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ABSTRACTS OF TECHNICAL PAPERS PRESENTED AT THE 1967 NSA CONVENTION

SEASONAL FACTORS RELEVANT TO FECAL COLIFORM LEVELS IN *MERCENARIA MERCENARIA*

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It is well known that during the winter in latitudes where the water temperature falls below 10° C for considerable periods of time, very few coliform organisms can be recovered from *Mercenaria mercenaria* even though they are growing in heavily polluted waters. This could be due to a more marked effect of low temperatures on uptake as contrasted to elimination, resulting in a gradual decrease of the organisms within the animal. A second possibility is death of the organisms within the animal. In this report the effect of seasonal changes on the presence of fecal coliforms in animals from a polluted area is documented. The more marked inhibitory action by temperatures of 10° C and lower and turbidities greater than 10 Jackson turbidity units (JTU) on uptake as compared to elimination of *Escherichia coli* by the quahaug is demonstrated. Data on the effect of seasonal changes on the experimental uptake and elimination of *E. coli* by the animal are presented. Possible bases for these differences are discussed.

PRESENT STATUS OF ATTEMPTS TO TRANSMIT *MINCHINIA NELSONI* UNDER CONTROLLED CONDITIONS

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The value of a method for producing infections of *Minchinia nelsoni* consistently under controlled conditions in the laboratory has been appreciated since the early stages of investigation of this disease agent of *Crassostrea virginica*. Attempts to infect have involved a variety of procedures. Among these are proximity to infected oysters or suspected alternate hosts, feeding with infected material both forced and as a suspension in the aquarium water, inoculation into various sites such as muscle, heart, visceral mass and mantle cavity, and implantation of infected gill and mantle tissue.

Failure to achieve infections has been a conspicuous feature of all of these attempts. In spite of failure in the primary objective, these experiments have contributed to our knowledge of *M. nelsoni*. It has been established that:

M. nelsoni plasmodia will persist in infected oysters kept in aquaria for at least 6 months though no multiplication was indicated.

Natural infections can occur in running seawater systems under some conditions.

M. nelsoni plasmodia can be transferred to a susceptible host and persist for as long as 8 days in the circulatory system.

Both normal and infected tissue may be transplanted into a susceptible host, the plasmodia appearing to remain viable up to 2 months, though infections do not spread to and develop in the host.

There is some indication of a toxic factor associated with infected tissue.

DEVELOPMENT OF A SALTWATER EMBAYMENT FOR MOLLUSCAN RESEARCH

A. Russell Ceurvels

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Department of Natural Resources
Boston, Massachusetts

The Massachusetts Department of Natural Resources, Division of Marine Fisheries, has recently acquired an 8-acre saltwater pool and 8 acres of upland. The Division plans to develop this area for a marine laboratory and shellfish culture station with emphasis on shellfish research. Larval shellfish will be available in limited quantities to

the cities and towns of Massachusetts for the rehabilitation of depleted shellfish areas. Particular consideration will be given to the science of aquaculture as a sound economic use of the resources.

RELATIONS OF SPOIL DISPOSAL TO SHELLFISH AREAS

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Natural Resources Institute
Chesapeake Biological Laboratory
Solomons, Maryland

Recent observations on the shallow-water disposal of dredged fine-grain sediments from the upper Chesapeake Bay provide information useful in the selection of spoil disposal sites. Field observations of the distribution of fine sediments in water, the deposition of sediments on the bottom, and the short-term spread of those sediments over an unexpectedly wide area was described. The possibile effects of such sediment on shellfish, and possibile responses of some organisms to deposition was discussed, with special reference to the upper Chesapeake Bay. Guidelines were suggested for assuring adequate protection of shellfish beds and other important benthic communities.

POTOMAC FISHERIES: POTENTIAL AND OPPORTUNITY

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During the four years since the implementation of the Potomac River Compact of 1958 by the formation of the Potomac River Fisheries Commission, the commercial fisheries of this largest tributary of Chesapeake Bay have been under joint Maryland and Virginia management. With the assistance of both states, the new agency has begun a progressive fisheries management program. Prior to this, disagreements between the states concerning the utilization of Potomac River fisheries had prevented effective management of these valuable resources. The Potomac River Fisheries Commission uses research and management advice from Maryland and Virginia as a basis for planning and operations. This cooperative effort offers the promise of increased seafood production in an area having a great fishery potential.

GONYAULAX WASHINGTONENSIS, ITS RELATIONSHIP TO *MYTILUS CALIFORNIANUS* AND *CRASSOSTREA GIGAS* AS A SOURCE OF PARALYTIC SHELLFISH TOXIN IN SEQUIM BAY, WASHINGTON

John L. Dupuy [1] and Albert K. Sparks

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Several aspects of the relationship of *Mytilus californianus* and *Crassostrea gigas* to the toxic dinoflagellate, *Gonyaulax washingtonensis*, from Sequim Bay on the Strait of Juan de Fuca have been studied using unialgal mass cultures for feeding experiments.

Five feeding experiments were conducted in which mussels (*M. californianus*) received *G. washingtonensis* at a concentration of 50 cells/ml. The feeding of these cells with a known amount of toxin per cell to the mussel has shown that when the standard extraction and mouse bio-assay is used, 75 to 92 per cent of the toxin is found in the shellfish meats. This relatively vigorous uptake of toxin by the mussel is similar to the uptake under natural conditions.

In contrast, feeding and water pumping rate experiments with the oyster (*C. gigas*) demonstrated no measurable uptake of toxin after 3 months. Two types of reaction have been observed to occur when the oyster was fed *G. washingtonensis*. The most common reaction was drastic curtailment of the volume of water filtered, with vigorous clapping of the valves or complete cessation of pumping activity. The second reaction recorded was a 30 per cent reduction in pumping rates with large amounts of pseudofeces being produced. No feces containing *G. washingtonensis* were observed. Concentrations of 20, 40, 80, and 120 cells/ml curtailed or caused cessation of the pumping activity. Culture medium, with cells removed, added to filtered sea water did not appear to affect the oyster's pumping rate. This general physiological reaction would tend to lend support to the hypothesis that oysters under field conditions refuse to accept *G. washingtonensis* initially but after acclimation partially accept this dinoflagellate as food. Results from sampling *C. gigas* at Sequim Bay has shown a 2 week delay in its uptake of paralytic shellfish poison in comparison to the California mussel. Furthermore, results from bio-assay of field samples have shown that the levels of toxin in *C. gigas* are 3 to 4 times lower than in *M. californianus*.

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[1] Present address:
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THE ENZYME HISTOCHEMISTRY OF THE SPORULATION OF *MINCHINIA NELSONI* IN *CRASSOSTREA VIRGINICA* [1]

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Five enzymes were surveyed for reactivity throughout the sporulation process of *Minchinia nelsoni* in *Crassostrea virginica:* NAD diaphorase, non-specific esterase, acid and alkline phosphatase and malic dehydrogenase. Plasmodia and sporonts were generally reactive for all the enzymes studied. There was usually a marked increase in enzyme activity in the sporont stage relative to the plasmodium; this was probably associated with an increase in metabolic activity as the sporulation process started. Early sporocysts manifested a strong activity for mitochondrial enzymes, NAD diaphorase and malic dehydrogenase, but appeared less reactive for the other enzymes. The cytoplasm of the later sporocyst became quite reactive for alkaline phosphatase, concentrations of which could be seen clinging to the cytoplasm surrounding the mature spore walls. Acid phosphatase present a similar picture. Mitochondrial enzymes appeared highly reactive in the cytoplasm of the sporocyst especially in the immediate vicinity of the spore, but sparsely distributed in the sporoplasm of the mature spore; usually 2-6 mitochondria could be observed per spore. Concentrations of non-specific esterase fell sharply as the sporocyst matured, although isolated granules were observed in the cytoplasm of the sporocyst as well as the sporoplasm of the mature spore.

Oyster tissues infected with the sporulating stages of *Minchinia nelsoni* (tubules of the digestive gland) appeared to maintain a near-normal level of enzyme activity; however, heavy concentrations of the parasite all but replaced host tissue, leaving little glandular tissue available for digestion.

THE MASSACHUSETTS ESTUARINE RESEARCH PROGRAM

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Since 1963, the Massachusetts Division of Marine Fisheries has been engaged in an extensive estuarine research program. This program was proposed by the Marine Fisheries Advisory Commission in December, 1960. Funds for the program were earmarked by the Legislature in 1962.

The objectives of the program as outlined in the Commission's advisory report were:

1. to provide comprehensive information about the current condition of the fisheries in the inshore waters of the Commonwealth,
2. to determine the factors responsible for the decline of fishing industries in certain areas, and
3. to suggest methods of developing or improving local marine fishery production.

With these objectives in mind, the Division is evaluating all of Massachusetts' major estuaries. To date, 9 estuary studies have been completed. Two more are presently in progress. The major study phases in each estuary consist of: 1) Review of the history of the marine fishery; 2) analysis of physical and chemical characteristics; 3) inventory of the distribution and utilization of shellfish, and 4) inventory of the distribution and utilization of finfish and crustacean populations.

In evaluating the shellfish resources of estuaries, primary concern has been given to determining the volume and value of shellfish harvest and, whenever possible, to define harvests in terms of acre production. For instance, in the Pleasant Bay estuary, a total of 11,255 bushels of quahaugs were harvested during a single year from about 640 acres of bottom. Acreage production was valued at about 199 dollars per acre. In another study area, the value of the blue mussel harvest was 371 dollars per acre. In contaminated estuaries, shellfish density studies have pointed out the extensive loss in resource utilization which occurs because of pollution. Biologists studying the Merrimack River estuary estimated that over 300,000 dollars worth of soft-shell clams were not being utilized annually because of pollution. In Quincy Bay, it was noted that with pollution abatement, the value of the soft-shell clam harvest could increase by about 35,000 dollars annually.

Published estuarine reports are serving as guideposts for the wisest management and utilization of our estuarine resources, as well as providing direction for future estuarine research in Massachusetts.

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[1] This research was supported by Contract 14-17-0003-111 with the U. S. Bureau of Commercial Fisheries.

PUMPING RATE OF *MERCENARIA MERCENARIA* AS A FUNCTION OF SALINITY AND TEMPERATURE

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Pumping rates of clams acclimated to temperatures ranging between 6 and 32° C and salinities between 15 and 40 ppt were measured by a metered stream of sea water, colored by soluble non-toxic dye, which completely replaced the incurrent flow. Determinations were made at the acclimation temperatures and salinities, and also after sudden changes of salinity to higher or lower levels.

The results indicated:

(1) At a salinity of 25 ± 2 ppt:

Pumping was completely inhibited at temperatures below 6° C and above 32° C.

A moderate and steady increase in the rate of pumping occurred as temperature was raised from 7 to 12° C.

No marked changes in the pumping rate were observed between 12° C and 18° C.

Maximum pumping occurred at temperatures of 24 to 26° C.

There was an abrupt decrease in pumping rate at temperatures above 26° C.

(2) At temperature of 25 ± 1° C:

Pumping was completely inhibited below 15 ppt and above 40 ppt.

Maximum pumping rate occurred in the salinity range of 23 to 27 ppt.

The lower and upper salinity boundaries for 50 per cent of the maximum pumping rate were 18 ppt and 31 ppt respectively for nonacclimated clams, 17 ppt and 34 ppt respectively for acclimated clams.

INFLUENCE OF SMALL QUANTITIES OF CORNSTARCH AND DEXTROSE ON GLYCOGEN LEVELS OF *CRASSOSTREA VIRGINICA*

Dexter S. Haven and Kenneth W. Turgeon

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Feeding experiments were carried out to determine the effect of cornstarch and dextrose on glycogen content and growth of *Crassostrea virginica*. Statistical analyses showed that starch-fed oysters had significantly greater glycogen content, wet meat weight, shell volume, total volume, underwater weight, and air weight than controls. Other supplements significantly increased glycogen content over the controls but had little effect on other growth parameters.

INSHORE SETTLEMENT OF *CRASSOSTREA VIRGINICA* IN DELAWARE BAY

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Factors were defined which may be important in producing the consistently heavy oyster settlement on the Delaware Bay tidal flats along the west coast of the Cape May Peninsula. A transect of 12 stations extending 4/5 mile offshore was measured for settlement over two seasons with cement-board panels placed throughout the water column. Oysters set heavily at the extreme inshore intertidal area and also 1/2 mile offshore at a slope area which separates the flats from deeper water. The slope settlement area coincided with high oyster larval concentrations. At the inshore area, however, similar setting rates were associated with only a tenth of the larval concentration, indicating existence here of factors promoting stimulation of set.

The offshore slope was the area of steepest gradient in current velocity. The most inshore water mass was characterized by relatively great salinity and temperature increases at early flood tides.

Laboratory-reared larvae, when subjected to similar salinity and temperature increases, were stimulated to settle in response to the temperature, but not the salinity factor. A gregarious tendency in settlement, however, reduced the statistical significance of these findings. Most of the set was generally received on one of several exposed cultch shells and often a heavy set was received which was not related to experimental treatment.

Further investigation of gregarious setting response revealed that individual cultch shells were not differentially preferable in oyster settlement. However, the presence of 24-hour spat and 2-month-old spat on cultch shells attracted set differentially and stimulated settlement.

The offshore slope area may be important in the primary concentration of larvae. Inshore settlement then probably is promoted by factors which may draw in offshore larvae, such as easterly

winds and spring tides, and factors which stimulate settlement, such as a temperature increase and the presence of oyster populations which may promote a gregarious setting response.

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TEMPERATURE MORTALITY STUDIES ON *MYA ARENARIA*

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Recent growth estimates of the steam electric industry reveal a 30 to possibly 256 times increase in electricity requirements by the year 2010. This industry has at present the greatest non-consumptive industrial demand for water as a heat transfer medium. The need for large volumes of cooling water is resulting in the location of many new installations on marine and estuarine environments. There is a growing need for ecological assessment of heated water discharges in the aquatic environment, especially the effects on important commercial and recreational species.

Studies of *Mya arenaria*, an important commercial shellfish on the east coast have been initiated. These studies have used bio-assay techniques to determine temperature mortality levels of *Mya* that were held at a series of acclimation temperatures ranging from 1° C to 30° C in 5° increments. Young-of-the-year clams and adults have been used and the results indicate that, in general, *Mya* are not temperature resistant under summer conditions. Increasing acclimation temperatures do not raise the lethal level to any extent. Young-of-the-year organisms are slightly more resistant than adults. These results were discussed in relation to thermal loading.

GROWTH OF YOUNG CLAMS, *MERCENARIA MERCENARIA*, IN TANKS OF RUNNING SEA WATER

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The well-tested mass culture method for rearing young oysters to metamorphosis is also the standard technique for rearing clam larvae. Unresolved, however, is the most dependable way to rear post-set clams to a size at which they have gained considerable immunity to predation by their principal enemy, the crab. Measures which will effectively protect recently-set clams in the field have not yet been developed. An alternative approach to the problem is to rear the young clams to the proper size in the hatchery. The following account describes our observations on the growth of young clams in sand in rectangular tanks 30 feet long and 4 feet wide through which sea water flowed continuously.

Small clams grew faster than did larger clams. Growth increments of equal volumes of clams 18, 13, 10 and 8 mm long for periods of 30 to 117 days produced the following growth ratios, respectively: 1.0, 1.9, 2.7 and 2.9.

Clams from 10 to 18 mm long grew equally well at planting densities of 8, 16 and 67 ml of clams per square foot of substrate. Expressed as number of clams per square foot planting density ranged from 8 clams 18 mm long to 428 clams 10 mm long.

Clams from 3 to 18 mm long grew equally fast at water flows from 15 to 56 gpm. At 9 gpm the growth rate of clams 8 to 18 mm long decreased by about 33 per cent and that of clams 2 to 5 mm long, over 50 per cent.

When the tanks were fully occupied with shellfish, growth of clams of all sizes was considerably better at all flow rates at the intake end of the tank than at the discharge end; for example, clams approximately 12 mm long, at times, grew almost twice as fast in the former location as in the latter.

Animal fouling in the tanks, especially by barnacles, mussels and tunicates, was severe. Irradiating the water with ultraviolet light before it entered the tanks controlled mussels but not barnacles or tunicates. Dense clusters of mussels which invariably concentrated near the intake of the untreated water tanks apparently reduced the growth of clams throughout the tanks. Growth in these tanks was roughly half as much as in tanks receiving irradiated water.

Growth of clams in the tanks compared favorably with growth of transplanted clams in Milford Harbor, Connecticut, in Wickford Harbor, Rhode Island (Landers, unpublished data), and in Home Pond, Gardiners Island, New York [1].

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[1] Carriker, Melbourne R. 1959. The role of physical and biological factors in the culture of *Crassostrea* and *Mercenaria* in a salt-water pond. Ecol. Monogr. **29**:219-266.

FEEDING RATES OF STARFISH, *ASTERIAS FORBESI* (DESOR), AT CONTROLLED WATER TEMPERATURES, AND DURING DIFFERENT SEASONS OF THE YEAR

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Starfish were held for 28 days in trays of running sea water maintained at a series of constant temperatures. Ample numbers of oysters were provided as food. During this period, at 5.0°C, each starfish ate an average of 2.3 oysters, at 10.0°C, 3 oysters; at 15.0°C, 4.2 oysters; at 20.0°C, 5.0 oysters; at 22.5°C, 2.8 oysters, and at 25.0°C, 1 oyster. Thus, the optimum temperature for feeding was 20.0°C. Starfish lost weight when held with oysters at temperatures above 23.5°C. Observations of variations in seasonal feeding rates were made from trays holding 20 starfish with oysters suspended in Milford Harbor. Starfish fed at low rates from mid-January to the end of March. From then until late June or early July, feeding increased rapidly. After spawning, starfish fed at about one-third of the rate recorded in late June. The period of low feeding, which appeared to be associated with both high temperatures (above 22.5°C.) and spawning, lasted from July through September. From late October, through December, the rate of feeding increased again to about two-thirds of its level in late June and early July. The rate again became low by mid-January.

HYBRIDIZATION IN SPECIES OF *CRASSOSTREA* [1]

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Attempts have been made to hybridize *Crassostrea angulata, C. commercialis, C. gigas, C. iredalei, C. rhizophorae,* and *C. virginica* in all possible combinations. Fertlization and cell cleavage occurred in all reciprocal crosses except when *C. commercialis* was one of the parents. The egg and sperm of this species were totally incompatible with all the other species. The larvae of all the crosses developed to the umbo stage and limited success has been obtained in securing attachment of the several hybrids. Meiotic chromosomes have been examined in all the species and mitosis in the species and hybrids. All species have a diploid number of 20.

[1] Supported by NSF Grant GB-5034

SOME CYTOCHEMICAL OBSERVATIONS ON *MINCHINIA NELSONI*, HASKIN, STAUBER, AND MACKIN, A SPOROZOAN PARASITE OF *CRASSOSTREA VIRGINICA*

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The cytochemistry of *Minchinia nelsoni* was studied in infected oyster (*Crassostrea virginica*) blood and in sectioned tissues. Nucleic acids were investigated with the Feulgen nucleal test, Azure B differential stain, and enzymic digestion. Three Feulgen-positive structures were observed in *M. nelsoni*: (1) A spherical, film-like (Feulgen-positive) structure containing evenly spaced, dense, Feulgen-positive concentrations on its surface. This structure was found in nuclei, generally at the nuclear-cytoplasmic interface, but often more centrally located in large nuclei. The Azure B stain and enzymic digestion confirmed the presence of DNA. (2) A dense, Feulgen-positive mass of variable size (0.2 - 4.0μ) and shape is often observed in a single parasite. During a three-month period, September-November, as few as 4 per cent and as many as 60 per cent of the parasites from individual oysters were found to have such structures. The Azure B stain and enzymic digestion confirmed DNA in this mass. (3) Intensely Feulgen-positive spheres of uncertain location, but apparently in the cytoplasm, were infrequently found. The spheres, when present, were in great abundance in individual oysters. They have been observed only in blood preparations of infected oysters, never in tissue sections from these same oysters. Because of this labile characteristic, it has not been possible to confirm DNA by enzymic digestion. The structures are thought to originate at the nuclear DNA concentrations at the nuclear-cytoplasmic interface, and may be the DNA of a newly-formed nucleus.

The presence of RNA was determined with the Azure B differential stain and confirmed with enzymic digestion. The cytoplasm of *M. nelsoni* varied from finely granular, rich in RNA, to fairly clear, containing coarse RNA granules.

The nuclear-cytoplasmic interface was rich in RNA, often masking the DNA found in this location. The most commonly observed nucleus in *M. nelsoni* was roughly spherical, about 1 - 2 μ in size with one prominent peripheral endosome and a variable number of small, secondary peripheral endosomes. The secondary endosomes correspond in size and location to the concentrations of DNA at the nuclear-cytoplasmic interface. The primary endosome is Feulgen-negative and rich in

RNA. In larger nuclei the primary and secondary endosomes are generally more centrally located.

The nucleoproteins were investigated with the Alkaline Fast-Green procedure of Alfert and Geschwind for the detection of histone, and the Picric Acid-Eosin Y procedure for protamine.

Histone was found in all confirmed DNA locations. The primary endosome and often the entire nucleus give a positive reaction for protamine.

The Periodic Acid/Schiff (PAS) test for carbohydrates was consistently negative for the nucleus. The cytoplasm generally contains fine PAS-positive granules.

SOME OBSERVATIONS ON THE DEVELOPMENT OF EARLY *MINCHINIA NELSONI* INFECTIONS IN *CRASSOSTREA VIRGINICA*, AND SOME ASPECTS OF THE HOST-PARASITE RELATIONSHIP

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Data from 4 lots of oysters (*Crassostrea virginica*) imported to the Cape Shore tide flats of Delaware Bay at various times during the summer of 1966 indicate that one lot became infected with *Minchinia nelsoni* sometime during the first 4 days of exposure (July 2 - 5). Another of the 4 lots imported on July 6 indicates that few, if any, new infections occurred for more than a month following this 4-day infection period. This chance occurrence provided the closest approach yet to a controlled infection experiment.

Individual oysters in the infected lot were compared as to number, location, size and shape of the developing lesions; the appearance and relative number of parasites in each lesion; and the host response to each lesion.

Minchina nelsoni infections originate from a small locus, possibly a single infective particle, in the water tubes of the gills. Only one lesion was observed per oyster with the exception of one oyster which had two separate lesions. This may indicate that the oyster encounters few infective particles or that few of the total number of particles establish an infection.

The fastest growth rate occurs in the earliest detectable lesions. A generation time (doubling of the population) of about 24 hours was calculated.

The parasite appears to be "epizoic" in these small early lesions, being restricted to the labyrinth of folds and crevices formed by the finger-like projections of the epithelium of the water tubes. The parasite spreads up and down the epithelium of the water tubes and enters the epithelium of intraplical water spaces, passes through the ostia and infects the epithelium of the gill filaments. At the base of an infected demibranch, the parasite continues its surface growth by infecting the base of adjacent demibranchs, the palps, or the mantle epithelium.

This epizoic stage lasted about one month after which some parasites penetrated to subepithelial tissues. The time of penetration varied from oyster to oyster and suggested the possibility of an "epithelial barrier". Epithelial sloughing is often associated with advanced lesions, and some lesions have large accumulations of leucocytes outside the gills, indicating that widespread ciliary paralysis may occur. Observable host response to the lesion is leucocytic infiltration of the infected area, often with swelling of the tissue and phagocytosis of the parasite.

In some oysters many of the parasites have a sharply delineated outer membrane, often accompanied by a withdrawal of cytoplasm from the membrane. This condition is associated with an increase in the size of the parasite nuclei and the appearance of coarse cytoplasmic RNA granules. When phagocytized, *M. nelsoni* generally appears condensed with densely staining nuclei and cytoplasm.

THE TOXICITY OF RHODAMINE-B TO EGGS AND LARVAE OF *CRASSOSTREA VIRGINICA*

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Rhodamine-b, a fluorescent dye, is being used extensively to trace water movements in estuarine environments. Since the larvae of various commercially important shellfish comprise an important segment of the biota of estuaries, it is worthwhile to determine the effect of this dye on their survival and growth.

Observations were made on the toxicity of the dye to eggs and larvae of *Crassostrea virginica*. Six decimal dilutions of Rhodamine-b, ranging from 100 ppm to 0.001 ppm, were tested in the laboratory. Eggs were exposed to the dye for 48 hours. Larval exposures were begun with 2-day-old individuals and maintained in the appropriate concentration of dye until all animals died, or metamorphosis began.

At 100 ppm Rhodamine-b, no eggs developed. At 10 ppm there was some development to the straight-hinge stage, but 27 per cent of the individuals were abnormal. Development at con-

centrations of 1 ppm or lower was comparable to control cultures.

Larvae exposed to 100 ppm Rhodamine-b died within 2 days. At 10 ppm they survived as well as those in control cultures but their growth was initially retarded by 10-17 μ. This retarding effect on growth was temporary, occurring during the first 2-3 days. Throughout the rest of the experiment their rate of growth was equal to that of the controls. Survival and growth of oyster larvae at concentrations of 1 ppm or lower was comparable to that in the control cultures during the test period.

INFLUENCE OF SELECTED ENVIRONMENTAL FACTORS ON THE ELIMINATION OF BACTERIA BY *CRASSOSTREA VIRGINICA*

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Studies were conducted to determine the influence of seawater flow rates, water temperature, salinity, and turbidity on the rate of bacterial elimination by the oyster, *Crassostrea virginica*.

Seawater flow rate study: Results indicated that with proper system design and physiologically favorable conditions of water temperature and salinity, comparable rates of coliform and fecal coliform elimination by the oyster could be obtained with seawater flow rates ranging from 0.5 to 5.0 liter/oyster/hr.

Water temperature study: Oysters acclimated to warmer waters (26.7-29.6°C) and transferred to cooler waters (18.9-23.4°C) eliminated coliform bacteria at a lower rate throughout 48 hours of exposure than oysters retained in the warmer waters. The rate and extent of fecal coliform elimination were comparable at both of the above temperature ranges.

Transferring oysters acclimated to cooler waters (16.3-22.0°C) to warmer water (24.4-35.1°C) had no apparent influence on the rate of bacterial elimination after the first 24 hours of exposure. During this 24-hour interval, oysters exposed to the warmer waters eliminated bacteria at a higher rate.

Experiments conducted to date have employed temperatures in excess of 16°C. Further studies are needed to determine the influence of lower temperatures on bacterial elimination rates by the oyster.

Salinity studies: Oysters exposed to salinities of 5.8 to 25.8 ppt eliminated coliform and fecal coliform bacteria at varying rates. The rate of coliform and fecal coliform elimination was much greater when salinities exceeded 16 ppt. With salinities below 7 ppt the rate and extent of coliform and fecal coliform elimination were greatly reduced.

Results of salinity studies indicated that the time required to achieve satisfactory levels of oyster depuration was dependent, to a great extent, on salinity. Development of a method(s) of salinity control, nondeleterious to shellfish activity, could be important in a plant design that would permit more precise prediction of acceptable endpoint depuration. Such a method(s) could also permit establishment and continuous operation of depuration plants in shellfish areas subject to recurring periods of low salinity.

Turbidity study: Data from this study demonstrated that the per cent reductions of *Escherichia coli* after 24 hours of elimination were comparable in oysters exposed to sea water having average turbidities of 8.8, 19.3, 22.3 and 69.4 Jackson Turbidity Units (JTU). The difference in reduction of *E. coli* in oysters exposed to 69.4 JTU and 8.8 JTU turbidities was 0.002 per cent. Results would indicate that a highly effective filtration system will not be needed for oyster depuration plants on the Gulf Coast.

THE REPRODUCTIVE CYCLE OF SURF CLAMS FROM THE NEW JERSEY COAST

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During the past 3 1/2 years nearly 2,500 gonad tissues of *Spisula solidissima* (Dillwyn) from the New Jersey fishery have been collected and processed for histological examination to determine the seasonal reproductive cycle. The adult clams, with a shell length of 120 mm or more, were caught by commercial hydraulic dredges in the vicinity of Barnegat Lightship, at water depths of 18 to 32 m. Samples of 25 clams were taken monthly in the winter and bimonthly during the spring, summer, and fall when active gametogenesis and spawning occur.

Five arbitrary categories were established to describe stages of the reproductive cycle. The time and duration of spawning is basic information necessary to understand the time of larval occurrence in the water and the eventual settling of juveniles on the bottom. The categories are: early active gametogenesis, late active gametogenesis, ripe, partially spent, and spent.

A biannual gamete maturation sequence cul-

minating in the spent condition indicated that the clams had 2 spawning cycles each year. Spent gonads were collected in July and August and in mid-October, 1962 and 1963, and in July and August and early November, 1964. In 1965, a single maturation and spawning occurred in mid-September to late October. The delay in spawning and single annual cycle was related to the colder environmental temperatures not observed in the 3 earlier years of the study.

WHAT'S WITH A SALT MARSH?

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The role of tidal marshes in estuarine productivity, particularly their impact upon shellfisheries, is not fully understood at this time. Nevertheless, certain undeniable trends — such as losses of shellfish growing areas due to encroaching pollution, the search for improved methods of shellfish culture, and the increase of research-generated information — are sufficient incentives, from a management viewpoint, to explore the possible significance of tidal marshes and comparable ecological units to shellfisheries.

A tidal marsh is a well-defined natural community and can be characterized by the kind and extent of its plant life. Attention is directed toward such communities because they provide food for shellfish. The nature of a middle Atlantic tidal marsh is considered in some detail, as is the relevancy of certain of its aspects to shellfish pond culture.

TRACE METAL ACCUMULATION BY THE OYSTER

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In 10-week and 20-week studies, *Crassostrea virginica* was subjected to nitrate salts of lead (0.025, 0.05, 0.1 and 0.2 ppm) and of chromium (0.05 and 0.1 ppm), copper (0.025 and 0.5 ppm), zinc (0.1 and 0.2 ppm), and cadmium (0.1 and 0.2 ppm), respectively. The 10-week study was conducted on summer-harvested oysters; winter-harvested specimens were used in the 20-week study. About 200 oysters were placed in each of several 50-gallon tanks in a flow-through seawater system maintained at a 2.5 gpm flow rate and water temperature at 20 ± 1°C; salinity varied, 31 ± 2 ppt.

The most obvious effects were observed in the oysters exposed to cadmium and lead. Cadmium-exposed specimens had very little shell growth, lost pigmentation of the mantle edge and digestive diverticulae, and suffered high mortalities. Comparable morphological data on the lead-exposed oysters were not obtained, but significant mortalities also occurred in that experiment.

FARMING OYSTERS IN ARTIFICIAL PONDS — ITS PROBLEMS AND POSSIBILITIES

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Four 1/4-acre artificial saltwater ponds were put into operation in the fall of 1964 at Oxford, Maryland. Three oyster culture studies were initiated in the ponds: production of seed oysters; comparative growth of 7 strains of Chesapeake Bay oysters, and response of oysters to 4 types of bottom. In addition, a 2-year ecological study was made of the invertebrate succession and pond colonization.

About 10 spat per 30 shells were caught in the first attempt to produce seed oysters in an artificial pond. The seed originated from the introduction of 4 to 5 million straight-hinged larvae (48 hours old). In the comparative growth study, all strains grew at about the same rate, but meat quality was lower than in similar groups suspended in a natural pond. Condition of oysters (percentage of solids) declined on all 4 types of bottom from an apparent lack of food; thus the effect of bottom type was overshadowed by other factors.

Future studies will refine methods of producing seed oysters, and develop methods of feeding pond-held oysters. We believe that artificial ponds have a commercial potential for culturing oysters which will be determined by further biological research.

THE EFFECTS OF ADDITIVES ON THE PUMPING RATE OF *MERCENARIA MERCENARIA*

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The effects of various additives on *Mercenaria mercenaria* pumping rate were investigated. The additives tested included simple organic com-

pounds (glucose and glycine), hormones (acetylcholine, adrenalin, and serotonin), and gonad extracts (male and female *Mercenaria* and *Mytilus*). Clams were maintained in sand in running sea water, and additives were administered directly into the incurrent siphon through a pipette hooked up to a flowmeter and reservoir.

The simple organic compounds and adrenalin and serotonin occasionally, but not consistently, stimulated pumping. Acetylcholine inhibited pumping at 10^{-7} — 10^{-3} M. Male and female *Mercenaria* gonad extracts stimulated pumping as well as spawning. As in the oyster the active principle of the male gonad extract is associated with the sperm and is not water soluble.

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SURF CLAM RESEARCH, 1966

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In our continuing study of surf clams (*Spisula solidissima*), four major research areas were investigated in 1966: fishery statistics, population densities and distribution, growth rates, and reproduction.

Port sampling, interviews with boat captains, and day trips aboard the fishing vessels provided information on fishing areas, catch, effort, clam lengths, and amount of small clams discarded at sea. The major fishing areas are off Point Pleasant and Cape May, New Jersey, in depths of 24 to 110 feet. Point Pleasant and Cape May-Wildwood are the principal ports. Average daily catch per boat was 328 bushels of clams and average catch per hour was 38 bushels. Boats fished an average of 9 hours a day. Average length of clams landed was 151 mm at Point Pleasant and 130 mm at Cape May-Wildwood. The amount of clams discarded at sea was negligible.

Two research cruises were made in 1966. The spring cruise assessed surf clam density and distribution in relation to bottom type and topography in the area from Montauk Point, Long Island, to Ocracoke Inlet, North Carolina. The fall cruise repeated stations established in 1965 between Montauk Point, Long Island, and Cape Hatteras, North Carolina. Only minor changes were found in the distribution or density of surf clams since the 1965 cruise.

A study to establish growth rates of young surf clams was completed in 1966. Marked and unmarked young clams in Chincoteague Inlet, Virginia, were observed from October, 1964, through March, 1967. The mean length of 1-year-old clams is 45 mm; 2-year-old, 69 mm and 3-year-old, 91 mm.

Surf clams in the major fishing area off New Jersey usually spawn twice a year, in summer and in fall, but spawned only once (late September) in 1965. Analysis of water temperature records from Barnegat Lightship indicated that the sum of daily temperatures above 0° C between spawnings may be more important to the spawning pattern than abrupt temperature changes.

NSA PACIFIC COAST SECTION

CHROMOSOMES OF OYSTERS, CLAMS AND MUSSELS

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Several species of bivalves from the Northwest Pacific coast of the United States have been examined for their chromosome complements and other aspects of chromosome cytology. The diploid numbers of some of the species examined are: *Ostrea lurida* 20; *Ostrea edulis* 20; *Crassostrea gigas* 20; *Saxidomus giganteus* and *S. nuttallii* 38 each; and *Mytilus californianus* and *M. edulis* 28 each.

Work on the hybridization of the 2 clams, *S. giganteus* and *S. nuttallii*, and the 2 mussels *M. californianus* and *M. edulis*, is in the preliminary stages. In the respective reciprocal crosses only a few eggs cleaved. Results of the experiments and morphology of the chromosomes are discussed.

A PARASITE FROM THE OVA OF *CRASSOSTREA GIGAS*

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During a recent (1966) summer mortality in *Crassostrea gigas*, 5 mature female animals were collected in Humboldt Bay, California and examined histologically. An unidentified parasite was observed in the cytoplasm of the ova of 3 of these oysters. Several different stages of the parasite were observed in the cytoplasm, but never in the nucleus. Single and multiple infections were observed, with as many as 10 parasites in an individual ovum.

Some infected eggs were necrotic and accompanied by an acute or chronic inflammatory reaction. However, even in the most heavily infected oyster, no evidence was seen of fibrous encapsulation which is frequently observed in oysters exposed to foreign bodies or pathogenic organisms. Neither an inflammatory response nor granulation tissue was observed among the eggs of the uninfected female oysters.

The relationship of this parasite to summer mortalities in Humboldt Bay is unknown — and probably unimportant, since the parasites appear to be confined to the ova.

BRITISH COLUMBIA CRUSTACEAN FISHERIES AND RESEARCH PROJECTS — PAST AND PRESENT

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The most valuable fishery in British Columbia is for Dungeness crab (*Cancer magister*). From 1961 to 1965 annual catches averaged 3.7 million pounds, with a landed value of 486 thousand dollars. Crabs are taken almost exclusively by traps, with a small amount caught incidental to fish trawling. The main fishery is near the Queen Charlotte Islands, and smaller areas are located around Vancouver Island and near the Fraser River mouth.

Trawling yields most of the shrimp production consisting of 5 species of *Pandalus*. Another species, the prawn (*P. platyceros*), is trapped. Important shrimp grounds are found around Vancouver Island and near Vancouver. Annual catches, 1961 to 1965, averaged 1.5 million pounds with a landed value of 238 thousand dollars.

Crab investigations, at first concerned with basic biology, started around 1917 and have continued with interruptions until the present. Recent crab work deals with population dynamics of Queen Charlotte Island stocks. Larval stages of shrimps were described around 1930, and growth rates and hermaphroditic reproduction determined. Since 1953 shrimp projects have included exploratory fishing, further life history studies, and a study of the dynamics of an inshore population. In 1964 an experiment to introduce Atlantic lobsters was started.

WOUND REPAIR IN *CRASSOSTREA GIGAS*

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University of Washington

An experiment was conducted to investigate histologically the wound healing process in *Crassostrea gigas*. Sterile wounds were inflicted in the visceral mass adjacent to the palps and the progression of healing was followed. Internally, the leucocytes rapidly filled the wound channel, elongated and formed a scar. At the surface, leucocytes formed a band under lying the mantle epithelium and replaced damaged epithelium. Eventually these leucocytes formed a pseudoepithelium which became indistinguishable from the adjacent normal columnar epithelium.

THE EFFECTS OF OIL FROM THE WRECKED TANKER, *TORREY CANYON*, ON SHELLFISHERIES RESOURCES OF ENGLAND AND FRANCE

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Crude oil released shortly after the tanker, *Torrey Canyon*, grounded on Seven Stones Reef off Southwest England came ashore along the coast of Cornwall, England and Brittany, France. Measures to control the spread of oil included spraying of 1/2 million gallons of detergents or solvent-emulsifiers at sea. In addition, over 2 million gallons of detergent were applied ashore in England to remove the oil from sandy beaches and rocky headlands. Adverse biological effects were increased by application of detergents which are much more toxic than oil.

In France, use of detergents was prohibited except on certain beaches important to tourism and unimportant to commercial fisheries.

Actual loss to commercial fisheries in both England and France was small but this was largely because of fortuitous circumstances of geography, currents and weather which prevented contamination of estuaries where oysters are farmed. Similar oil spills in other areas could have disasterous consequences for commercial shellfish resources.

SUBTIDAL SHELLFISHERIES DEVELOPMENT

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A survey of the subtidal hardshell clam populations of Puget Sound has recently been initiated by the Washington State Department of Fisheries. Actual field work started in July of 1967; therefore this report will be largely confined to objectives and methods.

The primary purpose of this program is to encourage the private development of commercial fisheries for subtidal clams. The study consists of 3 phases: (1) To conduct with scuba divers underwater surveys of hardshell clam populations in specific areas of Puget Sound; (2) to develop, in cooperation with industry, appropriate gear and harvest methods for clam species present; (3) to develop management practices for subtidal populations of commercial clams and geoducks.

Survey work completed thus far has been confined to Kilisut Harbor near Port Townsend. Buoys are first placed around the perimeter of the survey area and equally spaced transects are laid out. Divers take bottom samples of 2 sq ft each at equal intervals along each transect with a modified 4-in venturi gold dredge. Sample material is caught in baskets of the appropriate mesh size, hauled aboard the boat and clams are identified and grouped according to size.

Commercial quantities of geoducks *(Panope gererosa)*, native little necks *(Venerupis staminea)*, horse clams (*Schizothaerus nuttalli*), and butter clams (*Saxidomus giganteus*) have appeared in the samples. Wet weight of clams has varied from a few ounces to over 4 lbs/sq ft in samples taken in Kilisut Harbor.

From the limited data it appears that subtidal clams are most abundant in areas of tidal currents rather than the heads of bays. Subtidal clam populations are frequently composed of a single size group, apparently the result of a "good set year". Test diving in Hood Canal has shown commercial quantities of butter clams as deep as 60 ft below 0 tide level.

Dredging in Puget Sound has indicated that commercial dredges used on the East Coast are not suitable here because of large rocks in the substrate. Therefore, modification of these dredges or the development of new types will be necessary.

Information obtained in the study will be utilized to establish long-range management programs for subtidal clam resources in the Puget Sound area.

SEASONAL CHANGES IN THE CONDITION OF *CRASSOSTREA GIGAS* AND POSSIBLE RELATIONS TO NUTRIENT SOURCES

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A continuing study of Pacific oysters in Grays Harbor, Washington, in 1966 and 1967, involved sampling oysters from 1964 imported Japanese seed at two stations. Growth and condition of the oysters from average weights, and total solids and glycogen contents, are reported. Per cent total solids of oysters from both stations was lowest in winter and highest in summer. The weight of dried total solids followed a similar cycle except for pronounced drops which occurred after spawning. Very little weight recovery of total solids occurred during the subsequent fall and winter months. The level of glycogen storage in the oysters was highest in late spring and dropped to a minimum in summer.

Phytoplankton standing crops, and levels of particulate organic carbon, chlorophyll A, organic phosphate, and dissolved and particulate carbohydrate based on weekly sampling at both stations are also reported. These features are of interest since they may be utilized as food by oysters or may reflect levels of food utilized by oysters. Phytoplankton standing crops were relatively low in midsummer and winter with maximum abundance during the spring and fall blooms. On the average samples from the more seaward station had fewer phytoplankton organisms. Numbers alone, however, were of limited value for comparing plankton biomass from the two areas. Organic phosphate and chlorophyll A follows the phytoplankton cycle except in midwinter when chlorophyll A levels were lower and organic phosphate levels were higher.

Dissolved and particulate carbohydrate levels seemed correlated with phytoplankton abundance in late spring, summer, and early fall. Winter levels seemed more influenced by other environmental conditions. Conclusions based on the organic carbon data were limited because of the imprecision of the method. Relative levels of carbon, however, as well as those for chlorophyll A, organic phos-

phate, and particulate and dissolved carbohydrate averaged higher at the station located further upbay.

Of the five environmental features investigated, chlorophyll A levels correlated more significantly with weight changes of oyster total solids. For the more seaward station, $r = 0.62$ ($P<.01$); for the upbay station, $r = 0.57$ ($P<.01$). A better relation was obtained using the product of log temperature and chlorophyll A levels with weight changes of total solids expressed as per cent (relative growth). For the seaward station, $r = 0.79$ ($P<.001$) and for the more upbay station, $r = 0.75$ ($P<.001$).

DESCRIPTION OF LABORATORY-REARED LARVAE OF *PARALITHODES PLATYPUS* (BRANDT) [1]

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Larvae of the blue king crab, *Paralithodes platypus* (Brandt), were hatched and reared in the laboratory. All larval stages obtained developed in a manner similar to the development reported for other lithodid anomurans. In culture, *P. platypus* had 4 zoeal stages and a single glaucothoeal stage. The feature which distinguishes all zoeal stages of this species from zoea of the other two North Pacific species of *Paralithodes (P. camtschatica* and *P. brevipes)* is the presence of 9 pairs of telson processes (including a hair-like second process) rather than 8 pairs. Glaucothoe of *P. platypus* have one more pair of spines in the branchial region of the carapace than do those of *P. camtschatica.* Glaucothoe of *P. platypus* have 15 pairs of spines on the dorsal surface of the carapace — not including the spines of the frontal area (rostral complex) or the suborbital spines — whereas the glaucothoe of *P. Camtschatica* have 14 pairs of spines, and the glaucothoe of *P. brevipes* have 13 pairs.

[1] This paper appears in full in J. Fish. Bd. Bd. Can. 25(3):439-455, 1968.

THE PATHOLOGY OF "SPONGY" DISEASE IN FRESHWATER MUSSELS

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In March, 1967, 123 freshwater mussels (*Margaritana margaritifera*) were collected from the Ozette River in Washington. Seventy-five of these animals possessed large watery lesions on the foot, which have a resilient characteristic similar to a sponge; hence the name "spongy" disease. In some animals, the large spongy lesions were replaced by scarred wounds, in many instances bordered by multiple small papillary lesions that also had a spongy resilience.

Histologically, the large watery lesions are edematous areas in which some of the normal muscle tissue has been replaced by fibrous connective tissue. The epithelial covering over the afflicted area is disorganized, necrotic, lacking or reduced to a squamous lining. Those animals that possess scarred wounds and multiple small papillary lesions have a subacute inflammatory reaction with well-developed granulation tissue and collagen deposits. This disease affected only the foot of the mussels.

POST-EMBRYONIC DEVELOPMENNT OF LABORATORY-REARED "SPOT" SHRIMP, *PANDALUS PLATYCEROS* BRANDT

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"Spot" shrimp were reared successfully in the laboratory from eggs stripped from females caught in Dabob Bay, Washington. Eggs were incubated in circulating bubbling sea water at 53°F. Hatching occurred after 41 days while eggs on ovigerous females held at the same temperature took 22 days longer. Larvae were cultured in floating 800 ml beakers and were fed newly hatched brine shrimp nauplii. Six stages were recorded before the post-larval stage. The shape of the telson was characteristic for each of the six stages but was not of diagnostic value for older stages. Larvae were also kept at 51°, 53°, and 55°F. Survival was best at low temperatures in the early stages but larvae at higher temperatures survived the longest. Larvae at 55°F moulted 4.5 days ahead of larvae at 51°F. Highest mortality occurred during the moulting process.

LARVAL REARING STUDIES OF THE DUNGENESS CRAB, *CANCER MAGISTER* [1]

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The Dungeness crab is an important Oregon resource. Large fluctuations in annual landings prompted studies to determine the extent to which

selected environmental factors may limit crab abundance. Studies were conducted during the larval development, a critical period of crab life history.

Preliminary work developed culture methods for rearing crabs through the larval period with good survival. Crab larvae were reared in 250 ml Erlenmeyer flasks. The nauplius of the barnacle, *Balanus glandula*, was found to be a suitable food organism. Relatively equal numbers of barnacle nauplii were fed in each culture flask by using an automatic pipette. Good survival was maintained until crab zoea metamorphosed into the cannibalistic megalopa.

Subsequent work established the effects of combinations of temperature and salinity on survival and growth of crab zoea. Zoea were tolerant of greater ranges of temperature and salinity than are normally found in the ocean. Survival of zoea exceeded 70 per cent within temperature and salinity ranges of 50 to 64°F and 25 to 30 ppt respectively. Development time was inversely related to temperature.

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[1] Supported in part by the Commercial Fisheries Research & Development Act (P. L. 88-309)

THE SUPPLEMENTAL FEEDING OF *CRASSOSTREA GIGAS* IN THE LABORATORY

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Three laboratory experiments were conducted using cornstarch as a supplemental food for *Crassostrea gigas*. Starch was fed at 17 mg/l, 34 mg/l, and 68 mg/l. Water flow was maintained at 10 l/oyster/hr in a continuous flow system. Temperatures and salinities were recorded but not controlled during each 32-day experiment.

In Experiment 1, a noticeable increase in condition index occurred in oysters fed at 17 mg/l over nonfed laboratory control oysters, but greater increases occurred in condition index of field-control oysters over laboratory oysters. Results of Experiment 2 showed that supplemental feeding at 17 and 34 mg/l greatly increased condition of oysters over laboratory nonfed and field-control oysters. In Experiment 3, oysters were fed at 17, 34, and 68 mg/l with less conclusive results. Oysters fed at 17 and 34 mg/l did better than those fed at 68 mg/l, while the difference was highly significant between supplementally fed and nonfed oysters treated as groups.

It appears that supplemental feeding improves oyster condition at all times of the year, but that the optimum feeding rate varies by season or as a result of changes in physical parameters.

SOME EFFECTS OF A DENSE RAZOR CLAM SET IN WASHINGTON

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A dense set of razor clams, *Siliqua patula* Dixon, occurred on ocean beaches north of Grays Harbor, Washington in 1966. Patches of clams up to several acres in extent and 1 to 3 inches deep were noted for 2 to 3 weeks in June along 10 miles of beach. Set reached a record maximum of 13,600 clams per ft^2 in a single sample for this unusually early set.

The 1966 year class persisted at a high abundance level for 7 months, but grew at a greatly reduced rate. Growth of older clams was also adversely affected. There were indications that plankton concentration was depressed north of Grays Harbor during the period of dense set.

Availability of clams was adversely affected between September and December. Recovery of marked clams by standard digging methods averaged 5 per cent per dig north of Grays Harbor compared to about 20 per cent per dig at 2 beaches south of Grays Harbor.

BACTERIAL ACCUMULATION-ELIMINATION RESPONSE OF PACIFIC OYSTERS (*CRASSOSTREA GIGAS*) AND MANILA CLAMS (*TAPES PHILIPPINARUM*) MAINTAINED UNDER COMMERCIAL WET STORAGE CONDITIONS

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The accumulation and elimination of bacteria by Pacific oysters and Manila clams held in commercial wet storage was studied under varying hydrographic and climatological conditions. A series of 3 experiments was conducted in 2 locations in the southern Puget Sound area. In each experiment, samples of commercially harvested shellstock, depurated shellstock and overlying water were collected at various periods through a complete tide cycle. Bacteriological examination included the most probable number (MPN) of coli-

form and fecal coliform bacteria and 20°C plate counts using MacLeod's seawater agar. Water temperature, salinity, turbidity and precipitation data were also collected. Changes in coliform and fecal coliform levels in sea water correlated closely with the tidal cycle. Shellstock collected from various sections of the float showed similar rapid responses to changes in bacterial levels in the water. Examination of depurated oysters or clams positioned at various locations in the float indicated that these shellfish rapidly attained coliform and fecal coliform levels similar to those of the naturally harvested shellfish. This study is being continued to include other types of commercial floats in the Puget Sound area.

STUDIES ON THE JAPANESE OYSTER DRILL

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During the last 20 years, much research has been directed towards control of the Japanese oyster drill, *Ocinebra japonica*. In this report past research and plans for further research is discussed.

Past research on biological control has discovered the following vulnerable points in the life cycle and behavioral patterns of the Japanese drill: (1) Egg cases and juveniles are more susceptible to toxicants and environmental changes than adult drills or oysters; (2) male drills are attracted to water-born substance released by egg laying females, and (3) preliminary observations on sex of the drills indicate changes in relative abundance or distribution of male and females as the drills increase in size. If this last point is true, a control method may be possible by interfering with normal reproduction of the drill population. Future research will continue to examine these phenomena as basis for control methods.

Past research in chemical control of the drill has shown that, due to the physiological similarities of the adult drills and oysters, a complete chemical control may not be safe or economically feasible. Future studies in chemical control will test candidate chemicals: (1) against the more susceptible egg cases and juvenile drills, (2) as caustic poisons that would eliminate all drills from barren oyster ground, (3) as barriers to prevent reinfestation of oyster beds, and (4) for their ability to suppress the breeding potential of the adult population.

Past research in physical controls has shown that barriers are not effective unless used in conjunction with improved chemical repellants. Improved spawning site collectors and other means to collect egg cases or drills warrant further investigations.

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BCF ROLE IN FARMING OF THE SEA[1]

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There are many conflicting reports about the abundance of fish and shellfish in the oceans and their potential as a source of food for the millions of people existing on inadequate diets throughout the world. Some uncertainty also exists in the minds of many scientists about the role of aquaculture in augmenting this food supply.

It is not difficult to find articles written by "experts" who say that food from the sea is a myth, and that only through agricultural techniques can we hope to supply the food needed by our expanding population. On the other hand, many marine scientists have demonstrated through their own calculations that the oceans can supply the needed food. There is no doubt about my belief. I am convinced by the evidence presented that the oceans have been overlooked and neglected as a source of all-important animal protein.

At the Law of the Sea Institute meetings at the University of Rhode Island in June, 1967, several speakers presented some exciting estimates of possible annual yields of fishery resources from the oceans. The present world marine catch is approximately 46 million metric tons. Dr. M. B. Schaefer has estimated that the total annual potential is 200 million metric tons. Drs. W. M. Chapman and H. Kasahara estimate 2,000 million metric tons, while Dr. W. R. Schmitt's estimate is 4,000 million metric tons, or nearly 100 times the present world catch. Which of these predictions is the most accurate is of little significance, according to Dr. Schaefer, because an annual production of 200 million metric tons, if fed directly to people, would provide about 15 grams of fish per day for 6 billion people — twice the present world population. The total daily protein requirement of an individual is estimated at about 80 grams, of which 15 grams should be high-grade animal protein.

Only a portion of the potential annual world fish catch would be composed of edible species of types now accepted by the American people. In fact, Dr. Chapman breaks the potential catch down into three sizes of fish according to length: over 10 inches, 5 to 10 inches, and under 5 inches. Most species presently in demand in the United States, with the exception of mollusks and some crustaceans, are longer than 10 inches and are relatively expensive because the public wants particular flavor, texture, and appearance. The herring-like fishes which form a major part of the present world catch are included in the 5 to 10-inch category. The greatest volume of protein in the ocean, however, is in animals less than 5 inches long. These, for the most part, are not considered desirable as human food and therefore are inexpensive in the United States. Of course, shellfish whether mollusks or crustaceans, fall logically within the category of the more expensive species.

If wild fish and shellfish in the ocean can be harvested in the amounts predicted, why bother with fish farming? The answer is that aquaculture could provide a steady and probably increased supply of the high-priced seafoods now in greatest demand. My view is that industry has the potential for producing tremendous quantities of fish and shellfish in the relatively near future. I also see the possibility of producing large crops of less expensive fish through marine farming, but not within our lifetime. One of the reasons for this latter prediction is that we have far too little basic information on the techniques which must be used. Also, the cost of producing the less expensive fish would not permit us to compete with the cost of harvesting the wild species. For example, anchovettas are now landed in Peru for about $15 per ton. Hake, from the new resource discovered off Washington and Oregon, are harvested for approximately $18 per ton; and anchovies in California for about $20 per ton. It would be almost impossible for fish farming to compete with these costs in the United States.

With this background, let us look at some aspects

[1] Remarks of Mr. H. E. Crowther, Director of the Bureau of Commercial Fisheries, before a meeting of the 59th Joint Annual Convention of the Oyster Institute of North America and the National Shellfisheries Association at Boston, Massachusetts, on July 17, 1967.

of farming the sea by first drawing an interesting parallel with the development of agriculture.

The United States is the recognized world leader in agriculture, a position which has brought much prestige, and admiration from many countries. Agriculture achieved this status in the United States through simultaneous development of four courses of action: (1) improvement of the environment, (2) plant and animal husbandry, (3) agricultural engineering, and (4) product development. In addition, knowledge gained from research and development has been effectively transmitted to the farmer and agricultural industry through extension activities, including demonstration and training programs and nontechnical publications.

In developing commercial fish farming we can gain much by considering the history and accomplishments of American agriculture. In a sense, we have a blueprint to follow. The important lesson inherent in the history of agricultural development is that progress has followed sound and adequately financed research. Much aquacultural research is following this blueprint — but slowly. At present, aquatic husbandry and environmental improvement are emphasized, but we recognize the need for additional studies of engineering and product development. Such studies will lead eventually to the development of farms or factories to produce high-value, high-quality fish and shellfish to provide variety to our diet.

Now let me briefly review some studies of aquaculture being carried on by the Bureau of Commercial Fisheries.

Scientists at our Boothbay Harbor, Maine, laboratory are acquiring knowledge of the importance of suitable habitat to lobsters by using SCUBA divers to observe lobster behavior and the rate of colonization on a man-made reef. The relation between the surf clam and its ocean environment is being studied at our Oxford, Maryland, laboratory. At other laboratories, pesticide and radiobiology studies are adding to our knowledge of the accumulation and effect of these pollutants on commercial species.

At Milford, Connecticut, we have had some success in developing artificial culture methods for oysters and clams, some of which are being tested on a commercial scale. Our new laboratory at Milford, when in full operation, will carry forward this work more rapidly. Already we are investigating the genetics of mollusks, with a view to developing new strains and hybrids with more desirable characteristics.

Also at Milford we have developed some methods of protecting shellfish against predators in the natural environment. When perfected and approved, these techniques, coupled with an assured source of seed from hatcheries or other protected environments, can eliminate some of the major problems faced by shellfish growers today.

At Oxford, extensive studies in shellfish culture are also now underway. Techniques in catching seed oysters from shells suspended from rafts are being developed. In addition, off-bottom culture of oysters in both natural ponds and in man-made ponds are being investigated.

Also at Oxford, we hope eventually to extend our studies of disease to all marine animals, not just shellfish. As sea farming develops, problems with diseases undoubtedly will become more serious and it will be necessary to learn how to cope with them. The Bureau of Sport Fisheries and Wildlife has already made many important advances in knowledge and control of diseases of freshwater fishes.

At Galveston, Texas, our Bureau has made some promising beginnings at shrimp culture. Through the work being done in state and other laboratories as well as our own, we visualize eventually a thriving shrimp farming operation to provide select products for the gourmet trade.

These are the programs of the present. Now let us look at future possibilities.

SEA FARMING

There has been much talk recently about farming the edges of the sea. This approach has many attractive features, but we find the prevalent concept too narrow. Although there are intriguing possibilities for culture of marine animals and plants under some form of private ownership, there will always be resources to be harvested as common property and we see some exciting and completely untested avenues to be explored. These include (and we have broadened our definition of aquaculture to include such activities):

a. *Altering the Environment.* — Why do some relatively large areas of the ocean produce 300 pounds or more per acre per year, yet the average fishing yield of the world ocean is less than two pounds per acre? Can yields in "desert" areas be improved by altering the environment? The answer is yes, but we do not understand the mechanisms nor are we able to anticipate the costs at all well.

b. *Positive Approach to Waste Disposal.* — Domestic and many industrial wastes contain the essential nutrients for plant growth and increased production of animals. Yet our indiscriminate disposal of wastes into the natural environment brings out the harmful, rather than the potentially beneficial effects. Why not examine the feasibility of controlled waste disposal to enhance fishery production? This could be much cheaper than the

inevitable alternative, which is to remove these wastes entirely from the natural cycle.

c. *Hatchery Production of Ocean Fishes.* — Some of the earliest attempts to stabilize and improve marine fishery production were to rear large numbers of young in hatcheries and release them into the natural environment. Extensive effort in this direction was exerted for many years and on many species, among them; cod, flounders, and lobsters. The concept died slowly, but its demise was inevitable, because it produced no demonstrable effect and was not conducted scientifically. Our molluscan culture studies have provided a better understanding of what we must know to successfully operate marine hatcheries. We feel this idea should be reexplored and believe that many marine species can be reared through the critical early stages when mortality is high, if scientific methods are followed carefully. We have no estimates of the cost of large-scale culture, but we do know that the potential rewards are great. Such techniques might provide the only practical mechanism by which fluctuations in survival of abundant schooling fishes could be reduced or eliminated. This in turn could be the key to successful management of fisheries based on widely fluctuating resources like menhaden or, for that matter, almost any estuarine resource. Such developments could be fundamental to the success of a fish protein concentrate industry at home or abroad. A massive effort is necessary before we can evaluate the potential of hatchery techniques.

d. *Pond Fish Culture.* — An attractive alternative to all the difficulties inherent in achieving rational management of ocean resources would be to concentrate on scientific pond fish culture in fresh waters and in selected coastal areas. While we have been preoccupied with the hypothetical potential of the vast ocean, exciting developments have been going on almost unnoticed in Arkansas and neighboring states. Catfish production has been rising sharply and the potential exists in a five-state area in the Middle South to increase our total domestic fish production at least fivefold. In fact, if we set costs of production aside for the moment, there is no reason why we could not supply much of the increasing world requirements for animal protein well into the future from privately controlled waters in the United States. Private production offers attractive relief from most of the baffling and virtually insoluable problems of production from public waters. Private production could well be the key to survival of some of our commercial fisheries. There is no good reason to question the feasibility of private commercial production in fresh waters, but techniques, costs, and markets require careful examination. These questions deserve vigorous inquiry.

Let me close by reiterating my feeling that aquaculture of high-value fish and shellfish holds great promise for the United States fishing industry. In view of the increasing demands on the estuarine areas for recreation, urbanization, and industrialization; controlled farming of some species may be the only way we can maintain or increase production of the valuable species. There is no question that our expanding United States population will require more food; fishery products can supply some of this food. Aquaculture can assist in this increased production and provide the kind and quality of fish and shellfish that the public of the United States will be seeking.

For some species of fish and shellfish the rewards of research will come soon, since the states, industry and the Bureau of Commercial Fisheries have already accumulated a substantial amount of data on the production and management of clams and oysters. For others, the payoff may not be within our lifetime but the potential is so great that we owe it to future generations to begin the basic studies now. I hope budget conditions will soon be such that the Bureau of Commercial Fisheries can carry out its share of this needed research.

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THE STATES' ROLE IN MANAGEMENT OF SEA RESOURCES[1]

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"From a conservation viewpoint, the fisheries are perhaps the most poorly managed of all our national resources". Dr. Lionel Walford made the above statement more than 20 years ago; it's about time to take a hard look and see if he is right, and if so, to find out who's responsible and what can be done about it.

We inherited our basic philosophy of fisheries administration from the English, who, since the Magna Charta, have considered wild animals to be the common property of all citizens, held in trust by the Crown in its sovereign capacity. Following the Revolution, the sovereign power was transferred to the various States, and remains there today. Jurisdiction over game and fisheries resources, *per se*, has never been vested in the Federal government. The Federal government must derive what authority it has over these resources from the commerce clause, from broad interpretations of the "general welfare of the United States", from treaties, and from a liberal interpretation of its power to "make all needful rules and regulations respecting the territory or other property belonging to the United States."

There appears to be little doubt, then, that the States have the basic authority, and the obligation, to manage the marine resources within their boundaries. We hear quite a bit (from the States) about the States' rights and authority; we hear very little from the States about how well they have lived up to their obligations.

In all honesty, I think most of us will have to admit that the States, in general, have done a pretty miserable job. About the only management techniques that have been practiced (with the exception of oyster rehabilitation work) have been aimed at limiting the harvest. While these may have been effective in spreading the catch among more individuals, and in preventing gross overfishing, they cannot increase populations above the level already provided by nature. True management must have this as its goal — to provide a greater harvestable crop than would have been available otherwise — and we're a long way from achieving that goal.

Let's explore a few of the reasons why the States have not been able to do a decent job of managing these resources. First, the prime management tool that has been employed has been regulation of the catch. If overfishing is a significant limiting factor, then catch restrictions are biologically valid. If they are imposed in such a manner as to effect an increased monetary return per unit of fisherman effort, then they are economically valid. If a particular catch restriction is really worthwhile, its effect should be noticeable in the fishery after a reasonable period of time. Take a look at the catch restrictions in your State. How do they measure up?

A number of states have size limits on various species of marine fish. These regulations have apparently been enacted because of the feeling that "every fish should have the opportunity to grow up and reproduce." Whether or not reduction of the juvenile population is reflected in the size of spawning population, or whether the size of the spawning population is limiting the size of the succeeding generation, is rarely considered, and even more rarely known. Yet laws are enacted and officers are required to enforce them. Of course, the legal size of many species is adjusted downward through political manuevering until the restriction is essentially meaningless, or such qualifications as "unless obviously injured or dead" are written into the law. What fish could not become "obviously injured" when landed?

Many States have laws which restrict the size of certain units of gear (headrope of trawls, length of seines, number of dredges, etc.). These restrictions may reduce the harvest, and may be biologically justifiable, but they do it by reducing the efficiency of a commercial enterprise and placing it at an economic disadvantage in competition with other industries.

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[1] Remarks of Dr. David A. Adams, Commissioner of the Division of Commercial and Sports Fisheries, before a meeting of the 59th Joint Annual Convention of the Oyster Institute of North America and the National Shellfisheries Association at Boston, Massachusetts, on July 17, 1967.

My point is this. If there is some reasonable biological evidence that a population is being overfished, then the harvest should be reduced. Fishing should be regulated through the method that results in the greatest reduction in harvest with the least effect on fishing efficiency. Limits on entry into the fishery, seasons, catch quotas, selective gear (where possible), are all preferable to methods which cut into profits by reducing fishing efficiency.

There are a few examples of effective management through catch restrictions. The Pacific halibut fishery has profited through imposition of a quota-by-area system; the Alaska salmon fishery was probably saved by rather rigid restrictions backed up by biological monitoring (though the economics of some of the gear restrictions may be argued); the Hudson River shad run increased following restriction of fishing mortality. Let me emphasize, however, that only the last of these examples was a result of State effort; the halibut fishery is governed by international commission and Alaska was then a territory administered by the Federal government.

There is a biological principle which holds that the size of populations is limited by some single factor or group of factors working together. These limiting factors may change in time and space, and may be entirely different for different species. That is to say that the size of the east coast population of shad may be limited by the inaccessibility of sufficient spawning waters; that the west coast sardine population may be limited by the competition of anchovies; that the middle Atlantic coast menhaden population *may* be limited by overfishing on juvenile stocks; that the southeastern oyster population may be limited by insufficient cultch; and that the North Carolina estuarine population of penaeid shrimp may be limited by extreme winter temperature. However, the Hudson River example shows that shad in that river were at one time apparently limited by fishing pressure; anchovy competition apparently was not significant until the sardine population was reduced through overfishing; even if the fishing pressure on juvenile menhaden is reduced, destruction or deterioration of nursery areas may prevent this fishery from ever reaching its former level; adequate cultch may be available in middle Atlantic and New England waters, but oysters cannot increase because of disease problems and insufficient set; and winter temperatures may not be nearly as limiting on Gulf shrimp as they may be in more temperate waters. All I am trying to say is that there is always something or a group of somethings that holds a population at a given level. If we want to increase that population, we must first find out what that factor is and then we must be willing and able to do something about it.

Finding these limiting factors takes time, money, and trained manpower. At the present time, State agencies[2] are spending about $18 million per year, about a fourth of which is Federal aid, on research and development work, and have more than 600 persons employed in this area. All coastal States except Florida, Virginia, Mississippi, and North Carolina are now spending more for research and development than they are for law enforcement — a commendable reversal of past history. Only Virginia does no research and development within its marine fisheries agency (this function being carried out by Virginia Institute of Marine Science), while Texas spends almost $6 million per year on these activities. Intensity of effort, then, is not too bad — ranging from 0. 1 per cent to 15+ per cent of the value of each State's commercial catch — and is increasing each year. The time element is one which we can't do too much about, since it is difficult to reconstruct the past, but one which will decrease in importance in future years as backlogs of information accumulate.

Once the necessary information is available, utilizing it in a management program takes guts and, usually, money. There are few fisheries administrators who don't have guts — they can't last long without them. Most States now employ professional people in these positions, people who have at least some research training, are in the business as a career, and who are somewhat resistant to political pressure. It is not easy to face a group of irate fishermen who feel that a decision which injured them personally was a result of prejudice or politics, even when such a decision is backed up by reasonably adequate data, and would benefit the State fishery as a whole. Those of us who are unwilling to stand up to such groups had better get out.

Being willing to effect logical management programs is one thing, being able may be another thing entirely. Let us look first at the biological implications and then at the socio-political ones. In order for an agency to manage effectively a population of animals, it must have jurisdiction over the entire geographic range of the population. If Alaskan salmon are being taken in excessive numbers by foreign vessels on the high seas, there is little that the State of Alaska can do about it. If Louisiana's estuarine fisheries are being damaged

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[2] Does not include Alaska, Delaware, Massachusetts, New Jersey, and Washington, from whom information was not received in time for inclusion.

by pesticides carried down the Mississippi River, the State of Louisiana has little control over the situation. Things get even worse when parts of the same estuary lie in different states, as is the case in Long Island Sound, Delaware Bay, Chesapeake Bay, Mississippi Sound, and the mouth of the Columbia River. All this is merely to illustrate that, although the individual States have the responsibility for managing estuarine and marine fisheries, geography prevents their doing a decent job even if they could otherwise do so. The alternatives are interstate compacts or agreements of some sort (how effective have these been?) or Federal control through either constitutional amendment or some pretty far-fetched interpretation of existing constitutional law (how much chance does this have?).

The socio-political factors are even more complex than the biological ones. Let us take for an example a species which lives out its life entirely within a single body of water, within the jurisdiction of a single State, and one about which we have a considerable volume of biological information — oysters. You will remember that at the beginning I pointed out that fisheries are a public resource, held in trust by the State for the benefit of all its citizens. Yet there are among the citizens about as many points of view as there are people. Regardless of how well founded in fact a resource program is, it must have the support of a significant segment of the public or it is doomed. Distasteful as it may be at times, those of us in fisheries administration are painfully aware that individual people cast the ballots which elect the legislatures which appropriate the funds which sustain our programs, which pass the laws which we must enforce, and which, in many cases, can reshuffle agencies and personnel with an amazing amount of dexterity. Getting back to oysters, the State has an obligation to maintain a public oyster fishery. To do so it should employ the most efficient management tools applicable to the waters concerned, and it must weigh the effect of its management upon other, sometimes incompatible, uses of the basic fisheries resource. In some waters, natural reproduction and growth may sustain a fishery (with adequate protection) if cultch is available, and cultch may or may not be available; in others, seed must be brought in from other waters, and the users of those waters may be so vociferous in their objections to the removal of seed oysters that the State's efforts are effectively thwarted. Sometimes a local fishery must be sacrificed for the greater common good (as is the case with some development projects), and the ire of the local oystermen is something to be heard. Sometimes a proposed development project must be modified or eliminated in order to protect valuable nearby oyster resources (and land developers can get a bit excited at times, too). The same piece of bottom cannot support an oyster fishery, a haul seine fishery, be dragged by shrimp trawls, and serve as a bathing beach and recreational area — some decision as to its best use must be made, and any such decision will cause repercussions from the supporters of the losing cause. The State's responsibility to maintain a public fishery does not necessarily conflict with its right to lease, or otherwise grant franchises for private management of oyster grounds, but the proper balance between support and encouragement of private enterprise and maintenance of a public resource can be a pretty thorny problem.

So far, I've painted a rather bleak picture of State management of marine fishery resources. The picture is not entirely black; progress is being made and some promising courses of action are open to us. Once the limitations on State management have been realized, we can begin to do something about them.

A State may not have jurisdiction over all the waters entered by a given species during its lifetime, but it can do a decent job of stewardship during the time that species is in its waters. It can take steps to alter environmental conditions which are detrimental to its total fisheries. It can correct legal inequities which are based on prejudice rather than sound reasoning and fact. It can make certain that catch restrictions are first of all necessary, and that they are accomplished through the most efficient means biologically and the least disruptive economically. But it can only do these things if one important element, largely absent in past management, comes to the forefront. In the past, oystermen have spoken for oystermen, shrimpers for shrimpers, purse seiners for purse seiners, charter boatmen for charter boatmen, beach fishermen for beach fishermen, *ad infinitum*. As a result, much of the State agencies' efforts have been concerned with attempts to resolve disputes among these various factions, disputes usually based on selfish motives and short-sighted objectives. As long as fisheries administrators must serve as referees instead of being able to devote their time to consideration of the entire fisheries resources and plan for their present and future utilization, the resource is going to continue to slide downhill as it is doing now. As long as State legislatures are continually exposed to conflicts among many types of resource users, criticism of their State resource agency, and garbled assemblages of conflicting facts that are confusing to say the least, they will never see the need for a comprehensive program of resource utilization, nor will they see fit to fund adequately those agencies charged with this responsibility. The solution, then, is for each segment of coastal fishermen — clammers, oystermen, shrimpers, sport fishermen of all kinds — to see them-

selves as a part of a greater whole, and to support their State's efforts, through their legislature or other governing body, to such a degree that their State will be able to employ and retain competent men in sufficient quantities and with sufficient financial backing to initiate and implement sound, comprehensive programs for the total fishery. This takes a little personal sacrifice, for decisions of the agency will invariably benefit one segment more than another, or injure one segment more than another, and personal sacrifice is not too common a quality among modern man. Without such an approach, however, we will continue to be faced with a continual stream of petty bickering while the health of the basic resource upon which all segments are dependent continues to decline.

In summary, then, the individual States have the responsibility for managing their fisheries as a public resource. They are prevented from doing an efficient job because of both biological and sociopolitical reasons. The States are increasing their efforts toward sound management (within the above limitations and with the assistance of the Federal government). The true value and intelligent management of coastal fisheries will be realized only when competitive users are willing to compromise their differences and support a comprehensive resource program within their various States.

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OYSTER MORTALITY STUDIES IN VIRGINIA. VII. REVIEW OF EPIZOOTIOLOGY AND ORIGIN OF *MINCHINIA NELSONI* [1, 2]

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ABSTRACT

Intensive epizootics in Crassostrea virginica *caused by* Minchinia nelsoni *(MSX) show no signs of abating in lower Chesapeake Bay. Prevalences of the pathogen have commonly exceeded 50% in susceptible stocks during the first year of exposure; mortalities of 50 to 70% occurred during the first year and slightly lower losses in succeeding years. Disease activity increased in isolated lots of oysters from 1963 to 1966 during a drought period with high salinities. Native and planted oysters were extremely scarce in the lower Chesapeake Bay hence density of populations appears not to be important for disease activity. Seasonal patterns of death rates have remained stable through eight years of observations but morbidity has occurred earlier in drought years. These patterns are influenced by time of import of unexposed susceptible oysters to areas of disease prevalence.*

Laboratory-bred progeny from susceptible (unexposed) and selected (by MSX) parents were held to marketable size in areas where MSX is intensively active without serious losses (10 to 20% annually). Early exposure appears to be important for subsequent survival of large oysters; hence, acquired resistance is postulated.

M. nelsoni *has not produced epizootics of oysters in the high-salinity environment of the Seaside of the Eastern Shore of Virginia;* M. costalis *(SSO) regularly causes a sharp mortality there in May-June each year. Since these congeneric sympatric pathogens are very similar morphologically, it is postulated that their annual life cycles are similar, with June the period of sporulation. MSX appears to be highly virulent since death of the host usually occurs before sporulation which is rare.*

A hypothesis on the origin of a virulent strain which arose in Delaware Bay in 1957 and appeared to spread to Chesapeake Bay in 1959 is based on seed oyster transplantations between the areas.

INTRODUCTION

Nearly ten years of activity of the oyster pathogen, *Minchinia nelsoni,* in the estuaries of the middle Atlantic coast have been observed. Mortalities first appeared in Delaware Bay in 1957 and the pathogen was first observed by Stauber in 1958 (Haskin, Canzonier and Myhre, 1965). Early patterns of prevalence and mortality were reported by Haskin in 1959 (personal communication). The disease was found in Chesapeake Bay in 1959 (Andrews and Wood, 1967) where it quickly became epizootic. In 1966, the organism was named and described as a haplosporidian by Haskin, Stauber and Mackin (1966), and further linked with this group of parasites by Couch, Farley and Rosenfield (1966) on the basis of rare spores. The morphology and life cycle are incompletely known and sporulation is rare. Most information is based on field studies and collections. Laboratory cultures and infections have not been attained. The organism seems to be highly virulent and extensive damage has been inflicted on the oyster in-

[1] Contribution No. 263, Virginia Institute of Marine Science. Research supported in part by U. S. Fish and Wildlife Service, Bureau of Commercial Fisheries, Contract 14-17-0007-330, Subproject 3-6-R-1.

[2] Manuscript submitted for publication on July 26, 1967.

dustry of the middle Atlantic states, particularly in Chesapeake and Delaware Bays.

The epizootiology was described by Andrews (1966) from data collected through 1963. Subsequently, three dry years have brought some changes in the intensity and the seasonality of "MSX," as the organism is commonly called. Additional information on the effects of age and history of oysters is presented. The program of research has been broadened from epizootiology of susceptible oysters to include a search for resistant strains of oysters. A discussion of possible origins and life cycles is offered from the meagre clues provided by field studies. Finally, some findings that may permit oystermen to limit losses in afflicted areas are presented.

This paper presents evidence and discussion on three primary subjects: 1) confirmation, changes and refinements of epizootiological concepts given earlier; 2) evidence for innate and acquired immunities in oyster populations; 3) hypotheses on the origin and life cycle of the pathogen. The dramatic appearance and persistent depredations by *M. nelsoni* demand some attempt to rationalize its origin and history.

METHODS

Disease activities have been monitored with cohorts of oysters in plastic and wire mesh trays. Wild oysters transplanted from disease-free areas are referred to as "imports." The designation "progeny" is restricted to laboratory-bred groups spawned from parents chosen for their history of exposure to MSX. Groups of 300 to 1000 oysters have been held on oyster grounds in legged trays. Regular examinations and counts have provided accurate death rates and eliminated predation and physical causes of deaths. Little attempt has been made to control density of oysters. Each cohort was held as a separate population until numbers declined to about 100 oysters. Samples of live oysters were taken sparingly and all gapers (dead oysters with meats) were examined. Methods of handling trays and processing oysters have been described previously (Andrews, 1966; Andrews and Wood, 1967).

Several stations have been established in Virginia waters and often duplicate trays have been maintained at one or more stations (Andrews and Wood, 1967). "Off VIMS" refers to an oyster bed one-quarter mile offshore of the laboratory. Data from VIMS pier trays have been avoided because of *Dermocystidium* occurrence. Seasons of importation and histories listed in graphs are important factors for various cohorts. Seasonal patterns of morbidity and mortality were the chief parameters sought. Conclusions are drawn only when differences were large in magnitude. "Background" losses are acknowledged (usually less than 10 per cent annually) but are not explainable. Deaths from the fungus organism, *Dermocystidium*, were excluded for the most part by isolation of trays (Andrews, 1965). Many samples of live oysters were examined each year from private and public beds to follow disease activities in various commercial operations. These tests have aided greatly in confirming that tray data were representative of oyster beds.

Mortalities are expressed as death rates per month (30 days) regardless of period between examinations. In line graphs, the death rate is plotted at the end of the period of observation, hence refers to the period preceding the point. Death rates were obtained by dividing number of deaths during the period by number alive at the beginning of the period. Plotted death rates cannot be added to obtain cumulative mortalities. Total cumulative mortalities for peaks are often given below the curves with end points chosen as illustrated by vertical bars in Figure 1. Prevalences in all graphs are given as number of cases of MSX per 25 live oysters. No data are given for *Dermocystidium* because it was not involved in these studies.

In referring to the history of oysters, the terms early-and late-summer exposure (or infections) are used because a sharp break occurred in patterns of morbidity and mortality about 1 August (Andrews, 1966). Early-summer exposure applies to all oysters imported to an endemic area from November through July whereas late-summer infections were initiated in August, September and October.

The term "selection" is used in the phenotypic sense of choice of breeding stock by the activity of MSX and has no implications of phylogenetic changes because genotypes are unknown. The terms infectiveness or infectiousness refer to the ability of the pathogen to produce disease.

RESULTS

MSX in Susceptible Imports

The patterns of prevalences and mortalities for the years 1960-63 were derived from exposure of susceptible James River oysters. Each year new lots of the same oyster stocks were imported and observed until depleted. Intensity of MSX activity increased in the drought years of 1964 to 1966. Timing of events was earlier and death rates were higher but the basic patterns prevailed (Andrews, 1966). The following data confirm earlier observations under conditions of higher infective pressure by MSX.

Mortality data from spring and late-summer imports of susceptible oysters are compared for two-year periods in Figure 1. Spring imports are

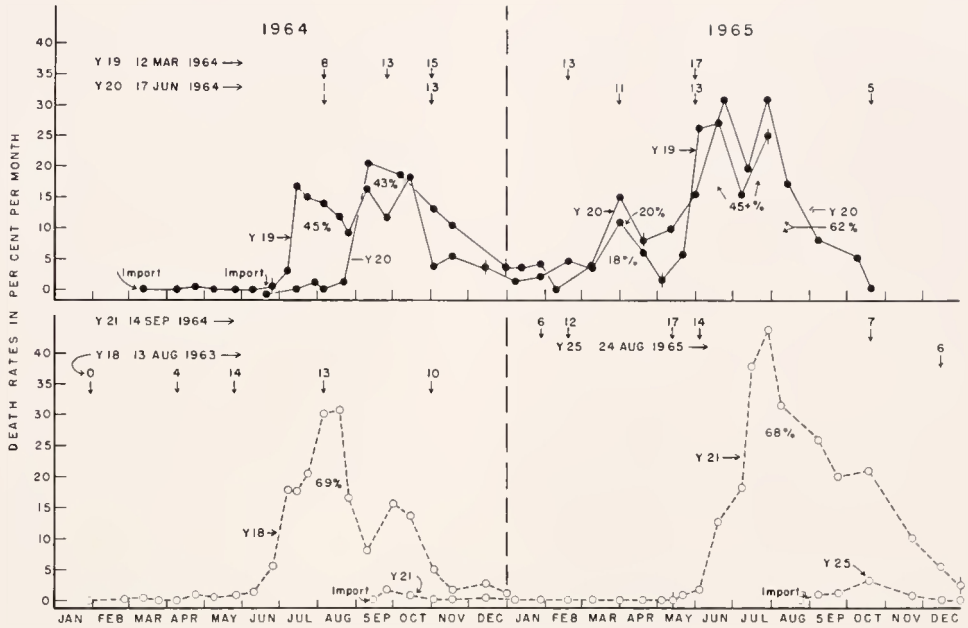

FIG. 1. *Death rates of susceptible oysters, imported 1) winter and spring (upper graph), 2) late-summer and fall (lower), for first two years. Cumulative mortalities for major peaks are given as percentages for periods delineated by vertical bars through points. Prevalences of MSX in cases per 25 live oysters are given above arrows designating dates of sampling. Station off VIMS.*

depicted in the upper half of the graph and fall imports in the lower half. The March imports (Y19) exhibited the earliest first-summer deaths in seven years of monitoring. Deaths began before mid-July whereas early August is typical. Oysters imported in mid-June (Y20) began dying about six weeks later, approximating the minimum period from infection to first deaths. Death rates were high in late summer and fall but declined as temperatures dropped. In the second year, a late-winter peak in March was followed by severe mortalities the second summer beginning in late May. Typically, death rates declined earlier in the second fall than in the first.

In contrast, late-summer imports of 1963 (Y18) began dying only slightly earlier than spring imports of 1964 but death rates were higher. Without the high prevalence in Y18 in May, one might suspect that infections occurred in both groups in May and June. It is possible that intensity of infection was boosted by infective particles acquired in early summer. A double mortality peak with the later one smaller is typical (Y18). The late-summer import of 1964 (Y21) repeats the pattern. Note that no mortality occurred for nine or ten months after import in either year. Infections were not clinical until May in the years 1960 to 1963 except for an occasional localized one. After 1963 deaths began as usual in June although infections began appearing earlier each year. In 1964, the first clinical infections were found in early April, whereas in 1965 infections appeared in January 1965 or earlier. The trend of late-summer infections becoming clinical earlier in drought years continued in Y25 which had 6 of 25 live oysters infected in December 1965 (Fig. 1). Many more cases had appeared by May 1966 (Fig. 2). Late-summer imports did not show mortality prior to June until 1967 (Y35, not shown).

A comparison of mortality peaks in 1965 (Fig. 1) suggests that most June deaths in Y19 and Y20 were from infections initiated in the early summer of 1964. These were apparently the last oysters to die of those first exposed and infected in May-June 1964. Perhaps late-summer infection pressure aided in their eventual demise. Y21 oysters imported in September 1964 died mostly in July 1965, matching the second peak in spring imports.

Prevalences of MSX are shown above arrows indicating dates of sampling (Fig. 1). Levels of infection in spring imports reached about 50 per cent during the first summer mortality peak and did not subside appreciably until after the second summer of losses. By this time some 75 per cent of

the imported stocks were dead. In late-summer imports, infections became clinical well ahead of deaths although this did not occur in earlier years (Andrews, 1966).

The same patterns occurred in 1966 with higher mortalities — particularly in spring imports (Fig. 2). Y28 and Y29 are duplicate trays except that the latter was located at AMOCO station three miles below Gloucester Point in the York River. It will be noted that the late-summer import (Y25), although showing patent infections in early winter, did not exhibit deaths until June of the year after import. Y35 had high prevalences in fall and winter and deaths began in winter and accelerated slowly through the spring of 1967 (not shown). By 1 June 1967, Y35 and a duplicate tray (Y34) had about 15 per cent mortality from MSX. Death rates increased steadily through cold winter and spring months without following the typical end-of-winter pattern. Both mortalities and prevalences in 1966 appeared to be higher than in 1964 and 1965.

MSX activity in 1966 is compared for several additional lots of oysters in lower Chesapeake Bay in Figure 3. Both time of import and source are variables in these groups although all were free of MSX initially. Horsehead oysters, imported in March 1966, were monitored in Mobjack Bay (MJ15) and Hampton Bar in James River (J14). Prevalences and mortalities were slightly lower at these stations than at VIMS but the timing and patterns were similar. A striking contrast was obtained with MSX-free Potomac River oysters imported 29 June 1966 during the period of early-summer infectivity (Fig. 3). Susceptibility is possibly higher than in James River imports because these Potomac River oysters were collected 50 miles above the occurrence of MSX. However, it is believed that the timing of the import is the major cause of the excessive activity by MSX. At the peak of this kill on 23 August 1966, 40 of 42 gapers had MSX and the other two were too rotten for diagnosis. After 70 to 80 per cent of the Potomac oysters had died (two trays with 700 oysters each initially), prevalences of MSX in the survivors

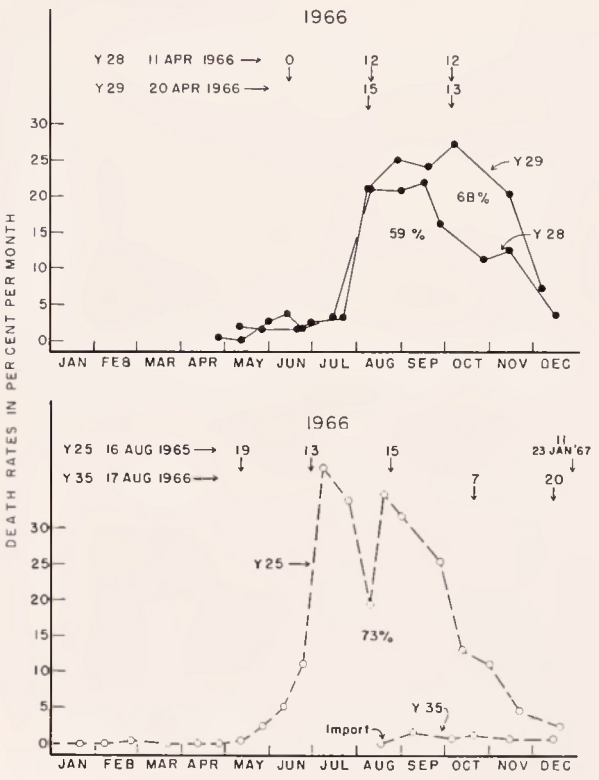

FIG. 2. *Death rates and prevalences of spring and late-summer imports in 1966. All were susceptible oysters imported from Horsehead Bar in James River to VIMS and AMOCO (Y29 only) stations.*

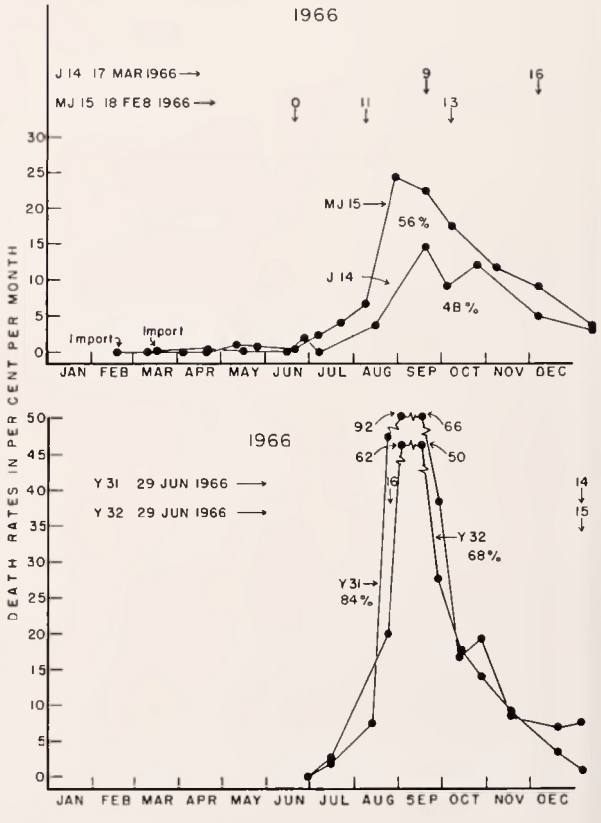

FIG. 3. *MSX activity in spring imports at Hampton Bar (J14) and Mobjack Bay (MJ15), and June imports of Potomac River susceptibles off VIMS. 1966.*

were over 50 per cent in January 1967. Note that prevalences were not much higher in Potomac oysters than in other groups but death rates were very high and more peaked in August and September. These groups of oysters illustrate the importance of timing and history of imports if severe losses are to be avoided, as the next lots further demonstrate.

MSX in Native Oysters and Progeny in Endemic Areas

One important factor in the epizootiology of a disease is the age of host organisms. It was noted early that young oysters had low death rates but it was assumed this was related to size and that MSX gradually selected populations more vigorously as oysters became larger. This was the experience with the disease caused by *Dermocystidium*. MSX infections in spat were observed in the 1958 epizootic in Delaware Bay (Haskin, personal communication, 1960).

For most studies of MSX patterns, James River oysters 2 to 3 inches in length were imported. James River is the primary source of seed oysters in Virginia although other seed areas have been developed since 1963. In lower Chesapeake Bay, small seed oysters are subject to predation; hence the use of large susceptible James River seed oysters is natural and practical.

In March 1965, Virginia opened, for the first time, public beds of the Piankatank and the Great Wicomico rivers for seed oystering. Beginning in 1963, these beds had been planted with reef shells obtained by a suction dredge. Samples of seed oysters in January and April 1965 showed the 1963 yearclass to be 60 per cent infected with MSX and small 1964 yearclass spat 20 per cent infected on the same bed. Due to crowding, few oysters were over an inch in length. A group containing both yearclasses was moved to VIMS in November 1964. Incidence of MSX declined in spring and summer of 1965 without much mortality and two years of monitoring revealed that losses were relatively low. These observations increased interest in the use of small, young oysters for rehabilitation of oyster beds although attempts to breed disease-resistant oysters were begun in the summer of 1962.

Other observations and reports indicated that native oysters in areas epizootic for MSX were producing some marketable oysters. The Nansemond Ridge area of Hampton Roads produced a sizable crop of oysters between 1960 and 1965. Deep Rock bed off the mouth of the Piankatank River was observed to have good survival and marketable oysters in the epizootic years of 1964 to 1966. Additionally, a native spatfall in September 1964 on pilings at Gloucester Point has grown nearly to market size without appreciable losses — in the same waters where imported susceptibles concurrently exhibited mortalities of 60 to 70 per cent per year. Fortunately, some data have been accumulated to explain these contrasting experiences with MSX.

Two groups of native trayed oysters exposed to the severe epizootics of 1965 and 1966 are shown in Figure 4. Y27 contained Deep Rock oysters almost marketable in size. It is clear that the 50 per cent prevalence of MSX when imported to VIMS in March was greatly reduced in April and May, and a light mortality of short duration indicates recovery of these oysters from infections. Tray Y33 containing VIMS piling oysters (1964 yearclass) showed no evidence of a typical summer mortality and prevalence of MSX was low. It is important to note that little selection of the piling population occurred unless it happened at a very small size when "boxes" (attached valves of dead oysters) were easily detached.

A comparison of susceptible imports and laboratory-bred progeny is given in Figure 5. Susceptible oysters are represented by trays of new imports in 1965 and 1966. The progeny group P10 was bred in the summer of 1964 and averaged three inches in the fall of 1966, hence represents MSX-exposed progeny as yearlings in 1965, two-year olds in 1966, etc. Death rates and MSX prevalences reveal dramatic differences in susceptible and exposed lots. P10 is perhaps the most resistant of 13 progeny groups of the 1964 yearclass being monitored. It was bred from rare survivors of millions of bushels of oysters decimated by MSX in Mobjack Bay. Tray P10 has not shown any of the mortality peaks typical of epizootics caused by MSX and prevalence has been consistently low (June 1967).

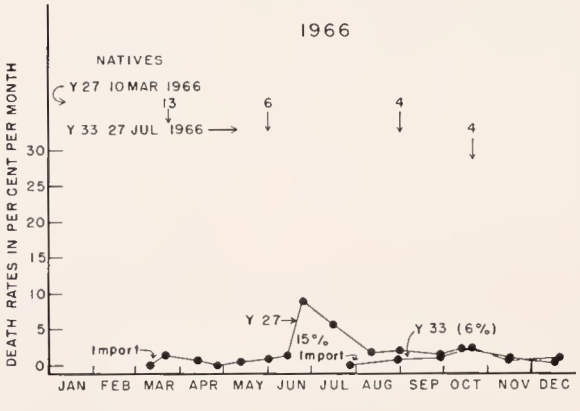

FIG. 4. *MSX activity in native oysters from Deep Rock (Y27) and VIMS pier (Y33) in 1966. Y33 oysters were placed in trays in July but had been exposed at VIMS from setting.*

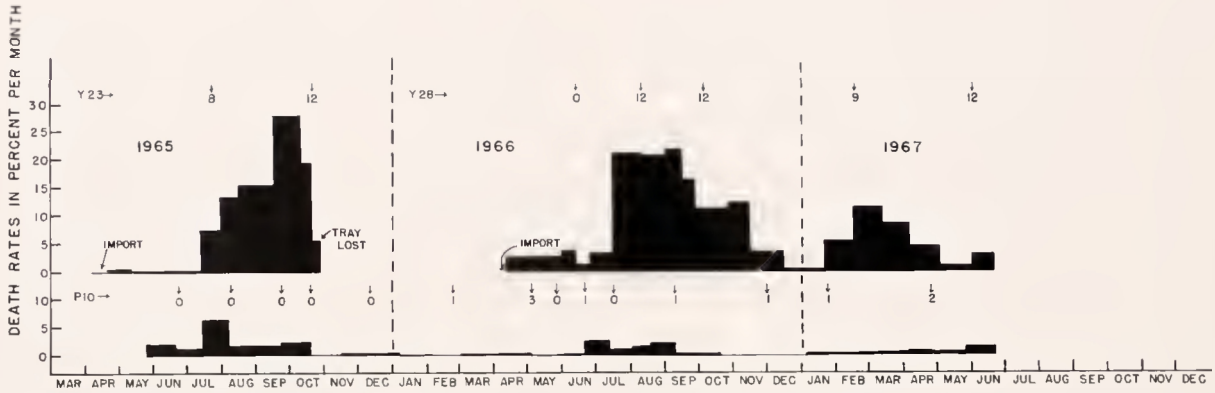

FIG. 5. *Susceptible oysters (Y23, 1965 imports and Y28, 1966 imports) versus laboratory-bred progeny (P10) off VIMS. Horseheads were older and slightly larger in both years but all selection by MSX shown on this graph. VIMS station.*

More typical of the level of MSX in progeny are groups P6 and P7 whose parents were the survivors of several years of MSX selection at VIMS in trays (Fig. 6). These groups show small mortalities at expected times of MSX-caused deaths, although the peak was very late in 1965 as yearlings, and peaks are barely detectable in 1966 as two-year olds. The winter loss of P7 is not typical of progeny, and factors other than MSX were involved. Prevalences, which usually were only one-third or one-fourth as high as in susceptible oysters, peaked in May (typical) but losses were not commensurate. Total losses in trays at two and one-half years of age and market size were about 35 per cent in each tray.

Low prevalences and mortalities were not confined to oysters reared from MSX-selected parents (survivors of epizootics). It is probable on the basis of population sizes alone that the native oysters which survived epizootics (in three separate river systems) were derived mostly from upriver breeding populations which had little or no selection by MSX. An example of a progeny group reared from unselected (susceptible) parents is P14 shown in Figure 7. These oysters bred in February 1965 from Horsehead parents are essentially comparable in size to 1964 progeny. Low prevalences and late moderate mortality were exhibited in 1966. The two groups of progeny depicted in the upper graph of Figure 7 are from MSX-selected parents. Both were held in Ames' Pond until March 1965, then moved to Horsehead Rock in the upper James River seed area where MSX is not prevalent. In April 1966 at a size of nearly three inches, P13A was moved to VIMS for exposure to MSX. Mortality and prevalences were surprisingly low although typical in timing. Background levels of mortality in disease-free low-salinity areas are exhibited by P13, which was a control group held at Horsehead Rock.

P14, raised from unselected parents, appears to have as good a record of resistance to MSX as progeny bred from selected parents. It was one of the few groups held at VIMS from time of setting,

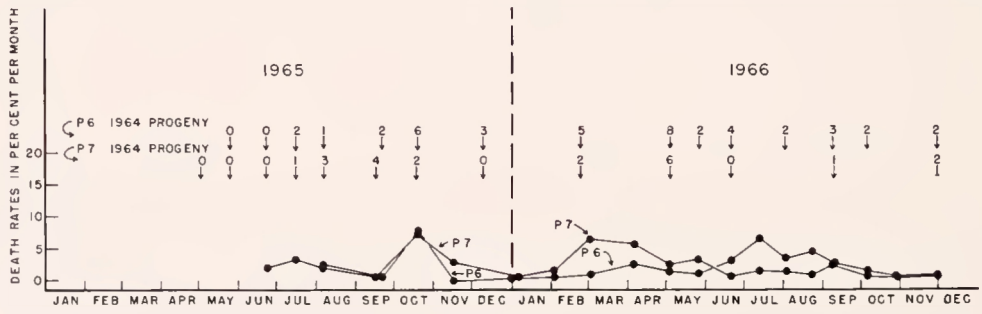

FIG. 6. *MSX activity in laboratory-bred progeny at VIMS (P6 and P7). Average size in 1966 was 65 mm in June and 75 mm in November.*

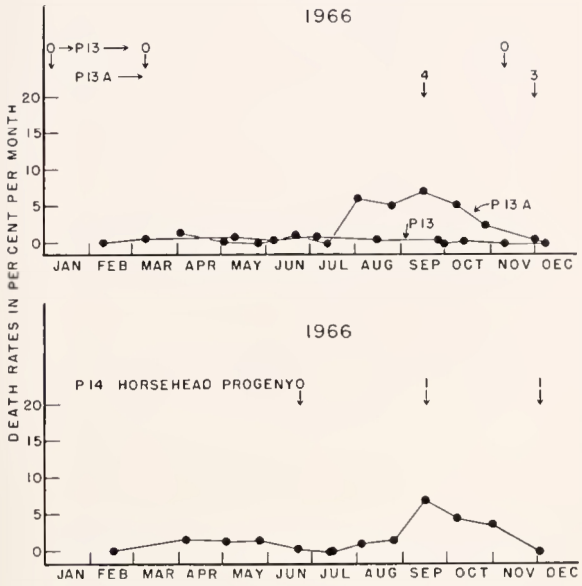

FIG. 7. *MSX activity in progeny from selected (P13 and P13A) and unselected (P14) parents. VIMS station except the control group P13 at Horsehead, James River, an area free of MSX.*

hence exposed intensively to MSX.

MSX Activity on the Seaside of Eastern Shore

Mortality patterns and prevalence data indicate very little MSX activity in the bays of Seaside of Virginia. Monitoring for MSX on the Seaside in 1959 and 1960 resulted in the discovery of Seaside Disease of oysters caused by *Minchinia costalis* (Andrews, Wood and Hoese, 1962). It was reported in 1962 that oysters on the Seaside did not exhibit the epizootic patterns of MSX and subsequent data have sustained this conclusion through 1966. In 1962, prevalence data for gapers and live oysters were presented indicating *M. costalis* (SSO) was much more active than *M. nelsoni* (MSX) in native oysters. Since there is a possibility that native oysters have developed some resistance to MSX, data from susceptible James River imports were chosen to demonstrate the inactivity of MSX on the Seaside. No attempt has been made to compare the level of activity of SSO in the six years illustrated.

James River oysters, imported to the Seaside in 1959, survived unexpectedly well for 12 to 14 months (Figs. 8 and 9). A sharp mortality, caused by SSO, occurred in May-June in 1960 but there was no evidence of epizootics from MSX in either year. Prevalences of MSX were low in live oysters. Fall samples are particularly significant because SSO is not clinically evident then. Four relatively isolated bays on the Seaside gave similar patterns of little mortality and low MSX morbidity during a two-year period. High prevalences of SSO were found in gapers during the May-June epizootics in 1960 as demonstrated previously for native oysters (Andrews *et al.*, 1962).

Two imports in later years are depicted in Figure 10. The mortality patterns and low morbidity from MSX again demonstrate the inactivity of *M. nelsoni* on the Seaside. The graphs also show that oysters go through their first SSO epizootic without losses and that this exposure apparently initiates infectons which result in an epizootic the next May-June period. The Long Island oysters were imported as spat hence were rather small and young for a full-scale SSO epizootic in 1963.

Mortality and prevalence data are given for two trays of native oysters in Hog Island Bay (Fig. 11). Mortalities were low and erratic except for the deaths caused by *M. costalis* each June. Deaths

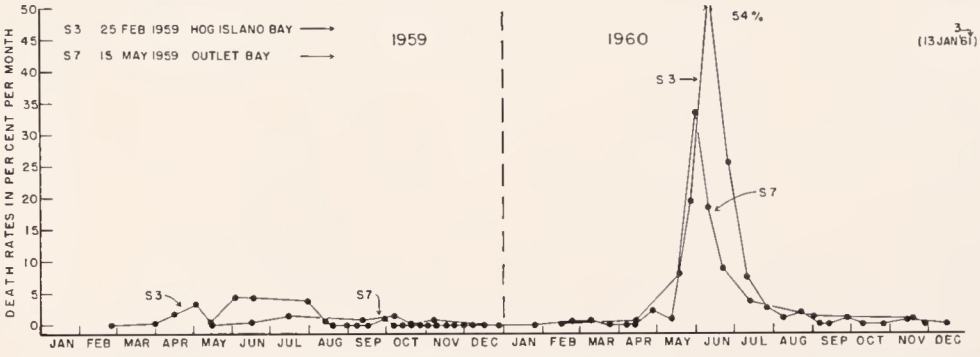

FIG. 8. *Susceptible Horsehead oysters on the Seaside of Eastern Shore Virginia (S3 and S7). The mortality peak in 1960 was caused by SSO. MSX was not active as indicated by absence of typical mortality peaks and scarcity of infected oysters.*

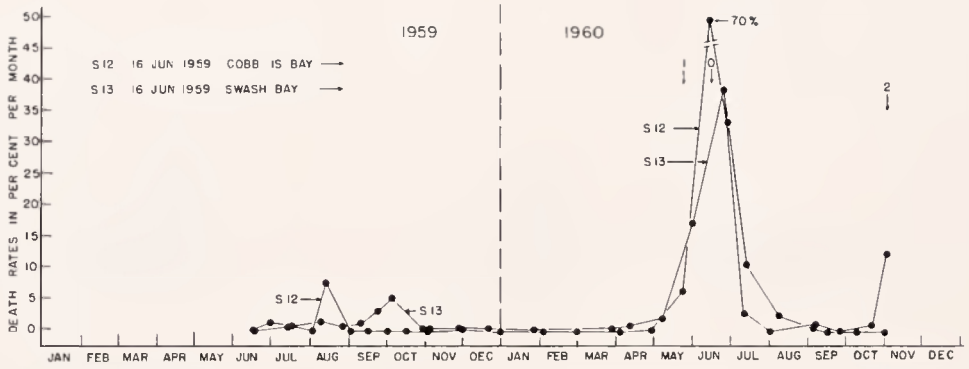

FIG. 9. *Horsehead oysters in two additional Seaside bays reveal little MSX but a strong SSO kill in 1960 (S12 and S13).*

in tray S49 for the period ending 30 September 1966 are of unknown accidental cause and should be disregarded. Prevalences in these groups, although low, suggest that MSX played a minor role in the 1966 deaths in S49. The summer death curve of this tray is spread much wider than in typical SSO epizootics.

MSX has not caused enough deaths in eight years of monitoring tray oysters on the Seaside to produce a recognizable peak in mortality curves. Exposure of susceptible oysters indicates that the environment is unsuitable in some way. The regular occurrence of MSX on the Seaside suggests that its failure to become epizootic may not be due to scarcity of infective materials although nothing is known of dosage. In contrast, SSO is an important cause of deaths but can be avoided for nearly two years by proper timing of imports.

DISCUSSION

The epizootics of oysters caused by *Minchinia nelsoni* show no signs of abating after eight years of devastation. My first description of the epizootiology of MSX was based on five years of field studies (1959-1963). The oyster industry in lower Chesapeake Bay was destroyed in these first years of MSX activity which were considered catastrophic. Yet, the past three years (1964-66) have brought increasingly intensive activity during a period of drought in the Bay. The range of MSX, or at least the area of damage, has been extended up the Bay and its tributaries in irregular patterns not associated with salinity alone. In 1964, the James River seed area was invaded seriously but not in later years. In 1965, the disease caused extensive damage in Pocomoke Sound, the lower Maryland tributaries of the Bay, and in the upper Rappahannock River — all of which had been marginal areas for MSX in earlier years. These extensions of MSX activity were not maintained in 1966 and 1967.

Epizootiology

The studies of recent years have essentially confirmed the patterns of morbidity and mortality re-

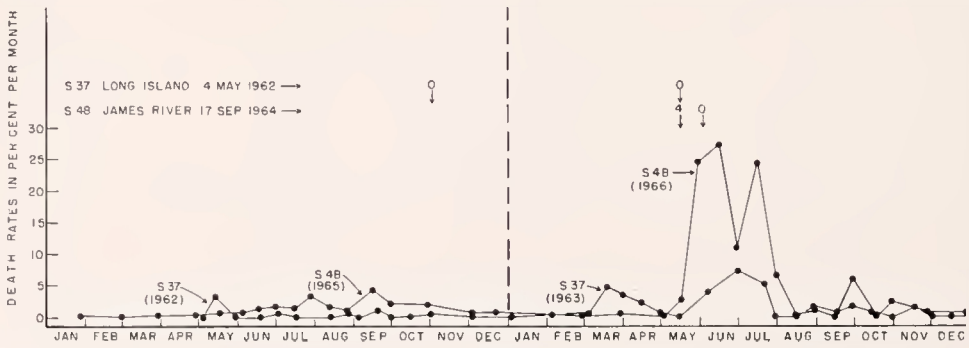

FIG. 10. *Imports monitored from 1962 through 1966 revealed typical SSO peaks but very little MSX activity (S37 and S48).*

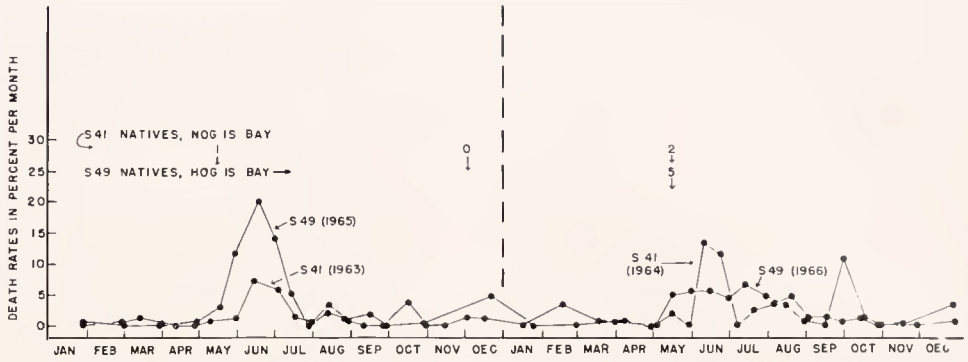

FIG. 11. *MSX activity in native oysters (S41 and S49) is similar to that in susceptibles—without clear mortality peaks from MSX and with low prevalences.*

ported for MSX earlier. A clearer understanding of the epizootiology has resulted from exposure of oysters of a greater variety as to age and history. Increasing intensity of the disease challenged susceptibles, resistant progeny and old survivors and provided new insights of timing of infections and sporogony. Sporulation, although still rare, has occurred more frequently in resistant progeny than in susceptible oysters. Mostly the susceptible oysters were monitored in earlier years to obtain patterns of mortality and morbidity. The life cycle is slowly being pieced together without the benefits of cultures and infections in the laboratory. MSX has been active in Delaware Bay for ten years. Delaware and Chesapeake bays are the two major areas damaged by MSX and massive collections of data are available for both. It would be very useful to have careful comparisons of data and of interpretations of epizootiology.

Patterns of Mortality

Patterns of mortality have remained essentially stable in timing but death rates have increased in recent years. Season of import is critical in subsequent time and level of deaths. Winter and spring imports begin dying from mid-July to late August. Oysters imported in winter and spring exhibited lower first-year deaths than those brought in during the early-summer period of infectivity (June and July). This was observed in earlier years but not appreciated fully until the 1966 experience with Potomac River oysters (Fig. 3).

For many years the absence of deaths (and patent infections) in late-summer (August through October) imports was an enigma. These groups usually lived for eight to ten months with deaths beginning in June of the second year. In recent years of increased MSX activity, this pattern has been broken. The change is more evident in prevalence data than in death rates but August 1966 imports began dying from MSX in November and December 1966. The death rates accelerated slowly from about 1 per cent per month in the late fall to 5 per cent per month in May without a distinctive pattern. These rates are very low for oysters 60 to 80 per cent infected with MSX, and most diseased oysters followed the usual pattern of high mortalities in June and July of the second year. The apparent occurrence of two separate types of infections seems to resolve now into a matter of intensity of infectivity or dosage followed by temperature reduction. The end-of-winter mortality peak is absent in late-summer imports despite high prevalences.

Patterns of Morbidity

Prevalence of MSX in live oysters has increased during the drought years of 1964-66. Susceptible Horsehead Bar oysters have been used for monitoring prevalence each year. In earlier years, 50 per cent prevalence was about maximum but in recent years samples exceeding this level are common in all seasons.

Winter and spring imports acquired typical June infections, evident in July, except that clinical infections have occurred slightly earlier in drought years — particularly in 1964 when deaths began before mid-July. The minimum observed period from import to deaths for populations has been six weeks; hence MSX infectivity must have been intense by 1 June in 1964. Imports in mid-June that year resulted in late infections and deaths beginning 1 August.

Late-summer imports have shown progressive changes in timing with increases in infective pressure as shown by susceptible controls. Previously, infections were apparently acquired promptly after import but were not clinically detectable until May of the following year. Beginning with the first of four drought years in 1963, a gradual change in the time of appearance of clinical infections was observed. In 1963, oysters imported 14

September had no infections in late January 1964 when first sampled. By early April 1964, infections were evident and prevalence reached 56 per cent in late May before deaths began. In 1964, mid-August imports already had 25 per cent clinical infections in late January 1965 and nearly 50 per cent in February. It is probable that some infections were clinical cases in the late fall of 1964 but no sampling was done because patent infections were not expected. In 1965, August imports showed 25 per cent infections in December and prevalence reached over 60 per cent in May 1966. Finally, in 1966 mid-August imports had begun to die in late October with 30 per cent infections and prevalence climbed to 80 per cent in December.

In recent years intensive MSX activity has demonstrated that throughout the warm season some susceptible oysters developed clinical infections in about two months after import. This implies that dosage of infective materials is variable by seasons and years. Early summer (late May to early July) appears to be consistently the period of most intensive infectivity and the shortest incubation periods follow. Incidence of MSX (percentage of oysters infected in a given period) is higher from early summer exposure than prevalence data indicate because deaths remove morbid oysters continuously. In late-summer infections, deaths are usually delayed for 8 to 10 months, hence the highest prevalences are observed in May. Cool temperatures intervene and delay the development of late-summer infections hence no major change in mortality patterns occurred even in the years of high MSX activity.

The conclusions from these many years of observations are that interaction of dosage, susceptibility of oysters (including effects of timing of importation) and temperature produced the patterns of infectivity and mortality described. These factors tend to obscure a pattern of continuous exposure at variable levels of infective particles. Apparently infections are established only during the warm season from May through October when oysters are dying most rapidly. This does not preclude exposure during the cold months of November to May at a low level of infective particles.

Susceptibility of oysters and dosage of infective particles are only very crudely measured in field studies by monitoring stocks of common history and origin at one station. I must assume steady virulence of MSX to conclude that dosage has increased during the drought years. Susceptibility has probably not increased, although it appears to be modified by time of import and age (size) of oysters at first exposure. Evidence on the importance of early exposure is presented below.

Contagiousness

Perhaps the most intriguing and dismaying features of MSX in lower Chesapeake Bay are the persistent patterns of widespread infections without regard for number or proximity of other oysters. Susceptible oysters held in trays with infected lots do not obtain infections earlier or in greater numbers than others grown in relative isolation. Most epidemiologists would probably regard this as evidence of another host whether alternate or one independent of oyster populations. The long period of infectiousness of MSX (about 5 months) and continuous infection pressure are factors of interest in this regard. The obvious dispersion of micro-particles in tidal estuarine waters makes it difficult to imagine direct transmission over widely spaced areas where oysters are relatively scarce. Yet the period of apparent infective pressure matches the period of oyster deaths from MSX rather closely. If another host is involved, it must release infective elements for a long period also. Large highly motile and widely distributed organisms, such as blue crabs and scianid fishes which are attracted to oyster communities, seem most likely as possible alternate hosts. The many varied situations where oysters have become infected tend to exclude fouling organisms and bottom infauna if proximity is required. Oysters essentially free of oyster associates have been imported and infected before fouling organisms could accumulate. Sandy, shelly and muddy bottoms show no apparent differential effect on infection activities.

Innate vs Acquired Resistance

Progeny of two types of parental populations were monitored in our program to select oysters resistant to *M. nelsoni*. Unexposed susceptible stocks and survivors of MSX-ravaged populations were chosen for breeding. The survivors from epizootic areas were estimated to comprise less than 10 per cent of the originial populations after five years of MSX activity. Evaluation of resistance in progeny tests was based on the phenotypic characters of mortality and prevalence rates. As controls and as indicators of MSX activity, native Horsehead oysters grown for two to four years in an area free of the pathogen were imported. In the years 1964 to 1967, MSX generated intensive infective pressure in these susceptible controls.

Progeny of both susceptible and selected parents exhibited low levels of MSX activity and of mortality. At first this was attributed to their small size and young age, but all progeny continued to exhibit resistance to MSX as they grew to market size at ages of two and three years. Progeny were reared in trays off VIMS in the same environment in which controls succumbed to MSX (Fig. 12). It is important to note that early selection of progeny did not occur. Spat were tested when moved from the pond to VIMS and MSX was

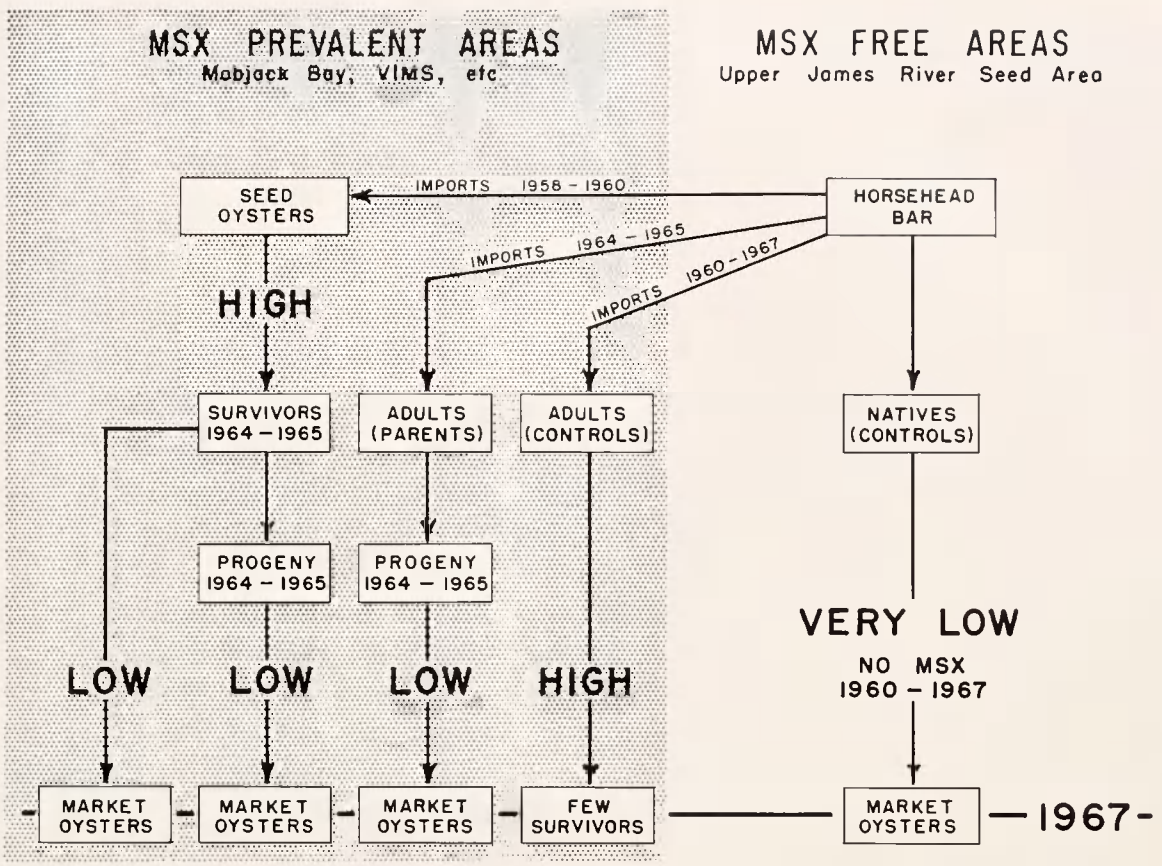

FIG. 12. *Flow diagram showing results of exposure of populations (rectangles) of oysters to MSX. The terms "low" and "high" indicate large differences in both rates of death and prevalences of MSX. All imports were of mature 2 to 3 inch oysters which were 2 or more years of age. Note that progeny, both of survivors and unselected parents, resisted MSX successfully whereas adult imports (controls) succumbed in the epizootic area at VIMS.*

absent. History and source of parental oysters was less important for survival than early exposure to an environment where the disease was active.

Native oysters of unknown parentage in MSX-prevalent areas also exhibited excellent survival. Native oysters from three rivers have been monitored in trays at VIMS with similar results. A wild set on VIMS pilings in 1964 was particularly impressive in the survival of oysters to market size. A trayed lot confirmed our observations that death rates were low and infections scarce in 1965 and 1966.

Survival of progeny and native oysters to market size in areas of intensive MSX activity where imported susceptibles had high death rates suggests that acquired resistance is involved. Horsehead susceptibles and their progeny -- from the same gene pool but reared in different environments — exhibited differences in MSX prevalences and mortalities, hence I presume that they had different immunological experiences. Small young oysters, regardless of history or source, seem to withstand attacks of MSX easier than large oysters. Size influences the responses of oysters to environment more profoundly than age. In an environment of known high infective pressure, it is presumed that high metabolic rates in small young oysters enabled them to withstand prevalent dosages of MSX infective particles. Without knowing the mechanisms involved, it seems probable that exposure of progeny to infective particles or occurrence of subclinical infections produced immunities. Lack of knowledge of mechanisms in invertebrates does not invalidate the hypothesis of acquired resistance. Stauber (1959), reviewing immunity in invertebrates, concluded that acquired resistance must yet be demonstrated in oysters.

These studies suggest that survival of early gen-

erations in MSX-prevalent areas may be enhanced more by acquired than innate resistance. The immediate practical applications of early exposure to reduce losses from the disease caused by *M. nelsoni* make it imperative that this aspect of resistance be explored and exploited fully. If effective in practice, it reduces the immediate need for producing seed from genetically resistant strains of oysters under controlled conditions of hatcheries and ponds. The effects of predation on spat and young oysters must be considered by oystermen in Chesapeake Bay where most seed oysters are large when planted. Most seed oysters originate in low-salinity sanctuaries where disease is absent or scarce, hence sources of stocks exposed to MSX must be considered.

Comparison of Life Cycles of MSX and SSO

SSO and MSX appear to be poorly adapted parasites of oysters. Both tend to kill their hosts before sporulation is completed. A carefully attuned parasite, such as *Bucephalus*, exhibits low pathogenicity and does not upset the feeding ability of its host — at least until late in the parasitic relationship when the parasite has had ample time to complete its reproductive cycle. Oysters show little reaction to *Bucephalus* although the parasites castrate their hosts and rob them of nutritive materials.

A comparison of the two *Minchinia* parasites of oysters reveals several important differences in adaptation of life cycles. These factors of seasonality, exposure, incubation period, intensity of infections and their implications about life cycles will be discussed. SSO is much more regular in its seasonal patterns of mortality and prevalence. This sharp seasonality was a primary reason for describing *M. costalis* as a separate organism in 1962. However, without sporulation stages and spores, which were common and regular in occurrence, the description of SSO as a new species would have been risky in 1962. Plasmodial stages were not easily separated from those of *M. nelsoni;* the characters used were recently described by Couch (1967). SSO disappears from oysters (clinically) in July and does not reappear usually until March and April, although occasional infections have been seen as early as December. Sporulation occurs rapidly in May and June and oysters die or recover in close synchrony. In contrast, MSX occurs in oysters throughout the year and kills them continuously in patterns related to time and intensity of exposure, susceptibility and temperature.

SSO appears to require exposure of oysters to an epizootic to initiate infections. MSX produces infections in newly-imported oysters for at least the five continuous warm months and probably oysters are exposed to infective particles throughout the year although this has not been demonstrated. Since oysters are dying most rapidly during these five months of demonstrated infectivity, this is indirect evidence that oysters are the source of infections. The problems of dosage and source of infective particles are not easily solved in field studies.

SSO exhibits a long incubation period (6 to 9 months) during which neither clinical evidence of the organism nor physiological evidence of reaction by the host can be observed. MSX studies have yielded a confused picture on period of incubation to clinical cases. Individual oysters exposed in early summer may show MSX in 5 weeks or 5 months, whereas oysters first exposed in late summer may show physiological effects in two weeks (under water weighing) but delayed appearance of clinical cases may extend to nine months. Again dosage and susceptibility are probably involved in ways now obscure and produce puzzling patterns of prevalences and mortalities.

Once patent, SSO infections develop rapidly, become intensive and kill oysters promptly. Tissues of nearly all organs are filled with sporocysts and are quite disrupted, but usually epithelia are left intact and not invaded. MSX can kill with equal rapidity but no clear relation between intensity of infections and deaths is seen. Susceptible oysters which die in the first summer often have light infections with little disruption of tissues. Those oysters which survive with persistent infections until late winter or early summer of the following year (one year after infection) usually have intensive infections and reactions to match. Yet tissue disruption is not as serious as in SSO cases.

In conclusion, it is postulated that the normal life cycle of *Minchinia* species in oysters is an annual affair with mortalities and new infections occurring in early summer (June mostly in Virginia). SSO is moderately adapted to its host in terms of seasonality, level of mortality and limitation of morbidity in oyster populations. This pathogen is limited to high-salinity areas in its environmental range. Its pathogenicity is high and a large proportion of infected host individuals die before sporulation is completed. Since oyster meats are rapidly destroyed after death, only a small proportion of gapers produces mature spores. The potential number of spores is very high.

MSX appears to be a closely related species parasitizing oysters in more brackish waters. Apparently, some recent event of mutation or hybridization has increased its virulence greatly. This virulent strain is so pathogenic that the annual cycle is interrupted very early in most oysters by death of the host. Sporulation is rare but it has been observed in nearly all months of the year. June appears to be the month when sporulation normally occurs. Timing of infections, deaths and

sporulation have all been disrupted by high pathogenicity. The usual fate of such a destructive parasite as MSX would be to decimate its host population, hence reducing its own occurrence. But MSX continues to thrive with greatly reduced oyster populations, and the source of infective particles and the mode of transmission remain a mystery.

Hypothesis on Origin of MSX

The origin of marine epizootics is always difficult to explain. However, it seems worthwhile to record the fragments of information available and to attempt an explanation of events. In Virginia we have positive evidence that MSX was not important as a disease agent in the decade of the 1950's prior to 1959. The preponderance of data consists of mortality records of many groups of oysters over many years. The patterns of oyster deaths were clearly linked to *Dermocystidium marinum*. Losses were confined to the late warm season each year. A few hundred permanent slides of gapers confirm the absence of MSX and high prevalence of *D. marinum*.

A tenuous but important link in the evidence on origin is the finding of two pre-epizootic cases of MSX — one in 1953 among 49 fungus-killed oysters six years before MSX became active. One must conclude that *M. nelsoni* was endemic but quite inactive in the 1950's. If this assumption is accepted, upon slender evidence, the question arises as to how MSX became epizootic. A theory of cyclic activity with fluctuations in susceptible host populations seems improbable with more than a decade of known inactivity when oysters were abundant followed by eight years of continuous epizootics.

In 1959, while monitoring for MSX, the haplosporidian parasite, *Minchinia costalis*, was discovered and later named by Wood and Andrews (1962). Seaside organism (SSO), as this pathogen was called, has never been found on the western shore of Chesapeake Bay although it appears to be endemic on Eastern Shore, particularly on Seaside. The pattern of a sharp peak of mortality in late May and June was a dominant reason for separating SSO from MSX. In addition, sporulation stages were observed progressing to typical haplosporidian spores. Considerable effort was expended in convincing colleagues that two separate organisms were involved, for the early plasmodia are remarkably similar.

In 1966, Couch *et al.* associated some rare *Minchinia* spores with MSX. These spores, found only in epithelia of liver tubules whereas smaller SSO spores occur in all tissues within epithelial walls, were observed first at VIMS in 1960. Occurrence of MSX spores has been extremely low in gapers and live oysters, but always typical MSX plasmodia have been present with spores (about 20 cases now on slides). Despite the rarity of spores (fewer than 1 per 1,000 cases of MSX prior to 1966), I believe the sequence of sporulation stages and invariable occurrence of MSX plasmodia are strong evidence of correct identification as a haplosporidian. The similarity of morphology and the placing of both pathogens in the genus *Minchinia* reinforce the concept of closely related species. I agree with Couch (1967), who described concurrent infections, that affinity of the two pathogens is close.

It is worth noting some other similarities and differences between MSX and SSO. Both exhibit a frequent failure to complete their cycle to a mature resting spore. However, SSO usually produces sporulation stages whereas MSX rarely does. In our paper on the epizootiology of SSO (Andrews *et al.*, 1962), we reported over half of the gapers with spores but our criterion was to report the most advanced stage seen. Many gapers had only immature spores and often few of these. Therefore, most SSO materials were being released to open waters as sporocysts without mature spores. In some recent years, spores have been comparatively difficult to find in smears and sectioned gapers during SSO epizootics although cysts of sporoblasts are common. A further similarity is the occurrence in both pathogens of a stage without distinct nuclei but with numerous punctate chromatin granules (probably schizogony) preceding formation of sporoblasts. This stage is distinctive in morphology but it is never observed unless sporogony occurs. Enlargement of plasmodia to sporocysts begins with this stage.

Field experiments by carefully timed imports indicate that periods of infectivity match those of oyster mortalities for both MSX and SSO. The infective period for SSO appears to be less than two months, with June the most important month. Although oysters are infected with SSO during a June mortality period, the pathogen is rarely found before March of the following year. In contrast, MSX kills oysters around the year but chiefly in the warm months, June through November, inclusively. Evidence has been presented that high levels of infection of MSX can be attained by exposure in any one of these six months except Novmber. MSX, too, tends to exhibit "hidden" infections for periods up to nine months.

The purpose of these discussions of similarities of MSX and SSO drawn from epizootiological studies is to demonstrate the close relationship of the pathogens. It is ironic that in the early 1960's my efforts were directed at proving differences in the pathogens whereas now I am emphasizing congeneric relationships. On Seaside, the pathogens are living sympatrically but only SSO produces typical infection and mortality patterns there.

The next episode of our reconstruction of the

origin of MSX is based upon well known events and their timing but presumes interbreeding of MSX and SSO for which there is no evidence. The history of seed oyster planting from the Seaside of Eastern Shore to Delaware Bay is well known. In the first half of the decade of 1950-60, over half the seed planted in Delaware Bay came from Seaside where SSO is prevalent. Although generally limited to environments nearly oceanic in salinities, SSO does live in Bayside creeks and lower Delaware Bay (Haskin, personal communication). In view of the sudden appearance of a virulent strain of MSX in Delaware and Chesapeake bays only two years apart, it seems quite logical to me that a new strain of *Minchinia* could have arisen in Delaware Bay from the proximity of native and Virginia oysters. No evidence that MSX was present in Delaware Bay prior to 1957 is available, but if endemic in Chesapeake, transplanting would almost surely have scattered it along the coast within its tolerance range.

If we assume that a new virulent strain arose, it could be expected to follow epizootiologically the patterns of a newly introduced disease. The patterns of kill are very similar in both bays (Haskin *et al.*, 1965). Mortalities began in late spring and early summer of 1957 and 1959 in Delaware Bay and Chesapeake Bay, respectively. The following summer severe mortalities occurred in all beds within the salinity tolerance of MSX. In both bays the initial large-scale infection occurred in late summer (well documented for Chesapeake Bay) and typical patterns of infection and mortality were established in both bays the second summer. In each bay a localized center of infection was observed from which the disease spread. In Delaware Bay this center consisted of imported Seaside oysters but in Chesapeake Bay it occurred in native transplants no different from beds in surrounding areas. It is hard to believe that this close parallel of events two years apart could be accidental or caused by natural changes or cycles.

Perhaps an important part of this hypothesis of a new virulent race of MSX lies in the environmental tolerances of the two pathogens — for lack of accurate knowledge of causes, often referred to as "salinity tolerance." It has been noted that SSO requires high salinity environments whereas MSX flourishes best in moderate salinities and does not produce typical epizootics in the optimum areas for SSO. The fluctuations in MSX activity at Cape May (Haskin *et al.*, 1965) are notable in comparison with persistent activity in lower Chesapeake Bay. I suspect lower Delaware Bay is marginal in some years for both MSX and SSO although the reasons are vague. One can only speculate that this environment provided suitable conditions for mutation or hybridization which gave rise to a virulent race.

ACKNOWLEDGMENTS

I am most grateful to Dr. J. L. Wood, Head of the Department of Microbiology at VIMS, and his Microtechnique Section for preparation of slides of oyster tissues. We now have a collection of 90,000 preserved oysters, most of which have been sectioned and stained on permanent slides. My appreciation is also extended to Mr. Michael Frierman who has diagnosed most samples in recent years for occurrence of MSX. The samples of James River oysters from the Seaside of Eastern Shore were obtained by Mr. Michael Castagna to whom I am indebted for monitoring trays and beds in that area.

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VITAL STAINING OF BIVALVE MOLLUSK SHELLS WITH ALIZARIN SODIUM MONOSULFONATE

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ABSTRACT

Alizarin sodium monosulfonate was used for vital staining of shells of larval and post-metamorphic hard- and soft-shelled clams, Mercenaria mercenaria *and* Mya arenaria. *In post-metamorphic clams a week-long immersion in 5 to 20 ppm alizarin resulted in peripheral and medial deposition of red shell material. Larval* M. mercenaria, *when subjected to 0.25 to 0.75 ppm alizarin for 48 hours, produced distinctive check marks on shells which may have value in marking. The long persistence (at least 18 months in the present study) of metabolically induced red shell coloration gives this method an advantage over more superficial marking techniques used in ecological research.*

INTRODUCTION

Shells of living mollusks have been marked for later identification in various scientific studies by painting (Carriker, 1955), tagging (Posgay, 1961), notching (Rounsefell, 1963), and application of fluorescent material (Tufts, 1967). Larvae of pelecypods have been marked by staining the soft tissue with neutral red (Loosanoff and Davis, 1947). These methods have been successful for their purpose to greater or lesser degree but all have disadvantages that limit their general applicability. Most require the handling of individuals, which precludes marking the great number of animals which are often necessary to obtain meaningful ecological data. A method such as painting may produce ephemeral marks or may cause mortality through injury, limiting its usefulness in studies requiring identification of marked individuals after a time lapse.

Alizarin sodium monosulfonate has been used in vital staining of vertebrate bone (Lillie, 1952). Also, Turner[2] used alizarin dye in marking the calcareous tubes of the marine polychaete *Hydroides dianthus*. These applications suggested that alizarin might also be used as a vital stain for the shells of bivalve mollusks.

The experiments reported here demonstrate the incorporation of alizarin red coloration in the deposition of new shell material in post-metamorphic hard- and soft-shelled clams, *Mercenaria mercenaria* and *Mya arenaria*. Also, alizarin has produced distinctive check marks on shells of larval hard-shelled clams.

MARKING POST-LARVAL BIVALVES

In the initial trials, post-metamorphic *M. mercenaria* were successfully marked by holding them in a solution of alizarin in sea water. Clams 2 to 3 mm in width across the shell were produced in the hatchery by spawning and rearing techniques summarized by Loosanoff and Davis (1963). One hundred clams were placed in each of several glass 1-liter beakers containing concentrations of alizarin of 0.25 to 20 ppm. Other beakers contained untreated animals as a control. All were reared for 7 days at 21° C in sea water that had been filtered and treated with ultra-violet light; the water and the alizarin concentrations were renewed at 2-day intervals. A mixture of live flagellates, *Isochrysis galbana, Dunaliella euchlora, Mono-*

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chrysis lutheri, and *Chlorella* 580, was fed every day at an approximate rate of 0.01 ml of packed cell volume per liter culture per day (Davis and Guillard, 1958).

Clams were active and feeding at all test concentrations of alizarin during the treatments. Shell exteriors soon became stained uniformly dark red.

After treatment, clams were held in screened cages in Milford Harbor for later observation. Under outdoor summer conditions all clams grew rapidly and soon lost the general dark red coloration of alizarin. The red margins of the shells, however, representing areas of growth during alizarin treatment were retained as red bands and had faded very little up to 18 months after treatment (Figures 1 and 2 show alizarin marks 6 months after treatment). The red bands were most distinctive at the higher dosage rates of alizarin, between 5 and 20 ppm, and appeared to be excellent marks.

In an attempt to place more than one red band on a single clam, the clams of the original experiment were subjected to a second 7-day exposure of alizarin (8 ppm in this trial) after a month's post-marking growth in the natural environment. The clams again acquired general dark red coloration. When returned to natural conditions the red color again faded, but a second red band was formed representing shell growth during exposure to the second alizarin dosage (Figs. 1 and 2).

Results of trials with *M. arenaria* were equally good. Juvenille *M. arenaria* were subjected to a 7-day treatment with 5 ppm of alizarin under conditions described above and then held under natural conditions for observation. Six months after treatment these clams had added new shell and had also retained red bands on the shells (Fig. 4). In addition to the persistent red bands, the general red coloration of the shell had not faded to normal coloration, but has remained light pink (Fig. 4). The pink shell, contrasting with the white color of shell deposited later also produced a distinctive marking effect. Similar results were obtained with the coot clam, *Mulinia lateralis*.

Cross-sections were made of shells to determine the site of alizarin deposition. Sections of *M. mercenaria* shells marked with a single red band revealed a red striation running with the laminae and extending through the complete thickness of the shell (Fig. 3). Sections of *M. arenaria* shells showed that the red coloration was deposited on the entire inner surface of the shell and extended to the outer surface in the area which was peripheral at the time of treatment (Fig. 5). Colorless shell beneath the red band represents additional medial shell that was deposited when clams were returned to natural conditions.

MARKING LARVAL BIVALVES

Larval *M. mercenaria*, when exposed to alizarin, produced a distinctive check mark on the shell. The larvae, like the post-larval animals, were initially treated for 7-day periods. Concentrations of alizarin below 0.10 ppm produced no ill effects or distinctive marking; concentrations higher than 0.25 ppm stopped growth and produced high mortality. If, however, larvae were subjected to 0.25 to 0.75 ppm alizarin for 48 hours and then returned to untreated sea water, growth of larvae

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FIG. 1 and 2: *External and internal shell surfaces of alizarin-marked juvenile* M. mercenaria, *6 months after marking. The smaller clam at right shows single red band after one 7-day alizarin immersion (5 ppm) whereas the larger individual at left shows two bands that represent two separate marking treatments; size bears no relationship to treatment. Shell length parallel to hinge line of largest individual — 6 mm.*

FIG. 3: *Cross-section of shell perpendicular to hinge line of juvenile* M. mercenaria *which received single alizarin treatment 6 months before sacrifice. Red striation reveals site of shell deposition during term of alizarin immersion. Shell length parallel to hinge line — 11 mm.*

FIG. 4: *External shell surface of alizarin-marked juvenile* M. arenaria *6 months after marking. Visible is red band and general red discoloration of the shell representing alizarin deposition with peripheral and medial shell growth during the term of alizarin treatment. Shell length parallel to hinge line — 2 cm.*

FIG. 5: *Cross-section perpendicular to hinge line of juvenile* M. arenaria *which received single alizarin treatment 6 months prior to sacrifice. Red striation reveals site of shell deposition during term of alizarin immersion. Shell length parallel to hinge line — 2 cm.*

FIG. 6: *Late stage larval* M. mercenaria *showing check marks produced by 48-hour immersion in 0.40 ppm alizarin. These shell marks are distinct from a later demarcation line between the prodissoconch shell of the larva and the dissoconch shell of the metamorphosed individual. Shell length parallel to hinge line — about 200 μ.*

FIG. 1

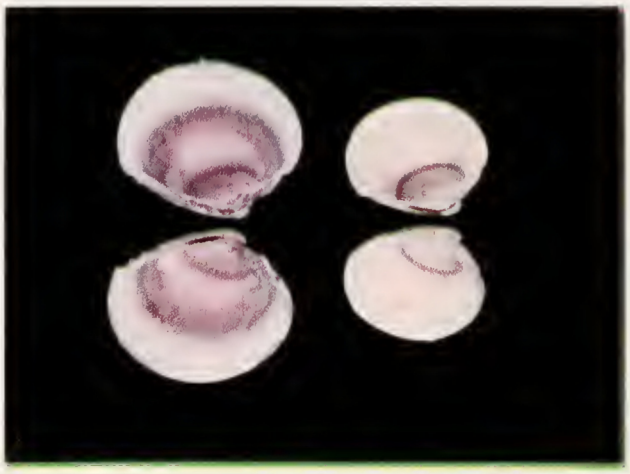

FIG. 2

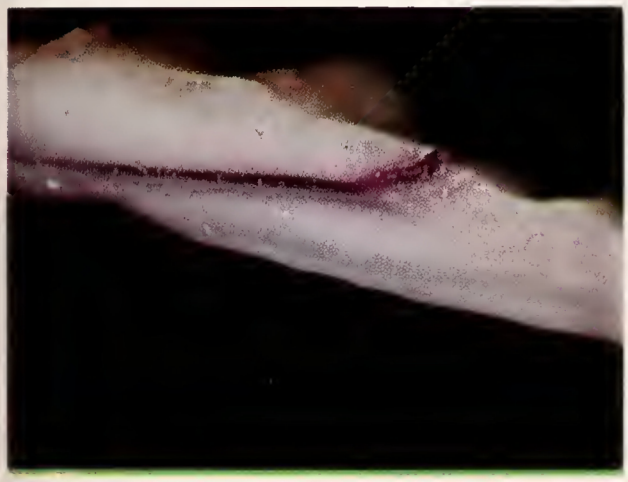

FIG. 3

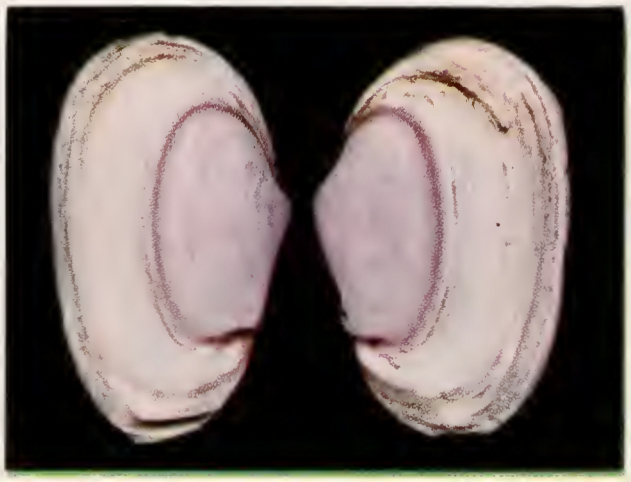

FIG. 4

FIG. 5

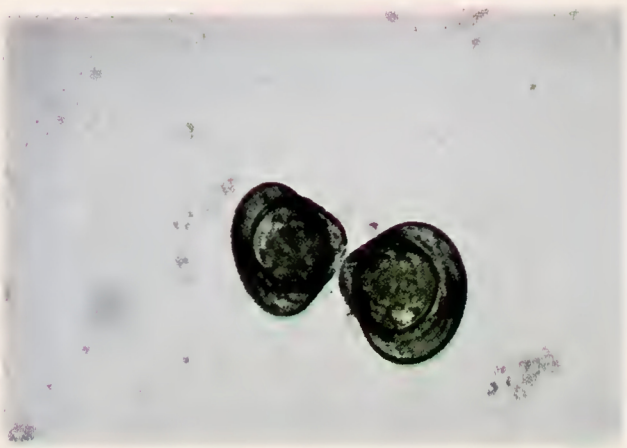

FIG. 6

resumed and a very distinctive check mark resulted (Fig. 6). Immersion in 0.30 to 0.40 ppm for 48 hours produced a light check mark recognizable in all individuals. With increasing concentrations check marks became more pronounced, until at 0.75 ppm larvae greatly indented the shell before normal growth was resumed. Larvae subjected for 48 hours to concentrations of alizarin above 0.75 ppm were unable to resume normal growth.

M. mercenaria larvae of all sizes from 115 μ straight-hinge veligers up to 225 μ pediveligers were marked in this manner, although success was somewhat more difficult to achieve than in the technique used for post-larval clams. Many cultures of marked clam larvae were reared past metamorphosis; the check marks could be readily identified as long as the larval shell itself was recognizable in the juveniles. Considerable work remains in testing the general value of this method of marking, but it probably could be used for marking other species as well.

DISCUSSION

The red bands in post-larval shells of *M. mercenaria* and the bands and pink discoloration of *M. arenaria* appear to result from the deposition of alizarin in the new shell added during treatment. The deposition of red shell was first suggested by the rapid fading of red coloration in all areas of the shell except in the area which was peripheral during the exposure to alizarin. Later, cross-sections of shells (Figs. 3 and 5) revealed sharp alizarin bands only on shell surfaces which were medial and peripheral during time of treatment.

An adequate food supply favoring shell deposition during alizarin treatment would appear to be the determining factor in the laying down of a distinctive mark, although present experiments have not conclusively demonstrated this. Adequate food may be particulary important in marking smaller juvenile animals because their growth rates may depend more on immediate food supply than on a stored food reserve (which might be more important for larger, more mature mollusks).

The alizarin marking method will be useful in ecological research requiring the marking of a large number of shellfish with a highly durable mark. The only limitations on the number of individuals that can be marked are the ability to keep them under such conditions that adequate shell deposit is formed while the animals are being treated and the number of animals that can be procured either through collection of wild stock or through hatchery rearing. Marks appear to be stable and are expected to persist for the life of the animal. After 18 months the marks had faded very little.

Certain difficulties might be expected in the field use of the alizarin technique. Marked animals released in natural water undoubtedly will become dark or discolored, thus obscuring the mark on the shell exterior. It may be necessary to hold the captured animals in sea water away from a substrate for several days to bleach shells sufficiently to distinguish marked individuals. Alternatively, shells of live mollusks could be filed lightly for examination of subsurface shell for traces of red marking. If the animals were to be sacrificed, the interior margins or complete cross-sections of shells could be examined.

M. mercenaria shells on occasion contain natural purple striae which might be mistaken for an alizarin mark. Nearly all natural striations of this type instead of running the complete thickness of the shell are confined to the medial third of the shell. The natural striae are probably variations of natural purple pigmentation of certain areas of the inner translucent region (Shuster, 1957), and seem to be associated with less than optimum growth conditions in *M. mercenaria*. Such coloration has not been observed in *M. arenaria*.

Trials are being continued to determine the general applicability and limitations of the method in molluscan research. No doubt other species of pelecypods and also gastropods can be similarly marked.

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RELATION OF HYDROGRAPHY AND *CRASSOSTREA GIGAS* SETTING IN DABOB BAY, WASHINGTON

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ABSTRACT

Studies have been carried out since 1952 on the relationship of the depth of the surface layer of warm water to the success of oyster setting in Dabob Bay. During this time it has been observed that depth of the water layer is just as important as numbers of larvae per sample in determining the intensity of oyster setting. Over the years information collected enables a close estimate of the probable number of spat per shell that will result from given numbers of oyster larvae in quantitative plankton samples.

INTRODUCTION

Since the first importation of the Japanese oyster *(Crassostrea gigas)* into Washington State, there has been interest in the possibility of local reproduction of this species. While the main source of seed oysters has continued to be from Japan, periods of seed shortage and the complete unavailability of oyster seed from Japan during World War II have focused increasing attention on local areas where reproduction of this oyster (now called the Pacific oyster) occurs. One such area, first recognized in 1936, is the Dabob Bay, Quilcene Bay area of northern Hood Canal.

Schaefer (1938) observed that successful setting occurred in Dabob and Quilcene bays with some regularity, and setting intensity was adequate for commercial utilization. Chapman and Esveldt (1943) made further observations of Pacific oyster setting in northern Hood Canel, and found setting intensity to be highest in Dabob Bay. Subsequently, Glude and Lindsay (unpublished manuscript) worked extensively on Pacific oyster reproduction and developed methods for prediction of time of setting.

Disappearance of oyster larvae from the water prior to setting was occasionally observed and posed a perplexing problem in the successful prediction of the occurrence of oyster setting. Westley (1956) found that the waters of the bay were thermally stratified and that the surface warm water layer which contained the larvae was moved out of the bay by the action of northerly winds.

Continued observations on spawning and setting of the Pacific oysters in this area have provided additional information on other aspects of oyster setting in northern Hood Canal. The purpose of this report is to summarize this information, to present a better understanding of the occurrence of oyster setting in Dabob and Quilcene bays, and to provide a better basis for interpreting the summer bulletins issued on spawning and setting of the Pacific oyster in Hood Canal.

DISCUSSION

Dabob Bay is a large, deep inlet located on the western side of Hood Canal (Fig. 1). The bay is about 10 miles long and 1 1/2 miles wide. Quilcene Bay is a branch of Dabob Bay opening about halfway down the west side of Dabob Bay at Whitney Point. During summer, waters of this area stratify thermally (Westley, 1956).

As part of the prediction service to the oyster industry, an effort is made to forecast both time and intensity of spatfall. Prediction of setting intensity is quite important to the commercial oystermen since it is generally believed that a minimum of 10 spat per adult Pacific oyster shell is necessary to make cultching (the placement of strings of Pacific oyster shell in the water to collect oyster spat) economically feasible. Since forecasting setting intensity is difficult at best,

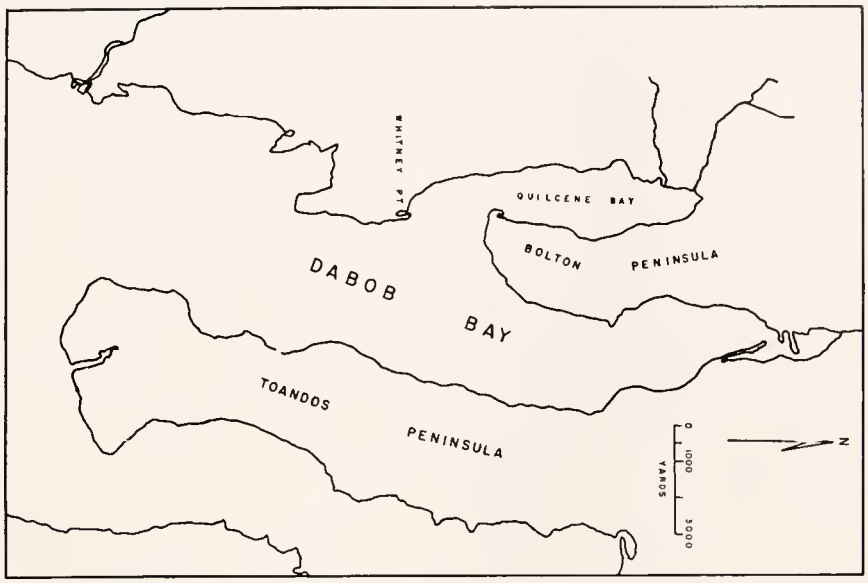

FIG. 1 *Map of Dabob Bay, Quilcene Bay area.*

these forecasts have been restricted to prediction of no setting (0 to 2 spat per shell), light setting (3 to 10 spat per shell), and commercial setting (over 10 spat per shell).

The procedure used in the past to forecast intensity of oyster setting was to collect quantitative plankton samples, determine the number of advanced larvae per unit of water, and relate this to subsequent spatfall (Lindsay, Westley and Sayce, 1958). In carrying out this procedure, it was observed that rather serious discrepancies sometimes occurred in northern Hood Canal between the predicted spatfall, based on the numbers of larvae in the water, and the actual set.

Evaluation of the available data indicated an apparent relationship between the depth of the warm water layer and the intensity of the oyster spatfall. As previously mentioned, removal of the surface warm water layer had been found to be partially responsible for the set failure in Dabob Bay. Thus extensive observations were made each year on the temperature and depth of this surface warm water layer.

Figure 2 presents vertical temperature observations in Dabob Bay each year from 1952 thru 1965, immediately prior to oyster setting. Table 1 presents the average number of advanced Pacific oyster larvae per 20 gal sample, and resultant set in numbers of spat per shell. It should be pointed out that the data presented on vertical temperature distribution is far from perfect. The depth of the surface warm layer is not always uniform over the entire bay, and at times the warm water may be occurring in pockets (Westley, 1956). In developing the information for Figure 2 every effort was made to present the average condition of the surface layer immediately prior to setting, but particularly in years of a limited surface layer, this information is not as representative of the entire bay as would be desirable.

Examination of the data in Figure 2 and Table 1 indicates a relation between the depth of the warm water layer and the intensity of spatfall, and shows that the deeper the layer, the greater the number of spat produced by the existing oyster larva population. These data indicate that depth of the layer is at least as important as number of larvae in determining intensity of spatfall. That such a relationship exists seems logical since in the case of thermally stratified waters, depth of the surface layer is another dimension of the oyster larva's environment, because the larvae cannot survive in the cold waters below. The data clearly demonstrate that any time the 17°C water is less than 5 ft deep, there is almost no possibility of successful commercial setting. The data also indicate that any time the depth of the 17°C water is between 5 and 10 ft, the chances of successful commercial setting are greatly reduced unless numbers of advanced larvae per 20 gal sample are 50 or more.

Table 1 shows that when the layer is over 10 ft in depth, good commercial setting can result from relatively low numbers of advanced larvae per 20 gal sample.

The depth of the warm water layer early in the

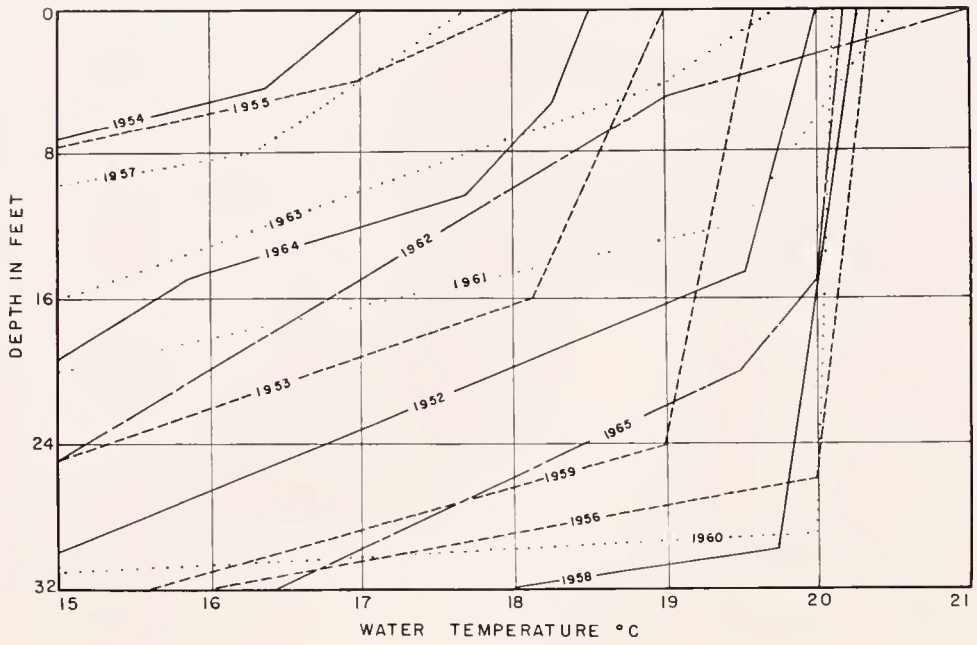

FIG. 2. *Vertical temperature distribution prior to oyster setting in Dabob Bay.*

summer is also an aid in forecasting the probable success of oyster setting for the entire summer. In the Dabob and Quilcene bay areas, oyster spawning often occurs in three waves during about a 40-day period. The first spawning is generally of low intensity but survival of the free-swimming larvae is often good. The second and third spawnings tend to be of greater intensity, but often occur so late in the summer that survival of larvae may be reduced due to the seasonal increase in frequency of northerly winds.

If oystermen are to cultch at all, it is often desirable for them to cultch in time for the first set in order to accumulate an adequate set from the successive spawnings throughout the summer. Therefore, some forecast of the probable survival of larvae populations from all spawnings during the summer is needed before the decision is made to cultch. The deeper the warm water layer is, the more resistant it is to total removal by northerly winds (Westley, 1956), and thus the depth of the warm water layer early in the season has been found to be a fairly good index of the probable survival of oyster larvae.

CONCLUSIONS

It has been found that two conditions are needed for oyster setting in the Dabob and Quilcene bay areas of northern Hood Canal. These are adequate numbers of oyster larvae, and a deep, stable, warm, surface layer of water. The fate of the warm water layer is greatly influenced by wind, but, when of sufficient depth (and breadth), it is able to withstand some north wind without complete removal. Knowledge of the depth of the warm water layer also allows more meaningful evaluation of the probable set that will result from any given number of oyster larvae in quantitative samples. Depth of the warm water layer has been found to be an aid in forecasting probable success of each year's setting early in the season when decisions relative to placing cultch material into the water must be made. Data in Figure 2 and Table 1 should be of assistance to oystermen in interpretation of the information presented in the summer bulletins on spawning and setting of the Pacific oyster in Hood Canal. It has been found that when the depth of the 17°C water is less than 10 ft, the chances of commercial setting are greatly reduced.

ACKNOWLEDGMENTS

The information presented has been gathered over many years by various staff members of the Washington State Department of Fisheries. The writer would also like to acknowledge the contribution of R. N. Steele of The Rockpoint Oyster Company, in collection of data.

TABLE 1. *Relationship of depth of water layer, numbers of larvae, and intensity of Pacific oyster spatfall in Dabob Bay.*

Year	Depth of 17°C water	No. advanced Pacific larvae per 20 gal*	No. spat per adult Pacific oyster shell**
1952	23 ft	7	100
1953	19 ft	1	30
1954	0 ft	2	3
1955	4 ft	0.5	0.1
1956	30 ft	99	250
1957	4 ft	4	2
1958	34 ft	22	405
1959	29 ft	90	200
1960	32 ft	425	800
1961	17 ft	50	50
1962	15 ft	20	15
1963	10 ft	500	20
1964	12 ft	0.25	0.5
1965	30 ft	250	400

* 200 μ or larger

** Floating cultch in Dabob Bay

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ACCUMULATION OF PARALYTIC SHELLFISH POISON BY THE ROUGH WHELK (*BUCCINUM UNDATUM* L.)

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ABSTRACT

Whelks fed for several weeks on molluscan tissue containing high concentrations of paralytic shellfish poison (P.S.P.) accumulated toxin in the digestive gland. This phenomenon probably accounts for the high concentrations of P.S.P. recorded in whelks from Quebec and is believed responsible for several cases of paralytic shellfish poisoning reported from the province following human consumption of whelks.

No toxin was found in extracts of the muscular parts containing the hypobranchial and salivary glands, intrinsic sites of poisonous secretions.

Starvation of whelks, or feeding on a non-toxic dict, resulted in rapid elimination of poison. The digestive gland/body weight ratio was found to be a sensitive indicator of starvation in Buccinum.

INTRODUCTION

Twelve mild cases of poisoning were reported by a fishery officer from the vicinity of Godbout, Quebec (Fig. 1), during the summer of 1966, which were suspected to be due to the consumption of whelks, *Buccinum undatum* L. (Table 1). The explanation offered locally was that beach seepage from a sawdust mill had contaminated the whelk beds. However, the symptoms shown by the victims closely resembled those of paralytic shellfish poisoning (P.S.P.) which often follows consumption of toxic shellfish (Medcof, Morin, Nadeau and Lachance [1]).

Shellfish poison is accumulated by bivalves as a result of feeding upon the dinoflagellate, *Gonyaulax* (Prakash, 1963). The poison stored in the digestive gland of the bivalve has been shown to be biochemically identical with that extracted from the flagellates (Schantz, Lynch, Vayvada, Matsumoto and Rapoport, 1966). The suggestion was made that whelk toxicity might have resulted from a diet of toxic bivalves, since *B. undatum* is known to feed upon shellfish, in addition to its normal scavenging habit.

An experiment was conducted to determine the accumulation of P.S.P. by whelks fed on toxic shellfish. Parallel experiments with whelks fed on non-toxic diets acted as controls.

Other possible sources of poison in neogastropods must be considered here: a toxin, acrylylcholine, was reported from *B. undatum* (Whittaker, 1960). This has some neuromuscular blocking action and is secreted by the hypobranchial gland. Other closely related gastropods have a toxin similar in action to P.S.P. and located in the salivary gland (Fig. 2). In testing for paralytic shellfish poisoning, the possible action of these toxins was excluded by sampling the digestive gland separately from these other "toxic" organs.

Bioassay technique for P.S.P. is based upon the effect of extracts of poisonous shellfish when injected into white mice. The lower limit of sensitivity of the bioassay technique occurs at 44 μg of poison/100 g of tissue. Any scores below this value will be referred to as negative. All extracts made during the course of the experiment were prepared from raw *Buccinum* tissue.

METHODS

Whelks used in the experiment were captured

[1] Medcof J. C., N. Morin, A. Nadeau and A. Lachance. MS, 1966. Survey of incidence and risks of paralytic shellfish poisoning in the province of Quebec. Fish. Res. Bd. Can., MS Rept. (Biol.), No. 886, 22 p.

TABLE 1. *P.S.P. scores recorded by Nadeau and Lachance for various parts of rough whelks expressed in micrograms per 100 g of raw flesh (after Medcof* et al.[1]).

Location	Date 1964	Whole body μg	Visceral mass μg	Muscular parts μg
Pentecote	25 May	< 32	< 32	< 32
Matane	25 May	157	464	< 32
Matane	1 June	512	1195	50
Matane	8 June	37	176	—
Pentecote	8 June	115	192	< 32
Godbout	22 June	69	400	53
Pentecote	29 June	< 32	< 32	< 32
Godbout	29 June	608	1600	93

in a baited hoop trap (Medcof[2]) set on the bottom from the wharf of the Biological Station, St. Andrews, New Brunswick. The net was baited with herring in the morning and hauled in the afternoon. Up to 200 animals were captured at a time. Only the larger specimens (over 6 cm long) were used in the experiment. Whelks were maintained in laboratory tanks of running sea water at 12°C on a diet of herring for several weeks prior to commencing the experiment.

A convenient source of toxic material was provided by the digestive glands ("livers") of sea scallops, *Placopecten magellanicus* (Bourne, 1965). Scallops were fished from the Mascarene area of Passamaquoddy Bay for several weeks until a sufficient amount of dietary material had accumulated. The scallop livers were separated from the rest of the body and frozen immediately. Frozen livers were stored in a cold room at —13°C. Before the start of the experiment this material was thawed, homogenized, and repackaged in 400 g aliquots, each sufficient for a single feeding. The purpose of this procedure was to eliminate possible variation in the toxicity of scallops fished from week to week during the experiment.

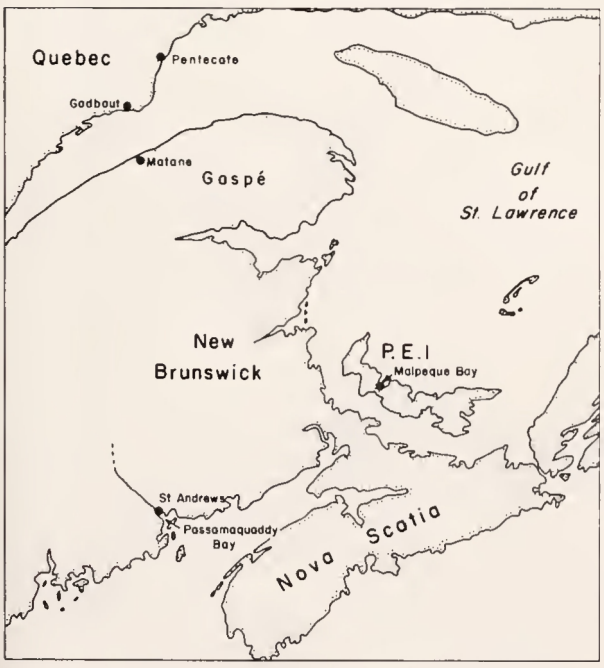

FIG. 1. *Map of the Canadian Maritimes and Quebec showing locations mentioned in the text.*

Preliminary analyses of whelk digestive glands gave a negative value for P.S.P. The initial toxicity of the scallop livers was 1672 μg of poison. Frozen storage at —13°C considerably reduced liver toxicity. After three weeks the score had dropped to 924 μg, and after eight weeks had fallen further to 550 μg. Thus the immediate objective of avoiding fluctuations in the toxicity of the diet was not achieved, and, in fact, the potency of the diet fell to less than half its initial value during the course of the experiment.

Four tanks, 3 ft square and 1 ft deep, each containing 200 whelks were set up to receive running sea water from a common mixing tank at 12°C. Whelks in tanks A and C were fed 200 g of whole scallop livers twice a week, and were sampled weekly for toxin accumulation. Whelks in tanks B and D were employed as controls, and fed 200 g of herring and 100 g of clams respectively at the same intervals. Animals in the control tanks were sampled for toxicity at the beginning and end of the experiment.

Clams, *Mya arenaria* L., were obtained from Malpeque Bay, Prince Edward Island, an area noted for its consistently negative records for P.S.P. Preliminary tests on these animals confirmed this negative score.

When sampling for toxicity, the whelks were all

[2] Medcof, J. C. Unpublished MS, 1966. The rough whelk fishery of Godbout, P.Q. Fish. Res. Bd. Can, Biol. Sta., St. Andrews, 5 p.

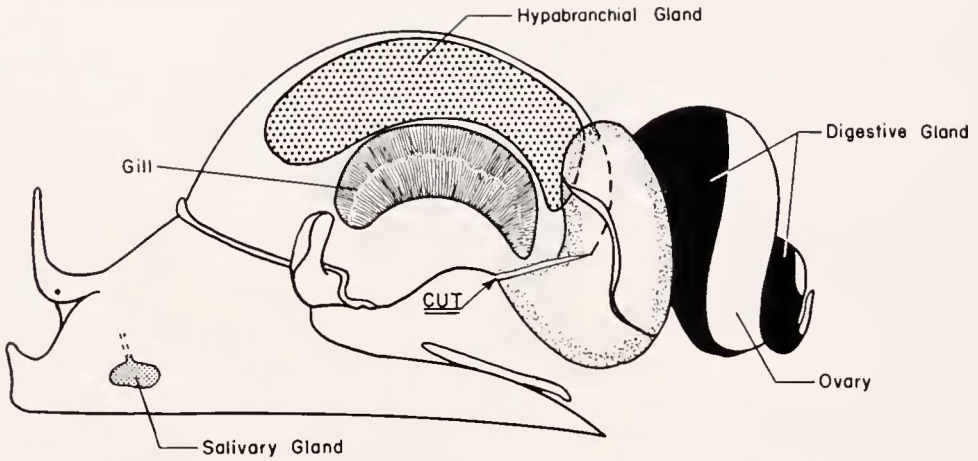

FIG. 2. *Point of bisection of the whelk used to separate the digestive gland from the muscular tissues (containing the salivary and hypobranchial glands).*

TABLE 2. *Toxicity values.*

Date	Sample	Scallop livers Score μg	Test tanks A — Score μg	Test tanks C — Score μg	Control tanks B — Score μg	Control tanks D — Score μg
Nov. 24/66	Whelk livers		neg.	neg.	neg.	neg.
	Rest of body		neg.	neg.	neg.	neg.
	Fresh scallop livers	1672				
Dec. 13/66	Whelk livers		neg.	neg.	neg.	neg.
	Rest of body		neg.	neg.	neg.	neg.
	Frozen scallop livers	924				
Dec. 20/66	Whelk livers		neg.	57	—	—
	Rest of body		neg.	neg.	—	—
Dec. 28/66	Whelk livers		75	48	neg.	neg.
	Rest of body		neg.	neg.	neg.	neg.
Jan. 4/67	Whelk livers		77	neg.	—	—
	Rest of body		neg.	neg.	—	—
Jan. 11/67	Whelk livers		54	64	—	—
	Rest of body		neg.	neg.	—	—
Jan. 18/67	Whelk livers		57	57	—	—
	Rest of body		neg.	neg.	—	—
	Frozen scallop livers	550				
Jan. 25/67	Whelk livers		110	79	—	—
	Rest of body		neg.	neg.	—	—
	Second phase:		(Fed herring)	(Starved)		
Feb. 1/67	Whelk livers		66	neg.	neg.	neg.
	Rest of body		neg.	neg.	neg.	neg.

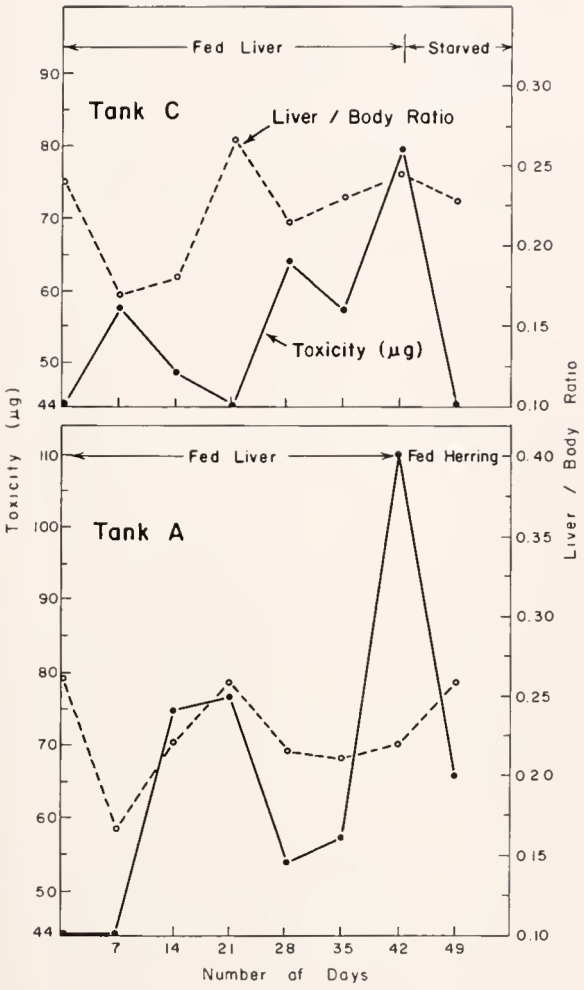

FIG. 3. *Fluctuations in liver toxicity and liver/body ratio for whelks fed a diet of toxic scallop livers over a six-week period. (Points lying on the abscissa correspond to toxicity values of 44 μg of poison, the lower limit of sensitivity of the assay technique.)*

detached from the sides of the tank and mixed thoroughly before the sample was netted out. Shells were broken by tapping gently with a hammer, and the soft parts carefully extracted. The whelks were bisected just above the mantle cavity in order to separate that part of the body containing the salivary and hypobranchial glands (possible intrinsic sources of toxin) from that containing the digestive gland (main site of P.S.P. accumulation in other mollusca). When 60 g of the digestive gland fraction ("whelk liver") had accumulated, both tissue fractions were weighed to 0.1 g. Individual whelk livers weighed approximately 3 g, so that at least 20 whelks were needed to provide each sample of digestive gland. Extracts of the muscular organs (including salivary and hypobranchial glands) were also prepared and tested for toxicity.

RESULTS

Preliminary tests of whelk livers for toxicity gave values below 44 μg of poison which is the lower limit of sensitivity of the assay method. After feeding one week on the toxic material, the whelks in tank C showed a measurable score for P.S.P., although whelks from tank A remained negative. Subsequent to the first week, all samples from the test tanks showed positive readings, except for one occasion (tank C, third week). Animals in the test tanks showed wide fluctuations in toxicity. No close correlation was found between toxicity readings for the two test tanks except that both showed maximum scores at the end of the feeding period. A maximum value for toxicity of 79 μg of poison was reached for tank C in the sixth week of the experiment; on the same date, tank A yielded a value for P.S.P. of 110 μg (Table 2, Fig. 3). Possibly even higher levels might have been attained if the numbers of animals had been sufficient to continue the experiment. Control tanks B and D gave negative readings for toxicity on all occasions tested.

An interesting side feature of these data was provided by the marked fluctuations noted in the ratio of the weight of the upper part of the body (including the digestive gland) to the rest of the body (liver/body ratio). This can be attributed to the changing volume of the digestive gland (or liver) under different conditions of feeding. Whelks in both tanks showed similar fluctuations. A fall in the average value of the liver/body ratio from 0.25 to 0.17 in the first week of the experiment may be assumed to reflect the initial reluctance of the animals to change over from a diet of herring. By the third week, liver/body ratio had returned to its initial value (Fig. 3).

Confirmation of the interrelationship between starvation and a reduction in the liver/body ratio was provided at the end of the experiment (Second phase, Table 2). At the end of the sixth week the toxic diet was terminated, tank A were fed herring and tank C starved. Tank A showed a pronounced rise in liver/body ratio, while tank C showed a corresponding fall. The toxicity of both samples fell away sharply.

All tests for toxicity carried out on extracts of the rest of the body gave negative results.

DISCUSSION

The results reported here are preliminary;

nevertheless they do establish certain points of current interest:

1. The experiment succeeded in demonstrating that toxin uptake may take place when whelks are fed a diet of toxic bivalves. There was a progressive though irregular rise in toxicity of the digestive gland during the course of the experiment.

2. No positive toxicity scores were shown by that fraction of the body tissues containing most of the muscular organs and the salivary and hypobranchial glands. This confirms the results of Medcof *et al.*[1] who found low toxicity scores for muscular tissues of toxic whelks collected in Quebec.

The irregular rise in toxicity of the digestive gland throughout the experiment may be due to one or both of the following factors:

1. The long intervals between feeding periods.

2. The small size of the assay samples. At least 100 g of tissue or a number of small samples should have been used on each occasion. However, this would have necessitated a large-scale experiment which was impossible under the circumstances.

Certain reservations must be made in applying our conclusions to the natural environment:

1. The highest level of toxin attained by whelks in the course of the experiment was 110 μg. This is considerably below the levels of toxicity encountered in nature (Medcof *et al.*[1] recorded scores as high as 1,600 μg for whelk digestive glands in the Godbout, Quebec, area).

2. Scallop livers were not highly favoured as a diet by whelks. There was always a proportion of the food left by the beginning of the next feeding period. A number of empty shells found in the test tanks at the end of the experiment suggests that some cannibalism occurred, although whether this was the cause of mortality was not established.

One curious feature noted was the presence towards the end of the experiment of several whelks in both test and control tanks which had abandoned their shells although still crawling actively.

The rapid fall in toxicity following both starvation and feeding on a non-toxic diet is particularly interesting, and suggests that prolonged storage of toxin as found in *Placopecten* (Bourne, 1965) may be absent in *Buccinum*. If this is true, whelks showing high toxicity scores in nature must have been feeding predominantly on toxic shellfish to maintain their levels of toxin. This throws an interesting light on selectivity of feeding in whelks. Other species of neogastropods have been shown to feed selectively on one prey species until it is no longer available: a period of starvation then precedes a changeover to another species of prey (Fischer-Piette, 1935). This phenomenon may also explain the drop in liver/body ratio observed at the beginning of the period of toxic diet.

The rapid elimination of toxin suggests a simple method of processing toxic whelks for human food, namely, maintaining them in running sea water for several weeks prior to consumption.

One incidental feature of the toxicity readings for the scallop livers was the progressive loss of toxicity when this material was maintained at —13°C. Further studies will be necessary to establish the extent of this phenomenon, but it is clear that the procedure of holding samples of material for toxicity analysis in a frozen state must be approached with some caution.

ACKNOWLEDGMENTS

Our thanks go to Mr. Hazen Boyd of the St. Andrews Fish Inspection Laboratory who prepared the extracts, and Dr. A. D. Tennant of the Public Health Engineering Division, Department of National Health and Welfare, Ottawa, who performed the bioassays.

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EPIZOOTIOLOGY OF *MINCHINIA COSTALIS* AND *MINCHINIA NELSONI* IN OYSTERS INTRODUCED INTO CHINCOTEAGUE BAY, VIRGINIA

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ABSTRACT

The introduction of Crassostrea virginica *into Chincoteague Bay, at Franklin City, Virginia, was studied in relation to haplosporidan epizootics from September, 1963 to September, 1966. The introduced oysters (1962 year class) were from Harris Creek, eastern shore of Chesapeake Bay, an area where* Minchinia nelsoni *and* M. costalis *(Haplosporida: Haplosporidiidae) have not been found. Eight introductions of about 1200 oysters each were made in September, December, March, and June during 2 successive years.*

Generally, mortality followed a similar course among oysters in all introductions. Oysters in the two quarterly introductions in September died during the following August-September, after 11-12 months of exposure, in numbers that indicated an epizootic. Other quarterly pairs of introductions in December, March, and June of both years also experienced similarly significant mortalities in the same August-September period, after 3-9 months exposure. Mortality was not significant during the winters following any of the introductions. The second mortality peak for all introductions came in May-June of the second summer of exposure. A relatively lower death rate persisted after the May-June epizootic until the end of the study. Total periods of exposure to Chincoteague Bay waters ranged from 16 months to 24 months and cumulative mortalities, to September 1966, ranged from 65.5 per cent to 76.5 per cent.

Among oysters introduced during the first year of the study, Minchinia nelsoni *was first observed in April of the first spring; its prevalence declined during May and June, then increased in July, August, and September preceding and concurrent with the first mortality peak. Among oysters introduced during the second year of the study,* M. nelsoni *was first seen in late July. Prevalence of the parasite increased during August and September, concurrent with the first mortality peak experienced by these oysters. The prevalence of* M. nelsoni *during the first and second winter of exposure was high in oysters examined, but accompanied by significant mortality. Prevalence of* M. nelsoni *in early May of the second spring dropped slightly but overlapped the first incidence of* M. costalis.

Prevalence of M. costalis *reached its peak (concurrent with heavy spore production) in May-June of the second year of exposure, during the second mortality peak, when* M. nelsoni *was also common. Concurrent plasmodial and spore infections of the two species were found during May and June. After early July,* M. costalis *was not found in oysters from any of the introductions. After the June mortality of the second year prevalence of* M. nelsoni *was low until the end of the study.*

The epizootiology of these parasites in Chincoteague Bay is compared with long-term studies by others in lower Chesapeake Bay, and Delaware Bay.

INTRODUCTION

After the discovery of the oyster haplosporidan termed "MSX" and later named *Minchinia nelsoni* (Haskin, Stauber, and Mackin, 1966), another haplosporidan, *Minchinia costalis* (Wood and Andrews, 1962), was described from oysters of Seaside Virginia. Subsequently, Andrews, Wood, and Hoese (1962) reported the epizootiology of *M. costalis* and *M. nelsoni* for sea-side areas of Virginia, and Andrews (1966, 1967) further described the effects of *M. nelsoni* on oyster populations of lower Chesapeake Bay, Virginia. Haskin, Canzonier, and Myhre (1965) described briefly the epizootics caused by *M. nelsoni* in Delaware Bay.

General epizootiological findings by these investigators were:

1) Two haplosporidan parasites of oysters exist in coastal waters of the eastern United States.

2) These parasites are found in high prevalences in certain oyster populations and may cause severe oyster mortalities.

3) Mortality patterns associated with these parasites are usually repetitious yearly and, to some degree, predictable.

4) A possible way to re-establish oyster populations in epizootic areas is to seek innate resistance in some percentage of the survivors and develop a resistant stock from the progeny of these survivors (Haskin, personal communication, 1967). Andrews (1966) expressed this concept as "raising herd immunity on a genetic basis."

Considerable work has been done on the history (Haskin, 1962; Haskin *et al.*, 1965; Mackin, 1961; Andrews, 1967), morphology (Haskin *et al.*, 1966; Couch, Farley, and Rosenfield, 1966; Couch, 1967), portions of the life cycle (Myhre, 1966; Myhre, personal communication, 1967; Farley, 1967), and histochemistry (Eble, 1966) of these parasites.

Previous studies (Wood and Andrews, 1962; Haskin *et al.*, 1966) suggested that Chincoteague Bay was an enzootic-epizootic area for *Minchinia nelsoni* and *M. costalis*. Our objectives were: 1) to determine annual and seasonal parasite prevalences in susceptible oysters; 2) to determine the relationships of season of introduction and length of exposure to detection of infections and mortalities; 3) to determine cumulative mortality in introduced populations after varying periods of exposure. This paper reports the results of this 3-year epizootiological study of *M. nelsoni* and *M. costalis* and compares the epizootiology in Chincoteague Bay with that in areas studied by others.

METHODS

Beginning in September, 1963, experimental groups of oysters were introduced at 8 quarterly intervals, over a period of 2 years, into Chincoteague Bay, Virginia, and held there for varying periods of time (Table 1). The first 4 introductions (September, 1963 — June, 1964) were duplicated chronologically by the last 4 (September, 1964 — June, 1965). A study year was from 1 September to 31 August. The oysters (1961 year set) were obtained from Harris Creek, a portion of Chesapeake Bay, where *Minchinia nelsoni* and *M. costalis* have not yet been found. A sample of 50 oysters from each introduction was examined microscopically before exposure in Chincoteague Bay. No haplosporidans were found in any of the 8 quarterly base samples, nor in any of the trayed control oysters kept in Harris Creek and examined periodically during the second year of introductions. Mortalities of these control oysters were extremely low.

Each introduction consisted of approximately 1200 oysters distributed among 4 wire trays—about 300 to 400 per tray. These trays were suspended about 1 foot above the bottom to reduce siltation and to prevent predation. Silt accumulations and fouling organisms were removed at each sampling period. One tray of each introduction was used throughout the study for mortality observations

TABLE 1. *Periods of exposure and cumulative mortalities of trayed Harris Creek oysters maintained in Chincoteague Bay.*

Introductions	Date introduced	Date terminated	Exposure time (months)	Cumulative mortality (%)
1	Sept. 1963	Sept. 1965	24	70.5
5	Sept. 1964	Sept. 1966	24	75.5
2	Dec. 1963	Sept. 1965	22	73.8
6	Dec. 1964	Sept. 1966	22	70.4
3	Mar. 1964	Sept. 1965	19	65.2
7	Mar. 1965	Sept. 1966	19	73.8
4	June 1964	Sept. 1965	16	76.5
8	June 1965	Sept. 1966	16	73.1

only, and was not sampled. Twice each month the observation trays were raised, and the dead oysters counted and removed. Total monthly (cumulative) mortalities of each introduction were determined. Dead oysters (gapers with remaining tissues) found in the observation trays were fixed, sectioned, and examined for haplosporidans.

The remaining 3 trays in each introduction contained about 900 oysters. Each month 25 oysters of each introduction were taken randomly from among the 3 trays. Oysters were fixed in Davidson's fixative, sectioned and stained with Harris' hematoxylin, eosin, and Ziehl's fuchsin (Farley, 1965). Single sections of 7 μ from the area of the gills, palps, and stomach of each oyster were examined for *M. costalis* and *M. nelsoni*. Percentage of infection of monthly samples (n—25 oysters per introduction) is expressed either as initial incidence (first level of infections for each introduction) or prevalence (monthly levels of infection).

Oysters of monthly samples were diagnosed according to the method of Ray (1952) for the presence of the fungal pathogen, *Dermocystidium marinum* (Mackin, Owen, and Collier, 1950), a species whose generic designation has recently been changed to *Labyrinthomyxa* (Mackin and Ray, 1966). No fungal infections were found in samples of oysters from this study.

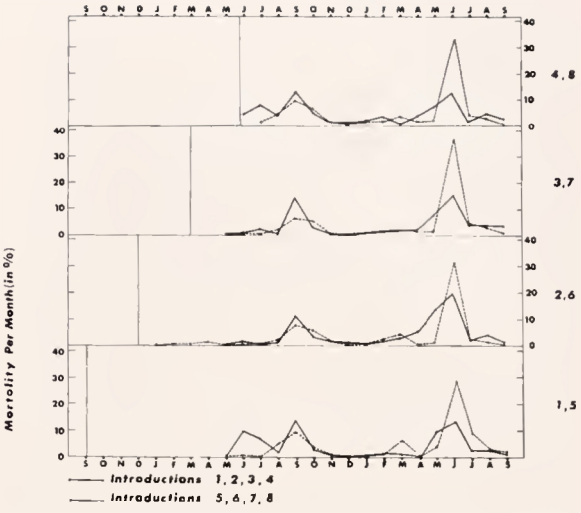

FIG. 1. *Mortality curves for 8 introductions of oysters in Chincoteague Bay, Virginia. Solid line represents introductions 1 through 4. Broken line represents introductions 5 through 8. Vertical scale is in per cent and mortality points are plotted as percentage dead each month of original number of oysters in each introduction.*

MORTALITY OF OYSTERS

The mortality patterns in introduced oyster populations are described first, followed by the incidences and prevalences of *Minchinia costalis* and *M. nelsoni* as probable causes of mortalities.

The quarterly introductions were numbered consecutively from 1 through 8. Curves of percentage monthly mortality for each introduction are presented in Figure 1.

Oysters introduced in September 1963 and 1964 did not die in significant numbers until the summer and early fall (curves 1, 5 — lowermost curves in Fig. 1). Oysters introduced in December, March, and June also experienced a similar period of first mortality (Fig. 1, curves 2,6; 3,7; 4,8). The first real peak of mortality occurred in September. During the winter after the initial summer-through-October mortality period, mortality was very low. A slight mortality occurred in March in the second-year introductions (Fig. 1, curves 5, 6, 8); this preceded by 1 month the major mortality period for these introductions (broken line). The period of high death rate (30-38 per cent) occurred in May and June. In 3 of the 4 first-year introductions (Fig. 1; curves 1, 3, 4 — solid line) the May-June mortality peaks approximately equaled their preceding September-October peaks.

Even though introductions 1 and 5 were exposed 4, 7 and 9 months before introductions 2 and 6, 3 and 7, and 4 and 8, respectively, all introductions experienced initial peak mortalities during the following September (Fig. 1). Susceptible oysters introduced in June (introductions 4 and 8) had mortalities in September (3-month exposure) comparable to mortalities in oysters introduced more than 9 months earlier (introductions 1 and 5). Therefore, as far as the first period of mortality is concerned, length of exposure was not as significant as seasonality of introduction.

Oysters in all introductions had to be exposed to Chincoteague Bay for at least 12 months (i.e. Fig. 1, introductions 4 and 8) before they suffered the peak mortality of May and June. Note that during the first May and June of exposure, mortality was relatively low in most introductions.

Cumulative mortality for each introduction of oysters is given in Table 1. All groups of oysters (except introduction 3) experienced over 70 per cent mortality. It is pertinent that the magnitude of cumulative mortality was not necessarily proportional to the duration of exposure. All introductions experienced approximately similar cumulative mortalities after two summers and one winter of exposure (e. g., compare introductions 1, 5 with 4, 8 in Fig. 1).

INITIAL INCIDENCES AND MONTHLY PREVALENCES OF *MINCHINIA NELSONI*

Initial incidence designates the first appearance

of *Minchinia* infections. Prevalence represents the level of infection (percentage of sample infected) in a given introduction at any given time after initial incidence.

Figure 2 shows initial incidences and monthly prevalences for *Minchinia nelsoni* (curves 1-8) and *M. costalis* (histograms) in living oysters. In the first year of introductions, *M. nelsoni* was first observed in March and April in exposed introductions (Fig. 2, introductions 1, 2, 3). These infections consisted of plasmodia largely restricted to the gill and mantle tissues. Initial infections were high in these introductions during these two months. The fourth introduction was made in June.

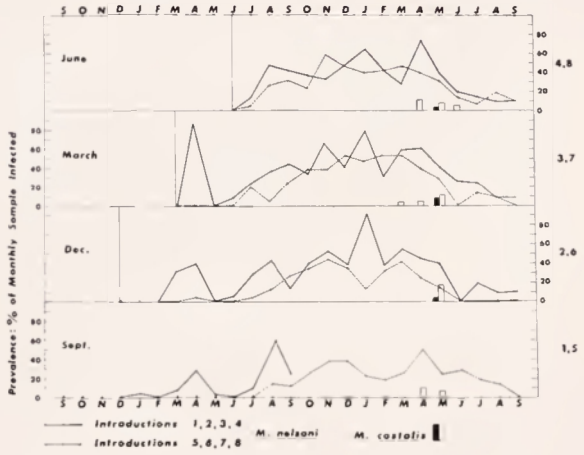

FIG. 2. *Incidence and prevalence data for* Minchinia nelsoni *(lines) and* Minchinia costalis *(histograms). Solid lines and histograms represent prevalences in introductions 1 through 4. Broken lines and stippled histograms represent prevalences in introductions 5 through 8. Vertical scale is in per cent and prevalence is expressed as percentage of sample of oysters infected per month. Note confined seasonal prevalences of* M. costalis *as contrasted to extended prevalences of* M. nelsoni.

In May and June the decrease of the prevalence to a very low level (5 per cent) in the first 3 introductions suggests that the original March-April infections became abortive or that stages of the parasite became cryptic. Prevalences of *M. nelsoni* in introductions 1, 2, 3, and 4 began to rise again in late July and by late August had reached 40 to 60 per cent preceding the first mortality peak in September. A drop in prevalences in 1, 2, and 4 in September possibly reflected infections removed from the introductions by the increased death rate in September. Monthly prevalences of *M. nelsoni* increased during the fall, however, in all introductions [1] and remained moderate to high in most during the winter. The apparent abrupt drop in prevalence during January of the first introduction (Fig. 2) may be a reflection of biased monthly sampling rather than a genuine drop in prevalence.

The high winter prevalence of *M. nelsoni* was not accompanied by any significant mortality (Fig. 1). Prevalence of *M. nelsoni* began to decline in the second spring and remained relatively moderate to low in the subsequent summer months until the end of the study. It should be noted (Fig. 2) that the prevalences of *M. nelsoni* in the spring overlapped the initial incidence of *M. costalis*.

INITIAL INCIDENCES AND MONTHLY PREVALENCES OF *MINCHINIA COSTALIS*

In Figure 2 the solid histograms represent prevalences of *M. costalis* in the first year introductions (1, 2, 3, 4) and the stippled histograms represent prevalences in the second year introductions (5, 6, 7, 8).

The earliest incidence of *M. costalis* was in March (Fig. 2, introduction 7) after a 12-month exposure. Introduction 8 (Fig. 2), however, had initial infection of *M. costalis* in April after only 10 months of exposure. The highest percentages of infections in all introductions occurred in May, after at least 12 months of exposure. In living oysters the apparent prevalences of *M. costalis* were low (highest: 18 per cent, introduction 6, May) in comparison with the prevalences of *M. nelsoni* in the same samples. *Minchinia costalis* was found in oysters only within the time period March to early July. The apparently low prevalences of *M. costalis* in living oysters is probably best explained by the observation that initial infections of the oysters by *M. costalis* slightly preceded and were concurrent with the second peak of mortality (Fig. 1). Apparently many newly infected oysters died quickly (May-June). This idea is supported by the high prevalences of *M. costalis* in dead oysters recovered during May and June from all introductions as discussed in the following section.

PREVALENCE OF *MINCHINIA NELSONI* AND *M. COSTALIS* IN DEAD OYSTERS

Mortalities occurred during warm periods, hence

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[1] Incidence and prevalence data for introduction No. 1 were not determined beyond 1 year of exposure September, 1963, to September, 1964 (See Fig. 2).

active predators and scavengers (i.e. small crabs and fish) quickly consumed the meats of dying and dead oysters in the trays. Table 2 shows prevalences of the two species of *Minchinia* in dead oysters recoverable during the two heavier mortality periods (these figures are combined data from all 8 introductions). Plasmodial infections of *M. nelsoni* were heavy in most of the oysters that died during September and October. There was much evidence of tissue replacement and damage caused by the plasmodia, particularly in weak and dying oysters. No *M. costalis* infections were found during the September-October mortality.

TABLE 2. *Prevalences of two species of* Minchinia *in dead oysters during the two peak mortality periods.*

	M. nelsoni	*M. costalis*
Sept.	26/30	—
Oct.	20/24	—
May	14/56	40/56
June	5/63	39/63

Both species of *Minchinia* were found in dead oysters from the May-June period of mortality. Concurrent or double infections with spores of *M. nelsoni* and *M. costalis* in individual oysters were also found during May and June (Couch, 1967): *Minchinia costalis* infections were highest during this early summer mortality. Well over 50 per cent of most of these infections were very heavy and usually occupied every tissue of the host. The prevalence of *M. nelsoni* in dead oysters during May and June was less than that of *M. costalis*. However, the prevalence of *M. nelsoni* was similar in living and dead oysters during May and June (compare Table 2 with Fig. 2).

SPORULATION OF *MINCHINIA NELSONI* AND *M. COSTALIS* IN RELATION TO MORTALITY

Diagnoses of *Minchinia nelsoni* in most cases were based on the identification of plasmodia. Sporulation of *M. nelsoni* has been found infrequently in living oysters and rarely in dying or dead oysters from Chesapeake Bay and Chincoteague Bay (Couch *et al.*, 1966; Couch, 1967). *Minchinia costalis* sporulated regularly, but spores of this species were found mainly in dying or dead oysters during the May-June mortality, and only rarely in living oysters. In the present study over 80 per cent of the diagnoses of *M. costalis* in dying or dead oysters was based upon finding the parasite's characteristic sporocysts and spores whereas almost all of the diagnoses in living oysters containing *M. costalis* were based upon recognition of its plasmodium.

ASSOCIATION OF MORTALITIES WITH *MINCHINIA NELSONI* AND *MINCHINIA COSTALIS*

In most introductions, serious oyster mortality was either preceded by or was concurrent with infections of *Minchinia nelsoni* or *M. costalis* or both (Figs. 1, 2). During the intervals between initial exposure of the oysters and initial incidence of *M. nelsoni*, mortality was not significant (see particularly introductions 1 and 5, Figs. 1, 2). The gradual increase in prevalence of *M. nelsoni* during August and September was concurrent with mortality. On the other hand, the high prevalences of *M. nelsoni* in winter was not accompanied by mortalities. Oysters are at their lowest level of metabolic activity during the winter months because of the lowered water temperatures (Galtsoff, 1964). Thus, even though oysters were heavily infected, it is possible that a potential mechanism of death was not operative until the general metabolism of the host increased with rising spring and summer water temperatures (compare Fig. 3 with Fig. 1). So all of the contributing factors necessary for parasite-induced death were present during the relatively high mortalities of May and June, including initial incidence of a second parasite, *M. costalis*. It is not possible to speculate on the role of salinity. However, salinities were relatively high during periods of mortality (Fig. 3).

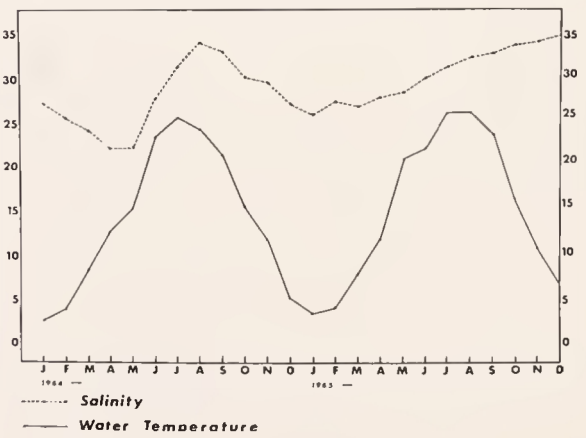

FIG. 3. *Seasonal salinity and temperature curves for Chincoteague Bay, Va. Data used to plot these curves were taken at the dockside tray station, U. S. Bureau of Commercial Fisheries Field Station, Franklin City, Virginia. Each point on the curve represents the average monthly salinity and temperature.*

Depending upon the season of introduction, oysters can become infected within 1 month and apparently mortalities can occur within 3 months (Figs. 1, 2).

It is pertinent to the present discussion to consider another parasite of the oyster that was once indicted as a pathogen, but was later found to have little effect on the host. Prytherch (1940) described *Nematopsis ostrearum*, a gregarine parasite (Eugregarinina: Porosporidae), and thought it was a serious pathogen of oysters. Spores of this parasite are found in tissues of the oyster, which acts as a vector to transmit the parasite to its definitive hosts, mud crabs, when the crabs consume infected oyster tissues. The stages (spores) of the gregarine do not multiply in the oyster. Sprague (1955, p. 102) stated: "Any effect of the parasite on the host must, therefore, be caused by these original invaders; there can be no effect (as in most Sporozoa and in pathogenic bacteria) attributable to multiplication of the parasites within the oyster." Therefore, intensity of infection by spores of *Nematopsis* is directly proportionate to the number of infective stages contacting the oyster, and not to proliferation, or metabolic increase, or activity of the parasite within oyster tissues. The passive spore held within oyster tissues is perhaps less pathogenic than a proliferating parasite that is actively metabolizing and increasing in large numbers. *Minchinia nelsoni* and *M. costalis* appear to have the necessary qualifications for pathogens. Each species apparently increases vegetatively within the oyster; distinct life-history stages are reproduced within the host. Host tissue is replaced drastically by the parasites. Prevalences of the parasites were substantial in living, dying, and dead oysters in epizootic areas during mortality periods. Thus, Andrews' (1966, p. 30) statement, "By reason of a very extensive experience with the distribution of MSX and associated mortalities, it is probable that the first postulate [Koch's], that of regular association, has been satisfied indirectly," is applicable to both *M. nelsoni* and *M. costalis* in Chincoteague Bay.

RELATION OF INFECTION TO REPRODUCTIVE CAPACITY OF OYSTERS

Little has been reported on the influence of haplosporidans on their host's physiology or development. One potentially detrimental effect a parasite may have upon a population is to reduce that population's reproductive capacity by directly or indirectly impeding gametogenesis. Some indirect evidence of this effect is presented in Table 3 for oysters infected with *Minchinia nelsoni* and *M. costalis*. These data were gathered from living oysters sampled from introductions 7 and 8 in April through September, 1966 (main period of gametogenesis) after at least 11 months of exposure. All of these oysters were adults. Before this period (April-September) oysters in these introductions had high prevalences of *M. nelsoni*, and during the period both *M. nelsoni* and *M. costalis* were present in living oysters in each group (Fig. 2).

The essential points to be inferred are: 1) Uninfected oysters (52.3 per cent of total) were most successful in producing gametes (fully developed sperm or eggs); 2) infected oysters were least successful in producing gametes (only 7 per cent), and 3) comparisons between infected and uninfected oysters showed that a slightly higher number of infected oysters were without gonadal development.

It is intriguing to speculate about the history of the gravid, uninfected oysters because the groups from which the above oysters were obtained had a previous high prevalence of *M. nelsoni* and an initial incidence of *M. costalis*. Did these oysters recover from infections in time (before April or May) to begin active gametogenesis? Was there

TABLE 3. *Relationship of* Minchinia *infections and host gonad conditions (1966).*

Months	Uninfected with ripe gonad	Infected with ripe gonad	Uninfected without gonad	Infected without gonad
April	1	1	10	12
May	32	8	12	26
June	22	0	1	4
July	26	2	1	4
August	19	3	6	5
September	12	1	6	0
Totals	112	15	36	51
N=214	52.3%	7.0%	16.8%	23.9%

a genetically controlled resistance mechanism in a certain percentage of the oysters of this group which was activated by physiological changes at the onset of gametogenesis? Had these oysters escaped initial infection completely? The first two questions cannot be answered with presently existing information. The answer to the last question is probably "no", because prevalences of *M. nelsoni* in the source introduction had been so high in the preceding winter as to insure that some of these oysters had been infected at one time.

COMPARISON OF DISEASE IN CHINCOTEAGUE AND DELAWARE BAYS

The patterns of mortality in introduced oysters in Delaware Bay (Haskin *et al.*, 1965) attributed to *Minchinia nelsoni* are, as in Chincoteague Bay, influenced largely by season of introduction rather than duration of exposure. Spring and early-summer introductions have first mortalities in the late summer and early fall of the same year. Late-winter mortalities, reported for Delaware Bay, were not found for oysters in the present study. Late-summer and fall introductions had first mortalities in the following late-winter and June respectively. This timing differs from the September introductions (1, 5) of the present study in that introductions 1 and 5 had first epizootic mortalities in September, 12 months after initial exposure. The sharp June mortality peak reported by Haskin *et al.* (1965) for fall introductions (8 to 10 months of exposure) was not found in Chincoteague Bay for chronologically identical introductions. The June mortalities were attributed to *M. nelsoni* in Delaware Bay, whereas most dead oysters from the June mortalities (12-22 months exposure) were heavily infected with *M. costalis* in Chincoteague Bay. *Minchinia costalis* occurs rarely in Delaware Bay (Haskin *et al.*, 1966) and therefore is probably not a significant cause of mortality there.

Generally the high prevalences of *M. nelsoni* preceding and associated with mortalities in Chincoteague Bay were similar to those in Delaware Bay. Haskin *et al.* (1965) and Haskin (personal communication, 1967) also reported that recently certain high prevalences of *M. nelsoni* in Delaware Bay were not paralleled by heavy mortalities. High winter prevalences of *M. nelsoni* in Chincoteague Bay also were not associated with mortality. The reasons for failure of mortality to coincide with epizootic prevalences of *M. nelsoni* in the two areas at certain times are not understood presently, and are possibly not indentical.

Seasonal mortality peaks in Delaware Bay are apparently higher than comparable peaks in Chincoteague Bay, at Franklin City, Virginia. Spring introductions in Delaware Bay lose from 65 to 85 per cent of the oysters by November (7 — 10 months of exposure) (Haskin *et al.*, 1965). These losses are higher than experienced by any single introduction in Chincoteague Bay during a comparable time period.

COMPARISON OF DISEASE IN CHINCOTEAGUE AND LOWER CHESAPEAKE BAYS

Andrews (1967) pointed out that after 8 years of high prevalences of *Minchinia nelsoni* in lower Chesapeake Bay, there seemed to be no sign that this parasite was diminishing in either prevalence or virulence. The same conclusion apparently held for both *Minchinia nelsoni* and *M. costalis* in Chincoteague Bay as late as 1966 (present study).

As in Delaware and Chincoteague Bays, patterns of oyster mortality attributed to *M. nelsoni* in lower Chesapeake Bay depended more on season of introduction than on duration of exposure (see Andrews, 1966, Figs. 3, 7, 8). Oysters introduced in early spring had first epizootic mortalities in September and October, second mortality in late winter (February), and heaviest mortality in June through October of the second year of exposure. This sequence was similar to that of mortalities in comparable introductions in Chincoteague Bay (Fig. 1) with the following exceptions: 1) Certain seasonal mortalities (i.e. September) in Chesapeake Bay were slightly higher than comparable mortalities in Chincoteague Bay; 2) the late-winter mortality in Chesapeake Bay did not occur in Chincoteague Bay (at least not to a recognizable degree, and 3) the second summer mortalities (Andrews, 1966. Fig. 7) were spread over a longer period (June-September) than those in Chincoteague Bay (May-July).

Slight differences in times of initial incidences of *M. nelsoni* have been found for lower Chesapeake and Chincoteague Bays. Initial infections in lower Chesapeake Bay usually occurred in May (Andrews, 1966), whereas in the present study the majority of initial infections came in late July and August (Fig. 2), except for the possible atypical aborted infections in introductions 1, 2, and 3.

A 2-year prevalence pattern for *M. nelsoni* in epizootic areas of lower Chesapeake Bay was presented by Andrews (1966, Fig. 8). These data can be compared with Figure 2 in the present study in Chincoteague Bay. Andrews presented an extrapolated curve of prevalences based on data collected over a 5-year period, whereas in Figure 2 of the present study actual monthly prevalences (percentage infected of 25 oysters per month) are given for 8 introductions. The actual seasonal prevalences determined in the present study do not differ radically from Andrews' curve of prevalence for lower Chesapeake Bay. Minor differences were: 1) The few high (abortive ?) prevalences of *M.*

nelsoni in Chincoteague Bay in March and April were not found by Andrews in newly introduced oysters in lower Chesapeake Bay; 2) winter prevalences of *M. nelsoni* in Chincoteague Bay seemed, on the average, to be higher than in lower Chesapeake Bay, and 3) spring and early summer prevalences of the second year were not as high in Chincoteague Bay as they were in lower Chesapeake Bay. The significance of these similarities and differences may be open to some questions; these studies are not entirely comparable because of differences in location and years of study, ages of oysters introduced, and origin and susceptibility of stocks and perhaps experimental methods.

Cumulative mortalities in the two areas for comparable periods of exposure were closely similar (Andrews, 1966, Table 5; present study, Table 1).

Minchinia costalis played no known role in lower Chesapeake Bay mortalities, but was found to be a significant contributor to deaths of oysters on Seaside Virginia (Andrews *et al.*, 1962). The mortalities and prevalences attributed to *M. costalis* for Seaside Virginia were essentially duplicated during the present study in Chincoteague Bay, a similar and nearby locale.

PRACTICAL IMPLICATIONS OF THE PRESENT STUDY

Apparently it would be possible to manipulate oysters introduced in Chincoteague Bay to avoid high mortality from haplosporidans. If trayed oysters were to be placed there for relatively short periods to improve their flavor or for fattening, it would be necessary to introduce them in the fall and harvest them before the following June to avoid the September mortality. If losses of from 10 to 30 per cent could be tolerated, oysters should be introduced in the fall (September), maintained through one winter and subsequent summer, and harvested the second winter without exposing them to the second high mortality period in June (Fig. 1). Exposure of oysters for two summers and a winter results in cumulative losses of approximately 70 per cent (Table 1; — also see Shaw, 1966).

Evidence for stocks of oysters resistant to *Minchinia nelsoni* (developed from naturally selected, innately resistant parents) has been reported by Haskin (personal communication, 1967). The present study was not planned to seek or demonstrate resistance in newly introduced oysters. The only suggestion of possible resistance in these oysters was highly indirect and of uncertain significance. The fact that a certain percentage of each introduction did not die (after 2 years of exposure) suggests that some oysters may be less susceptible to the lethal effects of the parasites than others. No observation on the possibility of acquired immunity was made. Until the microecology and host-parasite relationships are better understood, very little can be said definitely about resistance of any kind in the oyster.

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RELATION OF FECUNDITY AND EGG LENGTH TO CARAPACE LENGTH IN THE KING CRAB, *PARALITHODES CAMTSCHATICA*

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ABSTRACT

The fecundity of female king crabs, collected in Kachemak Bay, Alaska, in July, 1967, increased with carapace length, but egg length did not. For crabs of the same size, the mean number of eggs carried was significantly greater than for crabs in a similar study in Kachemak Bay in July, 1960.

INTRODUCTION

The U. S. Bureau of Commercial Fisheries Biological Laboratory, Auke Bay, Alaska, has started a long-term study of the reproduction of the commercially important king crab, *Paralithodes camtschatica* (Tilesius), in Kachemak Bay off Cook Inlet, Alaska. Female king crabs spawn in early spring and carry the developing embryos on their pleopods for about one year. It is expected that during this time fecundity (the number of eggs or developing embryos) will decrease. Therefore, fecundity is being determined periodically during the year to measure this decrease. These data will be used to derive equations for estimating the average fecundity of the population at each sample date and to predict the probable number of larvae that would result.

Although many authors have reported on the number of eggs carried by the king crab, only Sato (1958), Bright, Durham, and Knudsen (1960) and Rodin [1] determined the number of eggs over the entire size range of ovigerous crabs. Sato based his studies on king crabs from the North Pacific Ocean off Nemuro, Japan; Rodin used king crabs from the Bering Sea; and Bright *et al.* did their research on king crabs collected in July in Kachemak Bay — the same area and time of year that I sampled.

In this report I describe the relation of fecundity and egg length to the carapace length of female king crabs collected July 14-25, 1967, in Kachemak Bay. These data are compared with the findings of Bright *et al.* (1960) to determine any changes in these relations in 1960 and 1967.

SAMPLE COLLECTION AND TREATMENT

Crabs were taken in pots similar to those used in the commercial king crab fishery. The pots were set 30 to 40 m deep and were usually checked every other day. Carapace lengths (posterior margin of the right eye socket to the mid-point of the posterior margin of the carapace) of ovigerous crabs were measured to the nearest 0.1 mm. To ensure representation of all sizes of ovigerous crabs, 90 were selected on the basis of their carapace length over a range of 98 to 175 mm. The egg masses, with the pleopods attached, were removed from the females by excising the pleopods at their base and placed in plastic bags.

The number of eggs in each mass was determined in the laboratory by sampling. Each egg mass was weighed (after blotting) to an accuracy of ± 0.01g, and a sample of eggs (about 5 per cent of the total weight of each mass) was removed, weighed, and counted. The count-weight ratio was used to estimate the total number of eggs in the mass. For counting, the eggs in each sample were separated from the hairs of the pleopods by soaking the sample in a saturated solution of sea water and sodium hydroxide for about 24 hours, and then rinsed several times in 10 per cent formalin. Repeated counting of several samples indicated a counting error of 0.08 per cent.

[1] Rodin, V. E. 1966. Soviet works of 1965 on the distribution of Kamchatka crab supply conditions *Paralithodes camtschatica* (Tilesius) in the southeastern part of the Bering Sea. (unpublished manuscript)

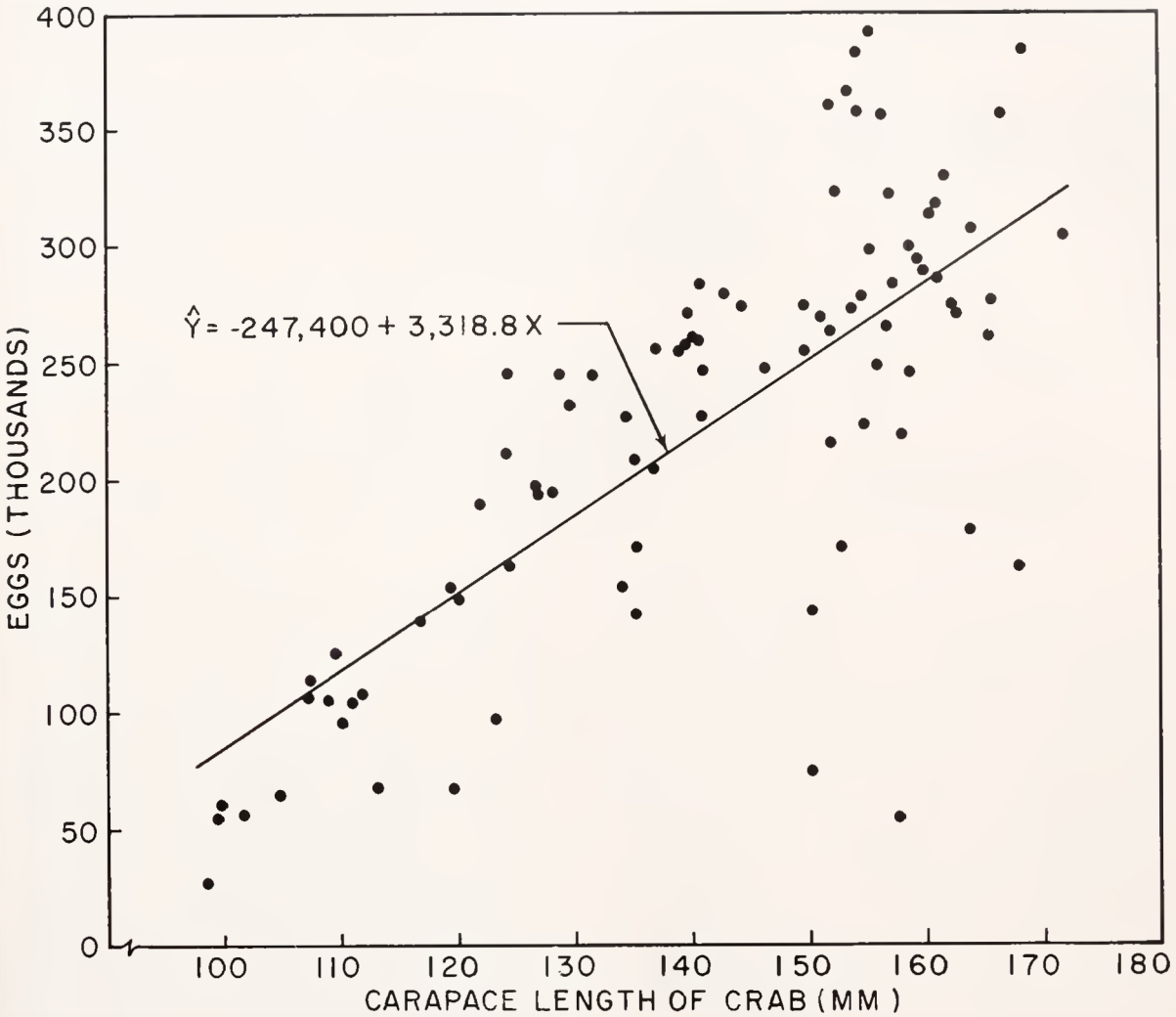

FIG. 1. *Estimates of fecundity of 90 king crabs collected in Kachemak Bay in July, 1967 and a computed linear regression of fecundity on carapace length.*

RELATION OF NUMBER AND SIZE OF EGGS TO CARAPACE LENGTH

In general, fecundity increased as carapace length increased. There was a wide range in the number of eggs carried by crabs of any given carapace length, and this range increased with carapace length (Fig. 1). The greatest range was in crabs whose carapace lengths were between 150 and 170 mm. Females in this size group had 25,030 to 390,000 eggs. To describe the relation of fecundity to carapace length, I computed a linear regression of fecundity (Y) on carapace length (X):

$$\hat{Y} = -247{,}400 + 3{,}318.8X$$

The cause of the wide range in fecundity is unknown, but it is possible that an exceptionally low number of eggs results when a female mates with a less than fully capable male. Powell and Nickerson (1965) found that, although a male king crab can mate successfully with at least five females, it may not be capable of fertilizing all the eggs of subsequent females. This inability may result in many unfertilized eggs, which do not adhere to the pleopod hairs. This possibility has an important bearing on the king crab fishery, because only males are harvested.

To compare my estimates of numbers of eggs with those of Bright *et al.* (1960), who presented their data as the mean number of eggs for each

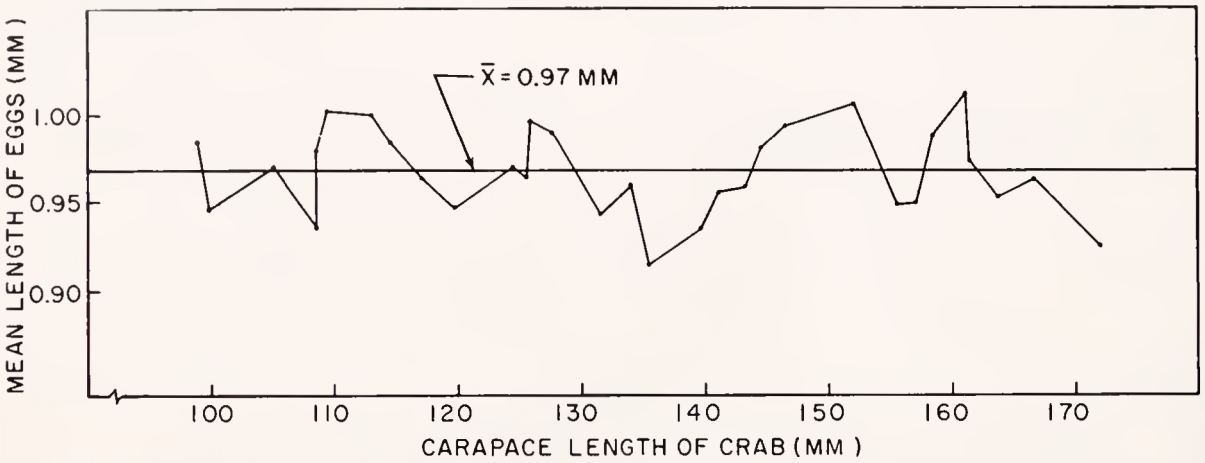

FIG. 2. *Relation of mean length of 50 eggs to carapace length for 31 female king crabs collected in Kachemak Bay in July, 1967.*

10-mm size class of crab, I computed a linear regression of the number of eggs (Y) on carapace length (X) for their data. Analysis of covariance was used to compare the regression coefficients and adjusted means of the two groups of data. At the 1-per cent level the regression coefficients were not significantly different ($F = 5.336$; 94 d.f.), but the adjusted mean number of eggs in my study was significantly greater — by about 11,000 eggs ($F = 751.6$; 95 d.f.) — than that calculated from the data of Bright *et al.* (1960).

The lengths of 50 unpreserved eggs from each of 31 crabs were measured (with an ocular micrometer) to determine if the size of the eggs increased with the size of the crab (Fig. 2). The mean egg lengths varied among the crabs but could not be related to carapace length. The mean length of 1,550 eggs measured in my study was 0.97 mm — almost the same as the mean of 0.98 mm calculated from measurements of 480 eggs by Bright *et al.* (1960).

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HERMAPHRODITISM IN THE SURF CLAM, *SPISULA SOLIDISSIMA*

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ABSTRACT

A single hermaphroditic surf clam, Spisula solidissima *(Dillwyn), was found among 2,500 clams collected to determine the seasonal reproductive cycle in the mid-Atlantic bight. Standard histological techniques were used to prepare stained sections of all gonads for detailed microscopic examination. The hermaphroditic specimen contained clearly distinguishable testicular and ovarian alveoli with developing germ cells of both sexes. The rarity of hermaphroditism in the surf clam is discussed in relation to this condition in other pelecypods.*

Hermaphroditism has not been reported for the the surf clam, *Spisula solidissima* (Dillwyn), a pelecypod that normally is dioecious (Belding, 1910). The simultaneous production of male and female germ cells within an individual is common in many gastropods, but is relatively rare in pelecypods (Coe, 1943, 1944; Galtsoff, 1961).

A hermaphroditic surf clam was collected on June 19, 1965, in an experimental jet dredge (Merrill and Webster, 1964) towed at a site 34 miles (ca. 63 km) east of False Cape, North Carolina (75° 10' W. Long., 36° 38' N. Lat.). Water depths ranged from 90 to 105 feet (ca. 27 to 32 m). This drag was one of many made during a study underway since 1962 to determine the seasonal reproductive cycle of surf clams. Eleven clams averaging 142 mm in shell length were taken in the 5-minute tow. The gonads were preserved in Bouin's fixative, and standard histological techniques were used to prepare 7 μ-thick sections for microscopic examination.

The hermaphroditic condition was not detectable in the freshly opened clam. Color differences between gonads of male and female surf clams are not obvious, but in the slide preparation a difference in stain intensity is apparent between the male and female alveoli (Fig. 1A). The darkly stained portions of the gonad contain male germ cells; the lightly stained portions contain female germ cells. The specimen is an example of bilateral hermaphroditism (Coe and Turner, 1938).

Both active gametogenic and ripe developmental phases are present in the testicular alveoli of the hermaphroditic clam. The tubelike alveoli are closely packed in the cross-sectioned gonad; interalveolar cells are sparse between the basement membranes of connective tissue. As is typical of active gametogenesis, primordial germ cells form a layer of several cells in one area of the gonad (Figs. 1B & 1C). Spermatogonia, and primary and secondary spermatocytes occur respectively from the basement membrane toward the lumina of alveoli, and spermatids and sperm are in the centers. In comparison with the ripe phase, however, the alveoli contain few germ cells, and especially sperm. Typical of the ripe developmental phase, sperm form a dense mass in the lumina of alveoli in another area of the gonad (Fig. 1D). Primordial germ cells lie nearest the basement membrane, and are less numerous than sperm.

The ovarian alveoli contain early developing ovocytes, typical of active gametogenesis, although a few ripe ovocytes are present in the lumina (Figs. 1B, 1C, & 1D). Early ovocytes protrude from the basement membrane, advanced ovocytes are attached by stalks, and ripe ovocytes lie free within the lumina and contain amphinucleoli (Allen, 1953).

Variability in development throughout a gonad is common; it was observed by Coe (1932) in the California oyster, *Ostrea lurida,* a species that undergoes a sex reversal and also has true hermaphroditic individuals in its populations. Coe (1943) hypothesized that in ambisexual oysters the sex-differentiating mechanism apparently fails to function normally. A tendency for sperm to ma-

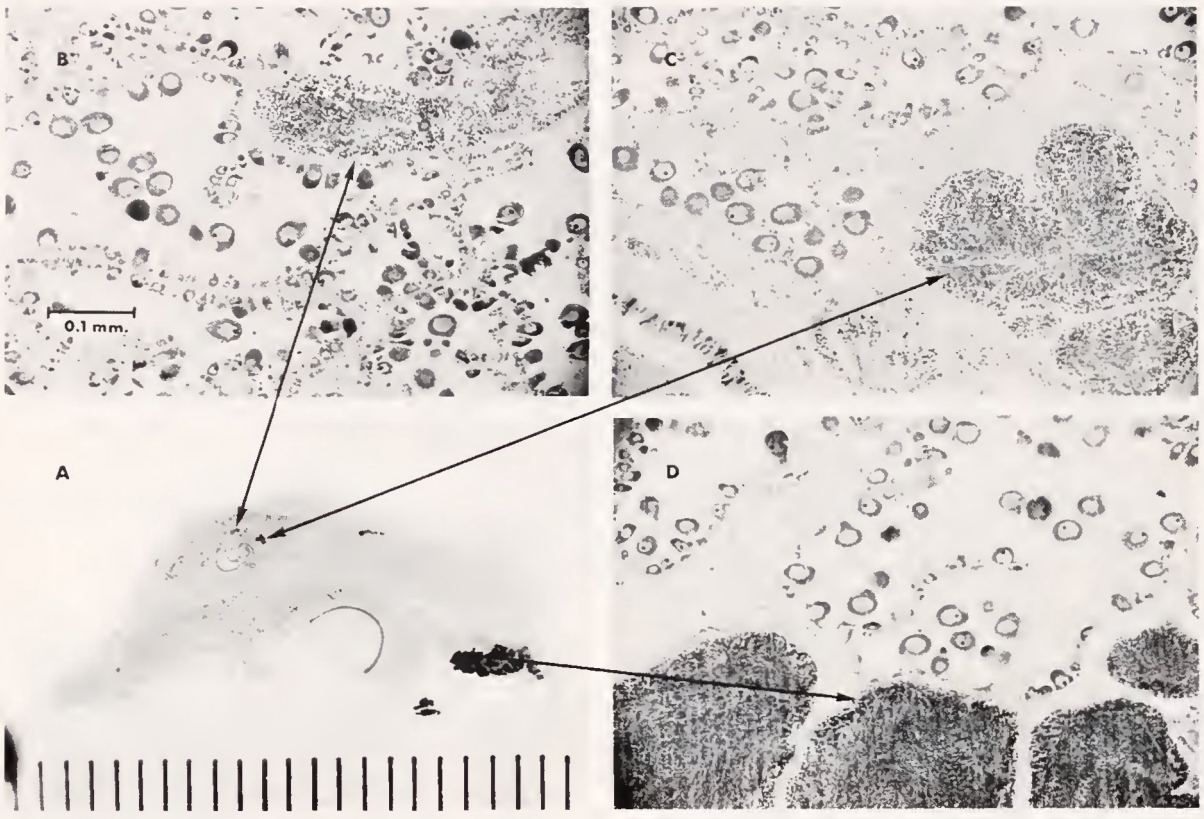

FIG. 1 *Sections of the gonad of a hermaphroditic surf clam,* Spisula solidissima. *(A) a macroscopic view of the section showing three areas of testicular alveoli stained more intensely than the adjacent ovarian alveoli. A millimeter scale is included in this photograph and arrows point to microscopic enlargements (100X) of areas containing testicular alveoli. (B & C) Two areas of testicular alveoli containing mostly primordial germ cells and primary and secondary spermatocytes. (D) Another area of testicular alveoli containing dense masses of mature sperm. Ovarian alveoli are adjacent to the testicular alveoli in all of the enlargements and contain developing ovocytes.*

ture in advance of ova in one area of the surf clam gonad (Fig. 1D), but at the same rate as ova in other alveoli (Figs. 1B & 1C) is perhaps a deviation from the normal developmental process.

Typically dioecious pelecypods produce few hermaphrodites. Coe and Turner (1938) found only three hermaphroditic soft-shell clams, *Mya arenaria,* in about a thousand obtained near New Haven, Connecticut. Shaw (1965) found no hermaphrodites in 800 soft-shell clams from Chesapeake Bay, Maryland, and Ropes and Stickney (1965) found no hermaphrodites in 1,400 *Mya* collected from nine areas along the New England coast. Loosanoff (1936) reported only two hermaphrodites in several hundred quahogs, *Mercenaria mercenaria.* The discovery of first one and then another hermaphroditic sea scallop, *Placopecten magellanicus,* by Merrill and Burch (1960) was interrupted by an inspection of about 3,000 normal gonads. The single hermaphroditic surf clam in a sample of nearly 2,500 must be an anomaly and would, then, be termed an "accidental functional ambisexual" by the classification of Coe (1943).

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DISTRIBUTION OF JUVENILE TANNER CRABS (*CHIONOECETES TANNERI* RATHBUN), LIFE HISTORY MODEL, AND FISHERIES MANAGEMENT[1]

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ABSTRACT

A large population of Tanner crabs was found at depths from 250 to 1,050 fm southwest of the Columbia River mouth. Analysis of trawl-catch data revealed that juvenile crabs were distributed deeper than the adult population. The adult population was not stationary. Females were most abundant at depths of 350 to 375 fm throughout the year. In contrast, the male population was centered at shallower depths during the spring and summer, shifting deeper during the winter breeding season. Adult females bearing eggs were found in catches made at all seasons and egg development proceeded on a yearly cycle, beginning in the winter. The biological and environmental factors responsible for the observed life history pattern are briefly discussed.

INTRODUCTION

During a cooperative investigation by the Bureau of Commercial Fisheries and Atomic Energy Commission of the deep-water demersal fish and invertebrate populations off the northern Oregon coast, a sizeable population of a large majid crab *Chionoecetes tanneri* Rathbun, was found at depths from 250 to 1,050 fm. Because this crab is found in relatively deep water, little was known of its distribution, abundance, or life history prior to this study. In two papers resulting from this study (Pereyra, 1966 and 1967) I reported on the distribution, abundance, and reproduction of adult crabs and their commercial potential. With the presentation of data on distribution and size of juvenile crabs[2] in this paper, it is now possible to develop a plausible life-history model for this species of deep-water crab and to elucidate the major environmental factors related to it.

METHODS

The study was made between 1961 and 1966 off the northern Oregon coast on a trackline running southwest from the mouth of the Columbia River. Stations were established at 25-fm depth intervals from 50 to 500 fm and at 50-fm depth intervals from 550 to 1,150 fm. A similar station pattern was used on all cruises. Stations from 50 to 475 fm were sampled four times a year with a modified 94-ft (400-mesh) Eastern fish trawl having a 1½-in mesh liner in the cod end (Greenwood, 1958). Stations at depths greater than 475 fm were sampled intermittently with a 43-ft flat shrimp trawl (Schaefers and Johnson, 1957) or a 70-ft semi-balloon shrimp trawl (Greenwood, 1959). The shrimp trawls were constructed of 1½-in mesh throughout. One-hour drags were usually made at depths from 100 to 475 fm, but drags were limited to a half-hour at 50 and 75 fm because of the large quantities of fish taken in shallow water. The trawling time varied between 0.6 and 1.5 hr for hauls made at stations deeper than 475 fm. Trawling time was considered as that time elapsing from the moment the desired amount of cable was out until retrieval of the cable was begun. All drags were made during daylight at an average vessel speed (relative to land) of about 2.6 knots. Throughout all drags, the gear was maintained at as constant a depth as possible.

Despite the diversity of fauna taken, all catches

[1] This work was supported in part by A.E.C. agreement No. AT(49-7)-1971-Mod. No. 1.

[2] Juvenile crabs are those which have not undergone their puberty molt.

were thoroughly enumerated. Tanner crabs in these catches were counted by sex and, when time permitted, weighed with hand scales. The sex of a crab may be determined by the shape of its abdomen regardless of the size of the crab or degree of maturity. A representative sample of Tanner crabs was taken, and the carapace width of each crab was measured to the nearest millimeter using Vernier calipers. Carapace width was considered as the greatest measurement across the branchial region of the carapace but did not include the lateral spines.

In this discussion, the calendar year is divided into four 3-month periods as follows: winter (January through March), spring (April through June), summer (July through September), and fall (October through December). This division is consistent with seasonal hydrographic phenomena, which show a lag effect; i.e., the greatest amount of incident energy reaches the ocean surface in the trackline area during June and July, but the highest surface temperatures occur during August and September (Tully, 1964).

DISTRIBUTION OF JUVENILE CRABS

Juvenile crabs were taken from 325 to 1,050 fm in depth with greatest availability occurring between 350 and 800 fm (Table 1). No seasonal availability patterns could be detected as sampling at depths greater than 475 fm was not conducted during the fall and winter periods.

All immature crabs found in the interval from 325 to 475 fm were less than 50 mm in carapace width. All subadult crabs (i.e., immature crabs greater than 50 mm) were taken at depths greater than 475 fm. The complete absence of the immature crabs larger than 50 mm in the interval from 325 to 475 fm suggests that, as development progresses, the smaller crabs are either lost, owing to predation or disease, or as a consequence of some type of "territorialism," move horizontally off the trackline or vertically down into the 500-to 650-fm zone.

As is shown in Figure 1, the size structure of the populations of immature male and female crabs over the two depth intervals 500 to 650 fm and 750 to 850 fm was not homogeneous. Larger immature crabs were relatively more abundant in the 500-to 650-fm interval than from the deeper interval (750 to 850 fm). Assuming that all crabs at depths greater than 650 fm do not eventually die, it seems reasonable to envision an immigration of the young crabs into the 500-to 650-fm zone as growth proceeds.

LIFE HISTORY MODEL OF THE TANNER CRAB

In this discussion I will draw extensively from recently published data from this study on the seasonal reproductive cycle and patterns of bathymetric distribution of adult crabs (Pereyra, 1966). This information together with the distributional

TABLE 1. *Numbers of juvenile Tanner crabs per 1-hour drag by depth, spring 1961 — spring 1966.*

Gear	Depth (fm)	1961			1962			1963		1964		1966
		Sp.	Su.	Fall	Wn.	Sp.	Su.	Wn.	Sp.	Su.	Sp.	Su.	Sp.
	250	0	0	0	0	0	0	0	0	0			
	275	0		0		0	0		0	0			
	300	0	0	0	0	0	0	0	0	0			0
Fish	325	0	0	0	1	0	0	0	2				
Trawl	350	0	36	11	1	0	300	31	15	12			
	375	0	30	0	3	96	20	14	23	0			3
	400-425	3	40	200	16	135	10	33	55	15	25	36	
	450-475		50	0	14	5		15	14	12			
	500										40		
	600					54	8				107		
	650									300	28		
Shrimp	750					45			6	21			
Trawl	800					36					65		
	850					6			3	1	54		
	900					0	0				0		
	1050					9					0		
	1150												0

Note: Shrimp trawl catches in 1962 were made with a 43-ft trawl; all others were made with a 70-ft trawl.

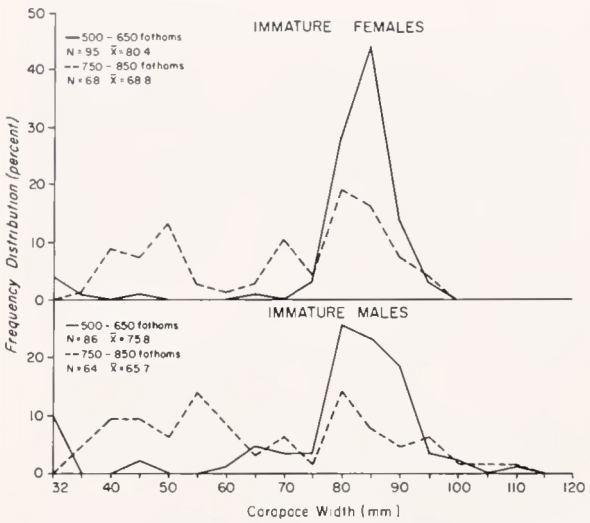

FIG. 1. *Percentage size composition of juvenile Tanner crabs taken in two depth intervals, spring 1964.*

data on juvenile crabs presented in the preceding section will be used to develop the life history model. Where data are lacking, such as for the larval period, the pattern will be inferred from the life cycles of other species of Tanner crabs. Throughout this treatment the reader is referred to Figure 2.

Pereyra (1966) reported that the population structure of the adult male and female Tanner crabs (*C. tanneri*) changes quite dramatically with depth. Females are fairly stationary, with the apparent abundance of the population centered between 350 and 375 fm. In contrast, the males are quite migratory and exhibit a seasonal movement pattern that repeats itself for all years studied. In the spring and summer the population of males is centered at 275 to 300 fm, but as winter approaches the entire male group shifts downward and occupies the same depth interval as the females. Following a short period of aggregation with the female group, the males return to the shallower environment, separating the sexes once again.

In shifting from 275 to 350 fm, the male mode,

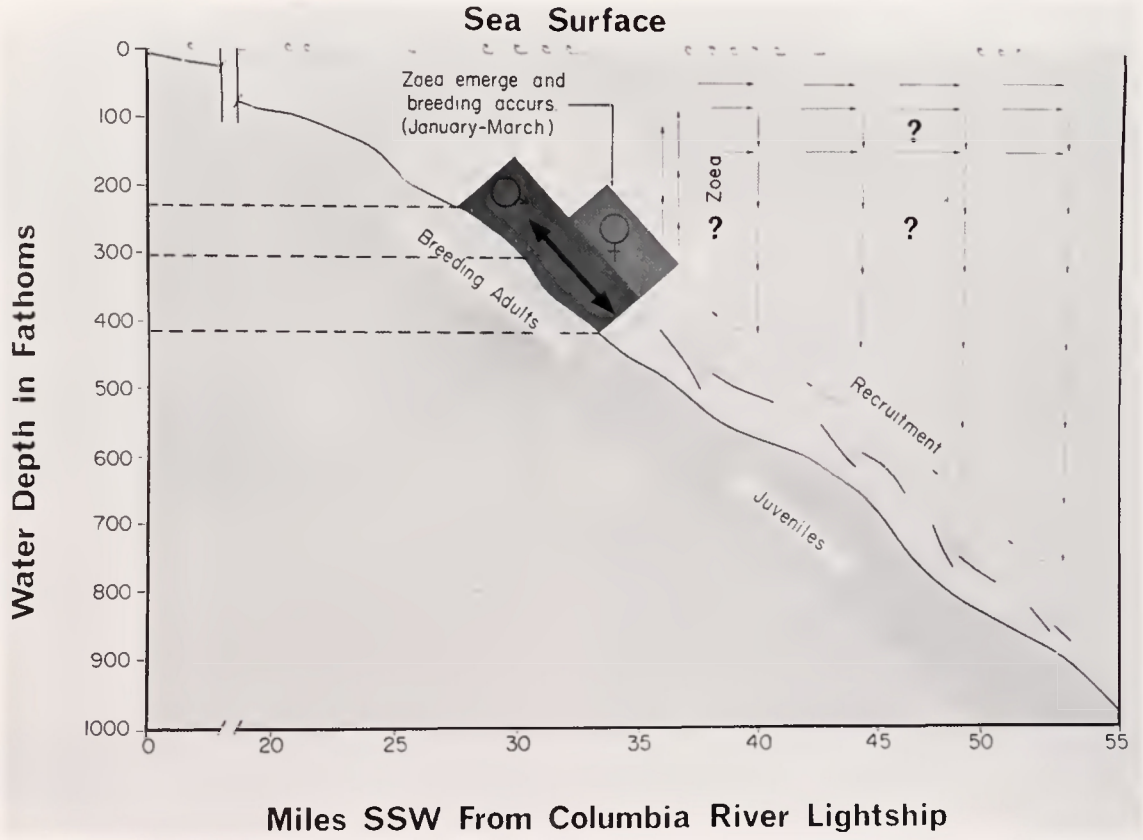

FIG. 2. *Schematic representation of Tanner crab life history model emphasizing migration and dispersion elements.*

i.e., the center of male availability, is displaced about 2½ nautical miles down the continental slope. If spread out over the 6-month period while this transition is occurring, the shift would average about 80 ft per day along the slope. Thus, one can see that on the average this displacement is quite gradual.

As the male population performs the seasonal shift downward, maturation of eggs, which are carried by the females, progresses. The process of maturation, which commences with the ovulation of new eggs in late winter at the time of intermixture of the male and female populations, also follows a yearly cycle. Hatching (i.e., emergence of the zoea) occurs during the winter, about one year after the eggs are ovulated.

The phenomenon of downward shift of the male population into depths of maximum availability of female crabs concurrent with the hatching of the fully developed eggs and the subsequent ovulation of new eggs is not a fortuitous circumstance. On the contrary, the sequence of events and their annual regularity strongly suggest that the downward movement of adult males is a prerequisite to breeding and thus can be called a breeding migration. Because of the slow rate at which this migration takes place, I strongly suspect that considerable random feeding movement is associated with it.

The abrupt change in the type of eggs carried by the female crabs during the winter is good evidence that ovulation takes place during this period of population overlap. At the beginning of the period, in January, 99.5 per cent of the females carry dark, fully-developed eggs, but at the end of the period, in March, only 20 per cent still carry dark eggs. The remainder are newly berried crabs.

Although the time of the year when the eggs are hatched is now fairly well known, the fate of the emerging zoea can only be conjectured. Assuming that the zoea of this species react similarly to those of *Chionoecetes opilio,* which were found by Matsuura (1934, *in* Katoh, Yamanaka, Ochi and Ogata, 1956) to lead a planktonic life near the sea surface, we might expect to find the newly hatched zoea of *C. tanneri* also situated in the upper water strata. Assuming that the upward movement of the emerged zoea to the surface is fairly rapid (by surface is meant the upper 25 fm), they should be present in this zone from January to March or into the summer, depending upon the rate of development.

The hydrographic structure of the northeastern Pacific Ocean during the winter should permit such a vertical movement to take place readily. Whereas a strong thermocline exists in the upper zone during the summer, this feature begins to dissipate with the onset of cooling processes during the fall and winter. At the time of zoea emergence during the January to March period, water temperatures are isothermal above approximately 55 fm (Tully, 1964). The only major discontinuity would be the permanent halocline, which is 40 to 85 fm thick, situated 40 to 55 fm beneath the surface. This halocline should not be an impenetrable barrier to active vertical movement.

Although the length of time that the larvae are in the surface layers is not known, it is assumed that they remain there well into the summer, growing and progressing through various molts in the zoeal and megalopal stages. With the attainment of some advanced larval stage, they would end their planktonic phase, settle to the bottom, and take up a benthic existence.

Since it has been assumed that the planktonic larvae would be in the surface layers throughout the summer, it is logical to assume that they would be subjected to the surface currents prevailing during that period. In the trackline area the northwesterly wind pattern, which dominates the offshore environment throughout the summer, causes surface waters to be directed offshore (Barnes and Paquette, 1957). Such a pattern would also distribute the developing larvae farther offshore, so that at the time of their descent to the bottom in late summer, the small crabs would be distributed at depths greater than the adult breeding population from whence they came some months before.

This conjecture as to the distribution of the juvenile crabs on the bottom is substantiated by distributional data presented in the preceding section. The fact that not all juveniles were found at greater depths and that some were taken at the deeper fringes of the adult group indicates that this dispersion pattern is more widespread than postulated. The variable nature of the offshore currents, as suggested by Barnes and Paquette (1957), may be partially responsible for the wide dispersal of post-larval crabs.

From the above it appears that recruitment to the adult population occurs from the depths. Differences in the size of immature crabs at depths greater than those at which the majority of adult crabs are found also support such a recruitment pattern.

The above life history model for the Tanner crabs appears to be consistent with the observed facts. The weakest aspect concerns the fate of the zoea from the time they hatch or are released by the female until they settle to the ocean floor. Lack of data makes discussion of this feature strictly conjectural.

MANAGEMENT IMPLICATIONS OF THE MODEL

If harvesting of the Tanner crab resource should

become economically feasible, information on the seasonal patterns of distribution of the various segments of the population as depicted in this model could be used to manage the fishery to permit maximum utilization at the minimum biological cost. The proposed management scheme would involve both time (season) and sex considerations.

During the spring and summer the adult male and female populations are fairly well segregated, with the males in shallower water than the females. By fishing only from April to September when the sexes are separated and keeping only male crabs, several beneficial results would be achieved. First, recruitment should not be impaired. The stock of males would be reduced by the fishery, but the suspected polygamous behavior of the males by multiple matings would maintain the female stock in a state of high fertility. Also, the fishing on male crabs would not be damaging to the juveniles because they occupy distinct areas. Second, males would be taken when they are concentrated over the shallow portion of their depth range (275 to 300 fm), and at a time of the year when the weather is most favorable for offshore fishing. These factors would facilitate their capture and reduce costs, thereby making the fishery more attractive economically.

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HOLDING AND SPAWNING DELAWARE BAY OYSTERS (*CRASSOSTREA VIRGINICA*) OUT OF SEASON 1. LABORATORY FACILITIES FOR RETARDING SPAWNING. [1, 2]

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ABSTRACT

Laboratory facilities and techniques for retarding the natural spawning of Crassostrea virginica *from Delaware Bay are described. Oysters were held in the laboratory from 11 May 1967 until September and October 1967 and January 1968 at which times spawning was successfully stimulated. This work demonstrates that oysters do not resorb even when held for periods up to 8 months in the laboratory if proper temperatures and quantities of water are provided the brood stocks. The importance of proper conditioning is stressed.*

INTRODUCTION

This study was undertaken to develop laboratory techniques for holding, conditioning, and spawning the American oyster, *Crassostrea virginica* (Gmelin), from the Delaware Bay region. Our main objective is to expand the spawning season in order to produce oyster progeny on a year round basis for research and hatchery requirements. Success in artificial spawning of oysters from waters of the southeastern United States has been limited (Andrews, Haskin, Hidu, Ritchie, and Shaw, personal communications). The purpose of this paper is to outline techniques and to describe laboratory facilities for holding oyster brood stock (from Delaware Bay) for 8 months and subsequently spawning them.

The important publication on rearing of bivalve mollusks by Loosanoff and Davis (1963) has served as a model for comparable studies throughout the United States. Their equipment, techniques, and experimental design have been followed or modified by many workers. Loosanoff and Davis (1963) were the first to publish results of oyster conditioning and spawning experiments from Delaware Bay. Haskin (unpublished report [3]) made reference to an unpublished report by Dupuy who had conducted spawning experiments on Delaware Bay oysters in 1960 and reported unpublished work on conditioning experiments by Hidu and Taylor. In other reports Haskin (unpublished report [4]) offered new experimental data on conditioning and rearing native oysters carried out by Hidu and Mulhern. Spawning experiments treated in Haskin's reports were concerned primarily with in-season spawning.

The basic factor involved in controlling spawning in oysters is temperature. That is, one can retard spawning with low temperatures and advance spawning with high temperatures (Loosanoff and Davis, 1963). *Crassostrea virginica* normally spawns in Delaware Bay sometime in midsummer when the water temperature has risen to around 26 °C. We were faced with having to hold large quantities of brood stock at relatively low

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[2] Contribution No. 49, University of Delaware Marine Laboratories.

[3] Haskin, H. H. Disease-Resistant Oyster Program, October-December 1964 Quarterly Report to U. S. Bureau of Commercial Fisheries.

[4] Haskin, H. H. Disease-Resistant Oyster Program, July-September 1966 Quarterly Report to U. S. Bureau of Commercial Fisheries.

temperatures in order to retard spawning and yet provide these oysters with enough food to promote proper gonadal condition. We therefore placed considerable emphasis on our brood stock holding room, the seawater supply, and refrigeration system.

Our results indicate that it is possible to retard natural spawning in Delaware oysters for at least 6 months and still cause them to spawn in a relatively normal manner in the laboratory. However, spawning responses of oysters in our experiments suggest the need for an additional period of conditioning at higher temperatures than our holding temperatures.

METHODS AND PROCEDURES

Facilities

The University of Delaware Shellfisheries Laboratory is located on the bank of the Broadkill River, adjacent to Roosevelt Inlet, within 3 miles of the mouth of Delaware Bay and near the town of Lewes, Delaware (Fig. 1). Within this building is the brood stock holding room. This room is rectangular (8 ft by 10 ft) with fully insulated walls 8 feet high. In the room are two 100 gallon plywood tanks (4 ft X 4 ft X 1 ft) which are insulated on sides and bottom with 4-in thick slabs of styrofoam.

A Teel submersible pump located 2 feet under the surface of the Broadkill River supplied continually running sea water to each tank through 1-in black plastic well pipe. Sea water is drained from the tanks by means of an overflow standpipe which maintains the water depth in the tanks at approximately 12 inches. A safety thermostat shuts the submersible pump off should the temperature in the brood stock tanks rise above the prescribed level. Water in the holding tanks is continuously aerated to reduce temperature stratification and to provide sufficient oxygen should the seawater supply be shut off.

When the water in the Broadkill River is warmer than the temperature desired in the holding tanks, the refrigeration system is activated. However, when Broadkill River water is cooler than the temperatures desired in the holding tanks, the heating system is activated. In order to produce 20 °C and 15 °C water when River temperatures are below 15 °C, the water is first heated to 20 °C. Half of this water is diverted to each of the two holding tanks and the water in one tank is cooled to 15 °C by the refrigeration system. Although this is inefficient, it was the only course of action open to us with our available equipment.

River water is heated by passing it through an epoxy-coated steel box containing two karbide heat exchangers (Union Carbide). The heat exchangers are connected to an oil-fired furnace (150,000 gross B.T.U. s/hr) which also heats the laboratory. Fresh water heated in the boiler of the furnace is circulated through the interior of the heat exchangers while the sea water flows over the exterior surfaces of the exchangers. The water temperature is controlled by means of a thermostat sensing the sea water as the water passes from the heat exchanger box to the holding tanks.

In each tank are refrigeration coils constructed of 1-in copper tubing that has been coated with 3 layers of epoxy paint. Each coil is approximately 46 ft long and is suspended 6 inches above the bottom of the tank at the upper edges of the plastic-coated trays in which the oysters are kept; i.e., the oyster trays are below the coils. The coils are connected to a 3-ton refrigeration compressor which is located outside of the Shellfisheries Laboratory. Water temperature in each tank is individually controlled by means of a solenoid valve in the freon line leading from the compressor to the coil. The solenoid valve is activated by a thermostat sensing the seawater temperature in the appropriate tank. A Dunham-Bush air cooler regulates the air temperature within the brood room.

Several important safety features have been built into the system. One is the insulated walls of the room and tanks. In the event of a complete power failure, water temperatures in the holding tanks would rise very slowly. Another feature is the safety thermostat controlling the seawater pump. In the event of a refrigeration compressor failure or malfunction of the seawater heating system, the safety thermostat would turn off the pump preventing warm sea water from entering the brood stock holding tanks and the water in these tanks would equilibrate with that of the brood stock holding room air temperature. Air temperature would of course be maintained at a relatively low level — about 15 °C. These safety features are intended to prevent a sudden heat shock (exposure to water of 28 to 30 °C) which might prematurely stimulate brood stock to spawn.

Holding and retarding brood stock

On 11 May 1967, 784 adult oysters were removed from the field and divided equally between the two brood stock holding tanks. Oyster stocks were segregated in plastic-coated wire baskets (Marlboro Wire Goods Co., Marlboro, Mass.) which have inner dimensions of 19 3/4 in X 17 1/4 in X 6 in. Four baskets containing oysters were placed on the floor of each tank. These oysters were held in the tanks until 6 September 1967 after which time small groups of oysters were periodically removed for spawning experiments until 15 January, 1968. Daily temperature ranges in each tank were

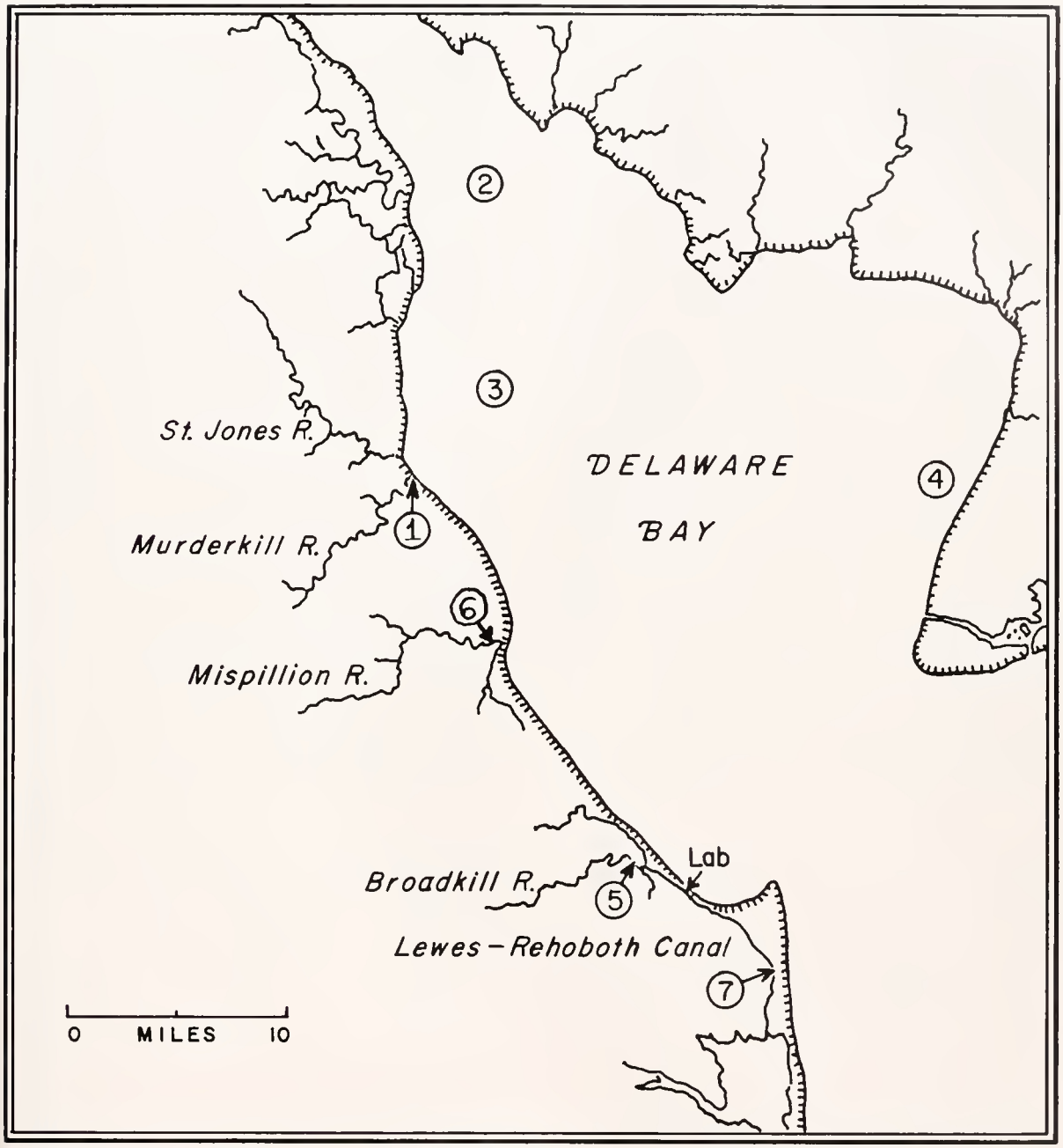

FIG. 1. *Locations of original sources of brood stock taken from Delaware Bay and its tributaries in relation to the University of Delaware Shellfisheries Laboratory. The numerical part of the brood stock code name indicates either the year the parents of these laboratory-reared stocks were initially acquired from these locations (1 — Bowers 62 and Bowers 63, 2 — Red Buoy 63, 3 — Clamshell 64, 4 — Cape May 64, 5 —Broadkill 64) or the year when brood stocks were acquired from these locations for inclusion in these spawning experiments (5 — Broadkill 67, 6 — Mispillion 67). Rehoboth 66 is laboratory-reared stock (from mixed Delaware Bay parents) which was placed in the field for 1 year at site 7 before inclusion in these spawning experiments.*

determined by means of a maximum-minimum thermometer. Oysters in each tank were provided with approximately 3 gpm of Broadkill River water on an almost continuous basis. Salinities at this location in the Broadkill generally range from about 14 to 28 ppt during a tidal cycle, although very heavy rains may push the salinity as low as 2 ppt for a short time at low tide. Oysters and the interior of the holding tanks were scrubbed by hand on a weekly basis to reduce fouling and siltation.

Nine stocks of oysters from different areas in Delaware Bay and adjacent areas were selected as brood stock (Fig. 1). These were Bowers 1962, Bowers 1963, Red Buoy 1963, Clamshell Delaware 1964, Cape May 1964, Broadkill 1964, Rehoboth 1966, Mispillion 1967, and Broadkill 1967. These stocks were selected on the basis of several criteria: 1) Field mortality studies, 2) presumed resistance or susceptibility to MSX infection. (The first 5 stocks are considered presumed resistant stock because they were collected from areas of high incidence of MSX infection. The last 3 are considered presumed susceptible stock because they were collected from areas of low incidence of MSX infection), 3) laboratory-reared progeny (Bowers 1962, Bowers 1963, Clamshell Delaware 1964, Broadkill 1964, and Rehoboth 1966 were reared and set in the laboratory).

Temperature exposures of oysters held for spawning varied somewhat due to minor inconsistencies in temperature control systems and to modifications applied by the investigators. Oysters in both tanks experienced an average daily range in temperature of about 4 °C which was due to lack of refinement in temperature control. The following temperature-exposure schedule was set up in a rather arbitrary manner: 1) Experiments initiated on 6 September 1967 — the holding tank was set and held at an average temperature of 16.1 °C for 118-147 days prior to experiments. 2) Experiments initiated on 18 October 1967 — holding tank readjusted by investigators and held at an average temperature of 18.0 °C for 8-15 days. The oysters had previously been held for 151 days at an average temperature of 16.1 °C. 3) Experiments initiated on 2 January 1968 — these oysters were placed in the holding tank on 11 May 1967. The temperature of the tank was set and maintained at an average temperature of 11.6 °C for 152 days after which time the tank temperature was increased by the investigators to an average of 15.0 °C for the next 47 days. The oysters were then divided into two groups. One group was kept at 15.0 °C for an additional 42-49 days while the other group was exposed to an average temperature of 19.4 °C for 36-49 days. The temperature history of each oyster stock is presented in the results section of this paper.

Spawning techniques

6 September — 9 October 1967: In general, spawning techniques followed Loosanoff and Davis (1963) with some modifications. Each group of 8 to 16 oysters was removed from the 16.1 °C holding tank and placed in a 10-liter battery jar. The jar was then filled with sea water that had been heated to between 26.5 and 31.0 °C. Occasionally, sperm or egg suspension was added to the jar immediately in order to induce spawning, but most often this was delayed until it could be determined if the thermal stimulus was having any effect.

18 October — 3 November 1967: Groups of 2 to 8 oysters were removed from the brood tank (18.0 °C) and emersed in heated, unfiltered, running sea water in 12 in X 12 in X 5 in plastic trays at temperatures of 28 to 30.0 °C. After 1 1/2 hours the water was turned off for about 30 minutes to allow for closer examination of oyster pumping action. This procedure was repeated several times a day and on consecutive days until spawning occurred. Between each day's experiments, the oysters were held in 12 in X 16 in X 12 in fiberglass aquaria. During these interim periods, the water generally ranged from 23 to 26.0 °C with occasional lows of 19.0 °C. Water was changed at least once daily and aerated continuously. In some experiments, the thermal treatment was combined with a chemical stimulus (sperm or egg suspension). After the 90-min thermal period, the water was stopped and the sperm or egg suspension was placed in the plastic trays. Sometimes several suspension increments were added before the warm water was again turned on. In a few cases, an oyster, induced to spawn by thermal stimulus, was placed in a shallow glass tray with oysters which had not yet spawned. The spawning oyster supplied natural sperm which is preferable to stripped sperm in such experiments. In the one case where spawning did not occur, the experiment was terminated after 7 days.

2 January — 19 January 1968: Groups of 7 to 10 oysters were removed from the brood tanks in pairs (a stock from the 19.4 °C holding tank was matched with the same stock from the 15.0 °C holding tank) and emersed in heated, unfiltered, running sea water in plastic trays. The water temperature was raised gradually from about 25.0 to 30.0 °C. After 90 minutes, the water was stopped for about 30 minutes. This procedure was repeated several times a day and on consecutive days until spawning occurred. Between each day's experiments the oysters were held in the plastic trays. During these interim periods the water generally ranged from 19 to 23.0 °C. Water was changed at least once daily and aerated continuously. If the oysters had not spawned, a chemical stimulus was

introduced on the fourth day of testing. In these cases, heated water was turned off periodically and sperm or egg suspensions were placed in the trays of standing water. Sometimes several gamete suspension increments were added before the warm water was again turned on. On the fifth day of testing, the experiments were terminated.

RESULTS

Apparently the brood stock holding tanks were a reasonably healthy environment since only 46 oysters died during the total laboratory holding period of 248 days. This represents a mortality of 6 per cent for the 8 months.

During September and early October at least one sex spawned in each of the 17 experiments involving Delaware Bay oysters (Table 1). Spawning occurred in both sexes in 12 of the 17 trials. Fertilized eggs were produced by all 9 stocks tested. The fertilized eggs were viable and several batches of larvae were carried to setting. Spawning occurred within 50 minutes of initial stimulation (thermal only in this case) in the most rapid response but did not occur until 120 hours and 25 minutes after the initial stimulation (both thermal and gamete stimuli were applied within the first hour) in one experiment. The average delay between initial stimulation and spawning was 32 hours and 48 minutes.

Table 2 indicates that spawning occurred in one sex in 15 of 16 experiments in late October and early November. Both males and females spawned in 7 of the 16 trials; females produced viable eggs. Fertilized eggs were produced by 4 of the 7 stocks tested. Several batches of larvae were carried to setting. Spawning occurred from 1 hour and 15 minutes to 128 hours and 30 minutes after initial stimulation, with an average elapsed time of 18 hours and 42 minutes.

Oysters held at relatively low temperatures (15.0 °C) did not seem to spawn as readily as those held at higher temperatures (19.4 °C) during January experiments (Table 3). Successful spawning in both sexes producing viable fertilized eggs occurred in 5 of 7 groups held at 19.4 °C, while males only spawned in the sixth group (Table 3). Larvae produced by the three stocks represented by these groups were successfully reared to setting. In all cases where spawning was successful, no sperm or egg stimulus was necessary. Spawning occurred from 1 hour and 30 minutes to 77 hours and 45 minutes with an average elapsed time of 41 hours and 36 minutes. Oysters held at 15.0 °C were spawned successfully in only 3 of 6 trials with both sexes spawning in just 2 trials (Table 3). Spawning occurred within 76 hours and 30 minutes of initial stimulation in the most rapid response but did not occur until 96 hours and 33 minutes after the initial stimulation in one experiment. The average delay between initial stimulation and spawning was 83 hours and 24 minutes.

The spawning response times given for September, October, and January experiments are provided to give some measure of spawning success. These times should not be used as a direct com-

TABLE 1. *Spawning success in Delaware Bay oysters* (Crassostrea virginica) *held in the laboratory from 11 May 1967 until 6 September through 9 October 1967.*

Stock	Number of Specimens	No. of days at 16.1 °C	Spawning Stimulus			Spawning Success	
			Temp.	Egg	Sperm	Female	Male
Broadkill 67	10	118	X	X	X	X	X
Bowers 62	10	126	X	X	X	X	X
Mispillion 67	12	127	X	X	X	X	X
Bowers 63	10	134	X			X	X
Cape May 64	10	134	X			X	X
Cape May 64	10	138	X				X
Bowers 63	10	138	X		X	X	X
Clamshell 64	10	144	X	X	X	X	X
Bowers 63	15	144	X	X	X	X	X
Broadkill 67	16	144	X	X	X	X	X
Red Buoy 63	10	144	X			X	X
Red Buoy 63	9	144	X		X		X
Cape May 64	12	147	X	X	X		X
Broadkill 64	8	147	X			X	X
Rehoboth 66	10	147	X			X	X
Bowers 62	12	147	X				X
Clamshell 64	12	147	X				X

TABLE 2. *Spawning success in Delaware Bay oysters* (Crassostrea virginica) *held in the laboratory from 11 May 1967 until 18 October through 3 November 1967. These oysters were held initially at an average temperature of 16.1 °C for 151 days before being exposed to 18.0 °C water for periods of 8 to 15 days.*

Stock	Number of Specimens	No. of days at 18.0 °C	Spawning Stimulus			Spawning Success	
			Temp.	Egg	Sperm	Female	Male
Clamshell 64	6	8	X		X		X
Broadkill 64	8	8	X			X	X
Cape May 64	6	8	X			X	X
Bowers 62	6	9	X				X
Bowers 62	2	9	X				X
Bowers 62	4	9	X		X		X
Red Buoy 63	6	9	X		X*		X
Red Buoy 63	4	9	X		X		X
Mispillion 67	3	10	X				
Rehoboth 66	8	10	X			X	X
Rehoboth 66	2	10	X		X	X	
Clamshell 64	8	14	X			X	X
Bowers 62	8	14	X				X
Clamshell 64	8	15	X	X		X	X
Broadkill 64	8	15	X			X	X
Cape May 64	8	15	X		X	X	X

* sperm may have been accidentally introduced in this group.

parison between these sets of experiments because the application of thermal and chemical stimuli varied among experiments except for the two sets of January experiments.

DISCUSSION

As evidenced by the average delay time after the initial spawning stimulus, these oysters were not conditioned to the desired spawning ripeness to produce totally reliable and immediate laboratory spawning. However, the spawning response might be considered satisfactory when the difficulties encountered by others working with *Crassostrea virginica* from the more southern latitudes of the eastern United States are enumerated (Andrews, Haskin, Hidu, Ritchie, and Shaw, personal communications, and Loosanoff and Davis, 1963). This is especially true since these oysters were held under laboratory conditions for 8 months and spawning retarded approximately 5 to 6 months relative to natural spawning in Delaware Bay. According to Haskin (personal communication) light spawning started during the first two weeks in July 1967 in Delaware Bay. We feel that an additional period of conditioning at higher temperatures than our holding temperatures would allow more precise control of laboratory spawning of Delaware Bay oysters. Experiments are now underway which will hopefully allow us to delineate the temperature-time regime required for conditioning our oysters for more successful laboratory spawning.

A number of people have alluded to the difficulty of spawning oysters from the southeastern United States (Loosanoff and Davis, 1963). We feel that the difficulty stems from the fact that southern oysters are exposed to relatively high water temperatures and oyster gametes in the water for much longer periods of time than oysters from the north. In other words, southern oysters become refractory to the two major spawning stimuli, gametes and high temperatures, due to prolonged exposure to these stimuli. Loosanoff and Davis (1963) also suggest that differences in spawning responses of *C. virginica* from different locales may be due to the existence of physiological races.

We feel that our successful laboratory spawning of *C. virginica* was due to several factors. 1) Oysters were removed from the field (11 May 1967) before prolonged exposure to water temperatures above 15.0 °C and therefore before gametogenesis was well underway. 2) These oysters were rarely exposed to temperatures below 9.0 °C and above 22.0 °C during the 8 months they were held. 3) These oysters were supplied with an adequate quantity of food during the holding period as evidenced by obvious shell growth and successful laboratory-induced spawning. 4) The brood stock holding tanks were a healthy environment since

TABLE 3. *Spawning success in Delaware Bay oysters* (Crassostrea virginica) *held in the laboratory from 11 May 1967 until 2 January through 19 January 1968. These oysters were held initially at an average temperature of 11.6 °C for 152 days before being exposed to water of 15.0 °C for 47 days. After that oysters were exposed to water of 15.0 °C or 19.4 °C as indicated.*

Stock	Number of Specimens	No. of days at 15.0 °C	No. of days at 19.4 °C	Spawning Stimulus		Spawning Success	
				Temp.	Egg Sperm	Female	Male
Bowers 62	10	42		X	X		
Cape May 64	10	42		X	X	X	X
Clamshell 64	10	42		X	X		
Bowers 62	10	49		X		X	X
Cape May 64	10	49		X			
Clamshell 64	10	49		X			X
Broadkill 64	9		36	X		X	X
Bowers 62	10		42	X		X	X
Clamshell 64	10		42	X		X	X
Cape May 64	10		42	X	X		
Bowers 62	7		49	X			X
Cape May 64	10		49	X		X	X
Clamshell 64	10		49	X		X	X

only 6 per cent mortality occurred during the holding period.

In reference to the above factors, Loosanoff and Davis (1963) suggest that *C. virginica* is compelled to complete a gonadal cycle which requires resorbtion of gonadal products after an appropriate build-up whether spawning occurs or not. Therefore, removing the Delaware Bay oyster from the field before prolonged exposure to temperatures above 15.0 °C, and subsequently maintaining the oyster at approximately 15.0 °C, catches the Delaware oyster early in its gonadal cycle and retards the cycle for up to 6 months.

When holding oysters in the laboratory for spawning, it is obvious that gonadal condition must be maintained. This can occur only if the oyster is supplied sufficient quantities of food. Otherwise glycogen reserves slated for gamete production are converted to maintain the metabolism of the oyster. We maintained successfully approximately 800 two-to-five-year old oysters by providing an almost continuous supply of Broadkill River water at 6 gpm to these oysters. This supply seems to be adequate here, but our location may be somewhat unique with respect to phytoplankton density. The Broadkill River is relatively rich in organic pollutents (sewage and food industry wastes) about 10 miles up river from our laboratory. Although undoubtedly the bulk of the organic material is degraded long before reaching our pumping site, the released inorganic nutrients produce an almost continual plankton bloom in our vicinity. Therefore, 6 gpm of sea water for 800 adult oysters may not be sufficient in regions where oyster food densities are low.

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Without the aid of Mr. Earl Greenhaugh, Resident Manager of the Shellfish Laboratory, this study would have been impossible. We are grateful to Mr. Theodore P. Ritchie for acquiring the initial research contract (BCF 3-49-R-1) and for selecting the stocks placed in our holding facilities. We are indebted to Mr. Phillip Cambell of Vanderborgh Oyster Co., Oyster Bay, Long Island for acting as a consultant on the design and operation of our holding and spawning facilities.

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SIZE COMPARISONS OF INSHORE AND OFFSHORE LARVAE OF THE LOBSTER, *HOMARUS AMERICANUS*, OFF SOUTHERN NEW ENGLAND

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ABSTRACT

Comparisons are presented of lobster larvae taken toward the edge of the continental shelf and larvae taken close inshore in southern New England. In the first stage the larvae from offshore, presumably spawned in that area, were significantly larger than those from inshore, presumably spawned near the coast. This consistant contrast in size was not found in the later stages.

The American lobster, *Homarus americanus*, occurs from the edge of the continental shelf well up into the estuaries. There is considerable interest in the degree of reproductive isolation between inshore and offshore populations. Tagging studies and both morphometric and biochemical analyses of the juveniles and adults are being applied by others in attempts to identify sub-populations. An exploratory effort to distinguish between larvae collected offshore and inshore off southern New England is reported herein.

The larvae measured were collected in the summers of 1965 and 1966 by the University of Connecticut Marine Laboratory and the Graduate School of Oceanography of the University of Rhode Island. All specimens were originally preserved in formalin; the majority were later transferred to 70 per cent alcohol. The locations of the stations and areas from which larvae were collected are shown in Figure 1.

Observations on surface circulation (Bumpus and Lauzier, 1965; D. F. Bumpus, personal communication; G. S. Cooke, personal communication) indicate gyres and thus some tendancy toward containment in Rhode Island Sound and Block Island Sound whereas from the edge of the Continental Shelf there is a net drift toward these inshore waters which may average as high as four miles per day during the larval period. Accordingly we have considered the larvae from stations in the sounds and bays and in the coastal gyres as inshore, while those from over the continental shelf well south of a line from Montauk Point to Gay Head are considered offshore. Among the larvae taken over the shelf south of this line, first-stage specimens were most abundant at those stations farther from shore while fourth-stage larvae were most abundant closer to shore, suggesting an onshore drift of the larvae with time.

Figure 2 shows the characters measured. Measurements were taken at a magnification of 100 using a dissecting microscope with an ocular micrometer. Counts were made on four meristic characteristics, i.e., spines on the two claws and the rostrum, and setae on the telson, but the results were inconclusive and are not reported.

A comparison between the means for the inshore and offshore larvae was made for each character measured for each of the four larval stages, using "Student's" T test. The measurements for inshore and offshore samples for each character within each stage were tested for equality of variance using an F test, and the appropriate calculation of the T statistic, contingent on the result of this test, was made. The hypothesis tested for each measurement was that inshore mean equals offshore mean; the alternate hypothesis is that inshore mean does not equal offshore mean. Table 1 summarizes the results of tests of this hypothesis for each of the 12 characters considered on each of the four stages.

In the first stage, 7 of the 12 means were significantly different between inshore and offshore larvae. Of these total length, carapace length and telson length are considered to be the most reliable because they are sharply defined. Since these characteristics are a function of overall size, it appears from the data that in the first stage, off-

FIG. 1. *Stations from which the larvae measured were taken.*

TABLE 1. *Means of morphometric measurements (in mm) on larvae of the lobster,* Homarus americanus, *collected off southern New England. Where the means of inshore and offshore groups differ significantly for a particular character, the level of significance(α) is shown in a third column.*

	First Stage			Second Stage			Third Stage			Fourth Stage		
	n=56	n=45		n=22	n=13		n=19	n=13		n=22	n=10	
Measurement	Inshore	Offshore	α	Inshore	Offshore	α	Inshore	Offshore	α	Inshore	Offshore	α
Total length	8.32	8.60	.01	10.36	10.76		12.86	13.12		15.64	15.87	
Carapace length	3.81	3.95	.01	5.09	4.98		6.34	6.65	.05	7.66	8.00	
Right claw length	1.70	1.78	.05	2.18	2.07	.01	2.23	2.22		4.66	4.87	
Left claw length	1.69	1.73		2.13	1.98	.05	2.25	2.30		4.68	5.09	.10
Right claw width	0.52	0.53		0.77	0.74		0.76	0.72	.10	1.14	1.19	
Left claw width	0.54	0.52	.05	0.75	0.73		0.74	0.72		1.10	1.21	
Eye diameter	0.75	0.75		0.84	0.85		0.84	0.90	.05	0.79	0.86	.05
Telson length	2.10	2.24	.01	2.42	2.47		2.48	2.43		1.85	2.00	.05
Telson width	2.18	2.18		2.40	2.23		2.14	2.19		1.37	1.53	
Length longest telson spine	0.28	0.32	.01	0.30	0.35		0.35	0.44	.05	0.24	0.22	.01
Length 1st abdominal segment	0.37	0.40	.05	0.51	0.54		0.66	0.77	.05	0.78	0.94	.05
Length 5th abdominal segment	0.62	0.62		0.68	0.74		0.90	1.01	.10	1.08	1.17	

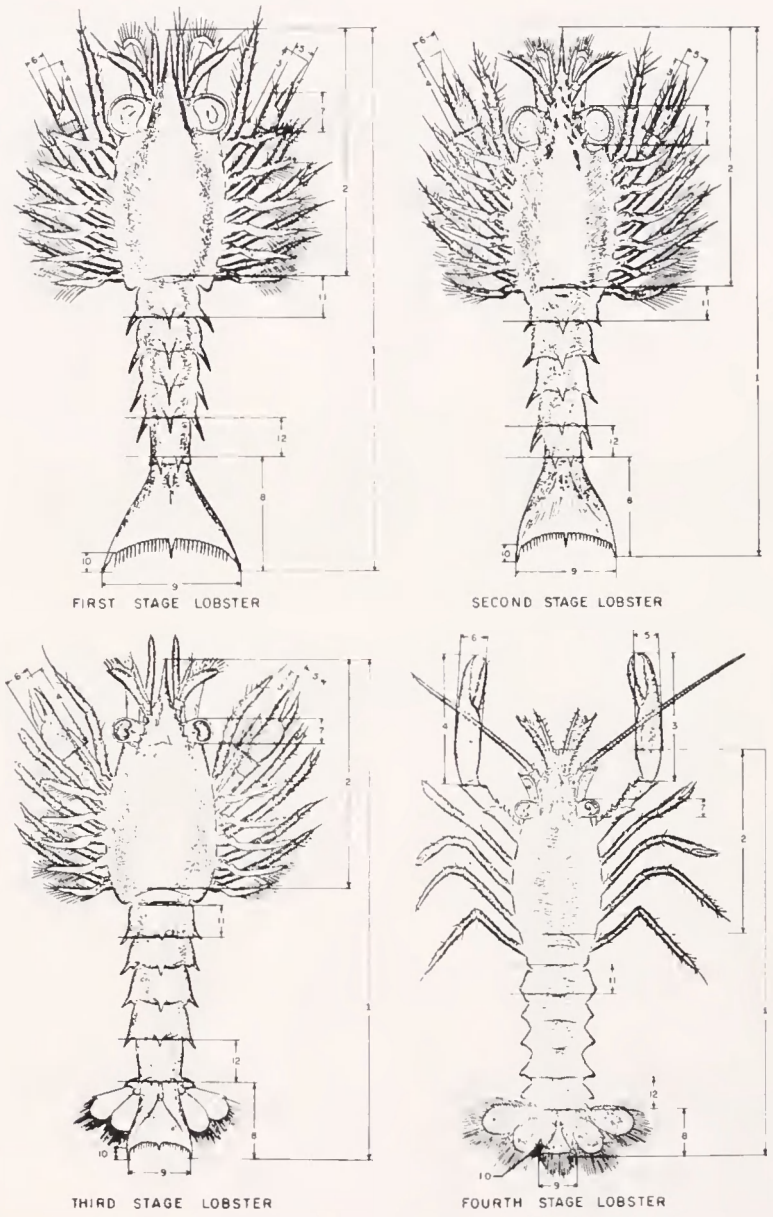

FIG. 2. Larval stages of the lobster, Homarus americanus *(after Hadley, 1906). Numbers indicate characters measured. For the first, second and third stages the end of the segment just anterior to the telson was measured at the base of the spines since the true end of the segment was difficult to discern.*

1 — *Total length*
2 — *Carapace length*
3 — *Length of right claw*
4 — *Length of left claw*
5 — *Width of right claw*
6 — *Width of left claw*
7 — *Eye diameter*
8 — *Telson length*
9 — *Width of telson*
10 — *Length of longest telson spine*
11 — *Length of first abdominal segment*
12 — *Length of fifth abdominal segment*

shore larvae are larger than those taken inshore. This consistent contrast in size was not found in the later stages though some of the comparisons suggest that meaningful patterns might unfold if more data were available. We assume that the larvae taken offshore are the progeny of offshore spawners and larvae from inshore are from inshore spawners. In fact for the first stage, showing the differences reported, we are quite confident of this, but we can only speculate as to whether differences noted are of genetic origin or reflect such influences as the size of the brood lobsters, the size of the eggs, or other environmental factors.

ACKNOWLEDGMENTS

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QUANTITATIVE AND QUALITATIVE COMMENSAL BACTERIAL FLORA OF *CRASSOSTREA VIRGINICA* IN CHESAPEAKE BAY

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ABSTRACT

The natural commensal flora of the oyster has been under study since July, 1966. Two areas of Chesapeake Bay were selected for investigation: Marumsco Bar, where severe mortalities occur annually, and Eastern Bay, a productive commercial oyster harvesting area. Water, mud, oyster mantle fluid and oyster gill tissue were examined for quantitative and qualitative estimates of total viable aerobic heterotrophic bacteria. In general, the two areas of Chesapeake Bay which were sampled are very similar in pH, dissolved oxygen, seasonal temperature range and salinity. Little quantitative variation was observed between samples within each area and between areas. The water counts ran at approximately 4 x 10^2/ml., the mud 10^4-10^5/ml, oyster gill tissue 10^3-10^4/gm and the mantle fluid 5 x 10^4/ml. Quantitatively, there was also little or no seasonal or environmental variation. From counts made on basal salts medium and on distilled water medium, salt-requiring bacteria appear to be a significant part of the bacterial flora of both areas. From the generic distribution of the bacterial types for the two areas tested, it was found that 81 per cent of the Marumsco Bar water, mud and animal samples consisted of Vibrio, Pseudomonas *and* Achromobacter *spp., whereas the Eastern Bay representation of these genera was 46 per cent.* Cytophaga/Flavobacterium *spp. was the dominant group in the Eastern Bay samples. The Marumsco Bar area harbors significantly greater numbers of* Vibrio *spp. Overall the Eastern Bay area represents a "balanced" microbial population.*

INTRODUCTION

The natural commensal flora of marine invertebrate animals has not been studied to any great extent. There is some published work on bacteria isolated from shellfish but the focus has been chiefly on the public health aspects of the animals and their environment. In 1959 the natural flora of *Crassostrea gigas* was investigated (Colwell and Liston, 1960). The research effort was directed primarily to obtain information which would aid shellfisheries technologists in establishing handling methods for prevention of product spoilage. It was subsequently established that the natural flora of *C. gigas* is composed of organisms representing the genera *Pseudomonas, Achromobacter, Flavobacterium* and *Vibrio* (Colwell and Liston, 1960). Dead and dying oysters in populations of *C. gigas* grown in Hood Canal, Oyster Bay and Willapa Bay, Washington, included a somewhat greater incidence of *Pseudomonas* spp. (Colwell and Sparks, 1967).

Several investigators have induced pathological conditions in oysters (Tripp, 1960; Pauley and Sparks, 1965), or succeeded in producing bacterial infection (Feng, 1966). *Pseudomonas enalia*, a bacterial pathogen for *C. gigas*, was recently described by Colwell and Sparks (1967).

Since July, 1966, we have undertaken a study of the natural commensal flora of *Crassostrea virginica* in Chesapeake Bay. Two areas were selected for our investigation: Marumsco Bar, where severe mortalities occur annually, and Eastern Bay, a productive commercial oyster-harvesting area. The work has been undertaken to obtain information concerning the microbial ecology of *C. virginica* in its natural habitat and possible involvement of

TABLE 1. *Environmental parameters — Marumsco Bar and Eastern Bay.*

Location	Date	Temperature °C			pH		Salinity (ppt)		Dissolved Oxygen (ppm)*		Depth (ft)
		Air	Sur.	Bot.	Sur.	Bot.	Sur.	Bot.	Sur.	Bot.	
Marumsco Bar	8-15-66**	—	25.8	—	8.1	—	19.5	—	—	9.0	8-9
Marumsco Bar	8-29-66	—	26.8	—	8.2	—	18.8	—	—	—	10
Marumsco Bar	9-19-66	—	21.5	—	7.8	8.0	19.8	—	—	—	10
Marumsco Bar	10-13-66	19.5	17.8	17.8	—	—	—	—	—	—	10
Marumsco Bar	3-21-67	9.0	3.9	4.0	—	—	15.6	16.2	12.24	12.39	—
Eastern Bay	11-7-66	10.0	11.7	11.3	7.5	7.6	17.2	17.3	—	10.40	10
Eastern Bay	1-17-67	9.3	2.7	3.5	—	—	15.8	—	12.69	12.67	—
Eastern Bay	3- 2-67	7.7	1.0	—	—	—	—	—	14.48	14.24	—

*Winkler method, in ppm.

**Dockside salinity = 15-17 ppt, dissolved oxygen = 3.5 ppm, pH = 7.3.

the commensal bacteria in Chesapeake Bay oyster mortalities.

METHODS

Both areas of Chesapeake Bay, Marumsco Bar and Eastern Bay, were sampled every 6 weeks. In the study reported here, water and mud from each area were collected aseptically in sterile containers. Five oysters, randomly selected from a trawl, were collected and, with the water and mud, stored separately on ice during transport to the laboratory. Initially, all samples of mud, water and oysters were analyzed on the boat (immediately after collection) and again back at the laboratory to determine the effect of time lapse and cold storage on the bacterial flora. The time lapse from sampling to analysis in the laboratory was, on average, 4 to 6 hours.

The animal, water and mud samples were analyzed by serial dilution, using sterile water collected from the sampling stations as diluent, and enumeration of bacteria by the aerobic spread plate count technique. Bacterial counts were determined after 5 days' incubation of the inoculated plates at 25°C. The oysters were examined by first placing the animals on sterile paper, cleansing the shell exterior and then opening the shell with a shucking knife which had been sterilized by immersion in alcohol followed by flaming. A sterile pipette was used to withdraw 1.0 ml mantle fluid. A composite of gill tissue excised from each of 5 oysters was also examined bacteriologically. The gill tissue (0.2 gm from each animal) was pooled and homogenized in a mechanical blender for 1 minute.

From each count plate 30-40 colonies were selected at random and transferred to a basal medium consisting of: 0.3 per cent yeast extract (Difco), 1.0 per cent proteose peptone (Difco), 2.4 per cent NaCl, 0.07 per cent KCl, 0.53 per cent

TABLE 2. *Total viable aerobic heterotrophic bacterial populations from water, mud and animals of Marumsco Bar and Eastern Bay. Figures represent counts in replicate.*

Location	Date	Water	Mud	Gills*	Mantle Fluids*
Marumsco Bar	8-15-66	4.0×10^2	8.2×10^4	5.2×10^4	5.0×10^4
Marumsco Bar	8-29-66	4.5×10^2	1.0×10^5	3.0×10^4	5.0×10^4
Marumsco Bar	9-19-66	7.3×10^2	6.1×10^5	7.0×10^3	3.5×10^4
Marumsco Bar	10-13-66	2.5×10^2	7.0×10^4	6.1×10^4	4.8×10^4
Marumsco Bar	3 21-67	6.0×10^2	9.0×10^4	2.5×10^3	3.5×10^4
Eastern Bay	11- 7-66	4.0×10^2	9.0×10^5	8.0×10^4	2.6×10^5
Eastern Bay	1-17-67	1.1×10^2	8.8×10^5	1.5×10^3	9.2×10^4
Eastern Bay	3- 2-67	4.6×10^2	4.2×10^5	4.3×10^4	9.2×10^4

*Average of 5 individual oysters.

TABLE 3. *Comparison of shipboard and laboratory total viable counts. Figures from replicate counts.*

Location	Date	Mud		Water		Mantle Fluid	
		Shipboard	Laboratory	Shipboard	Laboratory	Shipboard	Laboratory
Marumsco Bar	9-19-66	8.6×10^5	6.1×10^5	6.5×10^2	7.3×10^2	$7.2 \times 10^{3*}$	4.0×10^3
Eastern Bay	11-7-66	9.0×10^5	9.0×10^5	3.0×10^2	4.0×10^2	2.4×10^4	2.6×10^5

*Data for a single animal; other Mantle Fluid data are an average of 5 animals tested.

$MgCl_2$, 0.7 per cent $MgSO_4 \bullet 7H_20$, 2.0 per cent agar (Difco), pH 7.2-7.4. After purification, the cultures were subjected to a battery of tests using methods previously described (Colwell, 1964; Moffett and Colwell, 1968; Quigley and Colwell, 1968; Colwell, 1968). The taxonomic data were coded and transferred to IBM cards for computer analysis following the method of Colwell and Liston (1961b). Computer analyses were carried out on the IBM 1620 computer using Georgetown Taxonomy (GTP) programs GTP-2, GTP-4 and GTP-5.

RESULTS AND DISCUSSION

Selected parameters describing the areas of Chesapeake Bay under survey are presented in Table 1. A seasonal drop in temperature, accompanied by slight salinity changes, occurred. The temperature range for both areas was roughly 1-25°C and salinity *ca.* 15-20 ppt. Dissolved oxygen content ranged from 9 to 15 ppm.

Total viable aerobic heterotrophic bacterial populations from mud, water and animal samples taken from the two areas under study are listed in Table 2. A striking observation was the lack of fluctuation in the quantitative data. The total viable counts were found to be: water sample 4×10^2/ml; mud 10^4-10^5/ml; oyster gill tissue 10^3-10^4/gm, and mantle fluid 5×10^4/ml. Quantitatively there was, therefore, no seasonal or environmental variation.

Because it was possible that laboratory counts were not reflecting the natural situation, several bacteriological analyses were performed on the boat immediately upon sampling. No significant differences between shipboard counts and laboratory counts could be detected (Table 3). The counts obtained were well within statistical error expected for the bacteriological counting procedure.

A comparison of media for counts was also done. It is considered easier and more precise to use a synthetic medium, that is, in terms of ease

TABLE 4. *Comparison of media used in total viable counts of samples from Marumsco Bar (M. B.) and Eastern Bay (E. B.) areas.*

Area **	Date	Mud x 10^4/ml			Water x 10^2/ml			Gill Tissue x 10^3/g			Mantle Fluid* x 10^3/ml		
		ye-4			ye-4			ye-4			ye-4		
		Area[1]	salts[2]	yens[3]	Area	salts	yens	Area	salts	yens	Area	salts	yens
M.B.	10-13-66	7	16	2.8	2.5	2.2	0.3	61	50	6.5	48	41	0.6
E.B.	11- 7-66	90	100	1.2	3	2	0.3	80	45	3.5	260	25	4.7
E.B.	1-17-67	88	—	11	1	—	0.8	1.5	—	$<10^3$	92	—	33
E.B.	3- 2-67	42	—	14	4.6	—	1.8	43	—	2	92	—	7
M.B.	3-21-67	9	—	7	6	—	0.9	2.5	—	0.5	35	—	3.9

*Pooled data. Each figure represents an average of 5 animals tested.

**Samples plated on board collecting vessel.

(1) Water from the sampling areas as diluent.

(2) Yeast extract (Difco) 0.3%, Proteose peptone (Difco) 1.0%.
NaCl 2.4%
KCl 0.07%
$MgCl_2$ 0.53%
$MgSO_4$ 0.7%
pH 7.2-7.4

(3) Yeast extract (Difco) 0.3%, Proteose peptone (Difco) 1.0%, distilled water, pH 7.2-7.4 (Tris buffer).

TABLE 5. *Generic distribution of bacterial types in samples tested.**

Taxonomic Group	Marumsco Bay	Eastern Bay
Vibrio spp.	46%	20%
Pseudomonas spp.	22%	9%
Achromobacter spp.	13%	17%
Corynebacterium spp.	5%	7%
Cytophaga/Flavobacterium spp.	3%	30%
Micrococcus/Bacillus spp.	0%	6%
Enterics**	7%	3%
Other***	3%	8%
Total isolates in sample	125	104

*Total number of strains studied = 229

**Enterics: *Enterobacter* spp., *Proteus* spp.

***Other: *Caulobacter* spp., *Saprospira* spp., *Spirillum* spp.

of preparation, duplication of experiments, assembly of materials, etc. We attempted to simulate the salts of the environment in the medium by employing a Na^+, K^+ and Mg^{++} mineral supplement. From the data presented in Table 4, two points are clear. First, the counts on the distilled water-based medium were at least an order of magnitude lower than the counts on the medium prepared with water from the sampling areas or a salts base as diluent. Second, the medium prepared with a salts basal supplement did not always equal the medium prepared with area water as diluent in terms of total viable counts but better approximated the area water counts than did the distilled waterbased medium. Thus, a requirement for the cations, Na^+, K^+ or Mg^{++} (which constitute the basal salts supplement), was indicated for a significant portion of the aerobic hetero-

TABLE 6. *Generic distribution of bacterial types by area and month of sampling.*

		Taxonomic Groups*							
Date	Location	*Vibrio* spp.	*Pseudomonas* spp.	*Achromobacter* spp.	*Corynebacterium* spp.	*Cytophaga/Flavobacterium* spp.	*Micrococcus/Bacillus* spp	Enterics**	Other***
June, 1966	Marumsco Bar	57%	24%	4.7%	0	9%	0	0	4.7%
Aug., 1966	Marumsco Bar	30%	33%	18%	12%	0	0	6%	0
Aug., 1966	Marumsco Bar	30%	27%	11%	4%	8%	0	19%	0
Sept., 1966	Marumsco Bar	58%	17%	13%	5%	0	0	8%	0
Oct., 1966	Marumsco Bar	66%	5%	14%	0	0	0	0	14%
Nov., 1966	Eastern Bay	20%	9%	17%	7%	30%	6%	3%	8%
Jan., 1967	Eastern Bay	19%	5%	19%	3%	38%	11%	0	3%
Mar., 1967	Eastern Bay	17%	6%	17%	14%	14%	5%	8.5%	20%

*Total number cultures studied = 229

**Enterics: *Enterobacter* spp., *Proteus* spp.

***Other: *Caulobacter* spp., *Saprospira* spp., *Spirillum* spp.

TABLE 7. *Generic distribution of bacterial types in the mud, water and animal samples.**

Taxonomic Group	Mud		Water		Animals	
	Marumsco Bar	Eastern Bay	Marumsco Bar	Eastern Bay	Marumsco Bar	Eastern Bay
Vibrio spp.	37%	22%	56%	17%	47%	21%
Pseudomonas spp.	10%	11%	18%	0	27%	9.5%
Achromobacter spp.	16%	6%	13%	42%	11%	16%
Corynebacterium spp.	26%	22%	0	0	1%	4%
Cytophaga/Flavobacterium spp.	0	38%	6%	8%	3%	32%
Micrococcus/Bacillus spp.	0	0	0	0	0	8%
Enterics**	10%	0	6%	25%	7%	0
Other***	0	0	0	8%	4%	9.5%
Total in sample	19	18	16	12	90	73

*Total sample studied = 229

**Enterics: *Enterobacter* spp., *Proteus* spp.

***Other: *Caulobacter* spp., *Saprospira* spp., *Spirillum* spp.

trophic bacterial flora.

Since no significant differences were detected in quantitative distribution of the bacteria by season or by area, the qualitative distribution of bacterial types was examined. The generic distribution of bacteral types for each of the two areas is listed in Table 5. A very real difference in the types of bacteria isolated was noted. In the Marumsco Bar area, there was a much greater incidence of *Vibrio* and *Pseudomonas* spp. than *Cytophaga/Flavobacterium* spp. The *Achromobacter* and other groups were an approximately equal portion of the flora in both areas. The significance of this observation is enhanced when it is pointed out that the *Vibrio* and *Pseudomonas* spp. isolated were, in general, very active in their metabolic capacities, being lipolytic, proteolytic and saccharolytic.

Further examination of the qualitative distribution of the bacterial groups by season was carried out (Table 6). An indication of some fluctuation was noted for the Marumsco Bar area. *Vibrio* and *Pseudomonas* spp. appeared to be somewhat even more dominant in late spring and early autumn.

Finally, a breakdown of bacterial types by mud, water and animal was made (Table 7). In Marumsco Bar, the *Vibrio* and *Pseudomonas* spp. were dominant in all samples taken, especially in water and animal samples.

It is noteworthy that the results of the Chesapeake Bay study are in general agreement with the results of a similar study undertaken on the West Coast (Colwell and Liston, 1960; 1961a). The Eastern Bay data (Table 5) approximate the results obtained for Hood Canal, Willapa Harbor and Oyster Bay in Washington State. A notable exception is the high incidence of *Vibrio* and *Pseudomonas* spp. found in Marumsco Bar, Chesapeake Bay, which was not observed in any of the areas of Washington that were examined.

The quantitative data compare favorably with the results of Colwell and Liston (1960), since 10^4-10^5 organisms per ml oyster liquid was observed both for the West Coast animals and those of Chesapeake Bay (see Table 2).

CONCLUSIONS

The two areas of Chesapeake Bay which were investigated in this study show no significant quantitative difference in the natural bacterial flora of *Crassostrea virginica*. A relatively constant total viable population of bacteria was found in the Chesapeake Bay mud and water and on the animals. The quantitative estimates of the total viable aerobic heterotrophic bacterial populations for these samples were nearly identical to results obtained in a similar study of animals on the Pacific Coast, undertaken in 1959-1961.

The qualitative composition of the flora of the samples taken in the two areas of Chesapeake Bay, however, was markedly different. The Marumsco Bar area harbors significantly greater numbers of *Vibrio* spp. The Eastern Bay area, on the other hand, was similar to the Pacific Coast areas sampled, demonstrating a "balanced natural flora." Infection studies with the *Vibrio* spp. are now underway in the hope that their involvement in, or contribution to, the disease condition prevalent in Marumsco Bar, can be determined.

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DEVELOPMENTS IN THE METHODOLOGY FOR GLYCOGEN DETERMINATION IN OYSTERS

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ABSTRACT

Glycogen separation and assay time were substantially reduced by using the phenol method for colorimetric analysis. Na_2SO_4 addition prior to alcohol precipitation eliminates heating or overnight standing.

Tentative results on longer NaOH digestion show higher glycogen recovery. Samples were compared by using 1/2 hr and 1 1/2 hr tissue-digestion periods. Tissue frozen one week at — 7°C gave low glycogen recovery using the 1 1/2 hr digestion period comparison.

INTRODUCTION

In the general assessment of oyster condition a common method of analysis is the measure of oyster solids. Shaw, Tubiash and Barker (1967), Galtsoff (1964), Engle (1950) and others have proposed and used various methods for total solids determination.

Shaw *et al.* (1967) state that "the most precise measure of quality is determination of glycogen content in oyster tissue." In an assessment of our objectives for an oyster study, it was concluded that glycogen provided a better index of physiological condition. Total solids analysis provides a more rapid means of determining condition relative to commercial value. With use of the glycogen assay, the physiological state of the oysters can be related to the overall condition as determined visually or with total solids. For example, periods of lowest glycogen storage occur at spawning when the presence of sex products detracts from market acceptability. Relationships of glycogen and environmental factors have been shown by Herrmann (1965[1]) and Herrmann and Westenhouse (1966[2]).

Until recent years, glycogen assay (Calderwood and Armstrong 1941) had followed the classical pattern of alkaline tissue digestion, alcohol precipitation and end-product analysis. This procedure requires a relatively long analysis time. Literature examination showed that assay time can be reduced by colorimetric analysis of the separated glycogen.

A corollary requirement in changing methodology was data relativity. With carbohydrates, different methods measure different chemical species. Oyster glycogen assays of prior years must be related to newer techniques. Montgomery (1957), Kemp, Andrienne and Van Heijningen (1954), and van der Vies (1954) have used colorimetric assay of the separated glycogen. Fraser, Lo and Dyer (1966) used both the colorimetric and enzymic assay methods. In selecting the phenol method of Dubois, Gilles, Hamilton, Rebers and Smith (1956) it had already been shown (Montgomery, 1957, Fraser *et al.*, 1966) that the phenol method was directly related to alkaline prepared glycogen from cod muscle and guinea pig liver and muscle. Montgomery has compared phenol glycogen with concanavalin glycogen (Cifonelli and Smith, 1955). Fraser *et al.* (1966) used the glucose oxidase and phenol comparisons with good agreement on KOH prepared glycogen.

METHOD DEVELOPMENT

Newer colorimetric glycogen assay methods are conducted in acid systems. Our previous method

[1] Herrmann, R. B., Interim Report, Weyerhaeuser Company, 1965.

[2] Herrmann, R. B. and R. G. Westenhouse, Interim Report, Weyerhaeuser Company, 1966.

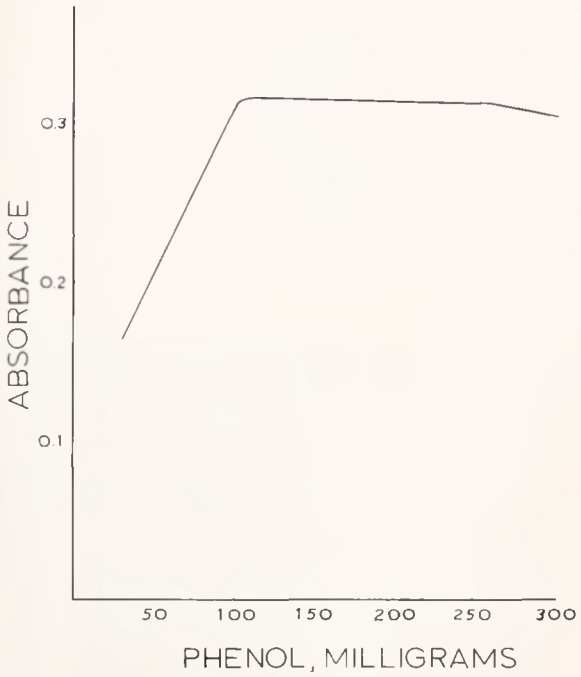

FIG. 1 *Absorbance change for 29 μg glycogen with the quantity of added phenol.*

utilized Cu_2O assay in an alkaline system. Johnson and Fusaro (1965) reporting on the alkaline conversion of glucose, refer to the following general reactions of aldoses in heated alkaline systems:

Aldose ⟷ Intermediate) ⟷ Enediol
⇖⟶ Ketose

With these potential alkaline conversion products as a reference, the acid phenol method of Dubois *et al.* (1956) was selected as the best approach to assay time reduction. The method of Kemp *et al.* (1954), while being specific for hexoses, was rejected because it lacked sensitivity and required a heating period for color development. Phenol assay allow ample dilution prior to analysis. This will minimize the effects of non-glycogen carbohydrate contamination. Either method eliminates the alkaline conversion products error.

Dubois *et al.* (1956), Handa (1966) and Montgomery (1957) used 1 or 2 cc samples with an experimentally determined weight of added phenol. It is often desirable to use larger sample volumes. A curve for phenol concentration was constructed using 4 cc samples, 1 cc of the phenol solution and 10 cc concentrated H_2SO_4. Figure 1 shows 1 cc of 15 per cent (w/v) phenol is adequate for these analysis conditions.

In changing the quantitative method of determining separated glycogen, results were evaluated on an acidified and pH 8.5-9.0 neutralized extract. The Cu_2O method was always applied to pH 8.5-9.0 extract. Table 1 indicates small differences due to pH of the glycogen extract prior to analysis, as well as the basic differences in alkaline or acid assay method used. The small differences could be due to contaminating low molecular weight carbohydrates or non-glycogen reducing groups. Separation procedures include sufficient washing to minimize the problem. Blanks carried through the entire procedure have consistently shown no filter paper contamination.

Paired sample results by the Cu_2O method have shown some divergence. Although data are not presented, the paired sample results from phenol assay have shown much better agreement. This evidence supports that shown by Johnson and Fusaro (1965) and is probably caused from localized heating during neutralization of the glycogen hydrolyzate.

Overall analysis time was reduced approximately 50 per cent by eliminating the hydrolysis step and use of the more rapid phenol analytical procedure.

There are many references to the anthrone (Viles and Silverman, 1949) method of carbohydrate determination. In a simultaneous comparison of this method with the phenol and Cu_2O assays the following data were developed (Table 2). Note the anthrone method shows a larger test range. It is

TABLE 1. *Method comparison per cent glycogen, wet weight.*

	Cu_2O	Phenol	Difference
No Acid to Glycogen Extract (10 tests)	0.97*	0.92	—0.05
Acid Added to Glycogen Extract (14 tests)	1.01*	1.07	*0.06

*Cu_2O method used as control; no acid added to any of these samples.

TABLE 2. *Tri method comparison — glycogen analysis.*

	Cu_2O per cent wet	Anthrone per cent wet	Phenol per cent wet
5 Sample Average**	1.50	1.42	1.41
Difference*	.00*	—.08	—.09
Range	±0.02	±0.21	±0.02

* Cu_2O method used for reference.

** Each sample run in duplicate.

the author's experience that the anthrone method is not as sensitive or reproducible as the other methods. In addition, the prepared reagent is not stable, causing problems in daily data comparisons. Under proper conditions (Westenhouse, 1964), however, anthrone can be suitable for carbohydrate and urea-formaldehyde analysis.

Van Handel (1965) reported a more rapid glycogen precipitation technique that yielded improved recovery after glycogen precipitation and separation. The basic change is the addition of saturated Na_2SO_4 solution to the KOH digest prior to alcohol precipitation. After standing 15-30 min the precipitated glycogen can be centrifuged or filtered. Comparative data by our older procedure of overnight standing and the more rapid Na_2SO_4-alcohol method are shown in Table 3. The phenol assay was used for these precipitation comparisons.

Use of the Na_2SO_4 modification allows an almost continuous procedure, eliminating the long waiting periods between analytical phases.

DISCUSSION

Use of the developed glycogen methodology has been centered on routine oyster glycogen assay. Through the time reductions afforded, additional studies have focused on the digestion phase of glycogen analysis.

Johnson and Fusaro (1966, 1967) have shown the necessity for careful interpretation of glycogen data in liver and muscle. Enzymic breakdown of glycogen to monose and oligoglucose resulted in lowered values.

TABLE 3. *Glycogen precipitation — per cent glycogen wet.*

Alcohol Precipitation Overnight wait	Alcohol — Na_2SO_4 15-30 Minute wait
2.99	3.16
3.14	3.22
2.71	2.72
2.98	3.01
4.07	4.04

An examination of our raw data has shown a cyclical variation in the analytical agreement between paired samples. These paired sample values (duplicate samples) tend to diverge in the winter-spring and converge in the summer-fall periods. To approach a resolution of this cyclic phenomenon, duplicate samples of tissue were digested in 30 per cent NaOH for the normal 1/2 and 1 1/2 hr periods. These preliminary results, shown in Table 4, indicate a higher percentage glycogen in samples digested for the longest period. This has occurred during the seasonal period when analytical agreement was poorest by the previous data analysis.

In a related experiment the same fresh homogenized tissue was frozen for seven days at —7°C. Analysis of this frozen tissue, shown in Table 4, gave low recoveries. One tentative criteria for glycogen assay may be the use of fresh tissue rather than frozen samples. This would apply only if the refrigerated temperatures cannot be maintained at dry ice values. A cursory assay of the low molecular weight carbohydrates washed from fresh tissue glycogen precipitates gave values of approximately 0.5 per cent as glycogen. Glycogen-wash solutions from frozen tissue have not yet been examined.

It cannot be concluded that less easily extractible glycogen forms are present in oyster tissue. Increasing the digestion period may be necessary for quantitative recovery, particularly during the winter-spring assay periods. Continued testing should clarify these points.

While performing a routine standard check with new reagent glycogen, the absorbency per unit of glycogen had increased over the original glycogen reagent. A thorough review of the data calculations and standard preparation has shown incorrect glycogen values were obtained. Subsequent evaluation of the glycogen used in preparing the original standards has shown it was partially degraded. New glucose and glycogen stand-

TABLE 4. *Tissue digestion — storage, per cent glycogen, wet.*

Month	1/2 Hour	1 1/2 Hour	7 Day Frozen, —7°C 1 1/2 Hour
January	2.44	3.05	—
February	3.06	—	2.50
March	2.75	3.40	2.53
April	3.29	3.35	—
May	3.02	3.11	2.98

ards, prepared with new chemicals, were analyzed by the phenol and glucostat methods. Both methods agree with the glucose: glycogen ratio of Johnson, Nash and Fusaro (1963); i.e., 1.11 x weight of glycogen = weight of glucose.

Enzymic carbohydrate methods (Johnson *et al.*, 1963, 1966, 1967; Passoneau, Gatfield, Shultz and Lowry, 1967; Kamel, Hart and Anderson, 1967) are being examined for use on oyster tissue. Our use of the glucostat method (Johnson *et al.*, 1966) on KOH glycogen has shown good agreement with phenol glycogen values on the few samples examined. The enzymic methods may ultimately provide a correction to the colorimetric assays currently used.

ACKNOWLEDGMENT

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APPENDIX

OYSTER GLYCOGEN DETERMINATION

Reagents, Special Equipment

30 per cent NaOH
Na_2SO_4, saturated solution
Ethanol, absolute
H_2SO_4, concentrated
Glycogen, highest purity available
Phenol, 15 per cent. Dissolve 37.5 g reagent grade phenol in 250 ml water.
Bausch and Lomb Spectrophotometer with 1 in cuvet

Method:

Homogenization, Sampling, Digestion

Remove the tissue from 20 oysters and let them drain 10 min. Add the drained oysters to a blender using maximum speed for 5 min.

Weigh to the nearest mg, a 5 and 10 g sample of homogenized tissue. Use a 10 ml plastic syringe for these transfers and weighings. Discharge the weighted tissue into 400 ml beakers containing 10 and 20 ml 30 per cent NaOH (2 cc NaOH for each gram of sample). When removing the samples for weighing, continuous agitation at very low speeds gives better duplication.

Cover the beakers with a watch glass and place on a pre-heated steam bath. When solution temperatures reach 80°C let the tissue digest for 30 min. Average tissue digestion temperatures are normally 88-93°C for most of the 30 min digestion period. Occasional stirring gives a more homogeneous mixture.

Precipitation, Washing

Remove the samples from the steam bath. Add 5 cc saturated Na_2SO_4 to each hot sample, mixing well. Add 30 cc water to the smaller sample, 15 cc water to the larger sample, giving a total volume of approximately 50 cc. After mixing, stir in 115 ml ethanol to each sample. Let stand 15-30 min.

Decant the supernatant liquid through a Whatman No. 54 filter paper. Wash the precipitated glycogen 4 times with 75 ml portions of 66 per cent ethanol. Decant with each wash step. Transfer the precipitate to the filter paper. Wash twice more with small volumes of 66 per cent ethanol.

Solution of Glycogen

Attach a small piece of tubing, 1-2 in long, to each filter funnel. Close a pinch clamp on each tubing extension. Fill the filter paper containing the glycogen with 75-90 ml boiling water. Let stand for 2 hr. Open the pinch clamp and allow the solubilized glycogen to drain into a 250 ml volumetric flask. Repeat addition of hot water after closing the pinch clamp. One hour standing is normally sufficient. Drain filtrate to the 250 ml volumetric flask. Wash the filter paper with small portions of boiling water until the total filtrate collected is about 240 ml. The washings at this stage should be clear and colorless.

If further analysis is to be delayed, add 2 cc concentrated H_2SO_4 to each flask and store in refrigerator. For immediate analysis, cool the flasks to room temperature and dilute to 250 ml.

Glycogen Determination

Pipet 5 ml of the well-mixed glycogen filtrate to another 250 ml volumetric flask. Dilute to mark and mix well.

Pipet 4.0 cc of this dilute glycogen to a 1 in B. and L. cuvet. Pipet 4 ml distilled water for a reagent blank. Add 1.0 cc 15 per cent phenol solution and mix well. Add 10.0 cc concentrated H_2SO_4 mix and let stand for 10 min. Measure at 485 μ using the reagent blank for reference.

Calculation:

$$\frac{(\mu g \text{ glycogen from standard curve})\ (0.3125)}{(\text{wet weight sample, grams})} = \text{per cent glycogen, wet}$$

A standard curve was prepared from oven dried glycogen. The calculation is based on glycogen values of 0.5 to 1.5 per cent glycogen, on a wet basis.

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APPLICATION OF THE BERGMAN-JEFFERTS TAG ON THE SPOT SHRIMP, *PANDALUS PLATYCEROS* BRANDT[1, 2]

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ABSTRACT

The Bergman-Jefferts ferromagnetic wire tag was used in two 50-day experiments on captive spot shrimp in saltwater aquaria. The tag was inserted into the abdominal musculature at the first somite.

Tagged, untagged and wounded shrimp were used in this study. The wounded shrimp were processed in the same manner as the tagged ones, including puncture by the needle, and differing only in the lack of tags.

A high incidence of mortality was generally observed among the test animals, but no marked difference was found between mortalities of tagged, untagged and wounded shrimp.

A mortality of shrimp occurred when the pH dropped below 6.6. The feeding habit and general activity of shrimp were considerably affected by low pH and newly shed shrimp appeared to be more adversely affected.

The percentage of shrimp that molted at least once was relatively high, 61.5 per cent in Experiment 1 and 72.5 per cent in Experiment 2, but only 2.5 per cent molted twice in both experiments and most of these were in the small size group ($\leq$20 mm carapace length). No difference in molting was observed between the tagged, untagged and wounded shrimp.

No abnormalities that could be directly attributed to the implantations of the wire tags in the shrimp musculature were noted. Hence, the wire tag has great promise in identifying shrimp and other crustaceans for migration, growth and population studies.

INTRODUCTION

Tagging and marking[4] methods have long been used in population and behavior studies of animals. In fisheries research, several fish-tagging and marking techniques have been developed and when properly designed and executed, such mark-recapture experiments provide valuable information on fish population movements, measures of population growth, fishing pressures and mortality.

Tagging studies previously conducted on crustaceans have met with many difficulties, since their exoskeleton is shed when molting occurs. Because of the loss of tags when these animals molt,

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[2] The work reported here was part of a thesis submitted by the senior author to the Graduate School, University of Washington, in partial fulfillment of the requirements for the Master of Science degree.

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[4] The term *tagging* is used to denote the application of a mechanical tag or other identification device. *Marking* refers to the use of mutilation, staining or other means of identification not requiring the attachment or insertion of mechanical devices.

tagging techniques involving the use of external tags are generally limited to short term experiments on larger specimens.

Because of their small size, shrimp are not suitable for tagging studies. In fact, previous tagging experiments on penеid shrimp indicate that tags increase the mortality rate (Lindner and Anderson, 1956); thus, the interpretation of the results may be open to inaccuracies. Other techniques used, such as marking with biological stains and dyes, also present problems. In addition to impairing the attractiveness of shrimp as food and rendering them more readily visible to predators, many stains and dyes often fade or are lethal within a short time after application (Klima, 1965) and fast stains, such as Trypan Blue and Trypan Red, become increasingly toxic with age (Dawson, 1957).

A possible solution to the problem of successful tagging of shrimp was recently developed by Jefferts, Bergman and Fiscus (1963) for Pacific salmon. This new tagging device permits the use of a ferromagnetic wire tag, commonly referred to as the Bergman-Jefferts tag, which can be implanted into the musculature beneath the exoskeleton of shrimp to preclude loss during molting. The magnetized tag can be readily recovered by detection of its magnetic field either by the simple coil detector developed by Jefferts, or an electrometer.

Following the work of Jefferts *et al.* (1963), the wire tag was employed on lobsters by Squires (Woodland, 1966), and Tutmark, West and Chew (1967) used it successfully on brachyuran crabs. The encouraging outcome of the latter preliminary experiment made it desirable to conduct the present study on the feasibility of using the Bergman-Jefferts tag on spot shrimp and to determine differential mortality caused by the tag, if any.

MATERIALS AND METHODS

Aquarium

This study was performed at the University of Washington College of Fisheries' saltwater aquarium using four similar aquaria in each of two experiments conducted on different dates. The inside dimensions of each aquarium was 137 cm long, 61 cm wide and 67 cm high.

The water level in each test aquarium was maintained at 56 cm throughout the study. Sea water from Puget Sound was recirculated through the aquaria at the rate of 5 l/min. The inflow and outflow of water were near opposite ends of each aquarium, about 128 cm apart.

Throughout the study, the water temperature was maintained at 51.5°F (± 0.5°F). This temperature is very close to the temperatures where the test animals were collected, as shown below.

Water samples were taken in the field when the test animals were collected to observe changes in hydrogen ion concentration (pH) and salinities to which the shrimp were subjected in being transferred from their natural environment to the aquarium. Water temperatures were also taken at the depths where the shrimp were collected. Results of the hydrographic sampling prior to the two experiments conducted are as follows:

Experiment 1

11/10/66 in field — pH 7.5, salinity 31.8 ppt and water temperature 50.0°F at 35 fm.
11/11/66 in aquarium — pH 7.2, salinity 29.6 ppt and water temperature 51.5°F

Experiment 2

1/13/67 in field — pH 7.8, salinity 30.1 ppt and water temperature 48.0°F at 38 fm.
1/14/67 in aquarium — pH 7.2, salinity 29.8 ppt and water temperature 51.0°F

The pH of the aquarium was determined daily and salinity once each week throughout the experiments.

A 25-watt incandescent lamp, placed over each test aquarium was turned on mechanically each morning (6:30 am) and off every evening (6:30 pm). It required about 35 min for the lamps to reach maximum illumination in the morning and to be completely extinguished in the evening. Thus, by means of this mechanical device described by Chew[4], the amount of light entering the test aquaria was controlled to simulate sunrise and sunset conditions.

Test Animals

The spot shrimp used for this study were all collected off Broad Spit of Dabob Bay, Washington, at depths varying between 30 and 40 fm. The test animals for Experiment 1 were collected on November 9 and 10, 1966, with a Gulf Coast shrimp trawl, and those for Experiment 2, on January 13, 1967, with the same gear.

Prior to the actual tagging, shrimp were conditioned to the salt water of the aquarium for about two weeks. During this acclimatizing period, the shrimp were all measured from the base of the eyestalk to the posterior mid-dorsal edge of

[4] Chew, K. K. 1958. A study of the food preference of the Japanese drill, *Ocinebra* (=*Tritonalia*) *japonica* Dunker. Master of Science Thesis, University of Washington.

the cephalothoracic exoskeleton (carapace length in mm).

The shrimp ranged in carapace length from 14 mm to 40 mm, and were divided into three size groups of small ($\leq$ 20 mm), medium (21 - 30 mm) and large (31 - 40 mm). In both experiments most shrimp fell within the medium size classification.

During the entire period of the study, the test animals were fed flatfish every other day. Minced mussels (*Mytilus* spp.), dead shrimp and other fish were used as food during the acclimatization period, but this practice was discontinued because of lack of constant supply. The flatfish, either fresh or frozen, was filleted and cut into small pieces. One piece per shrimp was dropped into each tank or compartment. Food supplied in this fashion is readily eaten and allows for even distribution.

Tagging Method

The coded wire tags were fabricated from cold-worked type 302 stainless steel and measured 1 mm in length by 1/4 mm in diameter. They were implanted with the Technical Research Company Wire Tag Injector, Model 3, into the musculature of the first abdominal somite, to the left side of the mid-dorsal portion in order to avoid damaging the centrally located dorsal abdominal artery of the shrimp.

The minute tags enter the injector in the form of a continuous stainless steel wire. The first abdominal segment of each shrimp is pressed firmly into the plastic mold which positions the segment on a hypodermic needle. This action triggers the injector which cuts one tag and places it in the shrimp. A push-rod inside the hypodermic needle forces the tag through the needle, which retracts ahead of the push-rod to avoid withdrawing the tag back into the needle. The implanting action of the needle takes only 1/4 sec. The actual sequence in operation is detailed in Figure 1.

Immediately after tagging, all specimens were examined visually for tag placement. Shrimp with misplaced tags were rejected and replaced. If the tag was properly positioned, or if no tag was visible, the shrimp was then passed over a horseshoe magnet to magnetize the tag (if present) and then through the sensing unit called the Jefferts electronic detector. If the detector was not actuated, then the individual was either rejected or retagged. The detector includes two connected parts — an audio unit and a sensing head. It

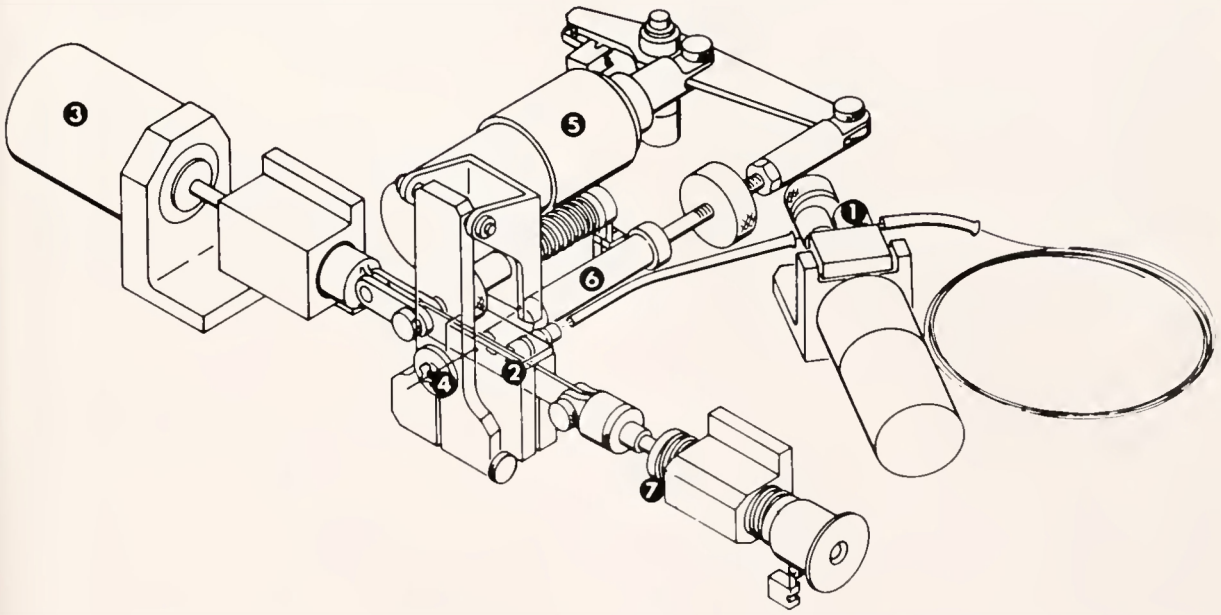

FIG. 1. *Operation of Wire Tag Injector. 1) The coil of color-coded wire passes through the friction feed-rollers on the motor. 2) The wire enters the hole in the cutter bar. 3) The cutter bar solenoid pulls the cutter bar to the left, shearing off a tag of wire as long as the thickness of the bar. 4) The hole in the cutter bar (carrying the tag) centers behind the hypodermic needle. 5) The rear solenoid drives a wire plunger through the hole in the cutter bar, pushing the tag through the hypodermic needle into the specimen. 6) The plunger retracts, leaving the tag embedded. 7) A spring pulls the cutter bar back to receive another tag, and the cycle is repeated.*

senses a magnetic tag in a shrimp passed through its sensing head and emits an audible signal.

The tags are coded with longitudinal epoxy color stripes. These will allow a large number of unduplicated tags since as many as 6 colors may be applied to the wire simultaneously from a selection of 12 distinct hues. This yields at least 8^4 different combinations if two stripes and two colors are reserved to denote the starting point and the direction of reading (Bergman *et al.*[5]).

Experimental Design

Two experiments, designated as Experiments 1 and 2, were conducted. Each experiment was carried out over a period of 50 days — Experiment 1, from December 1, 1966, to January 20, 1967, and Experiment 2, from February 2 to March 24, 1967.

In each experiment three categories of test animals were used. These categories were designated as tagged, untagged, and wounded with the latter two groups serving as controls. The wounded shrimp were processed in the same manner as the tagged ones. They were punctured by the needle but the tag was not inserted.

In two tanks (Tanks 2 and 4) a group of tagged (T) and untagged (U) shrimp were held together in a one-to-one ratio. A second set of tagged and wounded (W) individuals were placed together in two other tanks (Tanks 1 and 3). These were also in a one-to-one ratio. Thus, there was a duplicate set of tagged and untagged shrimp (TU1 and TU2) and one of tagged and wounded shrimp (TW1 and TW2), all in the same ratio. All four tanks contained equal numbers of shrimp (50). The shrimp in each experimental group were selected to reflect the average size composition of the population.

It was anticipated that molting shrimp might be eaten by the rest. To partially combat this possible problem, a plastic screen enclosure was installed in each test tank to protect newly molted shrimp. This, in addition to the constant feeding, helped control the post molting cannibalism that occurred among the shrimp.

Test tanks were checked at least twice daily for the presence of dead or molting animals and periodic counts were made of shrimp remaining in each experimental tank. Abdominal and cephalothoracic exoskeletons were removed whenever found. Shedding shrimp were observed to eat the branchial cast and associated structures, and these were consequently left in the tanks. An indication of the number of molting individuals was obtained by counting the cephalothoracic exoskeletons (carapace) found in each tank. When bodies were found, shrimp which had died from the effects of experimental procedures or other causes, were distinquished from those eaten by their fellows during or immediately after ecdysis, by the presence or absence of a firm exoskeleton.

[5] 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)

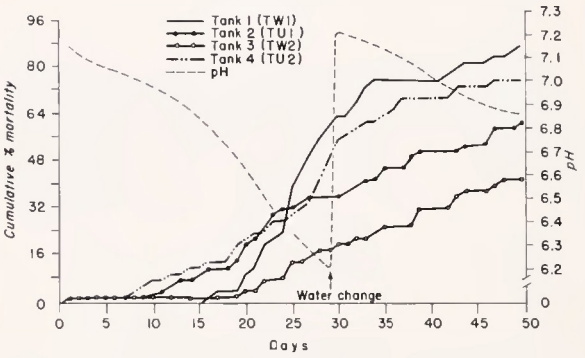

FIG. 2. *Cumulative mortalities of shrimp in Experiment 1 with corresponding pH fluctuations. TW=Tagged and wounded shrimp. TU=Tagged and untagged shrimp.*

RESULTS AND DISCUSSION

Mortality

The percentage of shrimp that died during the entire study was very high, ranging from 42.0 per cent in Tank 3 with TW2 shrimp in Experiment 2 to 90.0 per cent in Tank 1 with TW1 shrimp in Experiment 1. The cumulative mortalities of test animals in Experiments 1 and 2 are shown in Figures 2 and 3 respectively, while Figure 4 shows

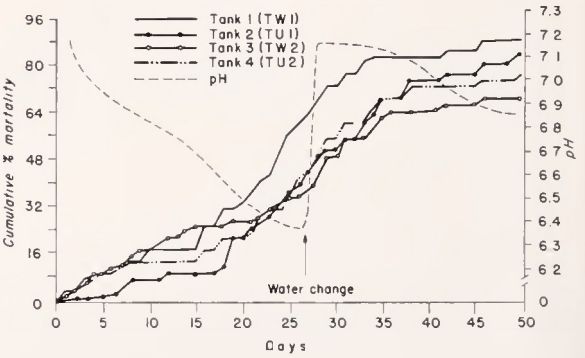

FIG. 3. *Cumulative mortalities of shrimp in Experiment 2 with corresponding pH fluctuations. TW=Tagged and wounded shrimp. TU=Tagged and untagged shrimp.*

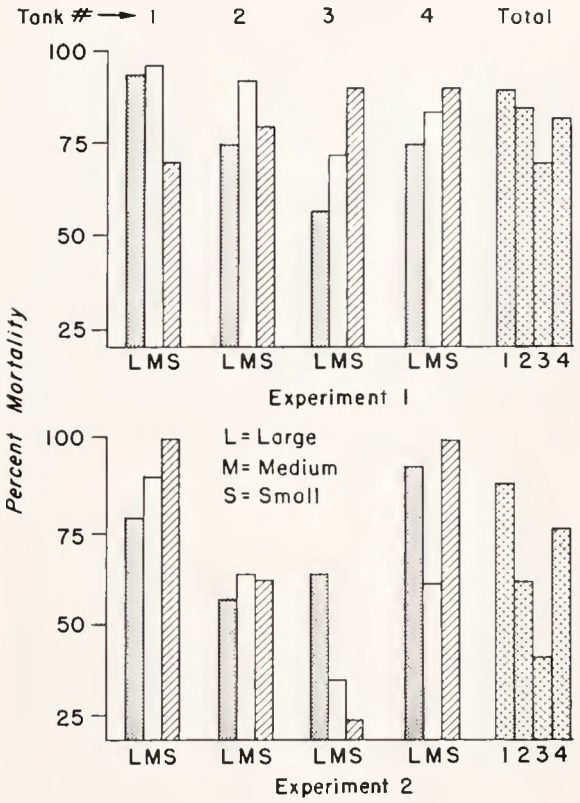

FIG. 4. *Percentage mortality of shrimp in Experiments 1 and 2.*

the percentage mortality within each size group in each tank. There appears to be no definite correlation between size and mortality as shown in Figure 4.

The high incidence of mortality during the entire study may be associated with several unknown factors possibly caused by the deficiency of the recirculating saltwater aquarium system where the study was conducted. From the data presented in Figures 2 and 3, there is an obvious relationship between mortality and pH; the mortality rate increases as the pH decreases. Until pH falls to 6.6 there seems to be little adverse effect on the shrimp.

It was also observed that at low pH, the test animals were relatively inactive. Usually, whenever food was introduced, the shrimp would actively compete for it, but this did not occur during periods of low pH, especially below 6.6 when their feeding ability seemed to be greatly affected if not completely inhibited. This peculiar behavior may be due to the fact that at a low pH the ability of these animals to extract oxygen from the environment is seriously affected (Pruthi, 1927; Tarzwell, 1957).

It was noted that newly molted shrimp were found to be more adversely affected during the period in which the pH was less than 6.6. In most instance, over 80 per cent of all deaths during low pH occurred among the "soft-shell". Of course, one must not discount the fact that these "soft-shell" shrimp may have also been subjected to other unknown conditions affecting their survival and well-being.

The differential mortality data are presented in Tables 1 and 2. To test possible differences in mortality due to treatment, size, replicate, and the interactions (treatment x size and treatment x replicate), a fully crossed, fixed model 3-way analysis of variance was conducted on the transformed data, using the Arcsin$\sqrt{\text{Percentage}}$ transformation (Snedecor, 1962).

Since the interactions (treatment-size and treatment-replicate) were not significant at the 25 per

TABLE 1. *Mortality in Experiment 1 between tagged, wounded and untagged shrimp of various size groups, held within four aquaria for 50 days.*

Tank	Tagged				Wounded				Untagged				Tank Total			
No.	L	M	S	T	L	M	S	T	L	M	S	T	L	M	S	T
1 (TW1)	7(8)[1]	11(12)	4(5)	22(25)	8(8)	12(12)	3(5)	23(25)	—	—	—	—	15(16)	23(24)	7(10)	45(50
%	87.5	91.7	80.0	88.0	100.0	100.0	60.0	92.0	—	—	—	—	93.8	95.8	70.0	90.0
2 (TU1)	5(8)	10(12)	5(5)	20(25)	—	—	—	—	7(8)	12(12)	3(5)	22(25)	12(16)	22(24)	8(10)	42(50
%	62.5	83.3	100.0	80.0	—	—	—	—	87.5	100.0	60.0	88.0	75.0	91.7	80.0	84.0
3 (TW2)	3(8)	10(12)	4(5)	17(25)	6(8)	7(12)	5(5)	18(25)	—	—	—	—	9(16)	17(24)	9(10)	35(50
%	37.5	83.3	80.0	68.0	75.0	58.3	100.0	72.0	—	—	—	—	56.3	70.8	90.0	70.0
4 (TU2)	7(8)	11(12)	5(5)	23(25)	—	—	—	—	5(8)	9(12)	4(5)	18(25)	12(16)	20(24)	9(10)	41(50
%	87.5	91.7	100.0	92.0	—	—	—	—	62.5	75.0	80.0	72.0	75.0	83.3	90.0	82.0
Total	22 (32)	42 (48)	18 (20)	82 (100)	14 (16)	19 (24)	8 (10)	41 (50)	12 (16)	21 (24)	7 (10)	40 (50)	48 (64)	82 (96)	33 (40)	163 (200)
%	68.7	87.5	90.0	82.0	87.5	79.2	80.0	82.0	75.0	87.5	70.0	80.0	75.0	85.4	82.5	81.5

[1] The numbers in parentheses represent the total number of shrimp in each size group.

L = Large S = Small TW = Tagged and wounded
M = Medium T = Total TU = Tagged and untagged

TABLE 2. *Mortality in Experiment 2 between tagged, wounded and untagged shrimp in various size groups, held within four aquaria for 50 days.*

Tank	Tagged				Wounded				Untagged				Tank Total			
No.	L	M	S	T	L	M	S	T	L	M	S	T	L	M	S	T
1 (TW1)	5(7)[1]	11(14)	4(4)	20(25)	6(7)	14(14)	4(4)	24(25)	—	—	—	—	11(14)	25(28)	8(8)	44(50)
%	71.4	78.6	100.0	80.0	85.7	100.0	100.0	96.0	—	—	—	—	78.6	89.3	100.0	88.0
2 (TU1)	3(7)	10(14)	2(4)	15(25)	—	—	—	—	5(7)	8(14)	3(4)	16(25)	8(14)	18(28)	5(8)	31(50)
%	42.9	71.4	50.0	60.0	—	—	—	—	71.4	57.1	75.0	64.0	57.1	64.3	62.5	62.0
3 (TW2)	5(7)	5(14)	1(4)	11(25)	4(7)	5(14)	1(4)	10(25)	—	—	—	—	9(14)	10(28)	2(8)	21(50)
%	71.4	35.7	25.0	44.0	57.1	35.7	25.0	40.0	—	—	—	—	64.3	35.7	25.0	42.0
4 (TU2)	7(7)	9(14)	4(4)	20(25)	—	—	—	—	6(7)	8(14)	4(4)	18(25)	13(14)	17(28)	8(8)	38(50)
%	100.0	64.3	100.0	80.0	—	—	—	—	85.7	57.1	100.0	72.0	92.9	60.7	100.0	76.0
Total	20 (28)	35 (56)	11 (16)	66 (100)	10 (14)	19 (28)	5 (8)	34 (50)	11 (14)	16 (28)	7 (8)	34 (50)	41 (56)	70 (112)	23 (32)	134 (200)
%	71.4	62.5	68.8	66.0	71.4	67.9	62.5	68.0	78.6	57.1	87.5	68.0	73.2	62.5	71.9	67.0

L = Large S = Small TW = Tagged and wounded
M = Medium T = Total TU = Tagged and untagged

[1] The numbers in parentheses represent the total number of shrimp in each group.

cent level of significance, a pooled sum of squares (treatment-size interaction sum of squares + treatment-replicate sum of squares + error sum of squares) was formed, and a pooled mean square computed from it. The latter was used as the denominator in testing for the variations due to the main effects (treatment, size and replicate) under the hypotheses:

1) H_1: no treatment effect;
2) H_2: no size effect;
3) H_3: no replicate effect.

The results of these tests are presented in Table 3. As shown in Table 3, within the limits of experimental error, there is no significant effect due to treatment, size, or replicate in the mortality of the spot shrimp used in this study. In fact, from the data analyzed, no initial mortalities or other abnormalities can be directly attributed to the implantation of the coded wire tags in the shrimp musculature.

Molting

Throughout the entire study, molting was never observed to take place during the daytime.

A high incidence of molting was observed in both Experiment 1 and 2. Out of the 200 shrimp used in each experiment, 123 (61.5 per cent) molted at least once in the 50-day period in Experiment 1, and 145 (72.5 per cent) in Experiment 2. The highest percentage (82.0 per cent) of molting surprisingly occurred in Experiment 2 in Tank 1, which, incidently, also had the lowest percentage (56.0 per cent) of molting in Experiment 1. In both experiments only 10 (2.5 per cent) succeeded in molting twice. Most of these were small (≤ 20 mm in carapace length).

Approximately 30 per cent of the shrimp that

TABLE 3. *Analysis of variance of mortality among tagged, wounded and untagged shrimp of various size classifications.*

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio (F)
Treatment	0.020	2	0.010	0.084*
Size	0.107	2	0.054	0.514*
Replicate	0.172	1	0.172	1.638*
Pooled Error	4.418	42	0.105	
Treatment-Size interaction	0.076	4	0.019	0.160**
Treatment-Replicate interaction	0.042	2	0.021	0.175**
Error	4.301	36	0.119	

* Not significant at the 0.05 level

** Not significant at the 0.25 level (hence pooled with error term)

molted at least once in each experiment did not survive the molt, and only 3 out of the 10 that molted a second time survived.

Perhaps owing to a deficiency of some unknown component in the environment, the exoskeleton of molted shrimp in this study did not attain firmness even weeks after exuviation. Tarzwell (1957) asserts that some of the essential minerals in sea water becomes unavailable at low pH levels. This could retard the rate of calcification of the new shell after molting. As mentioned before, the experimental shrimp have frequently been observed to eat their discarded exoskeletons immediately after molting, a habit which may be nature's device for furnishing the large supply of lime salts needed for rapid hardening of the new shell.

The numbers and percentages of shrimp that molted at least once during the 50-day period in each of the three treatment groups (tagged, wounded and untagged shrimp) are given in Tables 4 and 5. A fully crossed, fixed model three-way analysis of variance was again used to test the following hypotheses:

1) H_1: no difference in the molting between the three treatment groups;
2) H_2: no size effect;
3) H_3: no replicate effect;
4) H_4: no treatment-size interaction;
5) H_5: no treatment-replicate interaction.

The data used for the analysis of variance test were the transformed data of Tables 4 and 5, using the Arcsin $\sqrt{\text{Percentage}}$ transformation (Snedecor, 1962).

Before the F statistics were computed for the variations due to the main effects (treatment, size and replicate), the hypotheses for the interactions

TABLE 4. *Percentage of shrimp of various size groups within different treatment groups (tagged, wounded and untagged), held within four aquaria that molted at least once in 50 days in Experiment 1.*

Tank	Tagged				Wounded				Untagged				Tank Total			
No.	L	M	S	T	L	M	S	T	L	M	S	T	L	M	S	T
1 (TW1)	3(8)[1]	7(12)	3(5)	13(25)	5(8)	6(12)	4(5)	15(25)	—	—	—	—	8(16)	13(24)	7(10)	28(50)
%	37.5	58.3	60.0	52.0	62.5	50.0	80.0	60.0	—	—	—	—	50.0	54.2	70.0	56.0
2 (TU1)	2(8)	11(12)	2(5)	15(25)	—	—	—	—	3(8)	10(12)	5(5)	18(25)	5(16)	21(24)	9(10)	33(50)
%	25.0	91.7	40.0	60.0	—	—	—	—	37.5	85.7	100.0	72.0	31.3	87.5	90.0	66.0
3 (TW2)	3(8)	8(12)	5(5)	16(25)	4(8)	7(12)	4(5)	15(25)	—	—	—	—	7(16)	15(24)	9(10)	31(50)
%	37.5	66.7	100.0	64.0	50.0	58.3	80.0	60.0	—	—	—	—	43.8	62.5	90.0	62.0
4 (TU2)	4(8)	6(12)	4(5)	14(25)	—	—	—	—	3(8)	9(12)	5(5)	17(25)	7(16)	15(24)	9(10)	31(50)
%	50.0	50.0	80.0	56.0	—	—	—	—	37.5	75.0	100.0	68.0	43.8	62.5	90.0	62.0
Total	12 (32)	32 (48)	14 (20)	58 (100)	9 (16)	13 (24)	8 (10)	30 (50)	6 (16)	19 (24)	10 (10)	35 (50)	27 (64)	64 (96)	34 (40)	123 (200)
%	37.5	66.7	70.0	58.0	56.3	54.2	80.0	60.0	37.5	79.2	100.0	70.0	42.2	66.7	85.0	61.5

L = Large S = Small TW = Tagged and wounded
M = Medium T = Total TU = Tagged and untagged

[1] The numbers in parentheses represent the total number of shrimp in each group.

TABLE 5. *Percentage of shrimp of various size groups within different treatment groups (tagged, wounded, and untagged), held within four aquaria, that molted at least once in 50 days in Experiment 2.*

Tank	Tagged				Wounded				Untagged				Tank Total			
No.	L	M	S	T	L	M	S	T	L	M	S	T	L	M	S	T
1 (TW1)	4(7)[1]	12(14)	4(4)	20(25)	5(7)	13(14)	3(4)	21(25)	—	—	—	—	9(14)	25(28)	7(8)	41(50)
%	57.1	85.7	100.0	80.0	71.4	92.9	75.0	84.0	—	—	—	—	64.3	89.3	87.5	82.0
2 (TU1)	5(7)	12(14)	2(4)	19(25)	—	—	—	—	2(7)	11(14)	3(4)	16(25)	7(14)	23(28)	5(8)	35(50)
%	71.4	85.7	50.0	76.0	—	—	—	—	28.6	78.6	75.0	64.0	50.0	82.1	62.5	70.0
3 (TW2)	5(7)	12(14)	2(4)	19(25)	3(7)	10(14)	3(4)	16(25)	—	—	—	—	8(14)	22(28)	5(8)	35(50)
%	71.4	85.7	50.0	76.0	42.9	71.4	75.0	64.0	—	—	—	—	57.1	78.6	62.5	70.0
4 (TU2)	5(7)	11(14)	2(4)	18(25)	—	—	—	—	5(7)	8(14)	3(4)	16(25)	10(14)	19(28)	5(8)	34(50)
%	71.4	78.6	50.0	72.0	—	—	—	—	71.4	57.1	75.0	64.0	71.4	67.9	62.5	68.0
Total	19 (28)	47 (56)	10 (16)	76 (100)	8 (14)	23 (28)	6 (80)	37 (50)	7 (14)	19 (28)	6 (8)	32 (50)	34 (56)	89 (112)	17 (32)	145 (200)
%	67.9	83.9	62.5	76.0	57.1	82.1	75.0	74.0	37.5	67.9	75.0	64.0	60.7	79.5	53.1	72.5

L = Large S = Small TW = Tagged and wounded
M = Medium T = Total TU = Tagged and untagged

[1] The numbers in parentheses represent the total number of shrimp in each group.

TABLE 6. *Analysis of variance to test differences in molting between tagged, wounded and untagged shrimp of various size groups.*

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio (F)
Treatment	0.028	2	0.014	0.292*
Size	0.835	2	0.418	8.708*
Replicate	0.003	1	0.003	0.063*
Treatment-Replicate interaction	0.212	2	0.106	2.208*
Pooled Error	1.933	40	0.048	
Treatment-Size interaction	0.148	4	0.037	0.740**
Error	1.785	36	0.050	

* Not significant at the 0.05 level

** Not significant at the 0.25 level (hence pooled with error term)

(treatment-size and treatment-replicate) were tested. At the 25 per cent significance level,

H_4: no treatment-size interaction

was accepted and hence a pooled estimate (treatment-size interaction sum of squares + error sum of squares) was formed.

The F statistics were then computed for the remaining hypotheses using the pooled mean square as denominator.

From the results of the foregoing analysis, as shown in Table 6, the conclusion could be drawn that there is no significant effect on molting due to treatment, replicate and the treatment-size interaction, but that there exists a difference due to size. This highly significant difference due to size agrees with what should be expected since smaller shrimp are known to molt more frequently than larger ones (Berkeley, 1930; Hjort and Ruud, 1938; Storer, 1951).

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SURVIVAL TIME OF OYSTERS AFTER BURIAL AT VARIOUS TEMPERATURES[1]

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ABSTRACT

Experimental burials of oysters were made 3 inches deep in containers of soil held in running sea water at five temperature ranges from less than 5°C to over 25°C. Survival time varied from 2 days in summer to 5 weeks in winter, showing a direct relationship to temperature.

INTRODUCTION

Accidental burial of oysters can be caused by storms; siltation due to high run-off or channel dredging; oyster harvesting and planting activities; smothering by vegetation or other organisms; and probably by unknown agencies. Occasionally, oysters being held in trays on or near the bottom have been buried. Both natural and man-caused burial are widespread phenomena (Galtsoff, 1964).

Oysters were buried experimentally to study how burial affects them. One result of these studies is an indication of survival time and rate of decomposition at several temperature ranges.

METHODS

Oysters from the lower Patuxent River were used and all were held in running sea water until dredge and handling damage to the shells was repaired. In each series, 8 oysters were buried 3 inches deep in mixed sand and mud in each of 4 polyethylene trays. Control oysters were placed on top of the soil and the trays were immersed in a large running seawater aquarium.

Observations of the soil in the trays and in glass tanks showed the following conditions as indicated by the presence of reduced sulfur compounds:

1 inch below the soil-water interface — still aerobic,

1½ inches below the soil-water interface — transitional, becoming anaerobic,

2 inches below the soil-water interface — mostly anaerobic,

3 inches below the soil-water interface — entirely anaerobic.

This gradient is similar to conditions observed in natural bottom having the same type of soil.

In trial shallow burials, oysters buried 1/2 inch or less deep could usually clear their bills of sediment if the water was warm enough for active pumping. Thus the 3-inch depth employed in this experiment indicated survival under conditions that did not permit recovery from burial.

Initially, burial periods which would furnish the desired information on survival could only be guessed. The work of Lund (1957a, b, c) was suggestive, and Wilson[2] experimented with burials but these authors dealt primarily with aspects of survival other than temperature. After the first two series of burials were completed, it was possible to plan exhumations so that they were made during periods of moribundity. Later experiments thus show progressively increasing decomposition and deaths.

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[1] Contribution No. 356, Natural Resources Institute, University of Maryland.

[2] Wilson, W. B. 1950. The effects of dredging on oysters in Copano Bay, Texas. *In* Annual Report of the Marine Laboratory of the Texas Game, Fish and Oyster Commission for 1948-1949, p. 1-50.

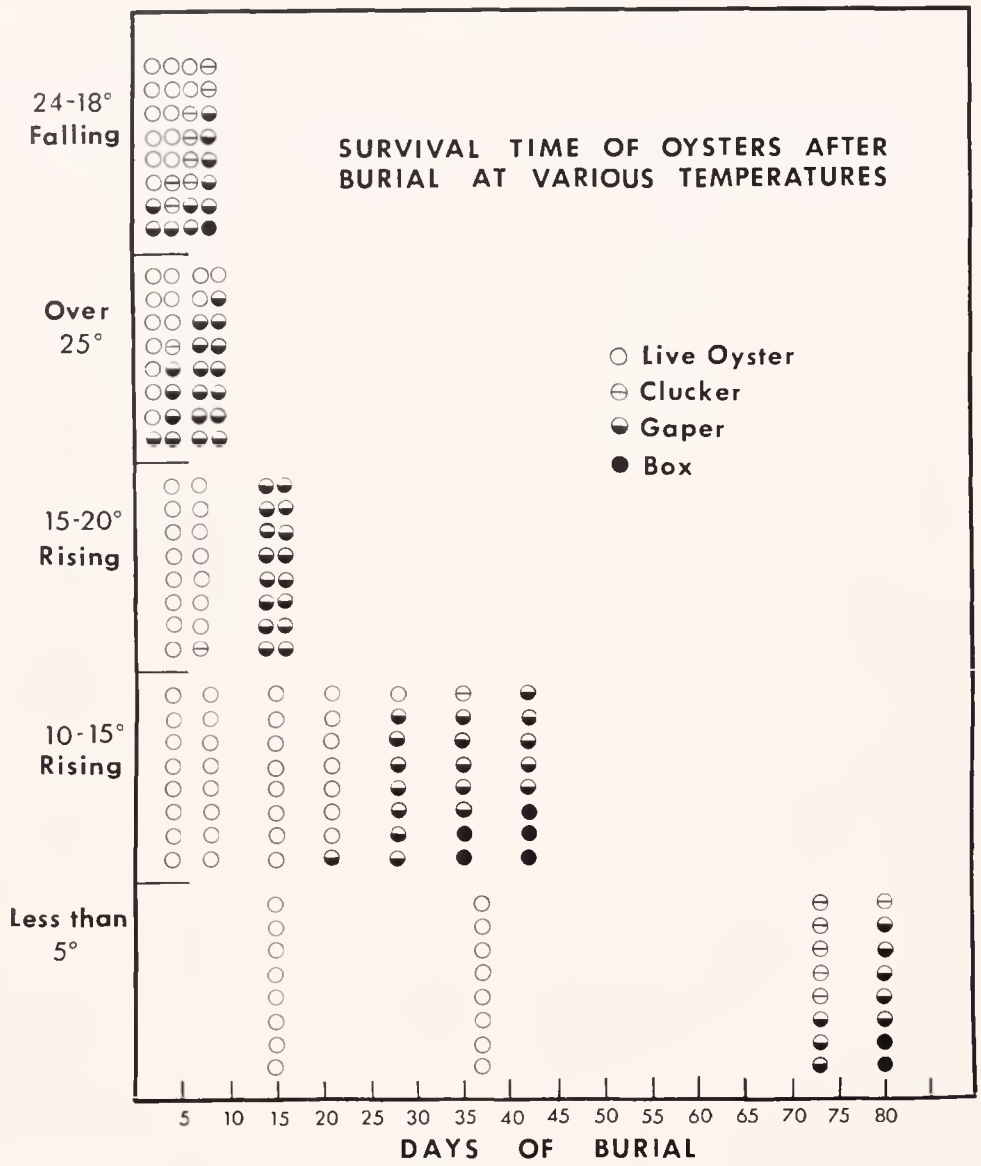

FIG. 1. *Condition of oysters at exhumation after various periods of burial. Each symbol represents a single oyster.*

RESULTS AND DISCUSSION

Figure 1 shows the results of burial at 5 different temperature ranges. At mid-winter temperatures of less than 5°C, oysters lived for over 5 weeks and decomposition was prolonged to over 10 weeks. Pumping would not normally occur when the water is this cold and experimental shell opening rarely happens at these temperatures.

In the 10-15°C series, the first dead oyster was found 3 weeks after burial. One week later, 7 were dead and later exhumations showed increasing decomposition.

At 15-20°C, most oysters survived for a week, but all were dead after 2 weeks. When this experiment was conducted critical moribundity periods were still not established.

During mid-summer temperatures of over 25°C, one buried oyster died in 2 days, 50 per cent were dead in 4 days, and after a week only one was alive.

In the fall with ambient temperatures declining from 24-18°C, a similar pattern was found,

with the difference that oysters died a little more slowly.

Although there are marked differences in survival times between the 10-15°C series and the 15-20°C series, it appears that within these ranges (below 20°C) once a certain threshold has been reached, death follows in about one week. At higher temperatures, this critical point is reached in less than a week.

All of the oysters were buried with the left side down. When they were exhumed and examined it was observed that the left side of the visceral mass decomposed before the right side and the posterior region decomposed before the anterior region. The exposure to the mud which occurred when they gaped and remained open seems to have hastened decomposition.

In several of the oysters that were exhumed alive, mud on the gills indicated that the valves were opened temporarily before they finally gaped and stayed open.

A direct relationship between temperature and survival time is shown by these experimental burials. Earlier death at higher temperatures may be due principally to a higher metabolic rate and more rapid consumption of reserves. The intrusion of toxic materials and bacteria from the mud when a buried oyster gapes may also be a significant factor in survival time and it probably hastens decomposition.

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ASSOCIATION AFFAIRS

ANNUAL CONVENTION

The National Shellfisheries Association 1967 Convention was held jointly with the Oyster Institute of North America and the Oyster Growers and Dealers Association of North America, Inc. from July 16 to 19 at Boston, Massachusetts.

Officers and Executive Committee members elected for the term 1967-1968 were:

President	Harold H. Haskin
Vice President	Albert K. Sparks
Secretary-Treasurer	Fred W. Sieling
Members-at-large	Sammy Ray
	Roy Drinnan
	James E. Hanks
Editor, NSA Proceedings	Arthur S. Merrill

John W. Ropes of the Bureau of Commercial Fisheries Biological Laboratory at Oxford, Md. was appointed Custodian of back issues of the NSA Proceedings.

A program of honors ceremonies took place, in which Dr. David L. Belding was elected to Honorary Membership in the National Shellfisheries Association. The Oyster Institute of North America presented plaques to Dr. L. Eugene Cronin and Dr. Leslie A. Stauber in recognition of their contributions to the industry through scientific research. Both the Bureau of Commercial Fisheries Biological Laboratory at Oxford, Md. and the Exploratory Fishing and Gear Research Laboratory at Gloucester, Mass. received plaques for sea clam biological studies and gear research respectively.

The Pacific Coast Section of the NSA met jointly with the Pacific Coast Oyster Growers Association on August 23-25, 1967 at Olympia, Washington. Officers of the Section elected for the term 1967-1968 were:

Chairman	Kenneth K. Chew
Vice-Chairman	C. Dale Snow
Secretary-Treasurer	John C. Hoff

The Pacific Coast Section has augmented its membership by the addition of a number of crustacean research scientists.

Regular membership to the NSA is $6.00, library subscription is $6.00, and patrons contribute $100.00 or more.