAGS ON CRABS [1]

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ABSTRACT

Bergman-Jefferts coded wire tags were employed for the first time in three species of crabs, Hemigrapsus nudis, H. oregonensis, *and* Cancer productus. *The tags were of type 302 magnetized stainless steel and measured approximately .04 inch in length by .01 inch in diameter. These were inserted into the body musculature through the ligament between the coxopodite and associated ventral plate of the right cheliped of the two species of* Hemigrapsus *and through the abdomen of* C. productus. *Being embedded in the musculature, these tags should not be lost when the crab moults. These tags hold great promise as a new method for marking and identifying crustaceans*

INTRODUCTION

This was a preliminary study initiated to explore the feasibility of employing the Bergman-Jefferts coded wire tags on three species of brachyuran crabs, *Hemigrapsus nudis, H. oregonensis,* and *Cancer productus.* The use of ferromagnetic wire in tagging is not an entirely new venture. Jefferts, Bergman and Fiscus (1963) and Bergman *et al.*[2], the originators of the tags, have successfully used it on Chinook salmon *(Oncorhynchus tshawytscha)*, in the laboratory and in the field. Dr. Hubert Squires of the Fisheries Research Board of Canada Biological Station at St. John's, Newfoundland, is currently using it on lobsters (Woodland, 1966). Also, the new system discussed here is similar in certain respects to other methods previously utilized. For instance, Wilimovsky (1963) employed a wire-form tag; LeCren (1954) and Butler (1957) used implanted tags; and metal detectors, although based on different principles, were employed by several investigators (Dahlgren, 1936; Tester, 1945; Moore and Mortimer, 1954; and others).

MATERIALS AND METHODS

As mentioned above, this tag was developed by researchers of the Washington State Department of Fisheries (Jefferts *et al.,* 1963) and is made of type 302 stainless steel, measuring approximately .04 inch in length by .01 inch in diameter. To aid in detection, these tags are magnetized. Information is encoded by the application of epoxy paint as colored stripes. Present equipment applies six longitudinal stripes to a .01 inch diameter wire and the colors are the same colors used in coding in the electronic industry. If two stripes and two colors are reserved to denote the starting point and direction of reading, this system will yield at least 8^4 different combinations (Bergman *et al.*[2]) Several devices for large-scale tagging have been developed. All employ spools of color stripe wire which is cut into tag-length sections immediately prior to injection. For this study, a manual injection device which utilized pre-cut tags was employed as shown in Figure 1.

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[1] Contribution No. 249 from the College of Fisheries, University of Washington.

[2] Bergman, P. K., K. B. Jefferts, H. F. Fiscus and R. C. Hager, 1966. A preliminary evaluation of an implanted, coded wire fish tag. Washington State Department of Fisheries, Olympia, Washington. (unpublished manuscript).

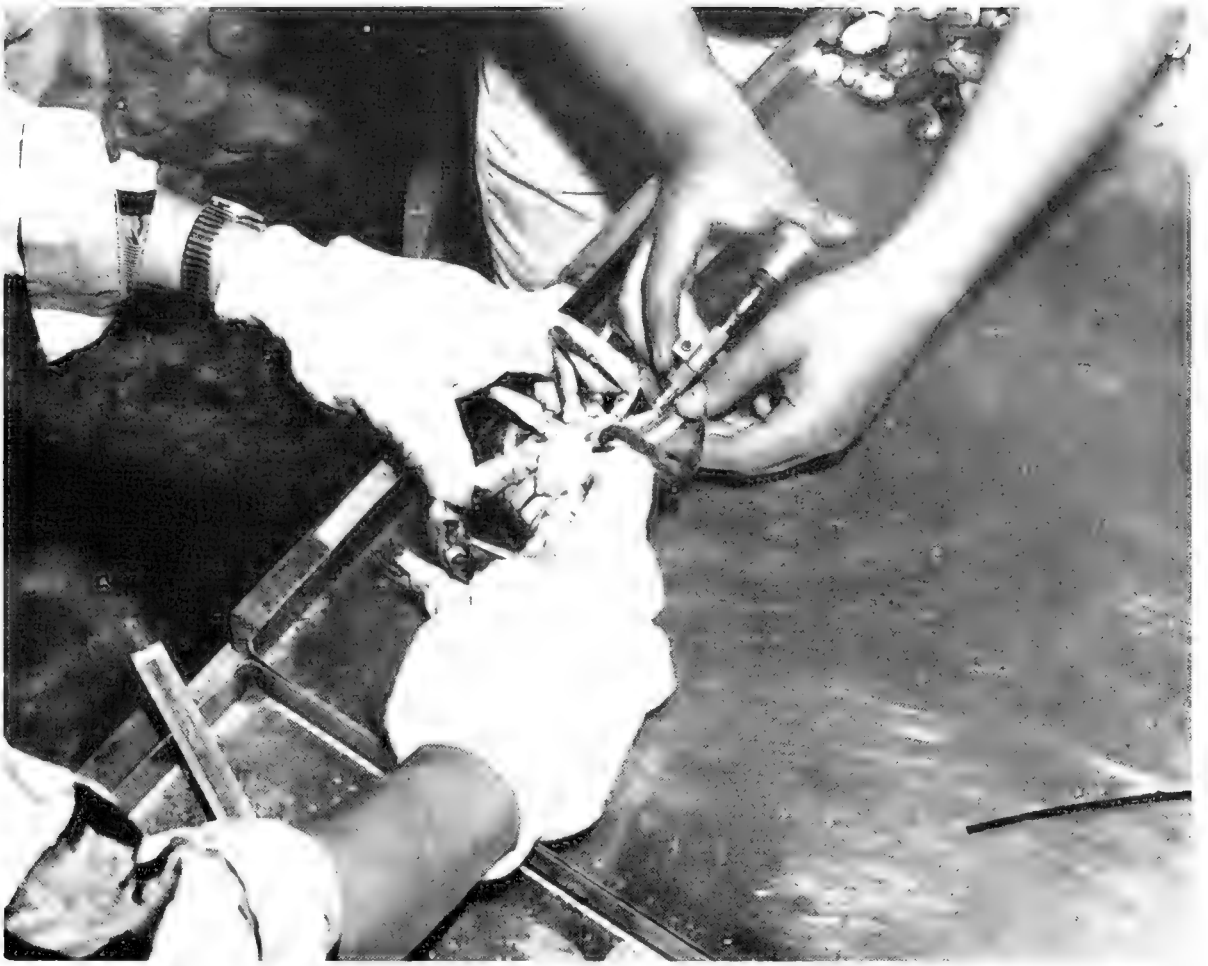

FIG. 1 *Manual injection device for the application of Bergman-Jefferts tag into the abdominal musculature immediately to one side of the intestine of* Cancer productus.

The principle of the tag injection devices is basically identical, whether the tag is injected manually or automatically. Individual tags are carried inside a 23-gauge hypodermic needle. Immediately behind the tag is a pushrod. The tag is implanted into the specimen by inserting the needle and then actuating the pushrod and thereby implanting the tag. Detection of implanted tags is accomplished through the use of a coil detector in the form of a large coil attached to a self-contained instrument package. This device will detect a moving magnetized tag at approximately three inches distance. The motion of the magnetized tag through the magnetic field of the coil is registered by the instrument package as an audible beep.

All crabs were collected from the waters of Puget Sound; both species of *Hemigrapsus* from Meadow Point and *Cancer* from Edmonds, Washington. The tagging was carried out in the University of Washington, College of Fisheries, marine aquarium. All crabs were given at least five days to adjust to the aquarium environment before tagging.

RESULTS AND DISCUSSION

The first application of coded wire tags was with 38 randomly selected *Hemigrapsus nudis* and *H. oregonensis* on 25 April 1966. In another aqarium an equal number of specimens, not tagged, were used as controls for mortalities. The tags were injected into the body musculature through the ligament between the coxopodite and associated

ventral plate of the right cheliped. Records were then kept on the tagged and untagged crabs for 37 days. Over this period of time there was no significant difference in mortality between the tagged and control crabs, nor did there appear over that time any impairment of function of the right cheliped or loss of the appendage.

Beginning on 8 July 1966 five male and five female *Cancer productus* were tagged by injecting the tag into the abdominal musculature immediately to one side of the intestine (Fig. 1). An equal number of untagged individuals were placed into another aquarium as controls. One-hundred-twenty days have elapsed with no tagged specimens moulting. These crabs are still being observed with the hope that some of the tagged specimens will later undergo ecdysis. As indicated by similar tagging studies conducted on lobsters (Woodland, 1966), the tags, being imbedded in the musculature, should not be lost when the shell is shed. As with both species of *Hemigrapsus*, *C. productus* exhibits no initial mortalities or other abnormalities that can be directly attributed to the implantation of coded wire tags in the musculature.

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SEASONAL OCCURRENCE OF LARVAL LOBSTERS IN COASTAL WATERS OF CENTRAL MAINE

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ABSTRACT

Seasonal occurrence of larval lobsters in coastal waters of central Maine was investigated in 4 years (1963-66). Larvae were most numerous in July and August.

The published record of larval lobsters, *Homarus americanus* Milne-Edwards, in coastal waters of Maine is limited to the report by Mendall (1934) of commercially important fish and shellfish in the stomachs of sea birds. The Bureau of Commercial Fisheries, in 1965, began a study of lobster populations in coastal and shelf waters off the northeastern United States. Our observations on occurrence of larvae were made as part of this investigation. Some information was available for earlier years from plankton collections made as part of other research.

Taylor (1950), using 10 years of hatchery records (1939-48), assigned June 1 to August 1 as the hatching period of lobsters held in tanks at Boothbay Harbor, Maine. Hatching in holding tanks begins earlier in southern New England and extends from late April to July (Herrick, 1911; Hughes and Matthiessen, 1962). These observations suggested that larvae could be present in coastal waters of Maine from spring through fall.

We examined zooplankton collected on bi-weekly cruises in the Boothbay Harbor area from May through October 1963, 1964, and 1965, for the presence of larval lobsters. The samples were originally designed to obtain larval herring; larval lobsters were captured incidentally. Samples were collected with a 1-meter net (0.36 mm apertures) towed just beneath the surface for 10 minutes at 2 knots. Of the 409 samples examined, 20 contained larval lobsters, but accounted for only 31 specimens. Although few larvae were collected, the occurrence within the season was similar in each of the three years. Larvae were taken most frequently during summer in July and August. No larvae were present in the May or October collections (Table 1).

The catch of larvae was increased in 1965 and 1966 by experimental towing at high speeds (4 to 6 knots). Three nets were used: a conical meter net and 2 rectangular trawls, one measuring 2 m x 1 m, and the other 1.5 m x 0.5 m at the mouth. Each was fitted with relatively wide-mesh netting (1-mm aperture). Surface tows were made with nets breaking the surface film. The rectangular trawls were towed with the widest mouth dimension in a horizontal plane. Duration of the tows varied from 10 to 30 minutes. A total of 257 surface tows were made at approximately bi-weekly intervals; 245 were from Casco Bay and the Boothbay area (Fig. 1), and 12 along the coast from Cape Ann, Massachusetts, to Machias Bay, Maine.

From June to September, 368 larvae were collected. Catches were highest in July and August (Table 1). This timing agrees with our findings from bi-weekly meter-net sampling. Evidence to show larval lobsters more abundant at the surface than a few feet below the surface has been presented by Templeman (1937), and Templeman and Tibbo (1945). Comparisons were made between catches with trawls at high speed (4 to 6 knots) at the surface and on oblique hauls through the water column (0-20 m) at 53 stations in 1966. Catches of larvae in the oblique tows were approximately 2.4 times less (17 larvae) than companion catches at the surface (40 larvae). It is likely that our increase in catch at high towing speeds in 1965 and 1966 was in part the result of towing at the air-sea interface.

The combined 1963-66 data agree with the July-August maximum of occurrence of larval lobsters in the Gulf of St. Lawrence (Wilder, 1953;

TABLE 1 . *Catches of lobster larvae from surface tows in central Maine waters, 1963-66.*

Year	Type of tow and number of larvae	Months May	June	July	August	September	October	Total
1963	Meter net[1]	12	35	24	14	26	8	—
	Number of larvae	0	1	3	5	0	0	—
1964	Meter net	20	18	20	20	24	37	—
	Number of larvae	0	2	4	4	3	0	—
1965	Meter net	43	14	27	33	10	24	—
	Number of larvae	0	0	5	4	0	0	—
1963-65	Number of tows	75	67	71	67	60	69	409
	Number of larvae	0	3	12	13	3	0	31
	Catch-per-tow	0	0.04	0.17	0.19	0.05	0	—
1965	High-speed[2]		10	97	24*	13	—	—
	Number of larvae		0	235	64	12	—	—
1966	High-speed	5	24	58	20	6	—	—
	Number of larvae	0	0	45	12	0	—	—
1965 66	Number of tows	5	34	155	44	19	—	257
	Number of larvae	0	0	280	76	12	—	368
	Catch-per-tow	0	0	1.81	1.73	0.63	—	—
1963-66	Total tows	80	101	226	111	79	69	666
	Total larvae	0	3	292	89	15	0	399

*12 of these samples were collected along the coast from Cape Ann, Massachusetts, to Machias Bay, Maine.

[1] Meter net tows were made with the nets just below the surface film.

[2] High speed tows were made with the nets breaking the surface film.

Corrivault and Tremblay, 1948), and with the finding of concentrations of larvae in coastal waters of Newfoundland in these same months (Templeman and Tibbo, 1945). Larvae also occur in offshore Gulf of Maine and continental shelf waters off New England in July and August. On a summer offshore cruise of the Bureau's research vessel *Albatross IV* (July 27 to August 4, 1966), 142 larvae were collected.

The number of days for the development of larvae from hatching to the end of the planktonic period at stage 4 varies with the temperature (Templeman, 1936; Hughes and Matthiesson, 1962). Average monthly surface temperatures in Boothbay Harbor ranged from 13.7°C to 15.0°C in July and August 1965 and 1966. At a temperature of 14.0°C the developmental period from hatching to stage 4 is 26 days; at 15.0°C, 22 days are required (Templeman, 1936). Larvae collected in the bi-weekly meter-net series from June through the first-half of August were all in developmental stage 1. Three stage 4 larvae were in later collections (August 26-September 2). The high-speed collections of 1965-66 contained stage 1 larvae most frequently in July, and stage 4 larvae in August and September (Table 2); apparently maximal hatching in coastal waters of central Maine was in July and early August.

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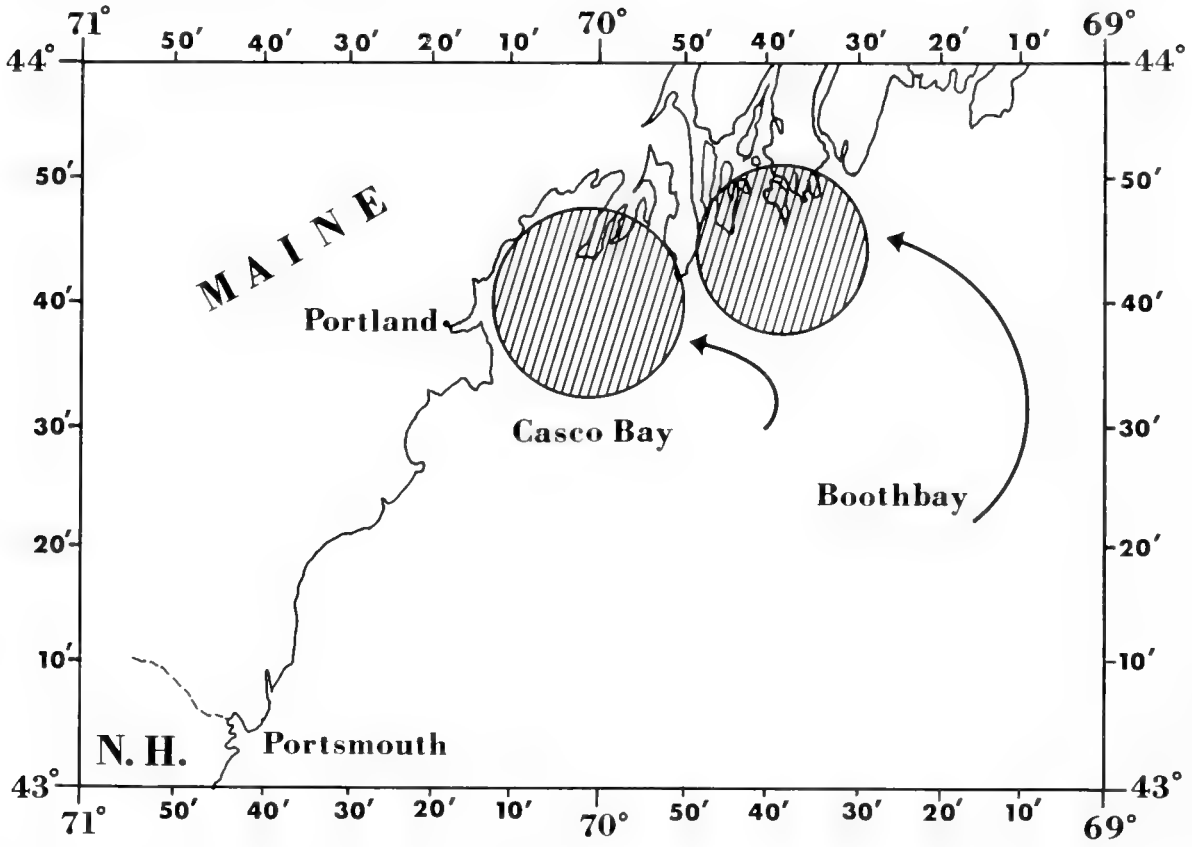

FIG. 1. *Coastal sampling areas for larval lobsters.*

TABLE 2. *Frequency of occurrence of developmental stages of larvae collected in high-speed tows, 1965 and 1966.*

Dates 1965-1966	Number of tows	Developmental stages* 1	2	3	4
July 1-14	73				
Number of larvae		77			
Catch-per-tow		1.05			
July 15-29	82				
Number of larvae		198	2		3
Catch-per-tow		2.41	0.02		0.04
August 2-16	32				
Number of larvae		15	5	1	7
Catch-per-tow		0.46	0.16	0.03	0.22
August 14-20	12				
Number of larvae		6			42
Catch-per-tow		0.50			3.50
September 1-3	19				
Number of larvae					12
Catch-per-tow					0.63

*As described by Herrick, 1911.

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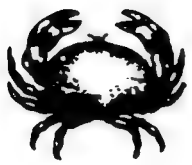

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USE OF ARTIFICIAL FOODS FOR LARVAE OF THE HARD CLAM, *MERCENARIA MERCENARIA* (L.)

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ABSTRACT

Artificial foods, consisting of nutrient solutions and suspensions of finely-ground dried, fresh and frozen materials in sea water were presented to hard clam larvae. Experimental foods were evaluated by comparing larval growth and survival to that in unfed cultures and in cultures receiving live unicellular algae.

Nutrient solutions were unsatisfactory foods. Some dried foods were satisfactory though larvae receiving them tended to metamorphose at larger sizes than larvae receiving live unicellular algae. Finely-ground fresh or frozen preparations, especially sea lettuce, Ulva lactuca, *were the most satisfactory foods. Clams were reared through pelagic stages to a maximum length of 450 μ on this diet without excessive mortality, but a major disadvantage of all particulate artificial foods was the clumping of food particles and a resulting accumulation of detritus in older cultures.*

INTRODUCTION

Laboratory-cultured algae are the most commonly used foods in experimental culture of lamellibranch larvae (Walne, 1964). However there are many disadvantages associated with the use of cultured foods. Adequate supplies of algae can be maintained only with large investments in time, man-power, space and equipment. In addition, most desirable algal species are difficult to culture.

Sporadic attempts to find an acceptable, convenient food have been made in the past with varying degrees of success. Davis (1950) was unsuccessful in attempts to rear oyster larvae *(Crassostrea virginica)* by feeding yeast, glucose and detritus. Loosanoff, Miller, and Smith (1951) reported that hard clam larvae *(Mercenaria mercenaria)* grew poorly and suffered a heavy mortality when fed detritus. Carriker (1961) raised hard clam larvae to metamorphosis by feeding extract of Pablum, but had "considerable" mortality. Hidu and Ukeles (1964) reared clam larvae to metamorphosis by feeding dried and freeze-dried unicellular algae and reported no unusual mortality. Ritchie (unpublished report at National Shellfisheries Association Convention, 1962) had similar success with dried sea lettuce *(Ulva lactuca)*. The use of artificial foods in larval culture has been briefly reviewed (Loosanoff and Davis, 1963b). The present study was undertaken from 1956 to 1960 to develop a satisfactory artificial food for bivalve larvae. Tested foods are evaluated and the preparation of the most satisfactory is described in this report.

MATERIAL AND METHODS

Artificial foods tested included basic nutrients (amino acids and protein solutions), materials similar to commonly used foods (multicellular algae) and preparations tested by other investigators. They were presented as solutions or suspensions. Suspensions of dried, fresh, or frozen material were prepared by grinding in a jar mill with water and removing large particles by a stainless steel screen with 50 μ mesh. It was necessary to wash thoroughly fresh and frozen algae with filtered sea water to remove attached organisms. Foods tested and details of preparation are given in Tables 1-3.

Larvae were obtained throughout the year by

TABLE 1. *Effects of liquid artificial foods on growth of* Mercenaria mercenaria *larvae.*

Food	Preparation	Evaluation
Bouillon	Boil 1 cube in 400 ml distilled water for one half hour. Make up to 400 ml with filtered sea water. Feed at 0.17, 0.33 and 1.0 ml/l.	Unsatisfactory though producing growth at 0.17 and 0.33 ml/l
Milk	Dilute fresh milk with sea water. Feed at 0.003, 0.03, 0.3 and 1.0 ml/l.	Unsatisfactory though producihg growth at 0.003 ml/l
Nutrient agar or lactose broth	Dissolve 2.5 g in 1 liter of sea water by boiling. Feed at 7.5, 15 and 30 ml/l.	Unsatisfactory
Bacterial media mixtures A and B	Dissolve 2.5 g nutrient agar (gelatin in mixture B) 2.5 g lactose broth and 2.5 g Bacto Peptone in 3 liters of water by boiling. Feed at 2.5, 5, 7.5, 10, 15 and 30 ml/l.	Unsatisfactory
Amino acid carbohydrate solution	Dissolve amino acids and carbohydrates in water in amounts and proportions as they would occur in a culture of *Monochrysis lutheri.* Feed at 2, 4 and 6 ml/l. (Parsons, et al., 1961).	Unsatisfactory
Amino acid carbohydrate solution plus corn starch	Same as amino acid carbohydrate solution but with 2.5 cc corn starch per liter. Feed at 2, 4, and 6 ml/l.	Unsatisfactory
Barley Pablum extract	Soak 15 cc Pablum in 100 ml of sea water for 2 hours. Filter through No. 41 Wattman filter paper. Feed filtrate at .01 ml/l of larval culture on 2nd and 4th day. Then 0.5 ml/l every other day.	Unsatisfactory
Extracts of rice, oatmeal or mixed Pablum	Same as barley Pablum extract.	Unsatisfactory though producing growth
Ulva lactuca extract A	Boil 1 g ground dry *U. lactuca* in 100 ml sea water for 1/2 hour. Centrifuge and discard residue. Add sufficient sea water to make 100 ml. Feed at 2 and 4 ml/l.	Unsatisfactory though producing growth at 2 ml/l
Ulva lactuca extract B	Mix 15 g ground dried *U. lactuca* thoroughly with 400 ml salt water. Centrifuge and use supernatant liquid. Feed at 2 and 4 ml/l.	Unsatisfactory though producing growth
Asterias forbesi extract A	Same as *U. lactuca* extract A, except use ground dried starfish. Feed at 2 and 4 ml/l.	Unsatisfactory
Asterias forbesi extract B	Same as *U. lactuca* extract B, except use ground dried starfish. Feed at 2, 4, 6-12 ml/l.	Unsatisfactory though producing growth at 2 and 4 ml/l
Laminaria agardhii extract	Same as *U. lactuca* extract B, except use ground dried kelp. Feed at 6-12 ml/l.	Unsatisfactory though producing growth

TABLE 2. *Effects of dried artificial foods on growth of* Mercenaria mercenaria *larvae.*

Food	Preparation	Evaluation
Corn starch or brewers yeast	Mix thoroughly in salt water and pour through fine mesh stainless steel screen. Feed at .01 cc solids per liter.	Unsatisfactory
Mixed Pablum A	Grind in jar mill in salt water with Burundum grinding cylinders for 1 hour. Screen through fine-mesh stainless steel screen. Feed at .01 cc solids per liter.	Unsatisfactory
Mixed Pablum B	Centrifuge mixed Pablum A. Discard supernatant liquid and resuspend in filtered salt water. Feed at .01 cc of solids per liter.	Unsatisfactory though producing growth
Isochrysis galbana	Centrifuge algal culture and dry residue. Resuspend residue in filtered sea water. Feed at .01 cc of solids per liter.	Fair
Ulva lactuca A, or *Laminaria agardhii*, or *Obelia commissuralis* or *Asterias forbesi*	Oven dry at 150° C and pulverize in a jar mill with Burundum grinding cylinders. Mix in salt water and remove large particles by pouring through a fine-mesh stainless steel screen. Feed at .01 & .03 cc of solids per liter.	Fair
Ulva lactuca B	Air dry, grind and store for several years. Some commercially ground so that 85% of particles 2-4 μ in diameter and less than .1% up to 15-18 μ diameter. Feed at .02 cc of solids per liter.	Fair
Fucus sp. (A)	Same as *U. lactuca* A.	Fair but associated with heavy mortality
Fucus sp. (B)	Air dry 40° C. Otherwise grind and prepare as *U. lactuca* A. Feed at .02 cc of solids per liter.	Unsatisfactory
Enteromorpha plumosa	Same as *U. lactuca* A. Feed at .01 & .03 cc of solids per liter.	Fair at .03 cc/l
Mixed Algae	Equal parts of *U. lactuca* A, *Fucus* sp., *L. agardhii*, *E. plumosa*. Dry and grind as *U. lactuca* A. Feed at .01 & .03 cc solids per liter.	Fair
Cyanea capillata or *Mytilus edulis*	Same as *U. lactuca* A. Feed at .01 & .03 cc of solids per liter.	Unsatisfactory

TABLE 3. *Effects of fresh or frozen artificial foods on growth of* Mercenaria mercenaria *larvae.*

Food	Preparation	Evaluation
Yeast	Suspend in sea water, or in 5% dextrose solution overnight, or freeze. Feed at .01 cc of solids per liter of larval culture.	Unsatisfactory
Ulva lactuca A	Wash. Use fresh or frozen. Grind in jar mill with Burundum grinding cylinders and sea water. Screen through fine-mesh stainless steel screen. Feed suspension at .01-.03 cc of solids per liter of larval culture.	Good
Ulva lactuca B	Same as *U. lactuca* A except place in 1 liter graduated cylinder and permit to settle 20 minutes. Divide into upper, 2nd, 3rd, and bottom 250 ml aliquots. Feed separately at .01 cc of solids per liter of larval culture.	Good but decreasing in food value with depth of food taken from cylinder
Boiled *Ulva lactuca*	Same as *U. lactuca* A except boil for 5 minutes before grinding. Feed at .01 cc of solids per liter of larval culture.	Unsatisfactory but producing some growth
Fucus sp. A	Same as *U. lactuca* A.	Good
Fucus sp. B	Same as *U. lactuca* A except grind in Waring Blendor instead of jar mill.	Unsatisfactory
Ascophyllum nodosum or *Codium tomentosum* or *Laminaria agardhii*	Same as *U. lactuca* A.	Unsatisfactory
Molgula manhattensis	Same as *U. lactuca* A.	Good

spawning clams in the laboratory (Loosanoff and Davis, 1951). At an age of two days 30,000 and 50,000 were placed in one-gallon polyethylene containers with three liters of filtered sea water. Sea water was filtered through a 1-μ orlon "Full Flo" filter with a polyvinyl chloride core and passed over an ultraviolet light to kill bacteria. Details of this equipment and procedure have been described by Loosanoff and Davis (1963a).

Duplicate or triplicate experimental and control cultures of larvae were established. Water in the cultures was changed three times a week but larvae were fed daily. Samples were taken from each culture, during water changes, by vigorously stirring a concentrated suspension of larvae and pipetting out a portion. Samples were preserved with 4 per cent buffered formalin. Mortality was estimated by counting dead larvae in the sample, and growth was estimated by averaging the lengths of 50 to 100 randomly selected larvae.

Foods were evaluated by comparing larval growth and survival in fed and unfed control cultures. Fed control cultures received unicellular algae (usually *Monochrysis lutheri* or *Isochrysis galbana*) at the rate of .01 cc of packed cells per liter. These clam larvae typically reached an average length of 200 μ in 8 to 14 days and began metamorphosing. Larvae in unfed controls normally averaged 120 to 140 μ long during the same period. Only rarely, even after several additional weeks, did unfed larvae metamorphose.

Foods have been classified as "unsatisfactory" when growth and survival are equal to or less than in the unfed control, "fair" when growth is more rapid than in the unfed control, but much less than in the live food control, and "good" when growth is almost as rapid as in the live food control.

All cultures were given either 50 to 100 mg streptomycin sulfate per liter or 0.2 ml Combistrep

(Pfizer) per liter at each water change since preliminary experiments demonstrated increased larval growth in streptomycin-treated cultures (Davis and Chanley, 1956).

Water temperatures were usually maintained between 24 and 26°C.

RESULTS

1. *Liquid foods* (Table 1).

Larvae receiving liquid foods either failed to grow more rapidly than unfed larvae or grew much more slowly than larvae receiving live food. The addition of corn starch to an amino acid and carbohydrate solution did not result in increased larval growth.

2. *Dried foods* (Table 2).

Larvae fed dried foods grew less than those receiving live unicellular algae but generally more than unfed larvae. Individuals metamorphosed in cultures receiving the following dried foods: *Ulva lactuca, Fucus* sp., *Laminaria agardhii, Enteromorpha plumosa,* a mixture of equal parts of these four species, *Isochrysis galbana, Asterias forbesi* and *Obelia commissuralis. Ulva lactuca* was consistently the best dried food tested and metamorphosis began in 13-17 days.

Larvae receiving corn starch, mixed Pablum or dried *Cyanea capillata* never metamorphosed and did not grow more rapidly than unfed larvae. Those fed brewers yeast, *Mytilus edulis* and blender-ground preparations of *Fucus* sp. suffered a heavier mortality and did not grow as rapidly as unfed larvae.

Dried food particles clumped together in all cultures, and accumulation of detritus made handling and examination of older cultures difficult. To combat this difficulty, sea lettuce was commercially ground so that 85 per cent of the particles ranged from 2 to 4 μ in diameter. Only 0.1 per cent were in the maximum size range of 15-18 μ (Personal Communcation, Pittsburg Plate Glass Co., Corona Chemical Division, Moorestown, N. J.). Nevertheless, these small particles clumped and could not be washed through a screen with openings 50 μ square. Constant rotation on a vertical wheel prevented settling, but did not prevent the aggregation of small particles.

Larvae fed dried foods were often pale, compared to the rich brown color of those in the live food controls, and metamorphosed at larger sizes. In cultures fed live unicellular algae, the smallest larva with a functional foot measured 175 μ long, while the largest with a functional velum was 225 μ. In typical cultures receiving ground dried algae, the smallest larva with a functional foot measured 225 μ while some measuring 250 μ were still actively swimming. This is unusual since slow-growing larvae generally metamorphose at smaller sizes than fast-growing larvae.

3. *Fresh and frozen foods* (Table 3).

The most rapid growth of larvae receiving experimental foods was among those receiving fresh or frozen *Ulva lactuca* or *Molgula manhattensis.* Although larvae receiving these foods did not grow as rapidly nor survive as well as larvae receiving live unicellular algae, they did survive satisfactorily and were reared to metamorphosis in 8 to 13 days. Eventually, they grew to lengths of 350 to 450 μ on these diets, and when later placed in running sea water, grew to several millimeters before being discarded.

Larvae fed frozen *Ascophyllum nodosum,* frozen *Codium tomentosum* or yeast grew no more rapidly than unfed larvae. Yeast cells were also kept in a dextrose solution to induce budding while others were frozen to rupture the cell wall, but neither preparation was utilized by larvae. Survival and growth of larvae fed *Laminaria agardhii* were less than in unfed larvae. Larvae grew when fed *Fucus* sp. that had been ground in a jar mill, but not when it was ground in a Waring Blendor. Apparently, toxic metallic ions were picked up from the blades of the blender (Hidu and Ukeles, 1964).

Accumulations of detritus from unused food occurred with fresh or frozen preparations as with dried food. Unsuccessful attempts to avoid this included the above-mentioned use of a rotating wheel, boiling food before grinding and allowing an *Ulva* suspension to settle for 20 minutes before using the suspensoid. However, larvae did grow more rapidly in the latter preparations.

DISCUSSION

One problem in evaluating experimental foods is the absence of a standard control. Larvae in both the live food and unfed controls grew at different rates during different experiments which were conducted throughout the year. These variations may have been the result of seasonal fluctuations in water condition, changes in growth phase of the algal food, variations in bacterial flora in either algal or larval cultures, and the use of different broods of larvae. Because of these fluctuations in rate of growth, direct comparison between experiments is impossible. Neither is it possible to evaluate foods on the basis of a proportion since factors influencing growth in the live food control may not affect the unfed control. Consequently, a food must be evaluated by comparing larval growth and sur-

vival in both controls within the same experiment. Only general comparisons are possible between experiments.

For practical purposes it is immaterial whether artificial foods are utilized directly or indirectly; however, it is recognized that addition of any food to larval cultures may enrich the water and result in a substantial increase in the bacterial populations. Larvae could feed on these bacteria rather than on the material added as foods. There are several reasons for rejecting this hypothesis.

1. Most investigators have found that bacteria have a detrimental rather than beneficial effect on larvae (Walne, 1964; Loosanoff and Davis 1963a; Guillard, 1958; 1959; Tubiash, Chanley, and Leifson, 1965). Furthermore, an attempt to use marine bacteria as a food for bivalve larvae was unsuccessful (Davis, 1953).

2. The selectivity of artificial foods as bacterial media would have to be extreme to account for differences in growth observed between larvae fed frozen *U. lactuca* and frozen *L. agardhii* or dried *U. lactuca*.

3. The best bacterial media tested (the liquid preparations) were consistently the poorest foods for larvae.

4. Larvae frequently acquired the color of some of the processed foods, indicating ingestion of the food (Loosanoff and Davis, 1963a; Hidu and Ukeles, 1964).

In combination these arguments strongly suggest that artificial foods were utilized directly.

No one class of food was superior to another and both satisfactory and unsatisfactory representatives of algae and invertebrates were found. The failure of larvae to grow satisfactorily when fed some artificial foods is not conclusive evidence that the foods are of no value. Possibly preparations of basically satisfactory foods were present in a state larvae were incapable of utilizing.

Artificial foods that are satisfactory for hard clams may not be utilized by larvae of other species. *Mercenaria mercenaria* larvae are known to utilize a greater variety of natural foods than larval *Crassostrea virginica* (Davis and Guillard, 1958).

CONCLUSIONS

Hard clam larvae grew when fed a wide variety of artificial foods. The most desirable artificial food developed in these experiments compares favorably with previously described artificial foods. It was prepared by grinding about 25 g washed fresh or frozen *U. lactuca* in about 300 ml of water in a jar mill with Burundum grinding cylinders for one hour. The resulting suspension was screened through a stainless steel screen with openings 50 μ square, and the residue discarded. After settling 20 minutes, the top half of the suspension was siphoned off as the food supply. Combistrep was added to the suspension so that 0.2 ml of Combistrep and .01 cc of packed solid food would be added per liter of larval culture at each feeding. As larvae began metamorphosing, a new food suspension was prepared so that larvae were given .03 cc of ground sea lettuce per liter without increasing the dosage of Combistrep. This preparation could be stored in the refrigerator for one week but, if refrozen, tended to clump. It was thoroughly stirred before each feeding. Some differences in sea lettuce quality were observed, probably due to seasonal and local variations.

Accumulation of unused food particles in larval cultures is a serious disadvantage of all particulate artificial foods. Future attempts to develop artificial particulate foods must consider this problem, especially if the food is to be used with late larval and recently metamorphosed clams that cannot be readily separated from bottom deposits.

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INTERACTION OF TWO DISEASES OF OYSTERS IN NATURAL WATERS[1]

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ABSTRACT

A localized epizootic caused by Dermocystidium marinum *was induced in oysters in the York River, Virginia, to simulate natural epizootics in timing of infections and mortalities. Relative isolation was achieved by use of trays located on barren bottom. Decimation by* Minchinia nelsoni *(MSX) since 1960 has insured low populations of oysters and a relative scarcity of* Dermocystidium. *Trays spaced 15 feet apart exhibited epizootics which were progressively earlier and more vigorous in proportion to the number of infected oysters added (foci of infection).* Minchinia *infections were severe and at least 50 per cent of the oysters died from this parasite.* Dermocystidium *spreads more rapidly and kills more quickly but requires dense populations and high temperatures. MSX is not localized and is much less affected by these limiting factors. In lower York River, new imports of disease-free oysters require one to three years to acquire* Dermocystidium *cases which initiate epizootics. During this period MSX decimates oyster populations, thereby preventing* Dermocystidium *from becoming epizootic.*

INTRODUCTION

Fifteen years experience in field and laboratory indicates that *Dermocystidium marinum* causes a typical infectious disease transmitted from oyster to oyster by close proximity. Isolation has proven to be an effective method of controlling the disease caused by this fungus pathogen. Nature has performed a large-scale isolation experiment on *Dermocystidium* in Virginia waters through the activities of *Minchinia nelsoni* (MSX), a virulent sporozoan pathogen which decimated oysters and forced oystermen to stop planting oyster beds in high-salinity areas. Before 1960 the fungus was common in oyster-planting areas throughout the lower Chesapeake Bay. Now it is rare except in areas where susceptible oysters are imported regularly and and where appreciable numbers of oysters survived the MSX epizootic.

Infection experiments carried out in natural waters before and during the *Minchinia nelsoni* epizootic have provided valuable information on transmission of *Dermocystidium* (Andrews, 1965). Failure to obtain timely infections in laboratory oysters, which were to have served as foci of infection, limited the value of the 1963 experiment conducted in a period of relative scarcity of *Dermocystidium*. A new proximity experiment conducted in 1965 is reported here. Continuation of the MSX epizootic insured low populations of oysters in the area of York River around the Virginia Institute of Marine Science (VIMS), hence low natural prevalence of *Dermocystidium* during the experiment.

METHODS

Methods and area of experimentation were the same as reported in the 1965 paper. Disease-free oysters (experimentals) were imported 27 May 1965 from Horsehead Rock, a low-salinity site in the James River. After 11 days at VIMS pier, four trays of oysters were placed about 15 feet

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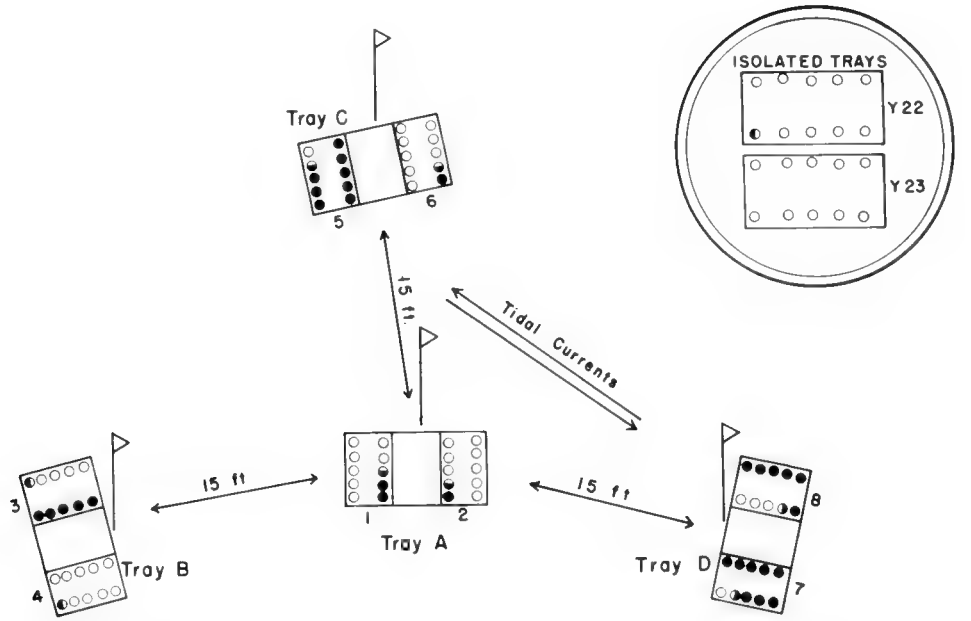

FIG. 1. *Percentages of live oysters infected with* Dermocystidium *on 8 October 1965. Each circle represents 10% of a compartment population with closed circles indicating fungous infections in experimental oysters.*

apart off from the pier on sandy bottom free of wild oysters. Each tray contained 400 oysters, segregated into lots of 200 in end compartments by two wooden partitions and monitored separately. Numbering of compartments in letter-designated trays is shown in Table 1 and Figure 1. The control tray (A) was placed by a center stake and three experimental trays by stakes arranged in a triangle around it at distances of about 15 feet.

On 16 June, 75 oysters marked with orange paint were fed macerated *Dermocystidium*-infected gapers in an aquarium. On 18 June these fungus-exposed oysters were added to compartments of the trays as follows: tray A—none; tray B, compartment 3—2 oysters; tray C, compartment 5—10 oysters; and tray D, compartment 7—61 oysters. Hence comparisons of prevalences and mortalities could be made between trays and between compartments in the same trays.

Gapers and boxes (dead oysters with and without meats) were usually collected from the trays by divers using SCUBA but occasionally, when the trays were brought up for cleaning, oysters were examined in the boat. All gapers and live oysters in samples were tested for *Dermocystidium* by the thioglycollate method of Ray (1952). All were examined also for MSX in stained tissue sections.

RESULTS

Monitoring of Artificially-Infected Oysters

Three paint-marked boxes were found in compartment 7 on 16 July. By 30 July, 12 more marked oysters were dead. Two gapers had heavy infections of *Dermocystidium* and nine live oysters revealed seven fungous infections, of which five were advanced cases. By mid-August marked gapers with heavy cases had been recovered from each of compartments 3, 5 and 7. These early samples indicated that laboratory infections had been successfully induced and that timing of infections was about right to simulate typical summer epizootics of the fungous disease. Epizootics of varying intensities were expected from the number of infected oysters introduced in some compartments and the absence of foci of infection in others. No attempt has been made to present complete data on marked oysters. Both MSX and *Dermocystidium* were found in gapers, with the fungus predominating. Nearly all of these oysters died before the experiment was ended.

Mortalities of Experimental Oysters

Deaths in the experimental oysters began in mid-July from MSX but less than 5 per cent had died by 30 July. By this date compartment 7 had

TABLE 1. *Accumulative mortalities in proximity experiment trays, 1965 (Initial count — 200 per compartment).*

Tray no.	Compartment	30 July	17 August	8 September	8 October	31 December
A	1	4	12	23	44	54
	2	2	11	26	47	63
B	3[a]	5	14	32	60	70
	4	5	16	43	60	72
C	5[b]	2	11	28	55	67
	6	4	12	16	36	54
D	7[c]	6	13	49	75	—
	8	4	11	24	40	65

[a]Foci of infection consisted of 2 fungus-infected oysters added.
[b]Foci of infection consisted of 10 fungus-infected oysters added.
[c]Foci of infection consisted of 61 fungus-infected oysters added.

one well-established infection of *Dermocystidium* in 25 live oysters. None were found in 25 live oysters from compartment 8. Eleven gapers from six different compartments of experimental oysters had no fungus on 30 July. By 13 August, 12 more gapers from seven different compartments revealed only one case of fungus (in compartment 7). Thus MSX had been killing experimental oysters for a month before *Dermocystidium* caused deaths.

Accumulative mortalities by dates of examination are given in Table 1. Mortalities began by 30 July and increased rapidly in August and September. By 8 September, compartment 7 had lost half its oysters and was ahead of other trays in cumulative mortality. A month later, compartmen 7 had reached 75 per cent mortality and other compartments had about 50 per cent dead. Losses in all trays for the six-month period ending 31 December were higher than those usually experienced for either MSX or *Dermocystidium* alone.

Prevalences of Diseases in Live Oysters

Prevalences of the two diseases in live oysters are shown in Table 2. Exposure to MSX infection can be assumed from 27 May, the day of import (Andrews, 1966). Gapers from fungus-inoculated oysters began to appear in late July at which time experimental oysters were first exposed to

TABLE 2. *Prevalence of diseases in live oysters, 1965 (Percentages in samples of 25 oysters).*

Tray	Compartment	17 August		8 October		31 December	
		MSX*	Dermo	MSX	Dermo	MSX	Dermo
A	1	64	0	48	24	56	0
	2	80	0	40	16	52	0
B	3[a]	40	0	28	52	64	8
	4	40	4	20	4	56	4
C	5[b]	32	8	56	88	40	8
	6	40	0	28	16	56	8
D	7[c]	36	75	52	88	—	—
	8	60	12	40	64	40	16

*An exceptional number of localized rare cases in gills in these samples.
[a]Foci of infection consisted of 2 fungus-infected oysters added.
[b]Foci of infection consisted of 10 fungus-infected oysters added.
[c]Foci of infection consisted of 61 fungus-infected oysters added.

Dermocystidium. This is typical timing of exposure for new imports in Virginia. By 17 August, two months after mixing with newly-infected oysters and no more than one month after first exposure to gapers killed by *Dermocystidium*, compartment 7 had 75 per cent infection of the fungus in live oysters. It had also appeared in three of the remaining seven compartments. At this time deaths from MSX were occurring rather uniformly in all trays. However, prevalences were more variable than usual for oysters of uniform history and exposure. This is due partly to an unusually high proportion of localized (very recently patent) cases. Finding of these by sections is somewhat fortuitous and samples of 25 oysters permit considerable variation by chance.

By 8 October, the compartments with fungus-inoculated oysters (3, 5, and 7) were showing high levels of infection compared to control compartments in the opposite ends of the same trays (Fig. 1). Apparently the trays were grouped too closely to prevent transmission to the control oysters in the center tray. However, fungous prevalence was no greater in compartments 4 and 6 adjacent to foci of infection in 3 and 5 than in the control oysters in tray A. Figure 1 shows the relative postions of trays and direction of currents but no attempt was made to keep compartments aligned as illustrated. Despite the prevalence of *D. marinum* in all compartments by 8 October, it is difficult to deduce that fungous infections had increased mortality rates differentially except in compartment 7 (Table 1). By this date all compartments had fungous infections. Despite the rising level of fungous infections, MSX was still causing most deaths in all compartments except 7. Curiously, tray A, with higher levels of MSX infection, had lower death rates than tray B. It is unfortunate that live-oyster samples were not taken about mid-September.

By 31 December, most *Dermocystidium* infections had disappeared from the populations, but about half of the surviving oysters had cases of MSX. Since deaths from the fungus usually stop about 1 November each year due to inhibition of multiplication by falling temperatures, samples should have been taken in November for post-mortality season prevalences of *Dermocystidium*. Oysters usually discharge those stages which respond to thioglycollate tests as soon as winter temperatures are reached in December. Furthermore, some oysters with mixed infections of MSX and the fungus were probably killed by the sporozoan parasite after 1 November.

A high level of initial infections by MSX from early-summer exposure can be deduced. Two-thirds of the oysters had died by the end of the year and probably 50 per cent were killed by MSX. Half the survivors were infected at the end of the year, hence a minimum of 75 per cent of the initial populations were infected. Unlike the fungus, MSX continues to kill throughout the winter at reduced rates, and infection levels remain high. Prevalences above 50 per cent in early winter of the first year of exposure (Table 2) are above usual levels of infection for this period (Andrews, 1966).

Prevalences of Diseases in Gapers

All gapers were examined for occurrence of *Dermocystidium* and MSX. Cases of the fungus are shown by date of occurrence in Table 3 to demonstrate timing and magnitude of activity in trays of similar history but variable exposure. The only known modification of the ecosystem of tray populations was the addition of varying numbers of fungus-inoculated oysters in compartments 3, 5, and 7. Usually oysters do not die from *Dermocystidium* until "heavy" cases are developed. These appeared first in experimentals on 27 August and by 8 September heavy cases had been found in nearly all trays.

Diagnoses of gapers for *M. nelsoni* are given in Table 4. No attempt has been made to list cases by intensities because all levels of infection were found in gapers. MSX infections were first observed in gapers on 16 July and continued in abundance through October. Prior to September nearly all gapers had MSX infections but thereafter through October a significant number of gapers with negative diagnoses were observed. These negative gapers were mostly killed by *Dermocystidium* as the data in Table 3 indicate. There was no pattern of variation of MSX infections among trays or compartments. All lots became infected and exhibited deaths with similar timing and intensities. MSX was diagnosed in 95 of 126 gapers (76 per cent), hence it was obviously the dominant mortality agent in these groups of oysters.

It is difficult to assign precise figures for the number of oysters killed by each disease. An attempt to summarize the prevalence of *Dermocystidium* and MSX in gapers is given in Table 5. For *Dermocystidium*, prevalence of serious cases (heavy and moderate infections) was related to the number of inoculated oysters added. In each comparison, oysters in compartments and trays with infected oysters added showed a greater number of serious infections than controls. Tray A, with no infected oysters added, had 6 serious cases of fungus in 27 gapers. Trays B through D, with increasing numbers of infected oysters added in the odd-numbered compartments, had: 6 in 29, 12

TABLE 3. *Chronological record of thioglycollate tests for* Dermocystidium marinum *in gapers* [1], *proximity experiment, 1965.*

Tray	Compartment	16 July	30 July	13 August	17 August	27 August	8 September	20 September	23 September	8 October	20 October	16 December
A	1			N		N		3N			N	N L
	2		N	2N	M*	4N	L* H	H	2N	3N	H M* L N	N
B	3		2N	N		N	H* M* N			H*	H* 2L**	M* 3N
	4	N	N	3N	2L**	2N	H* 3N			N	L*	
C	5	N	N			4N	H* N	2H* H		3H	2H	
	6		4N	N			N	3N	L*		2H L* N	H 4N
D	7[2]			M*	2N L*		3H**	4H** 3H	M*	3H*	2H M	
	8		2N	N	L*	N		3H**	2L* M*	N	H* M*	5H*** L*
Totals		2N	11N	1M	11N 1M 4L	12N	4H L 3N	9H M 4N	8H M 2L	8N 7H M L 5N	8H 2M 4L 2N	7H 2M 2L 9N L N

[1] Intensity of infections was rated heavy (H), moderate (M), light (L), or negative (N).

[2] A negative gaper on 18 June.

* Each asterisk designates a gaper infected with both MSX and *Dermocystidium*.

TABLE 4. *Diagnoses of* Minchinia[1] nelsoni *(MSX) in gapers*[1], *proximity experiment, 1965.*

Tray	Compartment	18 June	16 July	30 July	13 August	17 August	27 August	8 September	20 September	23 September	8 October	20 October	16 December	31 December	Totals	Per cent MSX by trays
A	1				P		P		3P		P	P	N		7P N	82
	2				4P	5P*	P*	N	P 2N	2P		2P* N	P		16P 4N	
B	3			P N	P			3P**		P*	3P***	P*			10P N	93
	4		N	P	3P	4P**		4P*			P*	3P			16P N	
C	5		P	P		2P	2P*	P* N	N	3N	N				7P 6N	72
	6			2P	P				3P	P*	2P* 2N	4P			13P 2N	
D	7	N			3P*	P*	2P** N	2P** 2N	P* 3N	P* N	3N				10P 11N	62
	8			2P	P	2P*		N	3P*** 2N	2P*	2P**	4P**** 2N			16P 5N	
Totals		N	P N	7P N	14P	14P	6P N	10P 5N	11P 8N	7P 4N	9P 6N	15P 3N	P N		95P 31N	

[1] Positive or negative

* Each asterisk designates a gaper infected with both *M. nelsoni* and *Dermocystidium*

TABLE 5. *Summary of diagnoses of diseases in gapers from proximity trays.*

Tray	Compt.	Number of gapers	Cases of *Dermocystidium**				Cases of MSX
			H	M	L	N	
A	1	8			1	7	7
	2	19	4	2	2	11	16
B	3	12	3	2	3	4	10
	4	17	1		3	13	16
C	5	16	9			7	7
	6	21	3		2	16	13
D	7	20	14	3	1	2	10
	8	19	10	2	3	4	16
Totals		132	44	9	15	64	95

* Intensity ratings: heavy (H), moderate (M), light (L), negative (N).

in 37, and 29 in 39 cases, respectively (Table 5). In all trays the most cases of fungous disease occurred in the compartment with infected oysters added.

Figure 2 is a graphic presentation of the data in Table 5 by trays only. MSX exhibited high prevalences in all trays but particularly those with less fungus. *Dermocystidium*, especially serious cases, increased in proportion to the number of artificially-infected oysters added. Concurrent infections varied from about 10 to 50 per cent, more or less in proportion to the amount of fungus present.

Figure 3 shows the percentages of gapers for each date that had *Dermocystidium* and MSX infections. Number of gapers varied from 8 to 20 except the first and last dates which had only 2 (see Tables 3 and 4). The graph shows that MSX preceded the fungus in occurrence in gapers. In mid-August 28 consecutive gapers were positive for MSX although five of these also had *Dermocystidium* infections. After the fungus appeared, MSX prevalence in gapers dropped from 100 per cent to about two-thirds. Fungous infections peaked in August through October and most cases were advanced ones. All trays were grouped for this graph regardless of degree of exposure to infection. During its active period, *D. marinum* probably caused half of the oyster deaths in the combined populations.

Figure 4 depicts chronological occurrence of cases of MSX, *Dermocystidium*, and concurrent infections. The vertical time scale is approximate except for the first and last dates which are "closed in." Each symbol represents a gaper, hence a gross scan of the graph conveys the timing and the proportion of deaths by each pathogen. MSX appeared in mid-July and occurred in 6 to 15 gapers per examination in a rather steady pattern. *Dermocystidium* appeared in mid-August and was found in about 5 to 14 oysters in each examination. Most fungous infections were advanced and all except three light cases were associated with MSX infections. Nearly one-third (29 per cent) of all gapers had mixed infections and two-thirds of these involved serious fungous infections (closed triangles). Thirty-seven of the gapers tested had infections of both MSX and *Dermocystidium*. Over half of these (19) were in tray D which received the greatest number of artificially-infected oysters. Serious infections of the fungus (heavy and moderate) occurred in 25 of the 37 gapers with concurrent infections.

From the thioglycollate tests, 53 of 132 gapers (39 per cent) had serious infections of *D. marinum*. With high prevalences of both diseases, a fairly large number of mixed infections could be expected. All combinations of intensities of infections occurred but usually it can be deduced which pathogen was the probable killer. Many light infections of the fungus were barely established and can be discounted as contributing to morbidity. Consequently, after subtracting serious *Dermocystidium* infections, at least 56 per cent of all gapers were probably killed by MSX. The percentage would be considerably higher if tray D, which had a large proportion of the fungous infections, were not included.

DISCUSSION

An attempt to induce and monitor a localized epizootic of *Dermocystidium* in the open waters of the York River was surprisingly successful in

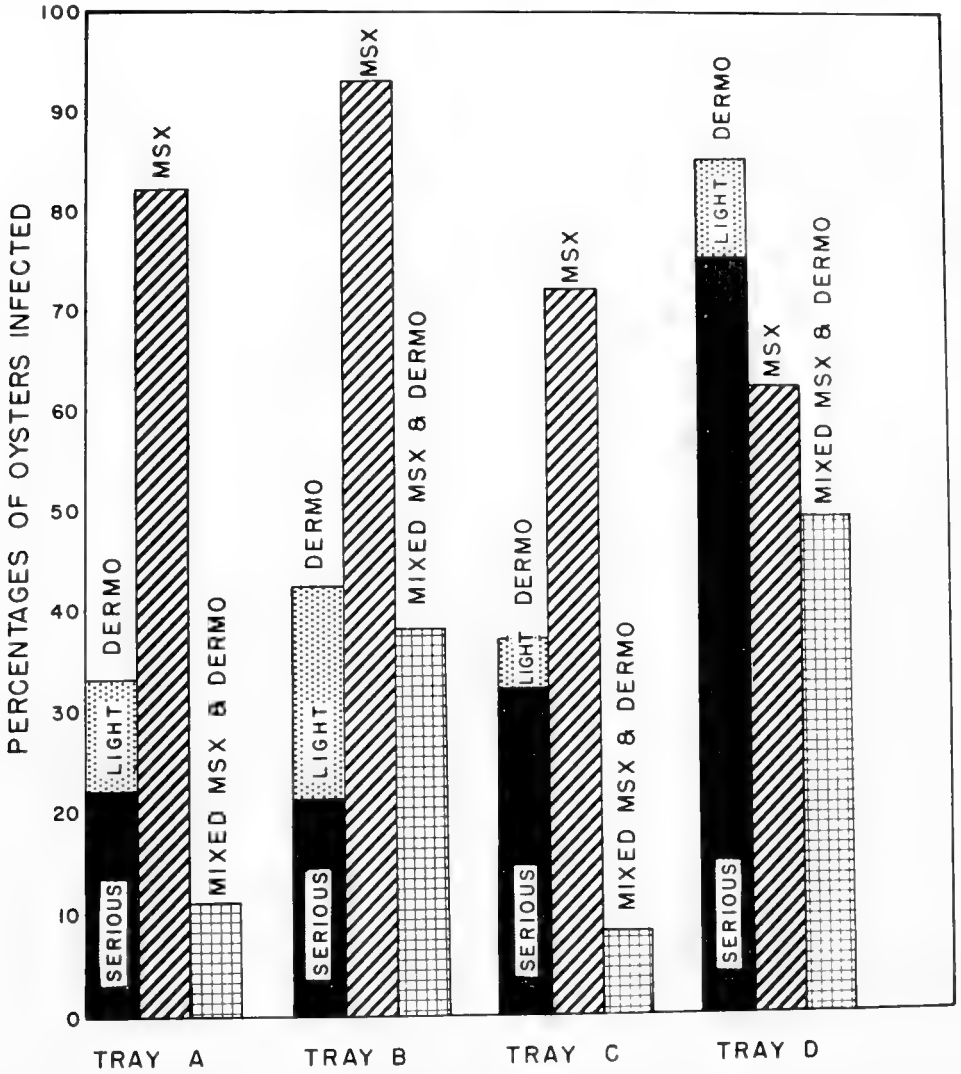

FIG. 2. *Comparison of* Dermocystidium *and MSX in gapers by trays. Concurrent infections are included in the percentages for each pathogen and also shown for each tray by separate bars.*

1965. Artificial infections have been induced many times in aquaria and in trays in open waters but limited circulation in the former and exposure to wild infections in the latter have complicated results. Since 1960, MSX has kept oyster populations severely reduced in the lower bay, hence *Dermocystidium* has been suppressed except in places such as VIMS pier where new populations have been imported regularly. Experience with trays of disease-free oysters on abandoned oyster grounds off VIMS has shown that from one to three years are required for an epizootic of the fungus to get started from natural sources.

The objectives were to initiate a localized epizootic which could be controlled in respect to timing and distances between various lots of oysters. One important but uncontrollable factor was continued epizootic activity of *M. nelsoni*. MSX appears unaffected by proximity of infected oysters hence it is assumed that all lots of oysters were affected about equally by this parasite. Although the data from individual samples or dates may not appear to justify this assumption, it seems to be verified by results of six-months' observations. Unfortunately, preoccupation with other work left three periods unsampled which

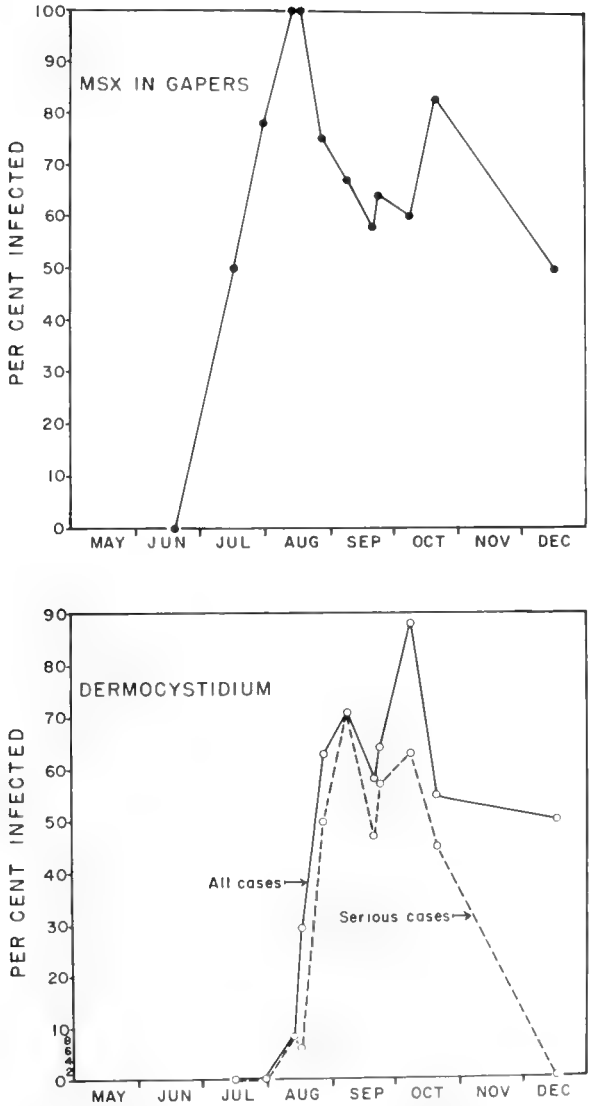

FIG. 3. *Percentage occurrence of MSX and* Dermocystidium *in gapers from all four trays. Number of gapers tested per date varied from 8 to 20 oysters.*

would have clarified the progress of the epizootics. Perhaps hindsight is involved, too, in the conclusion that samples should have been taken in mid-July, mid-September, and mid-November.

Experimental oysters were imported in late May, which is believed to be the beginning of the infection period for MSX. Six weeks later in mid-July, mortalities had begun and MSX-infected gapers were recovered. Both timing and prevalence of infections continued typically throughout the rest of the year. By mid-August prevalence reached levels of about 50 per cent which were maintained throughout the experiment, despite 60 per cent mortality. This straggling in the clinical appearance of MSX is characteristic, although all late-summer and fall cases can be traced to infections initiated in about a six-week period in June and early July.

The laboratory infections of *Dermocystidium*, induced in mid-June, began to kill marked oysters about one month later in mid-July. This is about typical of natural infections (over-wintering cases) which become apparent in late June and kill in July, thereby initiating a second round of infections. By mid-August, disintegrating marked gapers had initiated infections in 75 per cent of the experimental oysters in compartment 7. This rapid rise of *Dermocystidium* prevalence is noted also in compartments 3 and 5 between 17 August and 8 October. By 8 October it had spread to other compartments and trays including the control tray A in the middle of the cluster. The rapid climb in prevalence of the fungus at warm temperatures (about 25°C) is countered by an equally precipitous decline, usually in December. *Dermocystidium* spreads rapidly in crowded populations, once infected gapers begin dying. Even one gaper in a tray (only one of the two laboratory-infected oysters added to compartment 3 died by 17 August and the other much later) can initiate an epizootic.

It is presumed that the four clustered trays exhibited a self-contained epizootic of the fungous disease. This cannot be verified except by comparison with other trays introduced in the same year. Examples are trays Y22 and Y23, comparable to the proximity series except they were placed farther offshore of VIMS and at least 100 feet from other trays. Y22 had one light case in 25 live oysters on 20 October 1965 and Y23 had none (Fig. 1). One of 65 gapers collected from these two trays in 1965 had a light case of *Dermocystidium*. Furthermore, in 1963 natural infections were rare in a similar experiment at the same location (Andrews, 1965).

It is apparent from Table 2 that 15 feet was not an adequate distance to prevent infections being transmitted from one tray to another. Yet significant differences in prevalences were obtained in opposite ends of the same tray with only a pair of partition boards to prevent direct flow of water. The control tray in the center obtained as many infections as compartments 4 and 6 in trays with infected oysters added.

Interaction of Two Pathogens

It is impossible to integrate the many factors which contribute to deaths of oysters. Mackin and Sparks (1962) list over nine agents which cause

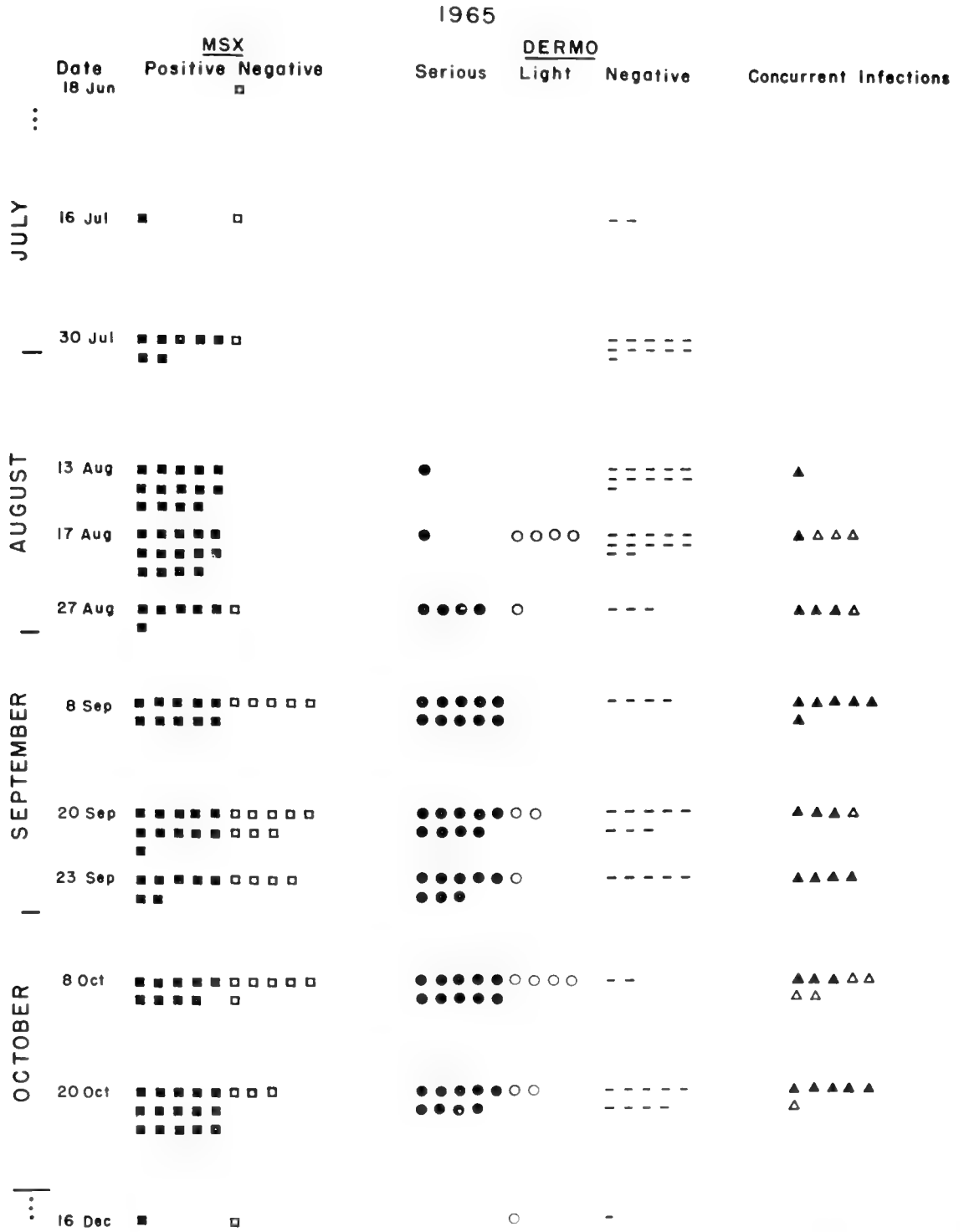

FIG. 4. *Occurrence of MSX and* Dermocystidium *infections in gapers. Each symbol represents one gaper. In concurrent infections, closed triangles represent serious fungous infections and open triangles light ones.*

oyster mortalities in Louisiana. Of those present in Virginia, only *Dermocystidium* is considered important in my studies. *Bucephalus* and *Polydora* species are minor agents compared to some undescribed pathogens, and *Odostomia* is not considered to be a cause of mortality. Blue crabs and mud crabs may kill spat occasionally but usually are scavengers. Boring sponges seem to play accessory roles to other agents in old oysters, including accidental breakage of shells. Predators were excluded and only diseases and parasites had free access to experimental oysters.

MSX and *Dermocystidium* were obviously the major causes of death, yet a few gapers exhibited neither parasite. A rough approximation of the roles of the two pathogens can be obtained in Table 3 by chronologically comparing the number of gapers diagnosed by intensity of infection as light or negative (probably killed by MSX) with those designated moderate and heavy (probably died from *Dermocystidium*). MSX predominated prior to about 1 September, whereas the two pathogens were about equally destructive from that time through October. Of 37 mixed infections, 25 had serious cases of the fungus, implying that *Dermocystidium* is quicker to kill when both pathogens have become established. In addition, MSX had nearly two months head start over the fungus in terms of exposure of experimentals to infection. Since both MSX and *Dermocystidium* may inhibit growth for two or more weeks prior to death (Andrews, 1963), some reduction of each disease can be expected from the presence of the other. If an oyster does not feed, it is unlikely to contract another disease, whereas if both diseases are already present, a synergistic lethal effect may occur. Mortality data imply that, except for compartment 7, exposure to *Dermocystidium* did not appreciably increase accumulative mortalities. Tray Y22 without the fungus had 60 per cent mortality from MSX (Andrews and Wood, 1967) which is about comparable to losses shown in Table 1.

Both pathogens are highly lethal to susceptible oysters given appropriate environmental conditions. It is extremely important therefore to know the epizootiology of both diseases as to seasonality of infections, duration of incubation periods, susceptibility of oyster populations, and inhibiting factors. With this knowledge, time and area of planting and harvesting, and choice of seed sources can be regulated to minimize losses. The interactions of two diseases in nature, one of which can be controlled by isolation whereas the other cannot, are important in management procedures. This experiment has provided some insight of these interactions.

One must conclude that MSX, within its range, is by far the most serious disease of oysters in Chesapeake Bay. Its ubiquity in most Virginia waters and tolerance of wide temperature range leaves little room for manipulation of oyster populations. Mackin and Sparks (1962) concluded that *Dermocystidium* overshadowed all other agents combined in Louisiana. Ten years ago, the fungus was the major agent of mortality in Virginia (Andrews and Hewatt, 1957).

Dermocystidium can be controlled by avoiding infested seed and by cleaning and fallowing oyster beds. It is subject to fairly rigid temperature controls. In short, being an infectious disease directly transmitted, it is amenable to manipulation of populations and planting grounds. The major purpose of this study was to demonstrate the importance of controlling or eliminating foci of infection for *Dermocystidium*. There is probably no "safe" distance for effective isolation but distances of 15 to 100 feet are useful in slowing the spread of epizootics. Probably the size of a bed of oysters is important in commercial operations in respect to distances needed for limiting infestations.

ACKNOWLEDGMENTS

The slides for diagnoses of MSX occurrence were prepared by the Microtechnique Section of the Microbiology Department of VIMS under the supervision of Dr. J. L. Wood. Grateful appreciation for this essential help is acknowledged.

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DISTRIBUTION AND EFFECTS OF THE ENDOPARASITIC COPEPOD, *MYTILICOLA ORIENTALIS*, ON THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*, ON THE PACIFIC COAST [1, 2]

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ABSTRACT

Mytilicola orientalis *was studied in relation to* Crassostrea gigas *from April, 1963 to March, 1965, in Humboldt Bay, California; Yaquina Bay, Oregon; Willapa Bay, Oyster Bay and Hood Canal, Washington.*

No short-term cyclic effects were noted in regard to the incidence or intensity of infestation, although the incidence of infestation at Willapa Bay showed an increasing trend throughout the study.

The infested oysters exhibited a lower Condition Index than the non-infested oysters, but there was little evidence of reduction of shell growth in the infested oysters. Survival of the infested oysters was not adversely affected.

INTRODUCTION

Mytilicola orientalis is an endoparasitic copepod found in the intestinal tract of mollusks. Its presence in Washington has been generally known for many years, but since it caused little if any apparent damage, it has been largely ignored.

This copepod came to the attention of public health authorities in California during a routine bacterial examination of Pacific oysters (*Crassostrea gigas*). Considerable interest was aroused both because of possible public health significance and the California Department of Public Health's pure food certification responsibilities. A meeting was held in San Francisco, California, to discuss this problem attended by representatives from the California Department of Public Health, U. S. Public Health Service, U. S. Food and Drug Administration, California Department of Fish and Game, Washington Department of Health, Pacific Coast Oyster Growers Association, and the University of Washington's Zoology Department and College of Fisheries (representing the Oyster Institute of North America). It was agreed that no risk of human disease was involved in the consumption of oysters infested with *Mytilicola*, but that the esthetic value of infested oysters was impaired. It was further agreed that the copepod might adversely affect the survival, fatness, and growth of infested oysters. It was suggested that the Invertebrate Section of the College of Fisheries, University of Washington, submit a proposal for a biological study on the relationship of this parasite to Pacific oysters. A proposal was forwarded to the U. S. Public Health Service and funds were granted effective September 1, 1962.

LITERATURE REVIEW

Mytilicola orientalis was first described from the intestinal tract of the oyster, *Crassostrea gigas*, and the mussel, *Mytilus crassitesta*, from the Inland Sea of Japan by Mori (1935). Wilson

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[1] Contribution No. 250, College of Fisheries, University of Washington.

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(1938) described *Mytilicola ostreae* from *C. gigas* in Puget Sound, but Odlaug (1946) pointed out that *M. orientalis* and *M. ostreae* are identical and that Wilson was apparently unaware of Mori's work. Wilson (1938) observed that the copepod maintained its position in the intestinal tract of its host by attaching to the gut wall with the aid of the distal segments of the second antennae which are provided with two spine-like setae and terminate in a stout, curved claw. He did not consider the copepod to be a true parasite since it did not appear to harm the host and its mouth parts were not suited for sucking fluids or biting tissue. However, Sparks (1962a) conclusively demonstrated that *Mytilicola* is capable of doing considerable damage to its host and described metaplastic changes in the gut of *C. gigas* associated with the presence of *Mytilicola*.

According to Odlaug (1946), *M. orientalis* is widespread in lower Puget Sound where it occurs in oysters (*C. gigas* and *Ostrea lurida*), mussels (*Mytilus edulis*), clams (*Protothaca staminea*), and slipper shells (*Crepidula fornicata*). The mussels were by far the most heavily infested species in Odlaug's studies. Chew, Sparks, and Katkansky (1964) recorded this parasite from the California mussel (*Mytilus californianus*) from Humboldt Bay, California.

Odlaug (1946) described a lowering of the Condition Index of native oysters (*O. lurida*) in Washington infested by this parasite. Rankin (1943) found infestations of *M. orientalis* in *O. lurida* and *M. edulis* in lower Puget Sound to be sporadic. He noted that infestations of less than five parasites per oyster (*O. lurida*) had little effect on the host; infestations of greater than five parasites per host resulted in weak, watery oysters and infestations of twelve or more parasites typically resulted in the death of the host. He speculated that *M. edulis* was the natural host of *M. orientalis* since no deleterious effects were noted in heavily infested mussels.

Sparks (1962b) stated that, in preliminary work in Washington and California, sharp peaks of infestation by *Mytilicola* in *C. gigas* appeared to occur in the spring and fall followed by a rapid loss of infestation. He further noted that reproduction of the copepod takes place in the fall and spring and that *Mytilicola* was incapable of maintaining itself in the gut of *C. gigas* for prolonged periods.

Preliminary work by Chew, Sparks, and Katkansky (1965) revealed two distinct size classes of *M. orientalis* in *C. gigas* from experimental stations in Humboldt Bay, California, and Yaquina Bay, Oregon. Those between 2 and 5 mm (total length) were mostly males with a few immature females in the 4 to 5 mm range; those between 6 and 11 mm (total length excluding egg cases when present) were females. Data from experimental stations in Washington, Oregon and California indicated that oysters (*C. gigas*) with below average Condition Indices had significantly higher infestation levels than uninfested oysters (with one exception where infestation rates were low). An inverse relation was found between an oyster's Condition Index and the number of parasites it harbored.

A closely-related species, *Mytilicola intestinalis*, is found in Europe. It was first described from the intestine of the mussel, *Mytilus galloprovincialis*, from the Gulf of Trieste by Steuer (1902). Pearse and Wharton (1938) reported a single specimen of *M. intestinalis* in the gut of an eastern oyster, *Crassostrea virginica*, from the Gulf of Mexico.

Mytilicola intestinalis is credited with causing catastrophic mortalities in European mussel stocks (Korringa, 1950). Korringa (1951) described the tissue of mussels dying from *M. intestinalis* infestations to be thin and watery, the byssus to be broken and the color of the digestive gland to have changed from a dark brown to a yellowish or cream color. No deleterious effects were noted when there were less than three copepods present per infested mussel, but mortalities occurred when the level of infestation reached a level of 5 to 10 copepods per infested mussel.

Andreu (1963) noted an inverse relation between the average meat weight of mussels and the number of parasites they harbored. No seasonal variation was observed in the average number of parasites per host.

Meyer and Mann (1956) found infested mussels to exhibit a loss of weight of the digestive gland. The characteristic appearance of the cells of the gland was lost and the capability of the gland to produce enzymes was diminished. A similar decrease in the weight of the digestive gland was noted earlier by Conteaux-Bargeton (1953).

Meyer and Mann (1951) reported physiological damage in infested mussels resulting in an acceleration in the digestion of albumin, an increase in the need for oxygen and lowered rates of filtration and nutrient absorption.

Mann (1956) noted damage to the gonads of infested mussels to be similar to that caused by trematodes.

METHODS AND MATERIALS

Five experimental stations were set up at selected sites in Washington, Oregon, and California. A floating station similar to one described

by Chew (1961)[4] was established at each site. The floats were modified slightly in that styrofoam was used for floatation instead of 55-gallon oil drums. Eight baskets were placed in each float (a double layer of four) to contain the experimental oysters. A plastic coating was baked onto each basket for resistance to saltwater corrosion. Bed stations were established at all sites except the Oregon station at approximately the plus 2.0 foot tidal level. A bed station consisted of a wooden rack approximately 12 feet long placed parallel to the beach with the legs driven 18 to 24 inches into the substrate; 8 baskets were lashed to this frame. A more detailed description of the experimental design by areas follows:

California

The station in California was established in Humboldt Bay. The float was anchored over the oyster beds of the Coast Oyster Company of Eureka; the bed station was located just west of the float. One-hundred-fifty Pacific oysters of the 1962 seed planting were placed in each basket. In addition, samples of Pacific oysters were collected for examination from the commercial planting near the bed station.

Oregon

The station in Oregon was located in Yaquina Bay. The float was anchored off the dock of the Oregon Oyster Company; since there was no suitable intertidal location for a bed station, Pacific oysters were taken for examination from a nearby subtidal bed. One-hundred-fifty Pacific oysters of the 1962 Japanese seed planting were placed in each basket in the float.

Washington

Stations in Washington were established in Willapa Bay, Oyster Bay, and Hood Canal. All shellfish used came from the beds of the Patterson Oyster Company at Oyster Bay. Each basket contained one-hundred-fifty Pacific oysters of the 1961 natural set. The float at Willapa Bay was anchored in the boat basin at Nahcotta, while the bed station was located on the State Shellfish Reserve. The float station at Oyster Bay was placed over the oyster beds of Mr. LeRoy Patterson; the bed station was also located on his oyster beds. The Hood Canal float station was located at the mouth of the Quilcene Bay near the Washington State Shellfish Laboratory at Point Whitney; the bed station was established on the public beach adjacent to the laboratory.

Before the shellfish were placed in their respective stations, samples were taken to determine the initial level of infestation by *Mytilicola*. Initial levels were: 14.1 per cent at Humboldt Bay, 32.9 per cent at Yaquina Bay, and 1.0 per cent at Oyster Bay.

The stations were located in their respective areas primarily because of the availability of facilities for sampling and relative isolation from the public and are not necessarily representative of the area. It would have been preferable to have had a common population of shellfish at all five stations, but this would have necessitated the interstate transplantation of shellfish with the inherent danger of introducing disease organisms or predators into new areas. It was decided, therefore, to populate each station with shellfish from the particular state in which it was located.

Since little information on the biology of *M. orientalis* was available, much of the preliminary work was based on the assumption that its biology is similar to its European counterpart, *M. intestinalis*. Since *M. intestinalis* has been found to exhibit positive geotaxis (Korringa and Lambert, 1951; Andreu, 1963), both float and bed stations were used in this study to ascertain whether the same condition exists with *M. orientalis*.

The stations were checked at approximately two-week intervals. In the float, each end basket on the bottom row was designated a "mortality" basket from which no shellfish were removed. In the bed station, the mortality baskets were placed at each end of the rack. At each station check, the shellfish in the baskets were examined and deaths recorded. The remaining six baskets were designated as sampling baskets and two oysters were removed from each basket at every station check. At Humboldt Bay a like number of Pacific oysters (12) were removed from the bed near the bed station. At Yaquina Bay, a like or greater number of Pacific oysters were tonged from the nearby bed.

At each check the surface temperature was recorded and a water sample was taken for salinity determination.

The individuals selected for gross examination were processed by first calculating a Modified Condition Index (MCI) on each individual. The MCI was determined by the wet weight of the meat (gms) to the volume of shell cavity (mls),

[4] Chew, K. K. 1961. The growth of a population of Pacific oysters (*Crassostrea gigas*) when transplanted to three different areas in the State of Washington. Ph.D. Thesis, Univ. of Washington, 178 p.

multiplied by 100. This is a modification of the usual procedure for condition determinations by other researchers who utilize the dry weight rather than the wet weight (Medcof and Needler, 1941). After weighing, the meat was packaged in cheesecloth and immersed in a solution of 9 parts 95 per cent isopropyl alcohol and one part glacial acetic acid. After one week, the tissue was firm and dissection for parasites was facilitated.

When designing this experiment, it was realized that there would be individual variation between oysters in a sample when the MCI was taken, but since it was desired to relate this variation to infestation by *Mytilicola,* the authors thought the procedure to be justified if a consistent relationship between the wet and the conventional dry weights for Condition Index determinations could be established. Before initiating the project, the wet and dry weights of several groups of Pacific oysters were determined. After each oyster was opened and excess moisture removed, the meat was weighed and then oven-dried until a constant weight was reached. The wet and dry weights were plotted and, as shown in Figure 1, a decided linear relationship resulted. Drying meats was impractical since the oysters were to be dissected.

The entire digestive tract was opened and any copepods encountered were placed in a petri dish containing a small amount of fixative. The copepods were measured and the length recorded to the nearest millimeter (excluding the egg cases of the female). Dissection was carried out under a low-power, illuminated magnifying lens to help locate the smaller copepods.

Incidence of infestation is defined as the per cent of the population (or sample) infested by *Mytilicola.* Intensity of infestation refers to the number of parasites per infested host.

INCIDENCE AND INTENSITY OF INFESTATION

Humboldt Bay

Pacific oysters in Humboldt Bay were most heavily infested at the float station (27.8 per cent; 5.3 parasites per infested host), next heaviest in the bed baskets (24.3 per cent; 4.6 parasites per infested host) and less heavily at the natural bed station (16.3 per cent; 2.2 parasites per infested host). Chi-square tests showed significant differences at the .05 level in the relatively low incidence of infestation which occurred at the natural bed station.

The incidence and intensity of infestation of *M. orientalis* in *C. gigas* is shown in Figure 2A for the float station, Figure 2B for the bed station,

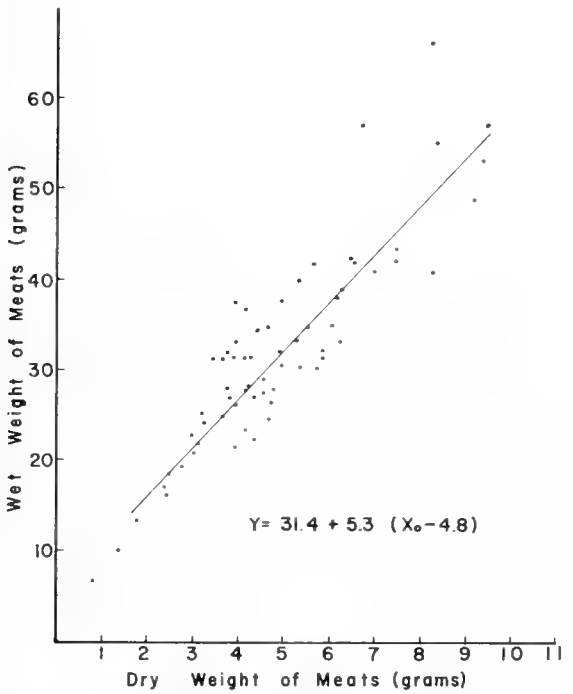

FIG. 1. *Relation between wet and dry weights of Pacific oysters.*

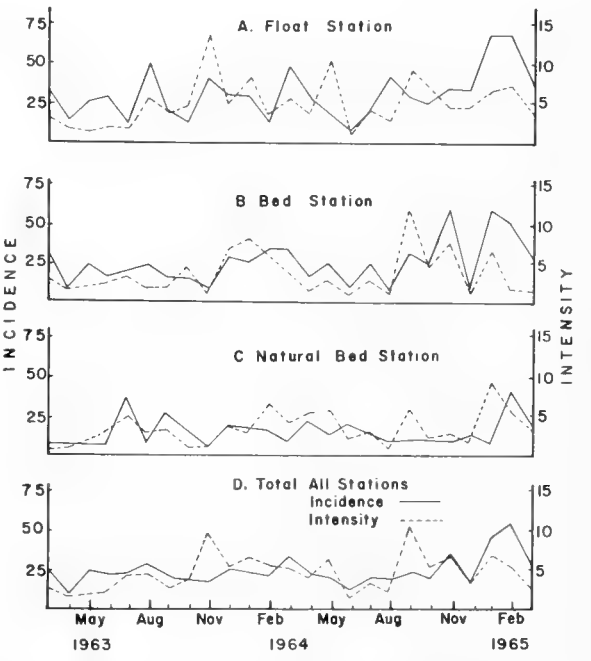

FIG. 2. *Humboldt Bay: Incidence and intensity of infestation by* M. orientalis *on* C. gigas.

Figure 2C for the natural bed station and Figure 2D for all stations combined.

Yaquina Bay

The most heavily infested oysters at Yaquina Bay were found at the natural bed station (32.7 per cent; 4.0 parasites per infested host) and least heavily infested in the float station (31.6 per cent; 3.2 parasites per infested host). The chi-square test showed the incidence of infestation at both stations not to differ significantly at the .05 level.

The incidence and intensity of infestation of Pacific oysters are shown in Figure 3. Considerable monthly variation exists, but no short-term cyclic relationships are evident. The intensity of infestation follows the incidence of infestation in magnitude.

Willapa Bay

At Willapa Bay the most heavily infested oysters (18.2 per cent; 6.1 parasites per infested host) occurred at the bed station while the less heavily infested oysters were found at the float station (16.7 per cent; 4.2 parasites per infested host). The difference in the incidence of infestation between the float and bed station, as confirmed by the chi-square test, is not statistically significant at the .05 level.

A rather definite increase in incidence of infestation is evident, especially at the float station (Fig. 4). The intensity of infestation built up steadily with the typical fluctuations from the beginning of the study until January, 1964, when an intensity of 22.5 parasites per infested host was reached (Fig. 4A), the highest recorded in any area during this study. The intensity then dropped off and continued at a rather steady level until the end of the study. The average intensity of infestation from April, 1963 to January, 1964 was 7.3 parasites per infested host and from February, 1964 to March, 1965, 3.3 parasites per host. The high initial intensity is explained by a combination of a relatively low incidence of infestation coupled with a few very heavily infested individuals.

The same trend in incidence of infestation was evident in the bed station until December, 1964, but then leveled off. The intensity of infestation built up from the beginning of the study until November, 1964 and then declined.

Hood Canal

At Hood Canal the bed station had the most heavily infested oysters (9.4 per cent with 1.7

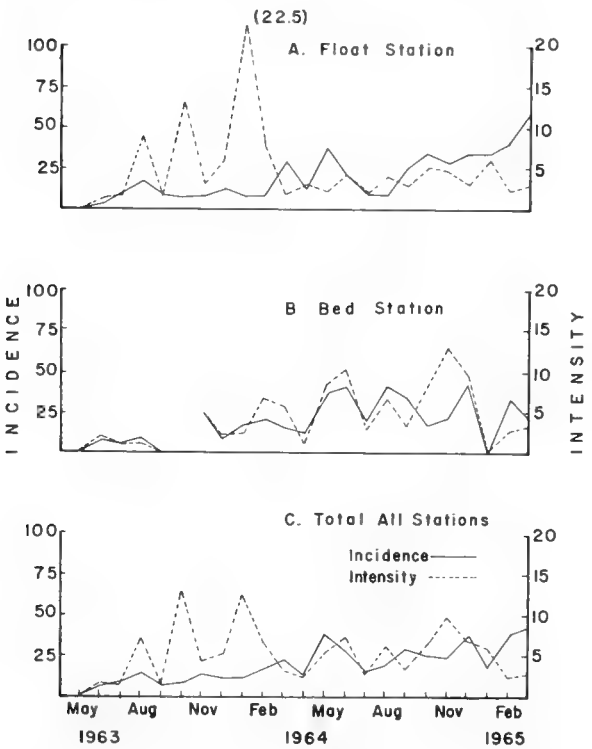

FIG. 3. *Yaquina Bay: Incidence and intensity of infestation by* M. orientalis *on* C. gigas.

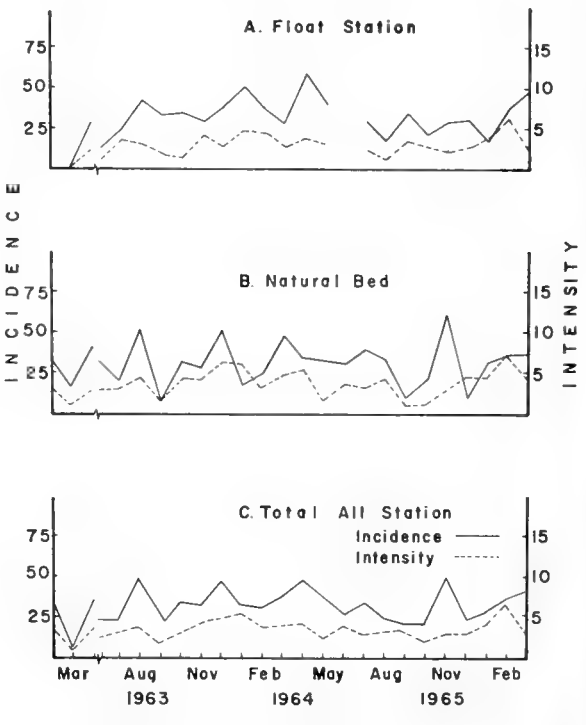

FIG. 4. *Willapa Bay: Incidence and intensity of infestation by* M. orientalis *on* C. gigas.

parasites per infested host) followed by the float station (6.4 per cent infestation with 1.4 parasites per infested host). The difference in the incidence of infestation between the bed and float stations is not statistically significant at the .05 level.

Figure 5 shows that the incidence and intensity of infestation at both the float and bed stations remained relatively low with minor fluctuations and with the maximums never exceeding 25 per cent. The intensity of infestation also remained low and followed the incidence of infestation rather closely. No cyclic trends of infestation were noted in either the incidence or intensity of infestation.

Oyster Bay

Oysters of the bed station at Oyster Bay were infested at slightly higher levels (4.5 per cent; 1.1 parasites per infested host) than those in the float (4.1 per cent; 1.9 parasites per infested host); however, these differences were not significant. Figures 6A and B show the low, sporadic incidences of infestation which occurred at both the float and bed stations.

Discussion

The natural bed station in Humboldt Bay is the only location where significant differences in infestation levels were found for Pacific oysters at the different stations within an area. Since the oysters in the float were always submerged and the oysters at the bed station were exposed at any tide below plus 2.0 feet, a first impression might be that exposure may account for the difference, but again, this difference was not found in any other area. The oysters in the bed baskets were suspended off the bottom and water circulated more freely around them than those of the natural bed and this possibly increased the chance of infestation. The natural bed oysters at Yaquina Bay showed no differences in infestation levels from the float station (however, the bed oysters were taken from a subtidal bed and were never exposed). It therefore seems rather doubtful that the differences in infestation levels in the float, bed, and natural bed sites in Humboldt Bay can be attributed to exposure. Since the natural bed oysters at Humboldt Bay are well delineated by year class, it is unlikely that more than one year class was sampled. It appears that this difference is a random one and could be expected in any area on occasion. It is a well-known fact that plankton tends to occur in patches (Sverdrup, Johnson, and Fleming, 1957) and it is possible for a group of infestive larvae to be concentrated near adjacent groups of oysters to a greater or lesser degree by currents or other hydrographic

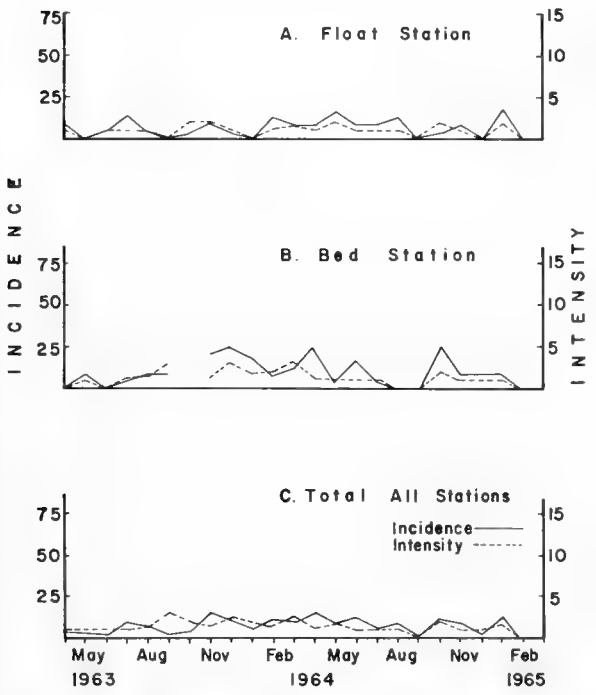

FIG. 5. *Hood Canal: Incidence and intensity of infestation by* M. orientalis *on* C. gigas.

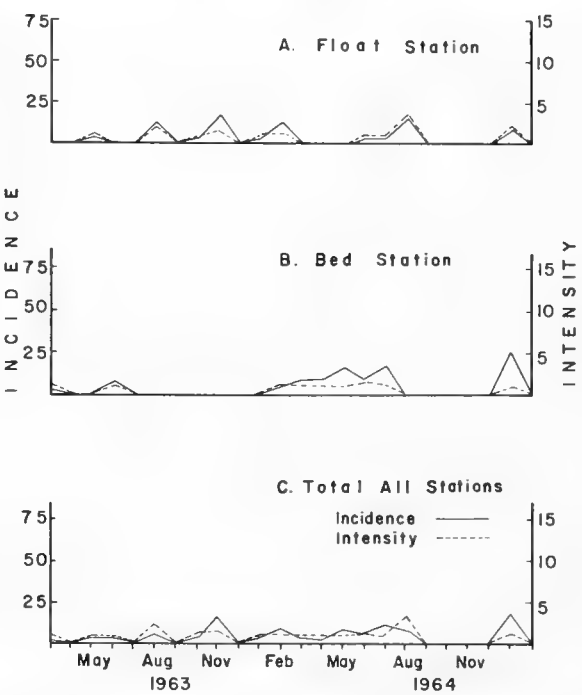

FIG. 6. *Oyster Bay: Incidence and intensity of infestation by* M. orientalis *on* C. gigas.

conditions. This effect would not be as apparent in the experimental baskets since the oysters are more closely associated than under natural conditions.

Sparks (1962a), using stated limited data, found sharp peaks of infestation in the spring and fall followed by a rapid decline of infestation rates. This was not found to occur in this study; instead, there was continuous infestation throughout the year with no short-term cyclic relationships. Variations in the incidence of infestations were noted from month to month at all three stations, but when the data were combined, these variations seemed to be somewhat smoothed out. The intensity of infestation appears to follow the incidence of infestation quite closely. This technique of combining data is valid when no significant differences are noted in the incidence of infestation between float and bed stations but perhaps is not valid when there are significant differences.

Since there is only one instance in which bed samples exhibited a significantly higher percentage of infested oysters, it is concluded that *M. orientalis* does not exhibit positive geotaxis (at least when considering *C. gigas)* as does *M. intestinalis.* Other data collected at these stations during the study period show the same pattern for *O. lurida;* however, experimental populations of *M. edulis* were almost without exception significantly more heavily infested at the bed station.

EFFECT OF PARASITISM ON PACIFIC OYSTERS

Of particular interest, especially from the industry's point of view, is the effect of parasitism by *Mytilicola* on the fatness of Pacific oysters. This factor was investigated using Pacific oyster data from Humboldt Bay, Yaquina Bay, and Willapa Bay. Since oysters from the other two stations were only lightly infested, it was decided to omit them from this analysis.

MCI of infested oysters in each sample was compared to the average MCI of the uninfested oysters in that sample and the direction of variation from the average MCI of uninfected oysters noted.

There were 1,123 uninfested oysters sampled for condition from the Humboldt Bay station, of which 583 (51.9 per cent) were below the average MCI for uninfested oysters; the chi-square test showed this ratio not to differ significantly from the expected 50 per cent—50 per cent ratio. Of the 324 oysters infested by *Mytilicola,* 222 (68.5 per cent) were below the average MCI for the uninfested group. In comparing this ratio to the ratio of oysters below and above the uninfested MCI, a value was found at the .05 level indicating a significant proportion of infested oysters below the MCI of uninfested oysters.

At Willapa Bay, 584 uninfested oysters were sampled, of which 293 (50.2 per cent) were below the average MCI; this ratio did not differ significantly from the expected 50 per cent—50 per cent ratio. Of the 152 infested oysters, 106 (69.7 per cent) were found to be below the average MCI of uninfested oysters. This ratio was significant at the .05 level, indicating, as at Humboldt Bay, that a highly significant proportion of infested oysters were below the MCI of uninfested oysters. Similarly at Yaquina Bay, 768 uninfested oysters were available of which 404 (52.6 per cent) were below the average MCI. The chi-square test showed this ratio not to differ significantly from the expected 50 per cent—50 per cent ratio. But of the 371 infested oysters, 230 (62.0 per cent) were below the average MCI of uninfested oysters which again, was of significance at the .05 level. It appears clearly evident that at the levels of infestations encountered at the above stations, infestation by *Mytilicola* definitely influences the condition of Pacific oysters.

Evidence that the MCI of infested oysters is lowered can be shown another way. Since wet weights of oysters had to be used in computing the MCI values, it may not be entirely accurate to compare MCI values or to combine these values from one sample to the next due to possible unequal draining and, therefore, variations in weight. Thus an average MCI was computed for noninfested oysters in each sample as well as an average MCI for infested oysters. The per cent change was then noted for the infested oysters and averaged over all samples.

There were 58 samples taken at Willapa Bay that contained infested oysters; of these, 45 showed a decrease in average MCI and 13 samples showed an increase in average MCI. The average change in MCI in all samples was a 9.5 per cent decrease.

At Humboldt Bay there were 112 samples of infested oysters, of which 80 showed a decrease in average MCI and 32 showed an increase. The average change in average MCI in all samples was a 5.2 per cent decrease.

Similarly, of the 80 infested oyster samples from Yaquina Bay, 55 showed a decrease in average MCI and 25 showed an increase. The average change over all samples was a 3.3 per cent decrease.

If infestations by *Mytilicola* affect the condition of oysters, it is possible that the growth of the oysters, as manifested by the increase in shell size, could also be affected. To test this hypothesis,

the Pacific oysters from Humboldt, Yaquina, and Willapa Bays were measured. Since the oyster exhibits compensatory growth (that is, grows wider when growth in length is physically retarded or vice versa), the product of the length and width was used as an indicator of shell size. The size of infested oysters in each sample was compared to the average size of the uninfested oysters in that sample and the results compiled.

It was found that at Humboldt Bay 50.1 per cent of the infested oysters were smaller than the average of the uninfested group. At Yaquina Bay, this ratio was 53.2 per cent and at Willapa Bay it was 63.6 per cent. The chi-square statistic showed that only at Willapa Bay were the infested oysters significantly smaller at the .05 level than their noninfested contemporaries. It should also be remembered that the greatest effect on the MCI was observed at Willapa Bay.

Apparently little if any effect on shell growth is caused by infestation with *Mytilicola*. When an effect is noted and the infested oysters are smaller, it is difficult to say whether the condition is a cause or an effect. The presence of the copepods in themselves might not cause a reduced growth rate as such, but the smaller, and possibly weaker, oysters may be the ones that are more susceptible to infestation.

THE EFFECT OF INFESTATION BY *MYTILICOLA* ON THE SURVIVAL OF OYSTERS

The effect of infestation of *Mytilicola* on the survival of oysters is especially important in light of the high mortalities caused by infestations of mussels by the related species, *M. intestinalis*, in Europe and the effect of *M. orientalis* on *O. lurida* described by Odlaug (1946) and Rankin (1943)[5].

The study was initiated by computing a mortality rate by a period of time and plotting it against the incidence of infestation for the same period. Each period was approximately one month beginning with the first sampling day early in 1963. When graphed, these data showed no definite pattern; the periods were combined in a variety of ways and an attempt was made to relate mortality with heavy infestations occurring at an earlier period but still the same apparent random scatter of points occurred. Finally, the data were subjected to analysis using a weighted regression computer program (FRG 703) available from the Fisheries Research Institute of the University of Washington. The incidence of infestation per period was designated the independent variable "X" and the mortality rate per period was designated as the dependent variable "Y". The program computes the "T" test to test the hypothesis $b_1 = B_1$ (that is, the slope of the regression line, b_1, is not significantly different than a designated value, B_1); as well as the parameters to determine the regression line to be fitted to the data. In this case, B_1 was set equal to zero to determine if the regression line had significant slope or was parallel to the X axis. If the regression line were indeed parallel to the X axis, it could be concluded that there was no evidence that infestation by *Mytilicola* had any effect on the survival of Pacific oysters. As was expected from the apparent random scatter of points when plotting incidence of infestation against mortality, no significant slope was found to any of the regression lines (since no effect on survival was found, the graphs were omitted in the text).

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EFFECTS OF REPEATED SHELL DAMAGE ON GAMETOGENESIS IN THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN) [1]

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ABSTRACT

A sample of oysters from an upper Potomac River oyster bar was randomly divided into four groups and held in the laboratory. The shells of groups 1 and 4 were repeatedly filed in order to force the continuous deposition of new shell. Groups 1 and 2 were held in running, unfiltered Patuxent River water. Groups 3 and 4 were held in running water filtered to severely limit their food intake.

Histological sections were made from 10 oysters of each group at the completion of the experiment eight months later, and levels of gonad development were compared. The fed oysters revealed considerable gonad maturity compared to the oysters with limited food intake. A less obvious difference was observed between the filed and the undamaged groups, although some retardation in gametogenesis was indicated when filing was accompanied by limited food intake. Both filed groups showed an unusually high ratio of males to females.

INTRODUCTION

The capability of the American oyster, *Crassostrea virginica* (Gmelin), to repair damaged portions of its shell is well known. Shell secretion is stimulated by mantle injury even at cold temperatures when normal shell deposition and growth are not occurring (Galtsoff, 1964). Removal of the recently formed growing edge initiates rapid replacement with new shell until original size is attained, whereupon shell growth slows to a normal rate (Loosanoff and Nomejko, 1955).

Defensive shell deposition required of oysters after infestation by foreign organisms, such as mudworms (*Polydora*) or boring sponges (*Cliona*) has been blamed for detracting from the oysters' condition in certain cases (Korringa, 1952). The continuous energy expenditure required to block the encroaching worm with new shell apparently reduces the ability of oysters to store food reserves and results in a thin watery appearance of the oyster tissue.

Since normal oyster gametogenesis appears to demand an extensive energy output which is expressed in a marked reduction of stored glycogen in early summer (Mitchell, 1917), it was theorized that the energy required to repair continuously damaged shell might slow or inhibit normal spring gonad development. The following work was initiated as a test of this hypothesis.

MATERIAL AND METHODS

During the second week of May 1964, oysters ranging in size from 50 to 100 mm in length were

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obtained from an upper Potomac River oyster bar and held for two months in a tray suspended in the Patuxent River at the laboratory at Solomons, Maryland. In July, they were introduced into the running seawater system in the laboratory.

On 6 August 1964, 240 of the oysters were selected randomly and divided into four equal test groups. Two adjoining 230-gallon tanks were set up to hold the experimental oysters, two groups to a tank. In the first tank, running Patuxent River water was supplied continuously — the rate varied from 1.5 to 10 gal/min, the average flow being closest to 1.5. In the second tank, the filtered water supply ran at approximately 1 gal/min. The filter was a No. 20 plankton net (mesh size 0.076 mm). Aeration was provided for both tanks. Filtration over a long period of time proved quite difficult and resulted in variations in the quality of the filtered water. The relative difference of available food in the two tanks provided enough contrast, however, to produce measurable growth variation.

On 10 August 1964, all oysters in one test population from each tank were filed along their growing edges in the manner of Loosanoff and Nomejko (1955), thus removing all recent shell accumulation. Care was taken not to remove too much shell, so that the oysters could still close effectively. Each of the four groups was assigned a number. The tank receiving unfiltered water held groups 1 and 2, and the tank with the filtered water held groups 3 and 4. Groups 1 and 4 were filed, so that the fed oysters consisted of a filed and unfiled group, as did the oysters with limited food. Groups 1 and 4 were refiled each week when active shell secretion warranted it. Refiling consisted of removing new shell back to the original filed edge. From the middle of October to the end of April, there was very little new growth and, therefore, no oysters were filed. Filing began again on 7 May 1965.

Oysters were removed from each group periodically so that the state of gonad development could be monitored. On 18 June 1965, 10 oysters from each group were fixed in Zenker's fixative for imbedding and subsequent histological preparation. Staining of the tissues was with iron hematoxylin and eosin.

RESULTS AND DISCUSSION

Marked retardation of the gonads occurred in both groups of oysters subjected to limited feeding. The two "fed" groups exhibited mature gonads with a slightly higher degree of development of filed over unfiled oysters. A comparison of the two starved groups suggested a slight difference in maturation in favor of the unfiled oysters (Fig. 1).

TABLE 1. *Observed sex ratios between experimental and control groups of oysters.*

	Male	Female	Undetermined
Undamaged Fed	3	7	—
Damaged Fed	8	2	—
Undamaged Starved	4	4	2
Damaged Starved *	7	1	1

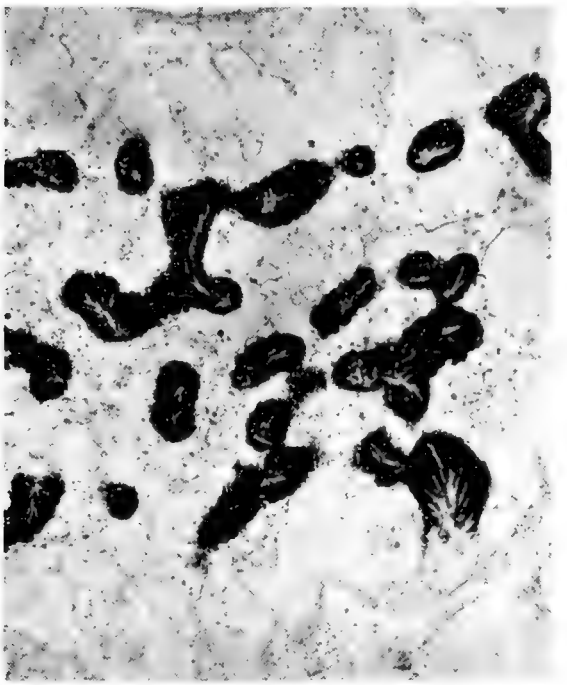
* One of these oysters was lost during histological processing.

An interesting relationship between filing and occurrence of males was observed. Examination of 10 oysters from each of the four groups showed that males seemed to predominate when the oysters are filed (Table 1).

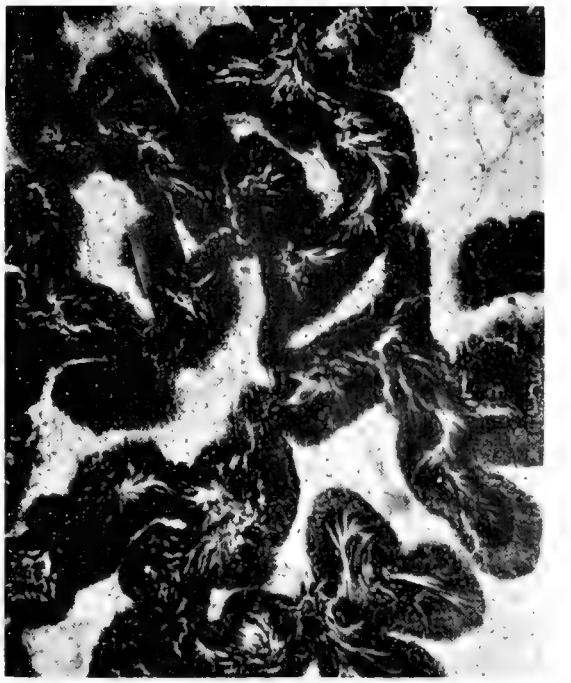
The results of this investigation do not necessarily bear out the hypothesis that energy will be expended in favor of repair of damaged shell over gonad development. That gonad development was greater in the damaged group when food was not a factor is quite interesting and can lead to speculations. It may be that the damage done to the oysters' shells represents a stress factor which stimulates a species survival mechanism leading to gonad maturation. If this is true, then perhaps gonad maturation at other than normal spawning seasons in mollusks being used in laboratory-rearing programs represents a response to an unrecognized stress.

The fact that there seems to be a predominance of males among the damaged oysters is also interesting, although not unprecedented. Galtsoff (1964) cites a paper by Amemiya (1936) in which it was demonstrated that an increased proportion of males of *Crassostrea gigas* resulted from the removal of one-third of the gill lamellae during the sexually indifferent phase after spawning. It can be reasoned that regardless of the amount of food available to an oyster, it can only utilize a given amount, and that its energy supply is therefore limited. Gametogenesis in both sexes demands nucleic acid synthesis, and oogenesis also requires mobilization of large amounts of lipid and protein for storage (Giese, 1959). Hence, de-

FIG. 1. *Histological sections through the gonads of representatives of the four groups of oysters showing degree of gonad development. All oysters shown here are males.* UF — *Undamaged, Fed;* DF — *Damaged, Fed;* US — *Undamaged, Limited Feeding;* DS — *Damaged, Limited Feeding.*



U F



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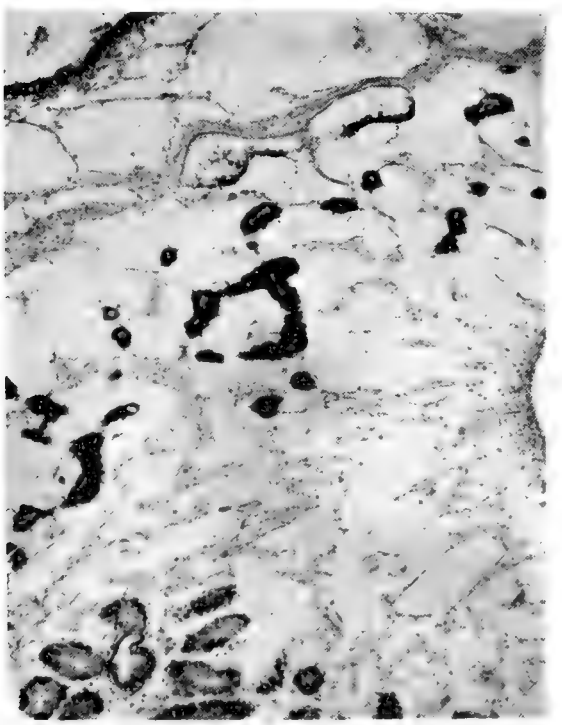

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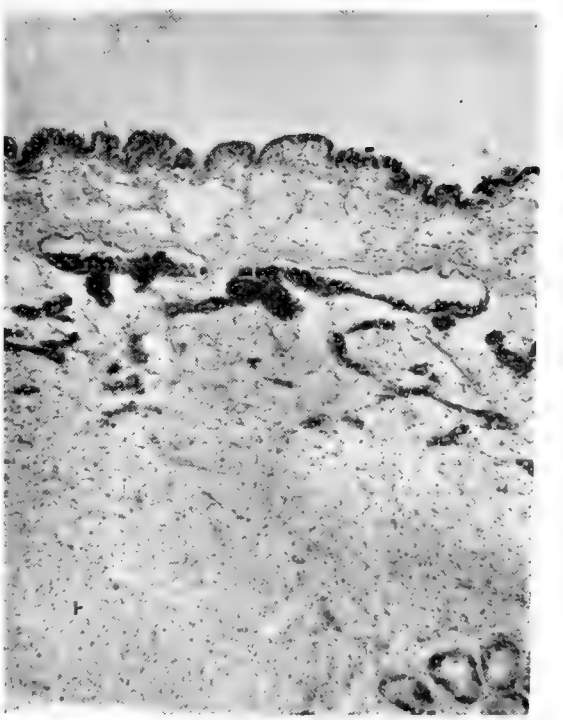

D S

velopment of the male gonad probably requires a smaller energy expenditure than that of the female. If the stress of damaged shell simultaneously initiates gonad maturation and shell repair, and the available energy is shared by these two processes, then the production of sperm would be favored. However, the role of sex hormones and the effect of stress upon them were not investigated in this study.

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

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ABSTRACT

Accidental and experimental infestation of the hard clam, Mercenaria mercenaria, *by the polychaete worm,* Polydora ciliata, *in the laboratory is described. Experimental infestation was obtained by putting free-swimming larvae of* P. ciliata *in pans with hard clams; here the larvae metamorphosed and built tubes on the clams' shells. Perforation of the shells of clams 5-8 mm long appeared within 18 days but took twice as long in clams 30-35 mm long. Calcified mud blisters appeared on the inner surfaces of the shells of all clams within 30 days after the shells were perforated. Despite considerable damage to the shells of the victims, especially small individuals, chi-square analysis of several groups of data did not consistently show statistically significant differences between the mortality of infested clams and controls.*

Clams in a sand substrate were not attacked by the worms. Several clams that became infested before they burrowed into the sand showed no signs of the worms when examined weeks later. It seems that, by burying, clams escape attack by P. ciliata *but exposed clams, especially small ones, can suffer considerable damage to their shells.*

INTRODUCTION

Several species of polychaete worms of the genus *Polydora,* including *P. ciliata,* have a well-deserved reputation as oyster pests (Korringa, 1952). *Polydora ciliata* have also been noted in other mollusks — for example, the sea mussel, *Mytilus edulis* (Field, 1922, from Lebour, 1907), the periwinkle, *Littorina littorea* (Thorson, 1946), and the bay scallop, *Aequipecten irradians* (Turner and Hanks, 1959). To my knowledge, however, this species has never been reported in the hard clam. The following account describes accidental and experimental infestations of the hard clam by *P. ciliata* in the laboratory.

During a recent winter, we found laboratory-reared clams of various sizes, from 3 to 16 mm long, infested with a species of *Polydora,* identified as *P. ciliata* by Dr. Marian Pettibone, Invertebrate Division, U. S. National Museum. The infested clams were being grown in trays of warmed, running sea water without a substrate into which they could burrow. At the time of the discovery, the infestation was well-established and most affected clams contained the U-shaped external burrows typical of *P. ciliata.* Also present, and covered with new shell material, were the mud-laden tubes of the worms which formed raised areas on the internal faces of the shells (Figs. 1 and 2).

All of the dead individuals from the largest size group (average length 13 mm) contained *P. ciliata.* Eighty-five per cent of the dead clams from the next largest size group (average length 10 mm) were also infested. Only 5 per cent of the dead clams from the 8 mm group contained *P. ciliata* and the worm was completely absent from dead clams of the smallest size group (average length 5 mm). These data suggested that the worm larvae were present in the seawater system, that they began to burrow into the clams soon after the clams were placed in running water and that the highest percentage of infestation was in the largest clams because they had been in running water the longest. It was apparent also that,

FIG. 1. *Typical U-shaped burrows of* P. ciliata *in live young clams.*

although the activity of the worms in the clams' shells may have contributed to the death of their hosts, many dead clams, especially of the smaller sizes, contained no *P. ciliata* and must have died from other causes.

These observations proved little, except that *P. ciliata* can attack clams. Left unanswered were several questions. How readily and under what circumstances does *P. ciliata* attack clams? What is the rate of penetration of the shell? How is the degree of infestation and damage to the clam related to size of the clam? Does the presence of *P. ciliata* contribute to the mortality of clams?

EXPERIMENTAL INFESTATION OF HARD CLAMS

Within 6 months after they were discovered in the clams, many of the worms became sexually mature and laid eggs. Development of the eggs and larvae followed Wilson's description (1928) for this species. Eggs were deposited in transparent sacs, each attached to the inner wall of the worm's tube by a stalk. There the eggs developed into ciliated larvae which moved about actively within the sacs. At a length of approximately 250 μ, when three setiferous segments had developed, the larvae left the egg sacs and became free-swimming. Contrary to Wilson's experience (1928), I had no trouble obtaining metamorphosis of the larvae and continued growth of the young worms.

Experiment No. 1

In the first attempt to induce experimental infestation, and thus to learn the sequence and timing of the events involved, I added early free-swimming larvae of *P. ciliata* to an enamel pan containing 6 liters of sea water and 100 clams 5 to 10 mm long. A pan of 100 control clams of similar sizes was also maintained. The water was changed 3 times a week. Mixed algae were added daily as food for the clams; it may also have been consumed by the worm larvae. In approximately 20 days, the larvae metamorphosed, constructed tubes on the outer surfaces of many clams, and began to erode the shell immediately under the tube. Within the next 10 days some worms had perforated the shell, stimulating the clams to deposit new shell material to wall off the intrusion. This group of clams was kept under observation 47 days. Erosion and damage

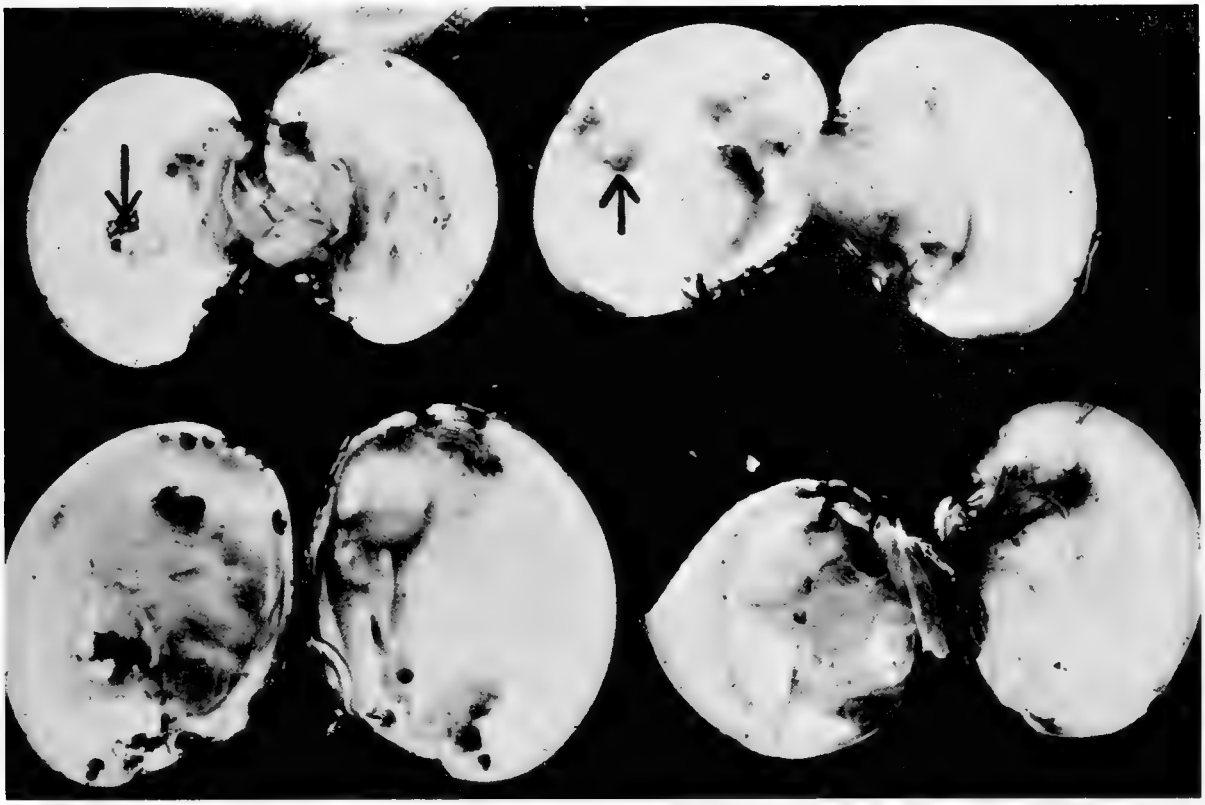

FIG. 2. *Live young clams with shell-covered mud-bubbles caused by* P. ciliata *tubes protruding through the clams' shells.*

to the clams' shells increased steadily; in a few clams the soft parts became visible through the damaged shells. During this period, 17 clams in the pan with *P. ciliata* died; all contained live worms and their muddy, shell-covered tubes. One clam died in the control pan. Chi-square analysis showed the difference in number of dead clams between treated and control groups to be significant at the 95-per cent confidence level.

Experiment No. 2

The second experiment was designed to learn whether *P. ciliata* attacks clams that have dug into the substrate. One hundred clams 5 to 10 mm long were allowed to dig into sand in a 6-liter enamel pan after which early free-swimming larvae of *P. ciliata* were added. A second pan of clams with worm larvae but without sand and a third pan with clams only were included in the experiment. By the fourteenth day worm larvae in the pan without sand had metamorphosed and had built tubes on 30 of the 100 clams. In the pan with sand, worm tubes were present on all of the clams which were still on the surface. Shortly thereafter, these 6 clams dug into the sand.

All of the clams were removed and examined at the end of 2 months. Of those in the pan with worms but no sand, 16 clams had perforated shells and at least one well-calcified mud pocket, each containing a worm. The remaining 84 clams were free of worms. Apparently, some of the worms that had originally set on the 30 clams not only failed to continue their attack but moved away. None of the clams in the pan with sand contained *P. ciliata* at this time and only 5 showed any sign of past infestation. Mortality was negligible in all pans.

Experiment No. 3

This experiment was performed to determine if the rate of infestation by *P. ciliata* or the extent of damage and death was related to the size of the clams. Twenty clams averaging 32 mm long, 100 averaging 8 mm long, and 100 averaging 5 mm long were placed in separate 6-liter pans together with equal numbers of worm larvae. Controls were maintained for each size-group of clams.

Shells of the 5-mm group of clams were perforated within 18 days after the worm tubes appeared; those of the 8-mm group were perforated within 20 days. In the 32-mm group of clams, however, shells were not perforated before the thirty-ninth day. The shell-covered, muddy bulges on the inner faces of the shells were present in all size groups within 30 days after perforation.

Six months later at the end of the experiment all 20 of the clams in the 32-mm group of controls were alive. Seventeen of those exposed to *P. ciliata* were alive and all contained at least 1 live worm, as had the 3 clams which died during this period. Most of the infested clams exhibited both shell perforations and the internal signs of worm activity but the areas of damage were a small part of the total shell area.

Eighty-three clams in the 8-mm control group and 71 clams in the group exposed to worm larvae were still alive at the end of 6 months. Twenty of the exposed clams contained *P. ciliata* or showed signs of former infestation. Those that did contain live worms also contained muddy areas newly-coated with shell material over a considerable part of the inner surface of the shells. The affected clams appeared to be healthy otherwise and siphoned as vigorously as the controls.

Of the smallest clams (5 mm), 84 controls and 71 of the clams exposed to worm larvae were alive when the experiment ended. Thirty-five of the exposed clams contained worms, all of which had internal, recently-calcified, muddy areas occupying more than half of the inner shell surface of the victims. In a few of the affected clams, the soft parts were visible through breaks in the shell caused by the burrowing of the worms. These clams, nevertheless, pumped actively and gave no sign of distress.

In none of the 3 groups of clams was the difference in number of dead clams between exposed and control lots statistically significant at the 95-per cent confidence level, although the chi-square value approaches significance in each group.

ROLE OF *POLYDORA CILIATA* AS AN EPIZOON

Polydora ciliata is known to be a boring species as contrasted to *Polydora websteri*, the common "mud blister worm" in oysters, which apparently is not (Korringa, 1952). Opinions in the literature differ, however, as to whether *P. ciliata* makes mud blisters on the inner surfaces of its hosts' shells. Korringa (1952) stated that *P. ciliata* does not make mud blisters in oysters. It burrows into the shell from the outside and only occasionally perforates it. The oyster seals off the occasional perforations by laying down small amounts of additional shell material which do not form blisters. Turner and Hanks (1959), on the other hand, found "calcified blisters characteristic of *Polydora* along the inner edge of both shells" in the bay scallop, *Aequipecten irradians*. My own observations of *P. ciliata* in clams show that the clams' attempts to seal off the extensive perforations produce large, raised, calcified, soft bubbles immediately over the worms' mud tubes on the inner surfaces of the shells; these structures can accurately be called "blisters".

The experiments reported here show that buried clams are not susceptible to attack by *P. ciliata*. The fact that worms leave infested clams that burrow into the substrate indicates that a subsurface existence is intolerable to *P. ciliata*.

The effect of the burrowing worms in the clams' shells on the well-being of the victims is not entirely clear. Statistical tests of the experimental data are inconclusive as to the significance of the differences between the death rates of clams exposed and those not exposed to *P. ciliata*. It is possible, however, that extensive shell damage, and the energy required to repair it, weakens the victims and may in time make them more susceptible to disease or predation.

ACKNOWLEDGMENTS

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POLYDORA INFESTATION OF ARCTIC WEDGE CLAMS: A PATTERN OF SELECTIVE ATTACK [1]

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ABSTRACT

The boring polychaete, Polydora websteri, *has been found frequently infesting the arctic wedge clam,* Mesodesma deauratum, *in the St. Lawrence Estuary. No similar infestation of the southern arctic wedge clam,* Mesodesma arctatum, *has been found, although fossil specimens of the latter bivalve found in Cumberland, Maine, were infested in Pleistocene times. Inasmuch as both bivalves have essentially identical habitats and the boring polychaete is known within the range of both clams, it seems possible that climatic and environmental variation may influence distribution, intensity and selectivity of* Polydora *infestations.*

INTRODUCTION

Two species of arctic wedge clams, *Mesodesma deauratum* (Turton) 1822, and *Mesodesma arctatum* (Conrad) 1831, are found in the western North Atlantic. The northern species, *M. deauratum,* has a range known to extend from Cape Gaspé, Québec, to the Strait of Belle Isle and may be found on some Newfoundland shores. The southern species, *M. arctatum,* occurs from Cape Gaspé, Québec, southward to northern New Jersey. These areas are shown in Figure 1. Distributional patterns have been determined by careful study of material from the National Museum of Canada, the Museum of Comparative Zoology, the American Museum of Natural History, the Academy of Natural Sciences of Philadelphia and the United States National Museum. This work has been supplemented by several collecting trips ranging from the north shore of the St. Lawrence Estuary to northern New Jersey. Although there is apparently little distributional overlap, the habitats of the two species are essentially alike — coarse, sandy bars and banks at the mouths of rivers, streams and tidal inlets. Neither species occurs in waters much deeper than 50 fathoms.

Separation of northern and southern species often seems indistinct, but studies conducted by the author since 1961 indicate that the two species can be separated by plotting dimension ratios using a straight-line regression analysis. Both species are characterized by relatively truncate posterior ends. This means that the posterior margin of the shell drops abruptly from the umbo to the ventral margin, creating a "sawed-off" appearance. The ratio resulting from comparison of total length to length of the portion posterior to the umbo is different in each species, and comparison of their respective ratios shows that the southern species, *M. arctatum,* is significantly more truncate than the northern species. Populations of the two species have been compared using this type of analysis. These studies have consistently shown a significant difference in the slope of the lines plotted for each species. Results have been conclusive enough to permit recent review of the taxonomy of each species coupled with descriptions of new lectotypes (Davis, 1964; 1965). These recent papers provide the basis for separating the northern and southern species at this time.

Early in these systematic studies it was noted that the northern species, *M. deauratum,* commonly showed extensive boring damage at its truncate posterior end. In 1965, living specimens

[1] Contribution number 249 from the Smith College Department of Zoology.

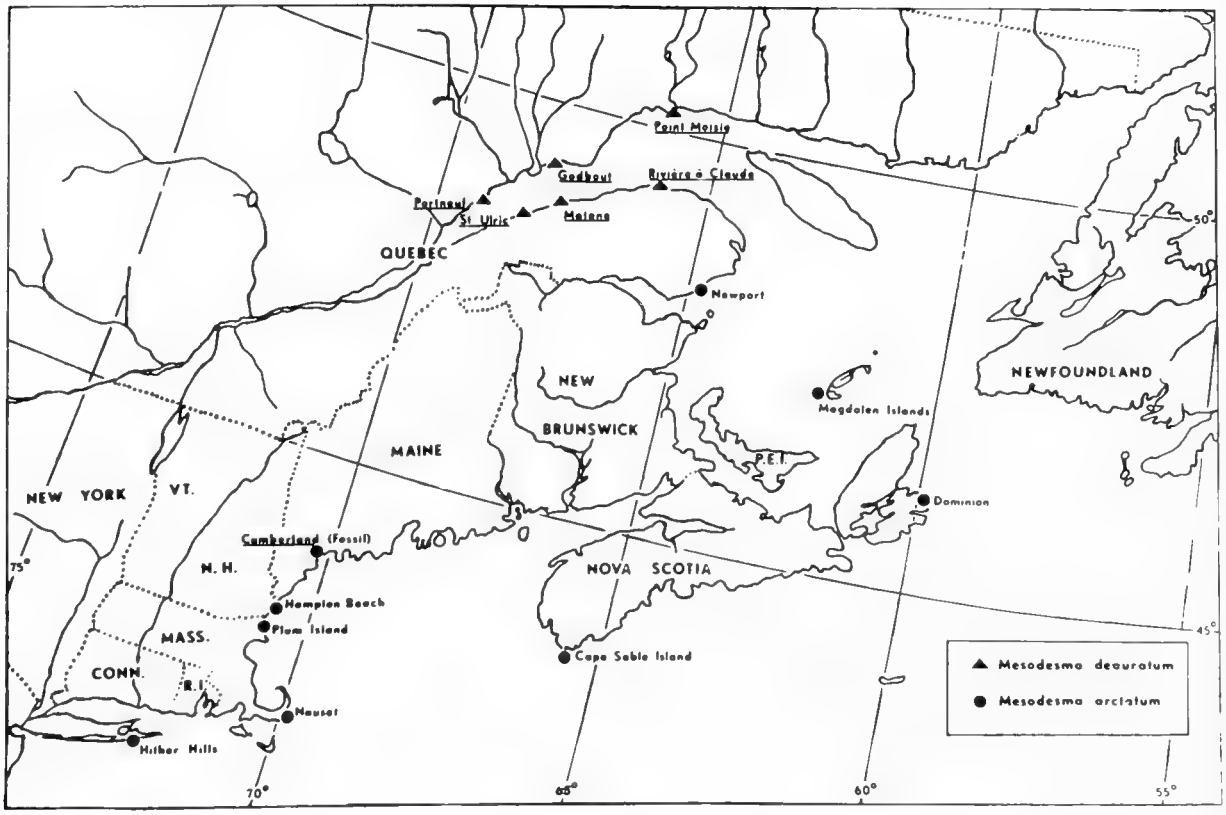

FIG. 1. *Map showing location of populations sampled and studied. Underlined names designate localities where evidence of* Polydora *infestation has been found. Species identified by symbols as indicated.*

of the boring polychaete worm, *Polydora websteri* Hartman 1943, were extracted from shells in a dense population of *M. deauratum* at Rivière à Claude, Québec, on the south shore of the St. Lawrence Estuary. No previous mention of this relationship was found in the literature although subsequent investigation has shown that the relationship is general throughout the Estuary. Its extent in the Gulf of St. Lawrence is unknown, but apparently *Polydora* does not ordinarily infest the southern species, *M. arctatum*, even though *P. websteri* does occur within its distributional range (the type locality of *P. websteri* is Milford, Connecticut — Loosanoff and Engle 1943). Nevertheless, examination of nearly 10,000 living specimens of *M. arctatum* since 1961 has not disclosed a single specimen infested by *Polydora*. Even an extremely dense population at Newport, Gaspé, only 80 air miles from Rivière à Claude, appears to be completely free from infestation.

NATURE OF THE INFESTATION

The host clams, usually less than 50 mm long, are found in dense populations (300-500 specimens/meter^2) at mouths of rivers and tidal inlets along the shores of the St. Lawrence Estuary east of Trois Pistoles and along the northern shore of the Gulf of St. Lawrence to the Strait of Belle Isle. The clams are most dense in coarse sand just below the mean low water level.

Infested clams are easily recognized by the presence of paired holes along both sides of the posterior margin and in some cases by short brown tubes extending from the holes. Each pair of openings leads to a U-shaped passage, usually somewhat twisted, extending anteriorly within the shell. It is within this excavation that the infesting worm lives. Ordinarily, the tunnels are hollowed out parallel to the surface and do not penetrate into the mantle cavity, but occasionally the worms in their tunneling efforts, do penetrate

FIG. 2. Mesodesma *shells damaged by* Polydora *infestation. Outer shell layers covering the burrows have been worn away or removed revealing the typical pattern of the excavation. Top — exterior of right valve of* Mesodesma arctatum *from a Pleistocene deposit at Cumberland, Maine; bottom — exterior of left valve of* Mesodesma deauratum *formerly infested with* Polydora websteri. *The latter valve is from a live specimen collected at Rivière à Claude, Québec, Canada, Sept. 1965. Scale in millimeters.*

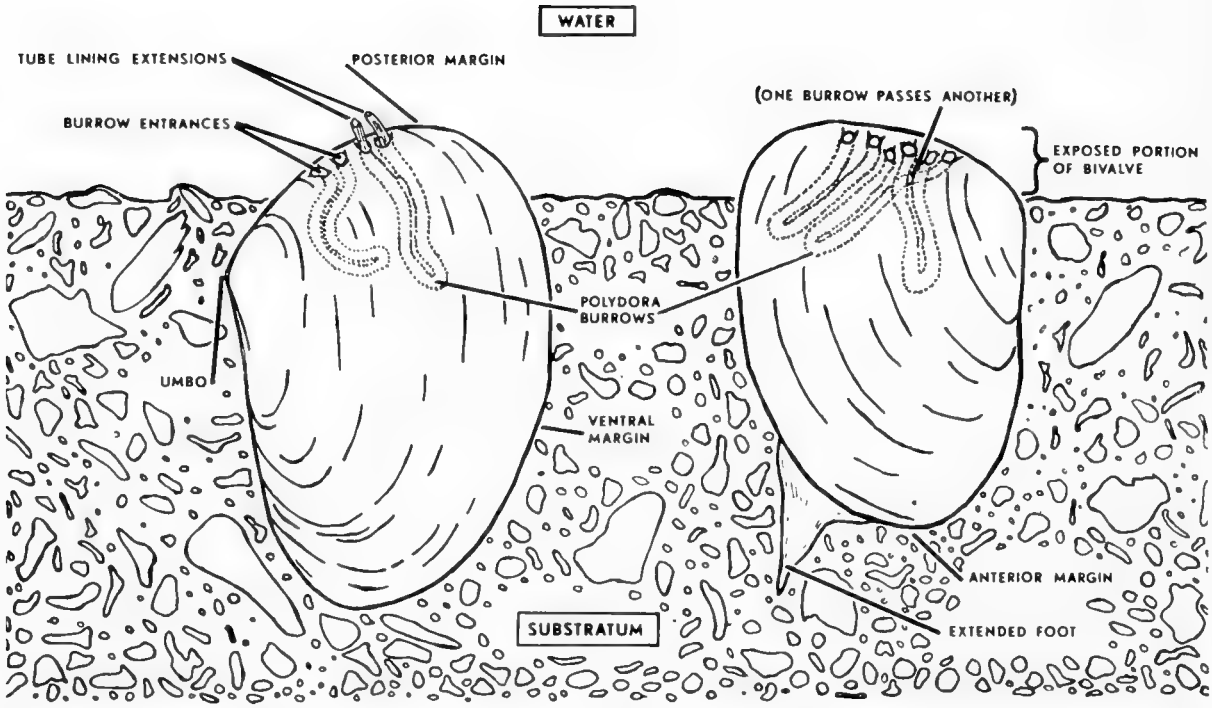

FIG. 3. *Typical position in the substratum of large specimens of* Mesodesma deauratum *showing arrangement of burrows created by the infesting* Polydora *worms. Posterior ends of the clams are uppermost and typically exposed as shown here. Larger specimens of* Mesodesma arctatum *position themselves in a similar manner in the substratum.*

through the inner surface of the valve. However, openings so formed are sealed by the clam, and neither the mollusk nor the worm appears to be harmed by the intrusion. In addition, outer layers of the valve directly over the burrow sometimes break away revealing its location. The valves shown in Figure 2 partially show this condition, but the remaining material covering the burrows was removed to display the nature of the excavations more clearly.

Examination of over 1,000 infested clams has shown all excavations confined to the area adjacent to the blunt posterior margin. Under normal conditions the bivalves position themselves in the substratum as shown in Figure 3, exposing only the blunt posterior region. Restriction of infestation to this exposed area indicates that these worms do not attack portions of clams buried beneath the surface of the substratum. The southern species, *M. arctatum*, resides within the substratum in the same fashion — with the posterior end exposed. Thus host-selectivity cannot be attributed to any difference in position of the two species within the substratum.

No evidence of infestation has been found on specimens of *M. deauratum* less than 20 mm long. This possibly indicates that small clams are immune to attack due to their relative instability in the sand. I have often watched areas densely populated with *Mesodesma* subjected to wave action. It was obvious that smaller specimens are much more easily dislodged by the incoming waves. Older, larger specimens hold upright positions more consistently than small ones. Hence, in all probability the bivalves do not suffer from attack by *Polydora* until they are large enough to hold positions effectively in the substratum.

FOSSIL EVIDENCE OF INFESTATION

In 1965 fossil specimens of *M. arctatum* were recovered from sand strata in Cumberland, Maine. The site is described in detail by Bloom (1960) who states that the area was under the waters of the Gulf of Maine during the period of marine submergence subsequent to the retreat of the Wisconsin glaciation probably somewhat

less than 10,000 years ago. A portion of this material is currently being carbon-dated at Yale University, and preliminary estimates place its age at about 8,000 to 10,000 years B. P. Most important, however, shells of *M. arctatum* from this material occasionally show boring damage identical to that seen on specimens of the northern species today. A valve from this material is shown in the upper photograph of Figure 2.

DISCUSSION

It is not known what species of polychaete worm attacked *M. arctatum* in Pleistocene times, but the similarity of burrows, as shown in Figure 2, strongly suggests that both the Pleistocene and current infestations can be attributed to the genus *Polydora*. Apparently, one or more species of *Polydora* infested *M. arctatum* in the western Gulf of Maine in Pleistocene times, while today no evidence of similar attack on this clam can be found anywhere between New Jersey and Cape Gaspé. Yet in the St. Lawrence Estuary the closely related bivalve, *M. deauratum*, is extensively infested by *P. websteri*, a species previously known to have a distribution extending only from Connecticut to Florida (Loosanoff and Engle, 1943).

Discovery of this new infestation extends northward the range of *P. websteri* but leaves unanswered the question whether it can be found in the waters between Long Island Sound and the St. Lawrence Estuary. Other *Polydora* infestations, mostly *P. ciliata* (Johnston), have been reported from these waters (Needler, 1941; Plaine, 1952; Turner and Hanks, 1959), but there are no additional records of *P. websteri* from New England and Maritime waters. This gap may be more the result of confused taxonomy than scarcity of the species. It is quite possible that when more collecting is done and distributional records reworked *Polydora websteri* will be found inhabiting these waters.

In recent years most workers have concluded that *Polydora* infestations usually do not cause excessive mortality among bivalve mollusks. Similarly, I have not observed any unusually high death incidence among populations of *M. deauratum* heavily infested with this polychaete. In fact, large living specimens of *M. deauratum* have been collected with as many as five or six *P. websteri* living within the valves of a single clam, and no apparent harmful effects could be detected.

Nevertheless, concern is expressed whenever high infestation levels are discovered among populations of commercially important clams. Such infestations, usually sporadic and often limited in range, probably are caused and controlled by local environmental conditions.

Based on faunal and floral composition, summer salinity levels and water temperature values, Bousfield (1956) has divided the shores of the St. Lawrence Estuary and Gaspé Coast into four main zones. The first two zones are freshwater and brackish areas extending downstream about 100 miles from Québec City and need not concern us here. The upstream limit of the third zone (where the second zone ends) is somewhat west of the mouth of the Saguenay River. Its outer limit is at Cape Gaspé, the outer extremity of the Gaspé Peninsula. Bousfield describes this third area as having (at the surface) summer salinity levels of 20-30 ppt and summer surface water temperature readings of 7-14°C.

The fourth region extends southward from Cape Gaspé and is characterized by surface summer salinities of 20-30 ppt and summer surface temperatures of 15-20°C. This last area blends into even warmer waters further south.

Bousfield's observations suggest that although surface salinities show little variation north and south of the Gaspé Peninsula, temperature ranges do. In other words, the waters warm considerably more south of Cape Gaspé during the summer.

The geographical-ecological pattern formed by these third and fourth zones does coincide rather well with the distributional limits of *M. arctatum* and *M. deauratum*. The line of separation, as noted earlier, lies roughly at Cape Gaspé with *M. deauratum* to the north and *M. arctatum* to the south. If these two species of clams can be set off so markedly, possibly *Polydora* distribution and/or selectivity could be affected somewhat similarly.

In addition, variability of attack appears to have existed over a considerable period of time. Evidence of Pleistocene infestation indicates that at that time ecological conditions in the Gulf of Maine permitted *Polydora* attacks on *M. arctatum* — just as *M. deauratum* is attacked today in the St. Lawrence Estuary. Subsequently, ecological conditions changed sufficiently in the lower Gulf of St. Lawrence and in the Gulf of Maine to discourage further attack, while, on the other hand, they permitted it to continue today in the St. Lawrence Estuary. In conclusion, it is suggested that differing ecological conditions, especially water temperature, may influence not only the density of boring polychaetes but also the intensity and selectivity of their infestation. It is further suggested that these variations exist in terms of time as well as in terms of distance.

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TOPICAL LABELING OF SHELLFISH [1]

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ABSTRACT

Three successful techniques for topical labeling of commercial pelecypod shellfish and certain gastropod species have been developed and tested, under controlled and simulated natural conditions. They involve labeling with 1) Epoxy glue, 2) Spectraglo Green Tracing Powder coated with Krylon, and 3) Krylon-sprayed acetone solutions of melamine sulphonamid formaldehyde resins. Tagged shellfish are detected by fluorescence of the label under ultraviolet light.

A durable marking method for shellfish would find several immediate uses, including detection of bootlegged shellfish [2], identification of growing areas and shellstock [3], and use as a tool in biological research (Ropes, Merrill, and Groutage, 1967).

Several different labeling methods have appeared from time to time in the literature. These include use of ink (Gustafson, 1954), shell grinding or engraving (Chestnut, 1952), identification by use of small celluloid tags (Loosanoff and Nomejko, 1949) (Loosanoff and Davis, 1947), et cetera, but in a given situation, perhaps, no one method may be able to satisfy all criteria for durability, speed and application facility, volume production, or other. Thus new approaches are constantly being sought as aids in biological problem-solving.

It is essential that such an identification methodology be:

1) non-toxic to shellfish or consumer, and must be water-insoluble so as not to introduce a radioactive or deleterious substance into surrounding waters or adjacent fauna;

2) reliable, practical, and lending itself to mechanization, amenable to coding, applicable to major commercial species, flexible enough so that both visible and invisible marking can be obtained by means of reasonably simple techniques, and with no decrease in marketability of the shellfish;

3) durable enough to withstand long periods of saltwater immersion, reasonable abrasion and handling, and, in some instances, cooking or steaming temperatures.

Representative commercial species of shellfish that were used to test the labeling procedures included: *Mercenaria mercenaria* (quahaug or hard clam), *Crassostrea gigas* (Japanese or Pacific oyster), *Crassostrea virginica* (Eastern oyster), *Mya arenaria* (soft shell clam), *Protothaca staminea* (Western littleneck), and *Mytilus edulis* (edible mussel). In addition, the following species of gastropods were labeled (according to methods 1 and 3): *Thais lapillus, Busycon canaliculatum, Littorina littorea, Nassarius obsoletus, N. trivittatus,* and *Urosalpinx cinerea.*

Ten specimens of each species were brushed and rinsed under fresh tap water to remove gross fouling. Sizes used were graduated, from approximately 2.5 cm to 7.5 cm in pelecypods, and field run in the gastropods. Oysters often presented heavy marine growth resistant to simple cleaning, but by striking off barnacles, a satisfactory clean area could be quickly prepared in the region immediately distal to the umbo. Each shellfish was allowed to drain, and all species but the oysters

[1] Contribution No. 22 from Northeast Marine Health Sciences Laboratory.

[2] Communicable Disease Center Hepatitis Report No. 19, June 30, 1964, PHS, DHEW, Atlanta, Georgia. Shellfish-associated infectious hepatitis, Part C.

[3] PHS files, 1965.

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were placed hinge-up in a slotted drying rack. Should identification of oysters destined for the half-shell trade be desirable, marking of the left valve or "cup" would be preferred. A bit of the periostracum of *Busycon* was removed to provide bare shell for labeling. Once the shells were dry, the following materials were used to mark each specimen:

1. Epoxy glue [4] was mixed with hardener and applied directly to the shells in selected areas of approximately one square centimeter. The bright blue fluorescence of epoxy under ultraviolet light was found to be due in major part to the hardener component. Thickness of the resin-hardener mixture made it difficult to apply a code or distinctive marking to the smooth-shelled species, but worked very well on the rough umbo regions of mussels, and was eminently satisfactory for spot-marking the rough shells of *Crassostrea* sp. and the gastropod specimens. Thinning the epoxy with acetone resulted in diminished fluorescent ability to a point not readily distinguishable from shell autofluorescence. At least two hours at room temperature (23°C) were required for the epoxy to harden sufficiently to prevent sand from adhering to the burrowing species. Oysters, mussels and gastropods could be placed in their habitat within an hour after applying the plastic.

2. Krylon 1306 [5] (a cellulose nitrate-dioctyl phthalate alcohol-ketone propellent spray) was applied to the lateral surfaces of mussel, clam and oyster shells, covering a central area of at least 2-3 cm. Care was taken not to strike exposed tissues of *Mya* to avoid possible irritation. Small amounts of the following six fluorescent powders were gently blown onto all specimens while the Krylon was still tacky:

"Dayglo" (fluorescent melamine-sulphonamid formaldehyde resins)[6]:
- Fire Orange A-14
- Signal Green A-18
- Horizon Blue A-19

Aminopyrine powder
Thymol iodide powder
"Spectraglo Green Tracing Powder" (zinc sulphide)[7].

This procedure required care if invisibility to the unaided eye was essential, as all but aminopyrine and thymol iodide were visible to the unaided eye unless applied in limited quantity. Any degree of visibility to the naked eye could be achieved by increasing the amount of fluorescent chemicals in acetone solution. A second coat of Krylon was sprayed on the marked areas to seal in the fluorescent chemicals. The Krylon spray dried rapidly, allowing nearly continuous handling of the mollusks, but because of the easy solubility of the Dayglo resins in it, two light applications were used rather than a single heavy coat to avoid washing off the marker chemicals.

3. An acetone solution of 0.025 per cent Fire Orange A-14 was prepared, sufficient to fluoresce brightly under black light when applied to a white shell, yet invisible to the unaided eye. The solution was easily and rapidly brushed onto the shells of each species of shellfish, with various coding figures possible, as in Figure 1, A-C. These acetone solutions dried quickly. (Similar solutions of Signal Green and Horizon Blue permitted color coding, but unless colors are closely related in the spectrum, the mixing of fluorescent pigments does not obey the standard laws applicable to the combining of ordinary colors. Rather, mixed fluorescent colors react according to the principle of mixed colored lights.) Two coats of Krylon were sprayed onto the marked areas, using care not to dilute the Krylon-soluble fluorescent resins. Another group of bivalves was prepared without the Krylon spray. These last unsprayed specimens were much faded at the end of six months, although still discernible under u.v., while the Krylon-coated shellfish retained brilliant fluorescence under activation.

In each experiment, the shellfish were checked under 3660Å ultraviolet light to verify marking success. If visibility to the unaided eye is desired, 3 to 4 times the recommended concentration of the Krylon-sprayed acetone solutions of melamine sulphonamid formaldehyde resins is suggested (method 3).

Half the pelecypods and all the gastropods were placed in tanks with running sea water at ambient Narragansett Bay temperatures varying from 2-19°C, with all but *Mytilus* and *Crassostrea* sp. allowed to burrow in sand. The gastropods were

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[4] "Elmer's epoxy glue": The Borden Chemical Co., Inc., New York, N. Y.
Use of the products in the study does not necessarily represent endorsement by PHS, nor were the experiments intended to constitute inventory or evaluation of other products that may be available.

[5] "Krylon No. 1306": Krylon, Inc., Norristown, Pa.

[6] "Dayglo" (Fire Orange A-14, Signal Green A-18, Horizon Blue A-19): Switzer Bros., Inc., Cleveland, Ohio.

[7] "Spectraglo Green Tracing Powder": Black Light Eastern, Division of Spectronics Corp., Westbury, L. I., N. Y.

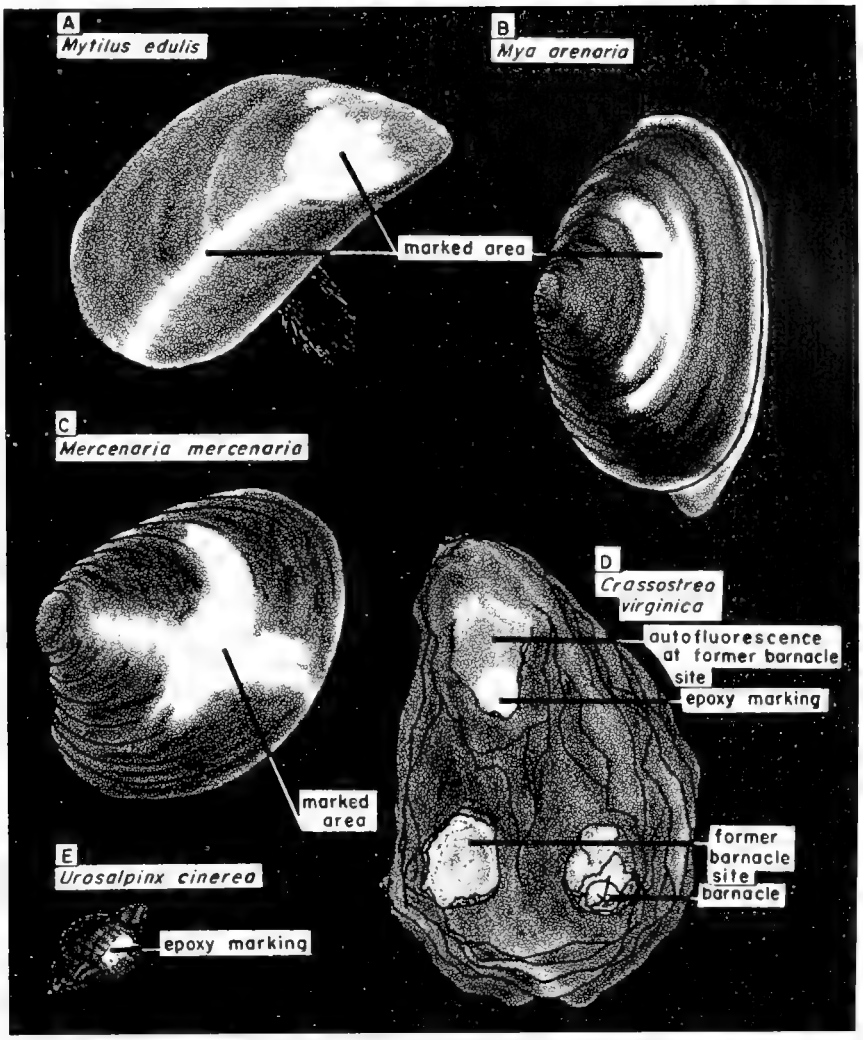

FIG. 1. *Appearance of mollusks with fluorescent markings under ultraviolet light.*

allowed to forage in the pelecypod tanks receiving ambient Bay waters, with the exception of *Urosalpinx* and *Busycon*. These two species were kept separate by plastic screens; *Urosalpinx* were supplied periodically with rocks encrusted with barnacles and small mussels, and *Busycon* were provided with freshly-opened bivalves and cut-up fresh fish.

The remaining half of the pelecypods were placed in 19-20°C running sea water, with sand for burrowing supplied for all but *Mytilus* and *Crassostrea* sp. Sulphide build-up within the holding sand for this group was minimized by weekly removal of the shellfish and re-bedding them in clean sand. To remove organic debris the oysters were lightly brushed at these times, while the mussels were rinsed with a stream of sea water.

RESULTS

Twelve months later, the marked shellfish were examined under 3660Å ultraviolet light. The epoxy-labeled specimens could be easily identified, and there was no difficulty in distinguishing this marker from the less intense pale blue autofluorescence of commensal barnacles. The method was well-adapted for use on *Crassostrea* of both species (see Fig. 1, D), and also for the gastropods (Fig. 1, E). On the smooth-shelled varieties, close

examination with the unaided eye often revealed the small straw-colored bead of epoxy, although it was never conspicuous. No appreciable differences in marking effectiveness of survival were observed between shellfish held at 19-20°C with regular cleansing and those left undisturbed at ambient Narragansett Bay temperatures that varied from 2-19°C.

Examination of the Krylon-sprayed powder preparations under ultraviolet disclosed that the best labels were Spectraglo Green, Fire Orange A-14, Signal Green A-18, and Horizon Blue A-19. The response of aminopyrine and thymol iodide to ultraviolet irradiation was not markedly different from that of barnacle and shell autofluorescence. The applications of Dayglo and Spectraglo powders to tacky Krylon, even without the sealing coats, were superior in longevity and brilliance to aminopyrine and thymol iodide but inferior to the Krylon-sealed powders.

The fluorescent melamine-sulphonamid formaldehyde resins in acetone solutions sealed with two light coats of Krylon 1306 spray retained bright fluorescence under u.v., with Fire Orange A-14 appearing to be the best of the three tested perhaps because of the contrasting bright orange color. The acetone solutions that did not receive the Krylon sealer coats were much faded but visible. Fire Orange again appeared to be the brightest under black light activation.

The three best markers were epoxy glue (method 1), Spectraglo Green Tracing Powder coated with Krylon (method 2), and the Krylon-sprayed acetone solutions of the melamine-sulphonamid formaldehyde resins (Fire Orange, Signal Green, and Horizon Blue, method 3). All three methods were nearly equal in durability and ease of identification. The Krylon-sealed acetone solutions of the Fire Orange group were easily made visible or invisible to the naked eye, easiest to apply, and permitted quick return of shellfish to habitat. Epoxy glue was nearly as efficient, especially on oysters or gastropods. All three types of fluorescent markers maintained the property of activity under ultraviolet stimulation after being subjected to 15 minutes of boiling in water. Colored letter, numeral, or other coding is easily possible with chemically similar products now on the market.

The criteria (first page) delineating the requirements for a successful shellfish marking methodology appear to have been fulfilled by each of the three procedures reported upon most favorably.

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AN ANALYSIS OF THE MOVEMENTS OF THE BAY SCALLOP, *AEQUIPECTEN IRRADIANS*, IN A SHALLOW ESTUARY [1]

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ABSTRACT

Displacements resulting from their summer swimming activity over periods of 3 to 6 days were recorded for tagged bay scallops, Aequipecten irradians, *on the shoals of the Niantic estuary. Analyses indicated that the slight directedness in their movement may be accounted for by tidal current transport.*

The bay scallop, *Aequipecten irradians*, propels itself in a zigzag pattern by jets of water produced by flapping its shell. On each "swim" this may carry the scallop two or more feet off the bottom; then the contractions of the adductor muscle which closes the shell subside and it sinks to the bottom. One readily observes that this activity is greatest during the warmer months.

To the casual observer it appears that, in this kind of swimming, the scallop flips about aimlessly in the water column for a few seconds; then settles to the bottom, only to repeat the aimless movement sometime later. It is possible, however, that the cumulative and net effects of this propulsion have some direction and distance components, either actively brought about by the animal or resulting from external influences such as currents which could transport these bivalves when they swim off the bottom. Further, the scallop has a thin, light shell adapted for swimming and has numerous and elaborate photo-and chemoreceptors at the edge of the mantle which may be associated with directed movements. Finally it is common knowledge that scallops can shift or get shifted in locale, although there has been no direct evidence of any long distance migrations and Baird (1966), in a brief summary of work on several scallop species, says, "the evidence against migration is convincing." The purpose of this study was to gather data on the short-term summer movements of marked scallops and to analyze these for directional components.

OBSERVATION PROCEDURES

Scallops were collected from the shoals of the Niantic River estuary in Connecticut. Each was marked by drilling a hole through the "ear" at the hinge line of the upper valve, and tying on a brightly colored fluorescent tag with nylon leader. From our experience watching tagged scallops in tanks we do not believe such a tag causes complications in the scallop's behavior. The scallops were then used for observations at the collection sites in about 2 to 5 feet of water (low to high tide).

Two methods were used to record net shifts in position. One involved an underwater compass rose and a measuring string marked off in 0.1 m intervals pivoted from the center of the rose. An improved method, freeing the observer from the effort of lining up each scallop with a string, involved a grid of 1 m squares plotted on the bottom, using the location of the scallops within the squares as the means for measuring how far and in what directions they had moved. In both methods scallops were released in a circle 1 m in diameter centered on the rose or the grid. Four releases were made and observed using 40 to 52 tagged scallops for each.

In each series, observations were made at successive but varying time intervals, seldom less

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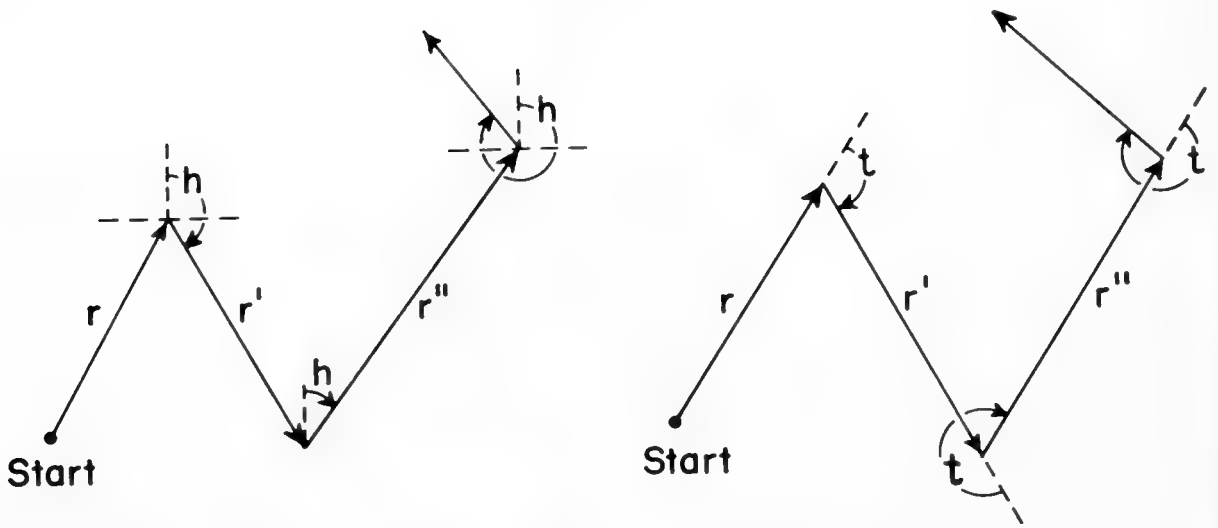

FIG. 1 *Angle of heading, "h", and angle of turning, "t". Leg or steplength, "r".*

than 8 and seldom more than 15 hours. A total of four series, lasting 6, 6, 3 and 3 days respectively, were run. When, during any one observation, a scallop was found 3 m or more from the central starting point, it was returned to the center. Such aids as mask, snorkel, and underwater writing tablets were used for observing and recording the scallop position. On the successive observations we did not always find all the individual scallops though often those "lost" would be recorded later.

ANALYSIS OF OBSERVATIONS

Curray (1956) details the Rayleigh test for randomness vs. directedness in a circle. The test assumes a circular normal or Mises distribution. The formula for this is: $P = e^{-L^2 n(10^{-4})}$

where, L = vector magnitude in per cent
n = number reported
P = probability of obtaining a greater amplitude by pure chance combination of random phases.

A P value of less than 0.05 indicates directedness at the 95 per cent confidence level.

Each series of observations can be examined in two ways. First the data can be considered as observations of groups. Each group is an array of scallops as observed scattered around a point of release after one of the time intervals. Here, the point is considered a circle of 1 m diameter. In series number 1 there were 10 such arrays observed; thus 10 opportunities to apply the Rayleigh test. Table 1 shows the data for each array in each of the four series.

The second way of analyzing the data is by following the movement of individual scallops. When a particular scallop has changed position during an observation interval, this is enumerated as one movement in the distance and direction involved, though it may be in reality a composite of several moves. A track is then plotted of the successive net movements of this scallop. For each such leg (r) of the plotting for an individual, two different angles were measured: the angle of heading (h) and the angle of turning (t) as illustrated in Figure 1. If each angle of heading is measured clockwise from north on the grid, the angle and the accompanying steplengths give data which can be treated similarly to the group array observations. In like manner, analyses of the movements of individuals can be made using the angles of turning and steplengths. Table 2 is a resumé of the results when the Rayleigh test was applied to those movements of individual scallops.

While individuals did not, in these analyses, show directional movement as frequently as groups, a directional tendency in their movement is readily seen by making log circular plots of the mean vectors for each (see Figs. 2 and 3 for examples from the first series of observations). These show a distinct north-south trend. Applying the Rayleigh test to check this trend, it is found that the mean vector angles of heading with the mean vector distances are directed but only at the 80 per cent confidence level with an average

TABLE 1. *Data obtained from arrays of scallops dispersing from a release point after successive time intervals.*

Series 1:											
Array number	1	2	3	4	5	6	7	8	9	10	Ave.
No. of individuals recorded	10	24	17	25	29	20	18	28	24	21	
Hours after release	3.5	24.0	29.5	49.0	55.25	80.5	95.5	101.75	121.0	145.0	
Mean vector angle (degrees)	163	172	197	153	143	173	153	134	173	174	163.4
Mean vector distance (M)	.6	1.0	.3	.6	.5	.7	.5	.6	.8	.8	.64
P (Rayleigh test)	.015*	.000*	.178	.000*	.000*	.000*	.025*	.000*	.000*	.000*	
Series 2:											
Array number	1	2	3	4	5	6	7	8	9		Ave.
No. of individuals recorded	31	32	37	35	38	36	36	38	30		
Hours after release	18.5	24.0	42.75	50.75	74.75	89.75	96.00	115.00	139.25		
Mean vector angle (degrees)	190	105	71	151	143	161	162	104	115		122.6
Mean vector distance (M)	.1	.3	.5	.5	.4	.2	.2	.4	.4		.33
P (Rayleigh test)	.692	.022*	.001*	.000*	.001*	.445	.182	.003*	.004*		
Series 3:											
Array number	1	2	3	4	5	6					Ave.
No. of individuals recorded	26	27	32	34	34	34					
Hours after release	18.0	23.5	42.5	48.5	67.25	87.25					
Mean vector angle (degrees)	155	164	164	174	174	173					167.3
Mean vector distance (M)	.7	.6	.8	.7	.7	.6					.41
P (Rayleigh test)	.000*	.000*	.000*	.000*	.000*	.000*					
Series 4:											
Array number	1	2	3	4	5	6					Ave.
No. of individuals recorded	16	15	20	21	22	22					
Hours after release	18.0	23.75	42.25	48.25	67.0	73.0					
Mean vector angle (degrees)	261	310	283	319	282	301					292.9
Mean vector distance (M)	.2	.4	.3	.4	.6	.5					.41
P (Rayleigh test)	.382	.121	.163	.022*	.000*	.001*					

*indicates directedness at the 95% level of confidence

mean vector angle of heading of 154°, not greatly different from the averages of vector angles in Table 1. The mean vector angles of turning with the mean vector distances are directed at the 99 per cent confidence level with an average mean vector angle of turning of 177°.

The mean vector angles given in Table 1 give a strong suggestion of a drift southward. Comparison with unpublished current data (N. Marshall) which indicate a north-south tidal flow and a net current drift southward directs attention to relationships with the tide. This is reinforced by Figure 3 which indicates a reversal of direction on successive observations possibly, in part, resulting from alternating tides. Two questions arise: 1) If such a tidal transport prevails, what action, if any, prevents the scallop population from being shifted toward the mouth of the estuary, perhaps in excess of 50 m throughout the six warmer months when they are most active; 2) is the directed effect entirely due to currents?

Perhaps the population does shift and, whereas

TABLE 2. *Results of the Rayleigh test on movements of individual scallops. Numbers indicate numbers of scallops whose movements were deemed random or directed on the basis of the Rayleigh test using the 95% level of confidence.*

	Using Angle of Heading	Using Angle of Turning
Series 1	Random = 35	Random = 29
	Directed = 3	Directed = 9
Series 2	Scallops not numbered; individual movement not recorded	
Series 3	Random = 33	Random = 31
	Directed = 2	Directed = 4
Series 4	Random = 22	Random = 21
	Directed = 0	Directed = 1
Total	Random = 90	Random = 81
	Directed = 5*	Directed = 14*

*Of the total of 19 directed individuals, 16 provided less than 5 moves each for the analysis.

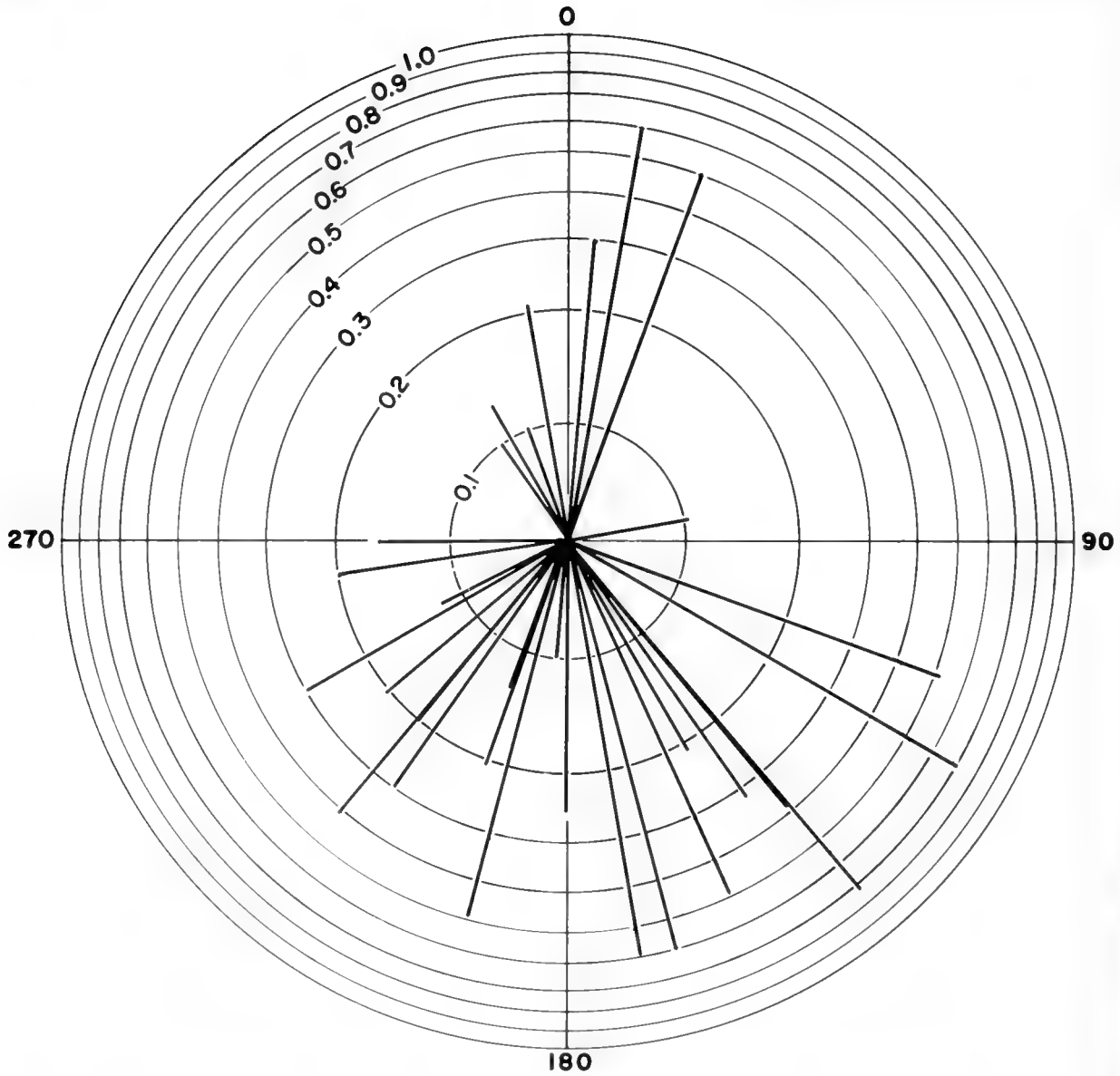

FIG. 2. *Log circular plot of mean vector angles of heading with mean vector distances.*

a few scallops may end up so far down-estuary as to be lost to an unfavorable habitat, the losses may be offset by replacements from up-estuary. On the other hand, the localized movement encountered in these observations does not preclude the possibility of migrations that would dwarf such a minor population shift.

With regard to the second question, some insight is gained from scallop activity data coupled with current data and estimates of possible tidal transport. Figure 4 represents observations on scallop activity in unmolested square meter plots during the summer months. There tends to be an increase in activity one to two hours after a tidal change. Since the tidal currents run to nearly full strength except within a period of some 15 minutes during the turn of the tide, it is clear that the swimming scallops are subject to the tidal drift. Unpublished data (N. Marshall) on the currents over the area of these observations show that the subsurface ebb current, from ½ hour after to ½ hour before the turn of the tide averages 12.1 cm/sec and the subsurface flood over a comparable span averages 8.6 cm/sec (observations on

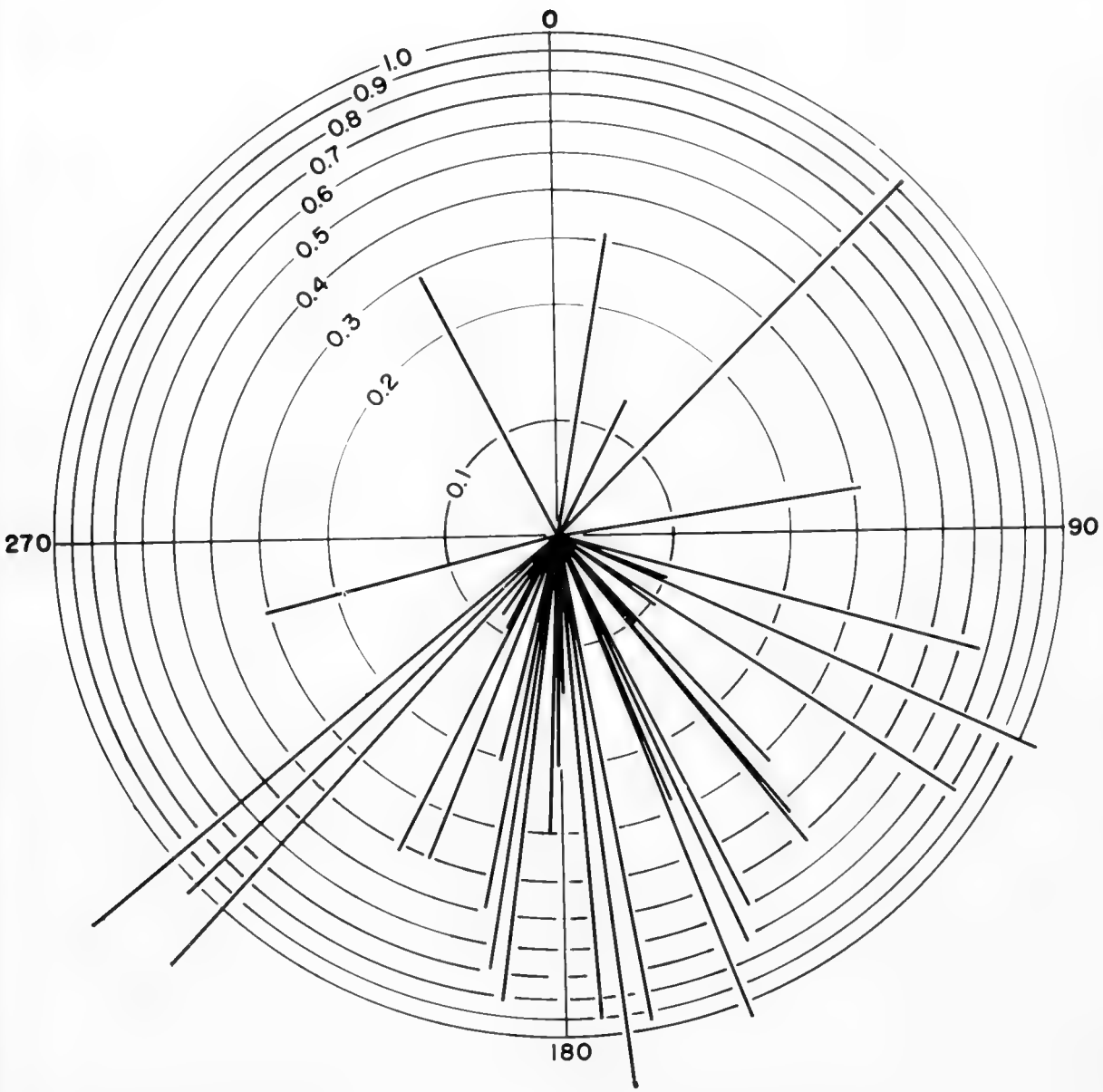

FIG. 3. *Log circular plot of mean vector angles of turning with mean vector distances.*

nine different tides). By dropping inert scallops into the water from 2½ feet off the bottom in the area under consideration and calculating the sinking rates, it was found that the drift in the average ebb flow would be about 0.2 m greater than the drift in the average flood flow. Assuming that a single scallop makes one swimming excursion on each tide and that during this excursion it is subject to the tidal flow for a period approximately equal to that of a falling inert scallop, the net movement resulting from tidal currents would be about 0.2 m/tidal cycle or 0.4 m/day in a southerly or seaward direction. This is a very crude analysis but it matches the observed net movement of the scallops (Table 1) and tends to support the interpretation that the directedness in the movement is due entirely or almost entirely to tidal current transport.

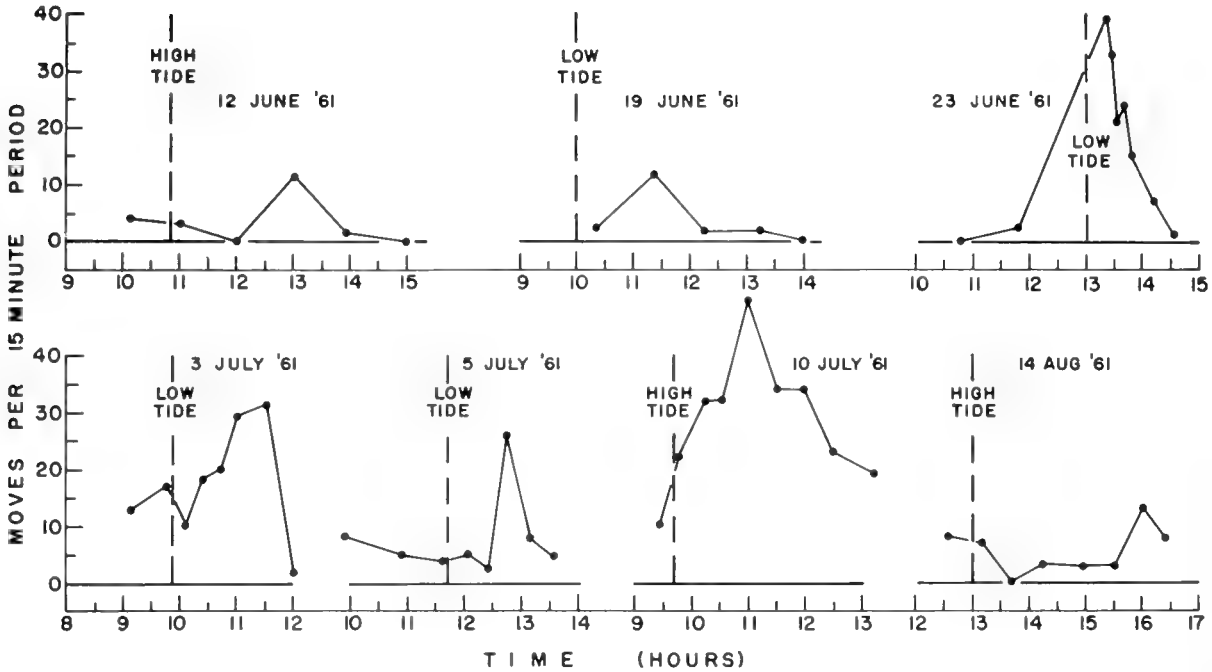

FIG. 4. *Observations on scallop activity in square meter plots containing 40-50 scallops.*

ACKNOWLEDGEMENTS

Professors Saul B. Saila and Harlan Lampe of the University of Rhode Island advised in the analysis of these data. J. M. Marshall did a large measure of the field work reported.

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FACTORS AFFECTING THE RELATIVE ABUNDANCE OF *MERCENARIA MERCENARIA* IN THE PROVIDENCE RIVER, RHODE ISLAND

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ABSTRACT

A simultaneous examination of sediment properties which included particle size, pH, cation exchange capacity, organic carbon, total nitrogen, available phosphorus, and water depth was made in an effort to discriminate between two classes of bottom samples in the Providence River. One class contained relatively large numbers of Mercenaria mercenaria *and the other had a low abundance. The linear discriminant function was found to be significant utilizing the eight measured variables. However, it was found that only two variables, organic carbon and particle size greater than 2 mm diameter, effectively contributed to the discrimination of the two levels of abundance. For the Providence River it was concluded from indirect evidence that much of the observed difference in abundance between samples may have been due to predation or other variables not measured in the study, and that sediment properties alone are not sufficient to predict levels of abundance with a high degree of precision in the area examined. The management implications of this study were briefly considered.*

INTRODUCTION

Data from sampling surveys as well as information from catch statistics have clearly demonstrated that the hard clam or quahog, *Mercenaria mercenaria* L., is a resource of major economic importance in Rhode Island. During recent years its annual value to local fishermen has approximated one million dollars. Annual yields during this time have been in the vicinity of 200,000 bushels. Pratt (1953) has shown that the hard clam is the most abundant animal of its size living in or on the bottom, and that it constitutes a significant portion of the total bottom-dwelling community in Narragansett Bay.

Population census surveys have been conducted at various times to determine the density and distribution of hard clams in various parts of the Bay. In general, it has been shown that large populations exist in the upper polluted portions of Narragansett Bay, such as the Providence River and Mount Hope Bay. Further, the major fishery for this species is located in close proximity to the polluted portions of the upper Bay.

From these as well as other studies, it has become evident that many aquatic species are not uniformly or randomly distributed in space. Instead, they are found in clusters or clumps exhibiting various degrees of contagion. The spatial relations of these clusters and gaps as well as their causation have eluded adequate explanation to date. Previous work by Saila, Flowers, and Campbell (1965) also indicated that the hard clam is contagiously distributed, and this was further confirmed by Saila and Gaucher (1966) for other marine pelecypods.

The study was undertaken to contribute to an understanding of factors affecting the small-scale variations in the spatial distribution of the hard clam in a relatively unexploited population from a restricted area. Specifically, seven sediment characteristics and one other variable (depth) were simultaneously examined to determine their role in discriminating between bottom samples which contained a relatively low abundance of hard clams versus those which contained greater numbers. An understanding of the significance of these variables is considered to be of interest in itself and also of possible predictive value in separating potentially good and potentially less valuable growing and management areas.

An initial census survey was made of the Providence River in 1956 by Stringer[1]. At this time the estimated total population of hard clams in an area of 3534 acres was 588,000 bushels. The Providence River within the area has been utilized for limited transplanting operations to clean waters in other parts of the Bay. Table 1 illustrates the number of bushels per year which have been transplanted from the Providence River. It is apparent that even in the years of greatest relaying activity, only a small fraction of the standing crop was removed. The maximum removed was considerably less than 10 per cent of the standing crop during the greatest relaying activity.

TABLE 1. *The quantities of hard clams transplanted from the Providence River during a ten-year period from 1957-1966.*

Year	Quantity (bushels)
1957	23,398
1958	40,576
1959	26,674
1960	19,257
1961	16,661
1962	23,781
1963	6,643
1964	42,538
1965	40,128
1966	17,566 (to April, 1966)

A second census survey of the Providence River was made in 1965, providing another estimate in the same area. The estimated standing crop at this time was found to be 1,260,000 bushels. This second estimate gives support to a premise made in this study, that the spatial distribution of hard clams in the area was probably affected very little by this limited exploitation for transplanting. It is suggested that some combination of environmental variables exclusive of exploitation accounts for the observed spatial distribution of the organism. Therefore, the analysis of sediment properties was considered desirable to determine their relative importance in relation to abundance in the sampling units.

[1] Stringer, L. D. 1959. The population abundance and effect of sediment on the hard clam. Appendix E. Hurricane Damage Control Narragansett Bay and Vicinity. (processed)

METHODS

The 1965 survey was utilized for the data analysis which follows. A total of 121 stations was selected by means of systematic sampling over a 300 yard grid interval in the sampling area. These stations were located in the field by triangulation with shore stations and calculated vessel running times. A ½ yard capacity construction bucket was operated from a double drum winch on a 32-foot dragger type of survey vessel to obtain bottom samples. The bottom samples measured 4½ square feet in area and sediment penetration was from ½ to 1½ feet depending on sediment composition. Samples of sediment for analysis were obtained by inserting 6 x 2 inch diameter core liners into the sample when it was taken aboard the vessel. The ends of the core liners were then sealed with plastic caps for sample storage. The remaining sediment was dumped into a wooden wash box equipped with a ¼ inch mesh hardware cloth bottom. The hard clams retained by this mesh after washing were counted, measured, and tabulated by station.

Sediment samples were prepared for analysis by air-drying on paper for at least two days with frequent mixing. The sample was mechanically fractioned on a 2 mm soil sieve. The pH measurements were made by mixing 10 g of air-dry sediment and 10 ml of distilled water in a 100 ml beaker, stirring for 20 minutes and reading on a Beckman pH meter. Cation exchange capacity was determined according to the method described by Jackson (1958, p. 95). Carbon was determined by wet combustion according to Jackson (1958, p. 219-221). Nitrogen was determined by the standard kjeldahl digestion technique, and available phosphorus analysed according to Black (1965). Water depths were measured at the time of sampling and were adjusted to mean low water by noting the sampling time with reference to local tide tables.

It is frequently desirable in marine biological research, when examining a sample, to be able to

decide to which of two groups the sample belongs. In this instance the problem involved deciding whether the properties of the sample could be used to discriminate between high and low numbers of hard clams per unit area occurring in the sample from Providence River. It is occasionally possible to make decisions based on a single variable, but most of the time the two groups differ in respect to several variables, each of which provides some indication as to the group in which the sample falls.

One approach to the problem in discriminating between two groups is to set up a function of the form:

$$Z = \lambda_1 X_1 + \lambda_2 X_2 + \lambda_3 X_3 + \cdots \lambda_k X_k, \quad (1)$$

where $X_1, X_2, X_3, \cdots X_k$ are the observed values of the k variables and $\lambda_1, \lambda_2, \lambda_3, \cdots, \lambda_k$, are the corresponding weighting coefficients. Fisher (1936) has provided a solution to this problem which involves determining the λ_k in such a way that, if an analysis of variance is made of the Z values, the ratio of the variance between groups to that within groups would be a maximum; i.e., maximize the discrimination potential. Several biological applications of the linear discriminant function have been made (Barnard, 1935; Baten, 1943; Day and Sandomire, 1942) but this is believed to be the first application to the separation of marine environments.

A systematic sampling scheme was utilized for the survey because clumping or aggregation of similiar units will tend to be more accurately represented than in a random sample. Of course, no estimate of the variance of the sample mean is available from such a sample. However, this applies only to the population estimate. The linear discriminant function is applied to two abundance classes divided on an *apriori* basis into high and low counts. Intermediate levels were discarded for analysis because the discriminant function, by definition, is designed to maximize separation between groups considered different on some arbitrary basis.

RESULTS

During the period 29 January 1965 to 6 May 1965, 121 samples were obtained from stations in the Providence River from which sediment analysis and hard clam counts were made. Figure 1. illustrates the configuration of the systematic sampling plan, and Figure 2 depicts the general distribution and density of the hard clams in the area. This distribution was drawn by estimating contours of equal abundance between stations shown in Figure 1. For purposes of discriminating between areas, the high density samples were arbitrarily defined as those which contained 25 or more hard clams per bucket sample and the low abundance samples as those which contained five or less per bucket. By partitioning the data in this manner it was found that there were 35 samples available with the lower level of abundance and 27 samples with adequate environmental data containing 25 or more clams per sample. The eight variables examined were defined as follows: X_1 = amount of sediment with a grain size less than 2 mm in diameter (per cent by weight), X_2 = amount of sediment of grain size greater than 2 mm in diameter (per cent by weight), X_3 = pH, X_4 = cation exchange capacity (me. cation/100g), X_5 = amount of carbon present (per cent carbon by weight), X_6 = total nitrogen content (per cent N), X_7 = amount of available phosphate (ppm P), X_8 = depth of water (feet).

A discriminant function was set up and solved by means of a FORTRAN IV program for the IBM 1410 Data Processing System. This program computes the least squares optimum, discriminant function between groups, and produces a stepwise solution which successively eliminates variables from the least to the most significant.

A matrix of correlation coefficients is presented in Table 2 to indicate the interrelationships among variables. The correlation of 1.000 is expected in the case of sediment particle sizes because one value is the complement of the other. The intercorrelations among some of the variables will be considered further in the discussion.

The discriminant function was found to provide a significant discrimination between the two levels of abundance using all eight variables ($F = 2.80$, d.f. = 8.53). However, it is not sufficient to calculate a discriminant function and determine its significance. We could have, for example, a case of 8 variables wherein some of the variables would not contribute anything of value to the function. In our case it was found that the contribution of several variables was negligible and the least significant were successively eliminated until a maximum discrimination, in terms of the highest variance ratio was achieved. The variables in increasing order of significance were: X_1 (grain size less than 2 mm diameter), X_4 (cation exchange capacity), X_6 (total nitrogen), X_8 (depth), X_3 (pH), X_7 (available phosphate), X_2 (grain size greater than 2 mm), and X_5 (organic carbon). The analysis of variance for the case of two variables, X_2 and X_5 was found to provide the

FIG. 1. *The systematic sample station locations for the 1965 survey in the Providence River. The void area corresponds to the channel which was not sampled.*

maximum discrimination. The analysis of variance is shown below:

	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Between groups	4.9525	2	2.0262	10.6841
Within groups	11.1764	59	0.1896	

There is no doubt as to the discriminating power of the function since $F_{.05} = 3.15$ for 2 and 59 degrees of freedom. The difference $\bar{Z}_1 - \bar{Z}_2$ between the mean of the discriminant for the low abundance and the high abundance groups is represented by D. In our example:

$$D = 0.1988 - (-0.0670) = 0.2658$$

The required discriminant function is:

$$Z = -0.01517\ X_2 + 0.10751\ X_5 \qquad (2)$$

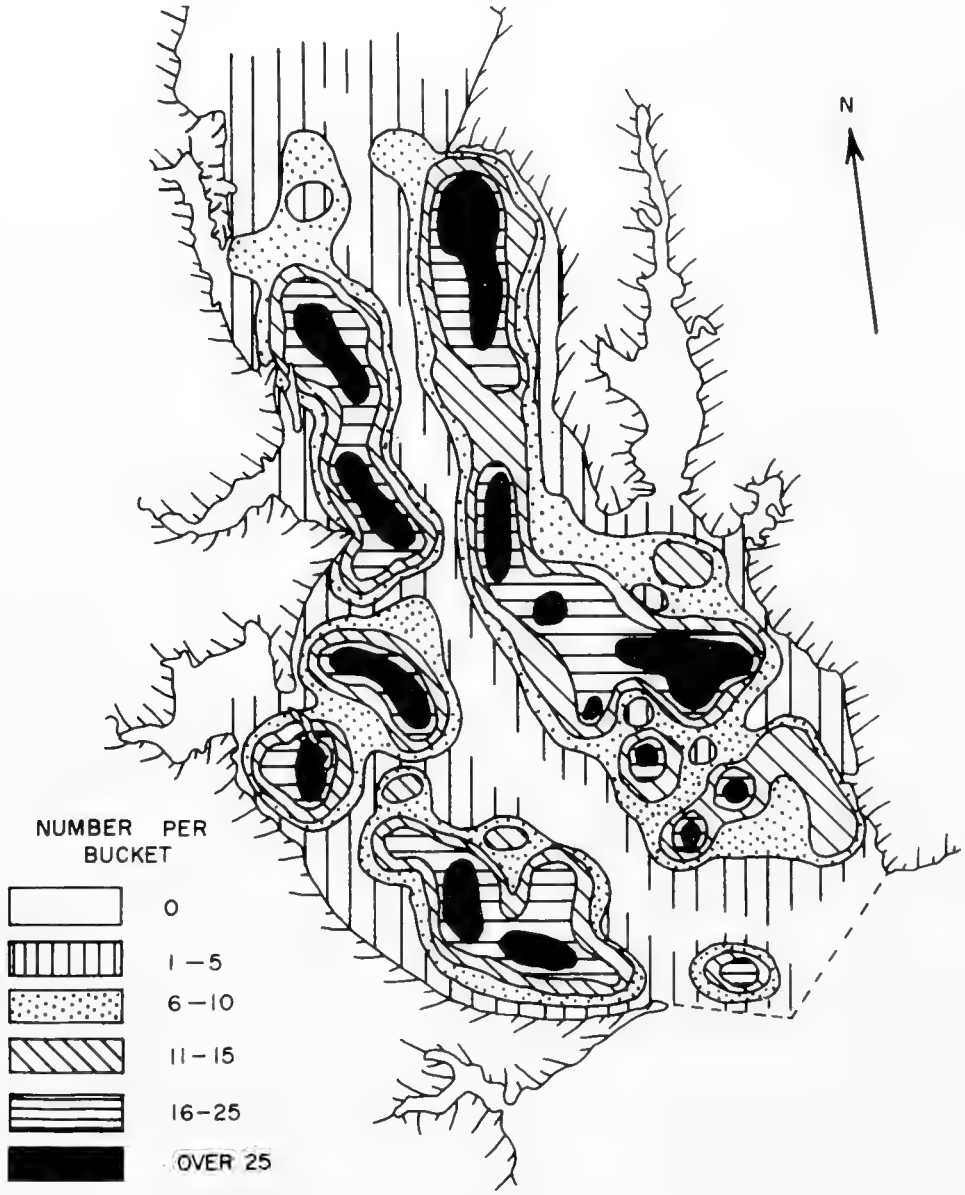

FIG. 2. ***Distribution of hard clams in the Providence River in 1965. Contours were drawn by interpolation from station data.***

For convenience we can divide by 0.01517 on the right, giving a new equation which we can put equal to Z because it is the final discriminant function:

$$Z = -X_2 + 7.08701\ X_5 \qquad (3)$$

Equation three shows clearly the relative values of the two characters in distinguishing the two kinds of abundance levels with organic carbon being of far greater discriminating power.

DISCUSSION

The major environmental factors affecting the occurrence and distribution of hard clams have not been clearly understood to date. The depth distribution of the hard clam has been described by

TABLE 2. *Matrix of correlation coefficients indicating the relationships among the eight variables utilized in the study.*

Variables	1	2	3	4	5	6	7	8
1	1.0000	—1.0000	—.1715	—.5880	—.0740	—.0978	—.0354	—.1127
2		1.0000	.1714	.0588	.0740	.0428	.1074	.1110
3			1.0000	—.7353	—.7240	—.3967	—.3642	—.2246
				1.0000	.8569	.5429	.5429	.6155
					1.0000	.8252	.5178	.4982
						1.0000	.2157	.3688
							1.0000	.4353
								1.0000

X_1 = grain size $<$ 2 mm diameter, X_2 = grain size $>$ 2 mm diameter, X_3 = pH, X_4 = cation exchange capacity, X_5 = organic carbon, X_6 = total nitrogen, X_7 = available phosphate, X_8 = depth

Belding (1931) as extending to at least 50 feet, and it seems reasonable to expect that the range of values in this study which extended to a maximum of 30 feet would not significantly contribute to determining areas of low versus high abundance. This was indeed the case in the analysis where depth (variable 8) was found to be the fourth variable eliminated because it did not add significantly to the discriminating power of the function. Pratt (1953) demonstrated that hard clams grow faster in sand than in mud when other variables are eliminated but also indicated a high level of abundance in fine sediments with larger particles such as shell and rock as minor constituents. The significance of particle size greater than 2 mm (variable 2) was clearly demonstrated in this analysis, and supports the previously completed growth comparisons mentioned above. Pratt and Campbell (1956) in summarizing observations on environmental factors controlling growth of the hard clam in Narragansett Bay stated that growth was not appreciably influenced by existing differences in current speed, dissolved oxygen content or the salinity of the bottom waters. However, the authors did indicate that growth rates were well correlated with the abundance of small diatoms in the overlying water column. Kerswill (1949) suggested that hard clams grow in proportion to the extent of water circulation. From previous unpublished work in this area, it seems reasonable to state that the Providence River area under discussion has a relatively high density of phytoplankton and relatively moderate differences in salinity, dissolved oxygen and current speeds. Thus it is not anticipated that these variables have significantly affected the observed distribution of hard clams in the survey area in spite of the fact that they were not directly measured at this time.

That shells and shell fragments contribute to the relative importance of variable 2 (particle size) is suspected, but no separation of particle size into shells and rock fragments was made during the study. It is suggested that this separation be made in subsequent work of this nature.

Bader (1954) has described the role of organic matter in determining the distribution of pelecypods in marine sediments, and this study reinforces his statements regarding the correlation between sediment organic matter and pelecypod abundance since organic carbon (variable 5) was the most significant variable studied. Another investigation by Bader (1955) concerning carbon and nitrogen relationships in subsurface marine sediments suggested that the association between carbon and nitrogen exhibits wide regional differences. In this study a fairly close association between carbon and nitrogen was found ($r_{5\text{-}6}$ = .8252) but the contribution of variable 6 (total nitrogen) to the discriminant function was found to be relatively insignificant.

It was also found that sediment cation exchange capacity (variable 4), pH (variable 3) and available phosphorus (variable 7) did not contribute significantly to the separation of the two levels of abundance. Finally, although Pratt and Campbell (1956) indicated slower growth of hard clams in sediments with a high silt-clay content, it is apparent that our mechanical analysis of sediments was not sufficiently refined to permit variable 1 (particle size $<$ 2 mm) to contribute to the discriminant function. The effect of the larger fraction ($>$ 2 mm) was significant and confirms the previous observations concerning the importance of larger particles even as minor constituents of the sediment.

Returning to the results of the correlation

analysis between paired variables, the high correlation ($r_{4\text{-}5} = .8564$) between cation exchange capacity and organic carbon seems to suggest that at least some of the cation exchange capacity was due to organic matter. Other reasonably high intercorrelations include that between pH and cation exchange capacity ($r_{3\text{-}4} = -.7353$) and between pH and organic matter ($r_{3\text{-}5} = -.7240$). There is no apparent explanation for these observed relationships at this time.

CONCLUSIONS

Since exploitation appeared to be relatively low and the sampling gear has been previously demonstrated to be efficient, it is reasonable to conclude that the observed differences in the abundance of hard clams in the sampling units were real. This indicates that the distribution of the clams was not uniform but instead showed aggregation. When the samples were partitioned into two groups (high and low abundance) it was found that only the organic carbon content of the sediment and particle size greater than 2 mm diameter contributed significantly to the separation of the samples in the two abundance classes.

It is also apparent within the limited range of observational data that the measurement of cation exchange capacity, available phosphate, and total nitrogen did not contribute to the separation of the two levels of abundance, and these time-consuming analyses are probably not of major consequence in separating abundance levels in areas such as this. It is suggested that a careful analysis of predation and setting patterns may contribute to a further understanding of spatial relationships.

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TOTAL SOLIDS AND LENGTH-WEIGHT RELATION OF THE SURF CLAM, *SPISULA SOLIDISSIMA*

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ABSTRACT

Commercial-size surf clams from New Jersey were sampled monthly to determine seasonal variation in percentage of total solids. Surf clams had a higher percentage of total solids than soft-shell clams, Mya arenaria, *and oysters,* Crassostrea virginica, *but the annual range of values was less.*

Ratios of shell measurements (height/length, width/length, width/height) for clams from Point Pleasant and Cape May were similar.

The relation of shell length to wet weight of meat for clams from Point Pleasant and Cape May was similar to that for clams from Long Island, New York.

Losses in meat weight from the industry's practice of removing stomach and gonad tissue varied from 11 to 20 per cent, depending on shell length and month of collection.

INTRODUCTION

The surf clam (*Spisula solidissima*) is the most valuable species in the commercial fishery of New Jersey; record landings of 42.3 million pounds of meats, worth 3.0 million dollars, were made in 1965 (LoVerde, 1966). This species attains a length of 8 inches and the meat may weigh 1 pound. Surf clams occur off the New Jersey coast from the intertidal zone to depths over 200 feet. They are taken commercially in depths of 20 to 110 feet by vessels using hydraulic dredges (Groutage and Barker, 1967). Clams are landed unshucked, and the entire body, except for the stomach and gonads, is utilized. Cleaned meats are minced for chowder and dips, or cut into strips for frying.

Purposes of this study were to: determine seasonal variation in percentage of total dry solids; establish the length-weight relation (total length of shell and wet weight of meat) for clams from Point Pleasant and Cape May; compare shell dimensions of clams from two areas of the fishery; and determine loss in meat weight during processing by the industry.

MATERIALS AND METHODS

Samples of surf clams were collected monthly in 1965, and less frequently in 1966, from a location off Point Pleasant, New Jersey; other collections were made in May 1965 and November 1966 off Cape May, New Jersey (Fig. 1). The live clams were brought to the laboratory and shucked. The entire meats were then washed, drained for 10 minutes, and placed on paper towels to remove surface water. Meat weight was recorded to the nearest gram, and shell measurements to the nearest millimeter. Shell measurements are defined as: length (L) — greatest dimension; height (H) — greatest dorsoventral distance; and width (W) — thickness of the clam with both valves in place.

To determine total dry solids, one clam was selected from each 10-mm group (120-160 mm). Meats from these five clams were homogenized in a high-speed blender and 20-gram portions were dried. Drying procedures followed the oven-dry method used by Engle (1958) and the freeze-dry method used by Shaw, Tubiash, and Barker

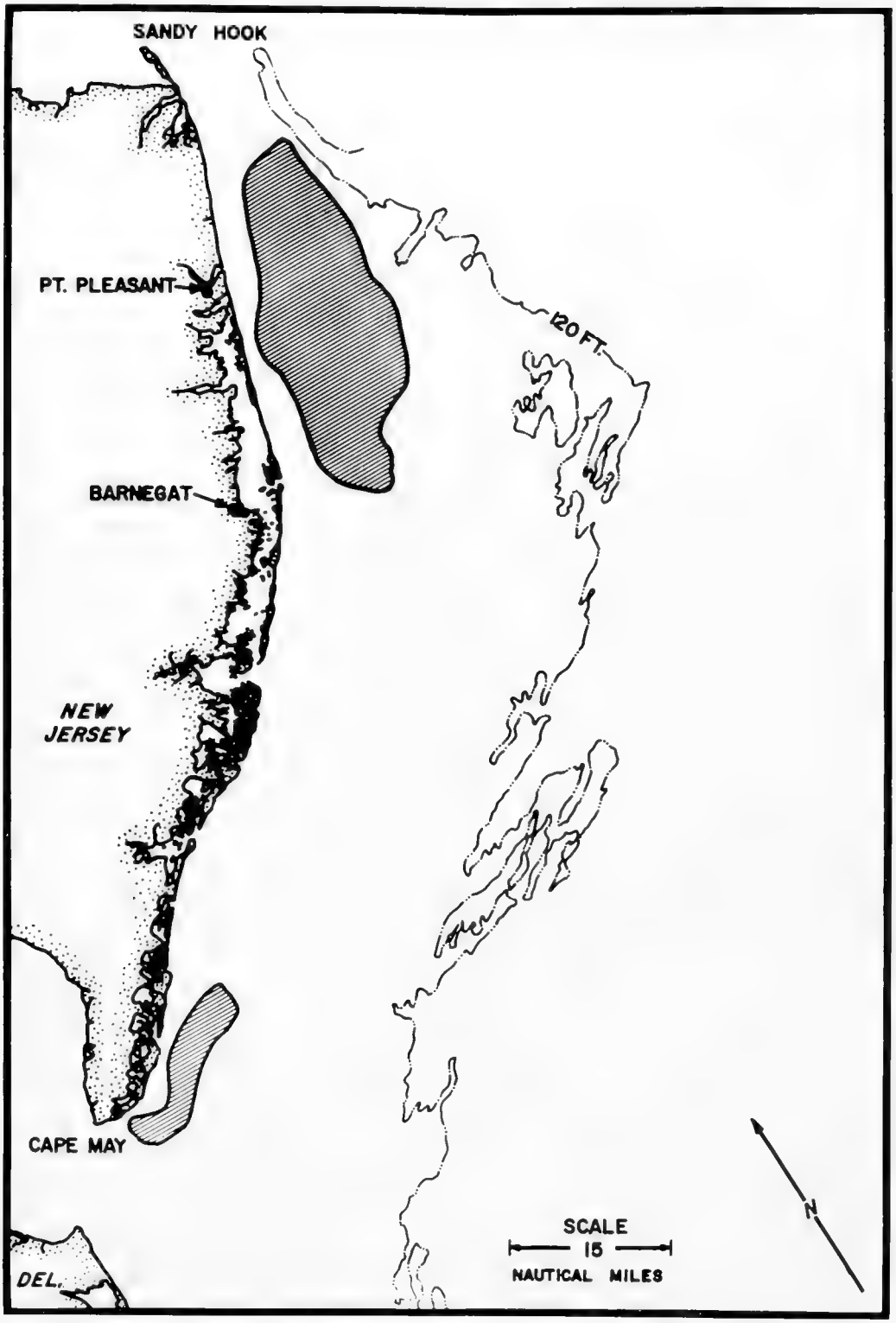

FIG. 1. *Areas of major surf clamming effort off New Jersey showing sampling locations in 1965 and 1966.*

(1966). Both methods gave essentially the same results. Percentage of total dry solids was obtained by dividing the dry weight of the meat by the wet weight, and multiplying by 100 (Engle, 1958).

Length-weight relationships were derived by measuring shell lengths and weighing the entire meats. Mean weights for length-groups were calculated by combining all samples from the same area.

Percentage loss in meat weight from processing was derived by weighing individual meats before and after removal of stomach and gonads.

TOTAL SOLIDS

Percentages of total dry solids derived by oven-drying Point Pleasant clams, collected in 1965, are presented in Figure 2. For 1965 the mean was 21.4 per cent and the range 18.1 per cent (in January) to 23.5 per cent (in April). A major fluctuation occurred during winter, when low water temperatures may reduce feeding and gonad development. Minor decreases in June and October coincide with the spawning periods. The large, muscular foot represents a high percentage of the total meat weight; here probably is the primary reason for the small seasonal variation in total solids.

The oven-dry method permitted comparison of percentage of total solids of surf clams with values found by other investigators for oysters, *Crassostrea virginica*, and soft-shell clams, *Mya arenaria*. The annual mean (and range) in percentages of total solids from selected areas were: surf clam, New Jersey — 21.4 (18.1-23.5); oyster, Chesapeake Bay (Engle, 1958) — 17.0 (14.0-20.0); soft-shell clam, Maine (Harriman, 1954) — 18.4 (15.2-21.5).

SHELL MEASUREMENTS

Shell shape may influence the volume of the shell cavity and, consequently, the amount of clam meat. The extent of shell variation must be known to allow valid comparisons of shell length-meat weight relationships of clams from different areas and of different lengths.

Shells of clams from inshore populations appeared to be more globose and less pointed than shells of clams from offshore areas. To determine if this was true, 2-bushel samples taken from deep (80 feet) water off Point Pleasant and shallow (22 feet) water off Cape May were compared.

Cape May clams were smaller than Point Pleasant clams. Lengths ranged from 102 to 133 mm (mean 119 mm) at Cape May and from 127 to 168 mm (mean 151 mm) at Point Pleasant. In Table 1, the data are arranged in centimeter length-groups, and ratios of the measurements (H/L, W/L, and W/H) are shown for these groups within each sample and between areas.

All three ratios were higher for Cape May clams. These ratios decreased gradually, however, as length increased. The larger Point Pleasant clams showed the same tendency — the longest length-groups had the lowest ratios. The greatest difference between two individuals in these samples was 0.12. Variation was much less, however,

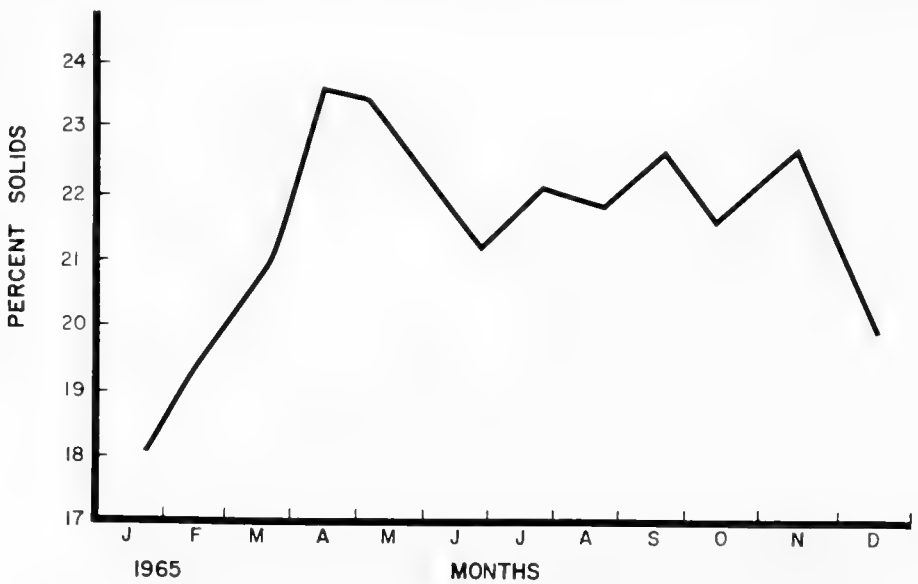

FIG. 2. *Percentage of total dry solids of surf clams from Point Pleasant, N. J., in 1965.*

TABLE 1. *Ratios of shell measurements, height/length (H/L), width/length (W/L), width/height (W/H) of surf clams taken off Cape May and Point Pleasant, New Jersey, in 1966.*

Length-Groups (mm)	Ratios		
	H/L	W/H	W/L
Cape May			
100-109	0.71	0.61	0.43
110-119	0.71	0.61	0.43
120-129	0.70	0.59	0.41
130-139	0.69	0.59	0.41
Point Pleasant			
130-139	0.70	0.60	0.41
140-149	0.69	0.59	0.41
150-159	0.68	0.58	0.40
160-169	0.68	0.58	0.40

when data from all clams of the same length were combined. The ratio decrease for each length-group for Point Pleasant and Cape May samples did not exceed 0.02 and for the combined samples 0.03.

The ratio variation between length-groups indicates that smaller clams are slightly more globose and less pointed than larger ones. The shapes of clams in these samples are a function of shell length; area of capture or water depth apparently had no influence on shape.

LENGTH-WEIGHT RELATIONSHIP

The shell length-meat weight relationship for 2,230 Point Pleasant clams is presented in Figure 3. Meat weights were generally constant throughout the year, although a slight decrease was evident during the spawning period. Weight increased rapidly in clams longer than 150 mm, which is the average length landed at Point Pleasant.

Meat weights of Point Pleasant and Cape May clams of corresponding lengths were similar. Shell length-meat weight relations for Point Pleasant and Cape May clams are also similar to the meat weight for surf clams from Long Island, New York (Westman and Bidwell, 1946).[1]

WEIGHT LOSS DUE TO CLEANING

Surf clams are shucked by hand in the plants, and stomach and gonad tissue is removed during cleaning. In this study, clams of all lengths from Point Pleasant and Cape May were cleaned to determine the relationship between shell length and percentage loss of meat weight. Two-bushel samples were obtained before and after the spawning period to evaluate the effects of gonad development on meat weight. About 150 clams from Point

[1] Westman, James R. and Milton H. Bidwell. The surf clam. Economics and biology of a New York marine resource. Mimeographed. Copy on file at Bureau of Commercial Fisheries, Oxford, Md.

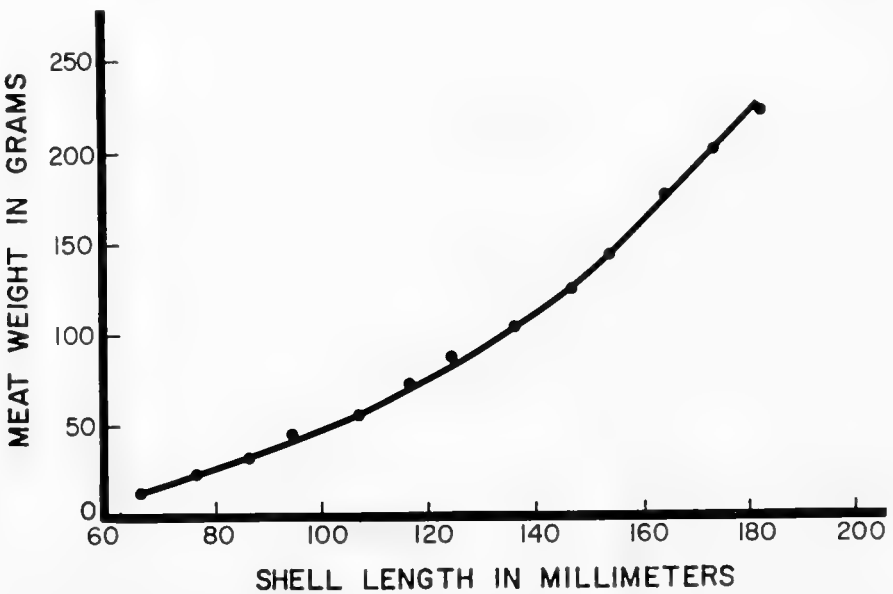

FIG. 3. *Shell length-meat weight relationship of surf clams from Point Pleasant, N. J., in 1965.*

TABLE 2. *Percentage weight loss due to removal of stomach and gonad tissue from meats of surf clams taken off Cape May and Point Pleasant, N. J., in 1965 and 1966.*

Length-groups (mm)	May 1965		November 1966	
	Point Pleasant %	Cape May %	Point Pleasant %	Cape May %
100-109	—	—	—	12
110-119	16	19	—	12
120-129	16	20	—	12
130-139	16	20	13	13
140-149	15	18	12	12
150-159	15	—	11	—
160-169	13	—	11	—

Pleasant and from Cape May were examined in May 1965, before spawning had started, and in November 1966, after spawning had ended. The May 1965 sample from Point Pleasant was collected at sea, aboard a commercial vessel, to obtain smaller clams for comparison with Cape May clams of identical lengths. Most samples were obtained directly from the boats when they returned to port with the day's catch.

Comparison of meat weights before and after cleaning revealed losses of 11 to 20 per cent; losses varied with shell length and month (Table 2). The weight loss was less for the largest clams from both areas in May, but decreases for all clams were similar in November. Point Pleasant clams had losses of 13 to 16 per cent in May and 11 to 13 per cent in November. Cape May clams had losses of 18 to 20 per cent in May and 12 to 13 per cent in November.

Since the annual mean weights of wet meats from clams of corresponding lengths at Point Pleasant and Cape May were similar, the greater loss from cleaning Cape May clams in May 1965 may be related to an earlier seasonal increase in gonad weight. In May 1965 bottom water temperatures were significantly higher in shallow water close to Cape May than in deep water off Point Pleasant. The mean temperature at five stations within the area of greatest clamming effort off Cape May was 10.0°C (range, 7.5 to 12.3°C) whereas the mean for five stations in the Point Pleasant area was 5.4°C (range, 5.1 to 5.7°C). In November 1966, bottom water temperatures were similar in both areas and all clams had recently spawned; thus, weight loss from cleaning was minimal.

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ASSOCIATION AFFAIRS

ANNUAL CONVENTION

The National Shellfisheries Association 1966 Convention was held jointly with the Oyster Institute of North America and the Oyster Growers and Dealers Association of North America, Inc., on June 5-9, 1966, at Norfolk, Virginia.

Officers and Executive Committee members elected for the term 1966-1967 were:

President ..... Jay D. Andrews
Vice-President ..... Harold H. Haskin
Secretary-Treasurer ..... Joseph H. Manning
Members-at-large ..... Sammy M. Ray, Albert K. Sparks, and Roy E. Drinnan

The Executive Committee now numbers eight, including Past-President John B. Glude and the Editor of the NSA Proceedings, Arthur S. Merrill.

The 1967 Nominating Committee consists of John B. Glude, chairman, Dana E. Wallace and R. Winston Menzel.

The newly organized Membership Committee will be chaired by Albert K. Sparks, with R. Winston Menzel, Clyde S. Sayce, and Dana E. Wallace as members.

The Pacific Coast Section of the NSA met jointly with the Pacific Coast Oyster Growers Association on August 25-26, 1966, at Olympia, Washington. Officers of the Section elected for the term 1966-1967 were:

Chairman ..... Clyde S. Sayce
Vice-Chairman ..... Kenneth K. Chew
Secretary-Treasurer ..... C. Dale Snow

A proposed revision of the NSA Constitution and By-Laws, prepared by the Executive Committee, was amended during the annual meeting and later accepted by the members through balloting by mail. Copies are available from the Custodian of the NSA Records, Oxford, Maryland.

Regular membership to the NSA is $6.00, library subscription is $6.00, and patrons contribute $100.00 or more. Twelve states are now patrons; these are Alaska, Califormia, Connecticut, Florida, Louisiana, Maine, Maryland, New Jersey, New York, Rhode Island, Virginia, and Washington.