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THIS VOLUME IS DEDICATED TO

THE MEMORY OF

DR. LESLIE ALFRED STAUBER

He will be missed by all who were fortunate enough to have known him.

M. R. Tripp
University of Delaware

PROCEEDINGS
OF THE
NATIONAL
SHELLFISHERIES
ASSOCIATION

CONTENTS

Volume 64 - June 1974

LIST OF ABSTRACTS BY AUTHOR OF TECHNICAL PAPERS PRESENTED AT THE 1973 NSA CONVENTION

ABSTRACTS OF THE NSA PACIFIC COAST SECTION

ABSTRACTS OF TECHNICAL PAPERS PRESENTED AT THE 1973 NSA CONVENTION

GROWTH AND MORTALITY OF TRAY-HELD OYSTERS IN THE PATUXENT RIVER, MARYLAND

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Three size classes of oysters, *Crassostrea virginica*, were held in trays for 27 months on the Patuxent River to determine differences in growth and survival. For 24 months seed and market oysters showed similar growth, but after 27 months differences could be seen. Differences in growth of spat were apparent from the beginning. Meat condition was similar throughout the study.

Two-year mortality was within a normal range. Following tropical storm Agnes in June 1972, a 69% mortality occurred in the Patuxent River. It is believed that low salinity during high ambient temperatures was responsible for the heavy mortality. These data were discussed in relation to changes in salinity of the Patuxent River noted since 1963.

PHYSIOLOGICAL RESPONSES OF THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA* GMELIN, TO SALINITY CHANGES

Roger D. Anderson and Jack W. Anderson

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Measurements of osmotic and chloride ion concentration of the pericardial fluid from *Crassostrea virginica* Gmelin showed that the fluid conformed to the ambient medium throughout the non-lethal range of salinities studied. The pericardial fluid remained very slightly hyperosmotic to the environment over the salinity range. Oysters moved to salinities below 4 ppt died before reaching osmotic equilibrium. Those animals transferred to salinities between 4 and 8 ppt reached a new steady state of fluid concentration at a slower rate than those moved to higher salinities. Analyses of chloride ion concentrations after transfer demonstrated a similar pattern of delayed conformity, but the resulting concentrations were slightly lower than the media. Changes in percent body water and percent ash as a result of salinity alterations occurred at slower rates than those of the pericardial fluid, but final values were proportional to the extent of sea water dilution.

UPTAKE AND DEPURATION OF PETROLEUM HYDROCARBONS BY THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA* GMELIN [1]

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American oysters, *Crassostrea virginica* Gmelin, were exposed to oil-water emulsions of selected crude oils and petroleum fractions. The rate of uptake and depuration of petroleum hydrocarbons was determined by gas chromatographic and ultraviolet spectrophotometric methods.

Oysters rapidly accumulated saturated and aromatic hydrocarbons from oil-water mixtures. Aromatic hydrocarbons were accumulated to a greater extent than n-paraffins relative to their

respective concentrations in the exposure water. Saturated hydrocarbons were accumulated in higher amounts from crude versus petroleum fractions. Accumulation of oil-derived petroleum hydrocarbons was not consistent when uptake of oil by oysters was measured over a period of several days. Following return to oil-free seawater, oysters depurated the saturated chains and most aromatic fractions rapidly. Depuration was nearly completed within 21 days.

Groups of oysters were exposed to oil-water mixtures then returned to bay waters for shell growth studies. Daily average growth of experimental and control populations revealed nearly uniform results. Growth of oyster control groups averaged slightly below most of the experimentals except for a slight difference in one test group.

[1] Supported by a contract from the American Petroleum Institute.

CRASSOSTREA VIRGINICA — MSX INTERACTIONS: CHANGES IN HEMOLYMPH ENZYME ACTIVITIES WITH *MINCHINIA NELSONI* LESION DEVELOPMENT [1]

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During the summer and early fall of 1971 an experiment was performed to determine the effect of *Minchinia nelsoni* lesion development on hemolymph enzymes of oysters *(Crassostrea virginica)* undergoing their first exposure to this pathogen. The enzymes examined were phosphohexose isomerase, a glycolytic sequence enzyme, and aspartate aminotransferase, an important enzyme in the metabolism of amino acids and Kreb's cycle intermediates. The data were grouped in four categories: (1) Normal; (2) Prepatent lesions; (3) Gill lesions; and (4) General infections. Changes in enzyme activities were interpreted as a reflection of the oyster's metabolism.

During the pre-patent stage there is a 50% decrease from normal in the activities of both enzymes. In the gill lesion stage there is a 100 - 120% activity increase over that of normal oysters. In the general infection stage activity levels are not significantly different from those of normal oysters.

The significance of depressed and elevated hemolymph enzyme activities is discussed with respect to host and parasite metabolism and also their relationship with possible host defense mechanisms.

[1] Supported under PL 88-309 contract 3-3-R-7 with the National Marine Fisheries Service and by the N. J. Department of Environmental Protection.

COMPARISON OF RESISTANCE TO DISEASE IN NATIVE DELAWARE BAY OYSTERS AND SELECTED LAB-REARED OYSTERS [1]

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For more than a period of 10 years members of this Laboratory have been reporting to the National Shellfisheries Association on developing resistance to MSX kill in native Delaware Bay oyster stocks and in oysters reared in the laboratory from parents previously selected for resistance to MSX mortality. Questions now asked are: (1) how much more resistant to kill are the present Delaware Bay stocks than those at the onset of the epizootic in 1957; and (2) what is the limit to which resistance may be developed.

Samples of newly-set lower Delaware Bay spat of the five year classes 1966 through 1970 have been exposed, in trays, on the Cape Shore tidal flats for test periods of approximately 3 years each. Mortalities in these stocks have been compared with those in (1) lab-reared spat of 9 susceptible progeny groups; (2) lab-reared spat of parents selected against MSX disease in trays at the Cape Shore for at least three years prior to spawning (first generation, selected); (3) lab-reared spat whose parents were first generation, selected, which in turn were selected against MSX disease in Cape Shore trays (second generation, selected) and (4) lab-reared spat whose parents were second generation, selected (designated 3rd generation, selected).

Results, after the approximately 3-year period of exposure to MSX, are in brief: (1) an average of 8% of the 9 susceptible progeny groups survived. These susceptible groups are believed to be closely comparable to the native susceptible Delaware Bay stocks present at the onset of the MSX epizootic.

(2) In comparison, an average of 22% of the five lower Delaware Bay native year classes survived the 3-year MSX exposure. (3) An average of 31% of the seven first generation resistant progeny groups survived. (4) An average of 44% of the eight second generation resistants survived. (5) Only one of the third generation resistant groups has been exposed for the usual test period to date, so it is not clear that its survival (50%) is significantly greater than that of the second generation groups. Rephrasing the results enables us to answer our two questions as follows. Over a three-year exposure period, compared with susceptible, control stocks, lower Delaware Bay native oysters will have about 3 x as many survivors; first generation lab-reared resistants about 4 x; second generation lab-reared resistants about 5½ x; and third generation lab-reared resistants may, or may not, show a slight increased resistance (6 x). This suggests that increased resistance within oyster populations exposed to the selective pressure of the lower Delaware Bay may approach a limit at 5 to 6 times the level of resistance in the old susceptible populations.

[1] Supported under PL 88-309 contract 3-3-7 with the National Marine Fisheries Service and by the N. J Department of Environmental Protection.

FACTORS IN THE RECRUITMENT OF EUROPEAN OYSTERS IN MAINE

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Ostrea edulis populations in Maine, introduced in the 1940's, today maintain marginal populations levels. However, hatchery-reared progeny from these stocks may exhibit superior overwintering qualities when compared to California hatchery-reared stocks. Thus preservation of this Maine-adapted gene pool is essential for use here in an intensive aquacultural development. A combined laboratory and field program is investigating factors important in recruitment.

In the laboratory the gregarious setting response is much in evidence. Larvae can be "triggered" to set by exposure to adult extrapallial fluid prior to exposure to cultch shells, indicating action of a waterborne pheromone. This contradicts the British view of "surface chemistry" response of the setting larvae. Extrapallial fluid of the American oyster stimulates setting in European oysters indicating an interspecific response. Other laboratory studies are investigating the biochemical natural of the setting pheromone in addition to describing the role and ultrastructure of larval sense receptors, particularly the eyespot and apical sense organ. A Latin-square field plot has been initiated in Boothbay Harbor to determine the importance of gregarious setting in field populations of European oysters. The presence of adult oysters appears to increase setting on nearby cultch shells but results are inconclusive at this point.

EFFECT OF SALINITY ON MUCUS IN THE MANTLE OF THE QUAHOG, *MERCENARIA MERCENARIA*

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The purpose of the study was to determine whether salinity changes had any effect on mucus secretions in the quahog clam, *Mercenaria mercenaria*. The specific tissues studies were located in the first fold of the mantle edge.

Six separate groups of clams were established. One group was held in seawater at 35 parts per thousand salinity, one at ambient salinity (approximately 30 ppt), one at 25 ppt, one at 20 ppt, one at 15 ppt and one at 10 ppt. After a week of exposure at the appropriate salinities, sections of

the mantle edge were prepared for histochemical studies of the quality and quantity of mucus.

There appeared to be a relationship between salinity and mucus production in the quahog in that as salinity increased so did the amount of reactive acid mucopolysaccharide.

BIOLOGY OF THE CLAM *RANGIA CUNEATA*: WHAT WE NOW KNOW AND WHAT IT MEANS

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Our laboratory studies have shown that *Rangia cuneata* juveniles and adults can live indefinitely (months or years) in salinities from near 0 to at least 32 ppt; can regulate internal salinity (in water of salinity below 10 ppt); feed on algae and detritus particles, and absorb glucose from dilute solutions, at rates unaffected by salinity; can live 2 weeks (at 22° C) anaerobically by using their large supply of stored glycogen and have other adaptations to extremely variable or otherwise adverse conditions. Nevertheless, they are ecologically almost entirely limited to the zone of 0.5 — 15 ppt salinity. The reason is their requirement for a change in salinity to stimulate spawning and requirement of eggs and early larvae for salinity between 2 and 10 (possibly 15) in order to survive and develop. After reaching setting stage, 6-7 days after fertilization of eggs, the juveniles can live and grow in salinities from 2 to 30 ppt, and perhaps in lower and higher salinities that were not tested. Adults can live for 15-20 years in salinities too low, too high, or too stable for reproduction. In such waters the entire population may be of one or two year classes. Presence of several to many year classes means that the conditions favoring reproduction and recruitment occur every year, or most years. This makes *R. cuneata* useful as an indicator of salinity climate, in addition to its commercial value for shell and meat and its ecological value as food for fishes, crustaceans, birds and mammals.

A PRELIMINARY ASSESSMENT OF THE EFFECTS OF ALASKAN NORTH SLOPE CRUDE OIL ON DEVELOPING LARVAE OF THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*

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The discovery of oil under Alaska's North Slope and proposals to transport the oil to the Puget Sound area for processing have precipitated public concern for the durability of the local marine biota. To help assess the potential danger of accidental oil spills, the toxicity of Prudhoe Bay crude oil to larvae of the Pacific oyster *(Crassostrea gigas)* is under investigation. Oyster larvae were selected as the test organisms for this study because their use is rapidly becoming a standard for the evaluation of environmental degradation.

Fertilized oyster eggs were subjected to graded doses of whole crude oil and to doses of two different kinds of seawater extracts of the oil. The extracts are subsequently being analysed for their content of small hydrocarbon compounds consisting of fewer than nine carbon atoms. Differences in the larval developmental responses to the various toxicants were discussed, and some potential biological repercussions of oil importation into Puget Sound were considered.

SUMMARY OF FLORIDA'S PENSACOLA AREA OYSTER CULTURE PROGRAM

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To offset effects of extensive September 1971 kills of *Crassostrea virginica*, the Florida Department of Natural Resources used a National Marine Fisheries Service grant to conduct public oyster culture programs in the Pensacola estuarine area.

Hydrographic and biological sampling during October 1971 through February 1972, led to selection of five oyster restoration sites in East and Escambia Bays. Beginning in April 1972, 50-100 yd^3 mounds of clam shells and oyster shells were

planted on firm mud bottoms. In addition, approximately 5,725 bu of live seed oysters were relocated to planting areas in Escambia Bay. At two areas spatfall on 100 cm^2 asbestos tiles was monitored and temperatures and salinities were recorded.

Spatfall on cultch plantings and on asbestos tiles was negligible except in September and October 1972. The effects of siltation, predators and fouling organisms were generally slight. Spatfall was better on East Bay plantings than on those in Escambia Bay and commercial harvesting during the 1973-1974 oyster season appears feasible.

ANNUAL PERIODICITY AND ITS REALTION TO THE INTERNAL SHELL MORPHOLOGY OF *MYTILUS EDULIS*

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Although daily or tidal periodicity structures are generally poorly preserved or lacking within the shell of the blue mussel, annual cycles are reflected in growth increment sequences in the innermost shell layer, facilitating age determination. Each valve of 24 specimens of *Mytilus edulis*, all of known or assumed ages, was longitudinally sectioned along the anteroposterior axis. Individuals having survived one, three and five winters show, respectively, one, three and five dark bands in inner nacreous layer of the shell. Spawning and disturbance lines, if present, are readily distinguished from annual bands and, therefore, present no problems similar to those encountered in classical age determination studies based upon surface shell morphology. Careful examination of growth patterns in the inner shell layer of other bivalves may facilitate age and growth rate determinations of many recent and fossil mollusks.

SYSTEMS ENGINEERING OF OYSTER PRODUCTION

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The possibility of producing oysters in a closed environment, away from the hazards of nature, has been discussed among people working in mariculture for a number of years. However, oysters produced in their natural environment still cost less than those that might be produced in the currently envisioned closed systems. The purpose of this paper is to indicate the important cost factors in the closed system, show some of the important developments needed and, finally, indicate where research effort might be expended in making the closed system competitive. The base system analyzed is that proposed in a study performed by the American Cyanamid Company for the Connecticut Research Commission in 1968.

The dominant system cost results from the pumping and heating of the mixture of salt and fresh water being delivered to the oysters. A recycle system, with at least 85% recycle, is necessary to bring the costs within range of the naturally produced oysters.

Developments beyond that of the partially recycled water are required to make the system economically competitive. A sensitivity analysis shows areas where major gains might be realized. An analysis of research costs, probability of success and relationship to the cost-sensitive areas shows that effort is justified for research in the following areas, listed in order of decreasing importance: heat recovery, improved growth rates in the hatchery, growing algae and the oysters in the same tanks, cross-breeding for more rapid growth, developing less costly tank designs such as PVC-lined artificial ponds and better definition of water requirements for the growing oysters. With the expected degree of success in each research task, the cost of oysters produced in a closed environment would be less than that for naturally grown oysters.

STATUS AND POTENTIAL OF OYSTER CULTURE IN PUERTO RICO

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Current production of the mangrove oyster, *Crassostrea rhizophorae*, in lagoons and small bays of Puerto Rico is limited. Historically, the greatest harvest area, Laguna Rincon, produced only 20,900 pounds of oyster, including shell, during 1972. Several factors are responsible for this condition, including primitive harvesting techniques, overexploitation, predation, lack of appreciable setting areas, competition for space on mangrove aerial roots and little knowledge of modern oyster growing techniques.

Approximately 776 acres are potentially available for raft and shoreline culture methods in four prime oyster-producing areas on the island. If one or more mariculture methods prove feasible and these areas are extensively utilized for oyster culture, oyster farming in Puerto Rico can be greatly enhanced.

To study the possibilities of augmenting production, oyster mariculture experiments are in progress. First, the growth, survival, seasonal sexual pattern and histologic condition of mangrove oysters transplanted from Laguna Rincon to several prime growing areas are being studied. Second, raft culture is being attempted in two suitable areas involving experimentation with various cultch materials such as rubber, asbestos and wood and varying the horizontal distances between strings in order to obtain optimum growth and maximum production. Growth in lagoons versus growth in open water areas are being compared. Third, disease-free seed of the Pacific oyster, *C. gigas*, and the eastern oyster, *C. virginica*, are being raised to planting size in a running seawater system and will be planted in key areas, protected from gastropod predation, and monitored closely for growth, survival and presence or absence of disease organisms. Although all oyster imports into Puerto Rico have failed thus far, it is thought that by closely monitoring these factors mortalities can be controlled and the introduced species survive and eventually compete favorably with local species.

Preliminary observations based on examination of 490 specimens show oysters from two typical growing areas, Puerto Real and Laguna Rincon, with an average length of 30.7 mm and a maximum length of 75.0 mm. Also, histologic analyses have revealed evidence of protandry in oysters from both areas and the presence of a gregarine parasite, *Nematopsis* sp. in the connective tissue surrounding the digestive tubules, gut and mantle, and in the gill epithelium. Also, a *Labyrinthomyxa*-like organism has been observed in the stomach, gut, and collecting duct epithelium, often accompanied by increased hemacytic diapedesis. These organisms do not appear to be harmful to the host.

UPTAKE AND DEPURATION OF PETROLEUM HYDROCARBONS BY THE ESTUARINE CLAM *RANGIA CUNEATA*[1]

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Clams, *Rangia cuneata*, were exposed to oil-in-water dispersions and water-soluble fractions of #2 fuel oil and South Louisiana crude oil or to sea water solutions of specific aromatic petroleum hydrocarbons. The rate of uptake of oil hydrocarbons by the tissues during exposure and rate of depuration when the clams were returned to oil-free sea water was determined by gas chromatographic and ultraviolet spectrophotometric techniques.

Clams rapidly accumulate oil-derived n-paraffins and aromatic hydrocarbons from oil in water dispersions and solutions. Aromatic hydrocarbons are accumulated to a greater extent than n-paraffins relative to their respective concentrations in the exposure water. The alkylnaphthalenes, 2 methylnaphthalene and dimethylnaphthalenes were the hydrocarbons accumulated to the greatest extent from the oil-in-water dispersions. Following return of the clams to oil-free sea water depuration of all classes of oil hydrocarbons was very rapid, though depuration rate was dependent on the hydrocarbon type. N-paraffins were depurated most rapidly followed by naphthalene and

[1] Supported by a contract from the American Petroleum Institute.

alkynaphthalenes. Alkyl benzenes and polycyclic aromatics appear to be depurated most slowly. Depuration is essentially complete within 1-2 weeks after exposure to oil.

AROCLOR® 1254, DDT AND DDD, AND DIELDRIN: ACCUMULATION AND LOSS BY AMERICAN OYSTERS *(CRASSOSTREA VIRGINICA)* EXPOSED CONTINUOUSLY FOR 56 WEEKS [1]

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Separate populations of oysters were exposed continuously for 56 weeks to 0.01 μg/l of Aroclor® 1254, p,p' -DDT and DDD, or dieldrin and sampled at 8-week intervals for residues. Maximum concentrations based on body weight (μg/g) occurred after 8 weeks of exposure, but maximum concentrations based on absolute amount of toxicant accumulated (μg) occurred after 56 weeks of exposure. After 8 weeks, average whole-body residues (wet weight) from five oysters analyzed individually were: Aroclor 1254, 1.65 μg/g, 4.0 μg; DDT (and metabolites DDD and DDE), 0.46 μg/g, 1.0 μg; and dieldrin, 0.08 μg/g, 0.2 μg. After 56 weeks, residues were: Aroclor 1254, 0.89 μg/g, 25.7 μg; DDT and metabolites, 0.37 μg/g, 7.0 μg; and dieldrin, 0.03 μg/g, 0.6 μg. Seasonal patterns of accumulation and loss of the three toxicants were similar. Residues based on body weight (μg/g) decreased 45%-81% in early July and late October, apparently as the result of spawning, and increased following these periods. This shows that the life history of oysters must be considered when evaluating residue data from monitoring programs. Growth rate (height and in-water weight) of exposed oysters was not different from that of control oysters (Student's t-test; $a = 0.01$) Mortality was not significant in any group.

[1] Contribution No. 174, Gulf Breeze Environmental Research Laboratory.

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BIOLOGICAL MAGNIFICATION OF DIELDRIN IN A TWO PART FOOD CHAIN

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This study explored the possibility of biological magnification of the chlorinated hydrocarbon insecticide dieldrin in a two member food chain consisting of the bivalved mollusk *Rangia cuneata* and the decapod crustacean *Callinectes sapidus.*

Clams were exposed to dilute solutions of dieldrin in seawater for 36 hours. At the end of the exposure time sub-samples of clam tissues were analyzed for residues of dieldrin and remaining contaminated tissues fed to blue crabs in a specially designed feeding apparatus.

Results of analyses of tissues by gas-liquid chromatography indicate a magnification factor of 33-35 times ambient water concentration in clam tissues and 3.9 — 6.8 times clam tissue residue levels in crabs.

Thus it is shown that dieldrin can be accumulated from water by bivalves and concentrated in predator tissues as a result of feeding.

A MANAGEMENT PROGRAM FOR THE OYSTER RESOURCE OF APALACHICOLA BAY, FLORIDA

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This study develops cost-benefit data on management practices suitable for protection and enhancement of the oyster producing environment of Apalachicola Bay as one aspect of development of an overall management plan for the oyster resource. Detailed data have not yet

been released but should be available to researchers very soon. Some of it may be of interest to marine biologists.

Another product of this research of possible interest to biologists is the development, from commercial sources, of a statistical series showing monthly averages of oyster meat yields in gallons per Florida barrel. This series is continuous from 1959 to the present. Recent analysis of this data by the author and a colleague, Dr. Warren F. Mazek of Florida State University, indicates that changes in atmospheric temperature and fresh water inflow into Apalachicola Bay explain as much of the variation in yield of oyster meats to the Florida Barrel as is explained by seasonal fluctuations. Work on establishing the reliability of this statistical series and further efforts to explain the observed variation in meat yields are continuing.

A third aspect of the research effort of possible interest to biologists is the diminished need for estimates of the maximum sustainable yield for various fisheries and the heightened need for estimates of optimum harvestable size by species and area. The unreliability of maximum sustainable yield estimates makes these calculations of lessened value to economists. By contrast, since minimum legal harvestable size is a principal element of much of shellfish regulation economists are in great need of additional biological data which would help establish the minimum appropriate. Biological calculations needed are life cycle growth rates by geographical area and incidence of loss through predation and disease at various life cycle stages, again by geographical area.

THE U. S. REGIONAL OYSTER PRODUCT FLOW

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Two aspects of the inter-regional marketing problem of particular interest in this study were the regional destination of the oyster product after it leaves the processor, and the volume marketed between regions. To facilitate the study, the United States was divided into the nine geographic regions used by the Census Bureau. The data required for investigation were generated by stratified random sampling. Each of the selected firms were contacted either by telephone or personal interview.

SUBMERGED PLASTIC NET STRUCTURES FOR OYSTER PROPAGATION

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The work presented in this report is an experimental investigation of the performance of submerged plastic net structures (S.P.N.S.) for oysters. The experimental model is described. There are three variables involved in this study: net mesh size, population density and oyster initial length. Floatation was added to the structure so the unit floated off the bottom and below the surface to avoid both bottom predation and surface freezing. Data gathered from the experimental models were used to make cost/benefit comparisons.

Graphs were presented which showed various relations between growth rate, mesh size, population density, initial size, final size and total cost-benefit relations, the S.P.N.S. system appears to be an attractive possibility for a future low investment shellfish aquafarm. However, other biological aspects of the concept require investigation before the feasibility of this system is firmly established.

SETTLEMENT AND SURVIVAL OF *CRASSOSTREA VIRGINICA* ON DELAWARE BAY SEED OYSTER BEDS[1]

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The seed oyster beds of Delaware Bay are located in low salinity areas. These beds have long been considered a sanctuary for young oysters because oyster drill populations are restricted by the lowered salinity. Since 1952 extensive data has been collected on the spat potential and seed production of these beds.

During the past three years two of these beds were studied to determine the factors which are important in settlement and early spat survival. Cohansey Bed, with an average salinity of 12 ‰ , has been a commercially producing seed bed, but New Beds, with an average salinity of 16 ‰ , had not been in production during the preceding 20 years. These beds were chosen for study because of the presence of a reproducing drill population on New Beds and the absence of drills on Cohansey.

The three years of study were anomalous years. The spat potentials as determined by clean test shells were some of the highest recorded in 20 years. Total seed bed production has increased and New Beds has begun producing seed oysters.

Spat potentials recorded with clean test shells over 20 years have been the same for these beds, but tests in 1972 with fouled bottom shells have shown that only a fraction of these spat potentials are realized on each bed. The realized potential is greater on the less fouled Cohansey shells than on the New Beds shells.

Lab-reared spat have been used to monitor early mortalities on the beds. The mortality from August to December was 10 - 20% greater on the lower salinity bed. Large initial mortalities of 50 - 70% occur on both beds during the first two weeks of spat exposure but can be reduced to 30 - 40% if the spat are protected from bottom predators. Quantitative estimates of predators have been made with a diver-operated suction dredge. Though drill populations in the bay have declined over the period of the study, the Xanthid crab populations on both beds are prominent at 150-250 crabs per square meter.

Seed oyster production of these beds appears to be related to the differential setting pattern of the larvae as influenced by space competitors, and the number of predators present.

[1] Supported jointly by a grant from the Water Resources Research Institute and by the State of New Jersey.

A MODEL RELATING MOLLUSK FOOD UPTAKE, METABOLIC WASTES, AND WATER FLOW, AND AN APPARATUS TO TEST THE CONCEPT

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One major problem in the design of commercial shellfish raising systems is the lack of feeding information which is useful to the design engineer. In commercial systems economics dictate that some of the shellfish in the system will receive the water before others. The first individuals to receive the water will remove some of the food and add their own body wastes. Therefore, later individuals will receive water which has a lower food concentration and a higher waste concentration. These later animals will not grow as fast. Optimum utilization of the food supply can be enhanced by a knowledge of the effect of these feeding parameters.

Chemical kinetic techniques are used to determine the relationship of food and waste concentrations in the water, water flow rate, and various water distribution patterns to the rate of shellfish growth. Included are a theoretical analysis of these feeding parameters, and the design of an experimental apparatus to test the technique.

NSA PACIFIC COAST SECTION

PREDATION OF JUVENILE BIVALVES BY THE SHORE CRABS *HEMIGRAPSUS OREGONENSIS* AND *H. NUDUS*

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Predation by two species of shore crabs, *Hemigrapsus oregonensis* and *H. nudus* on juveniles of three bivalve species, *Saxidomus giganteus*, *Venerupis japonica* and *Mytilus edulis*, was studied under experimental conditions. Crabs ranging in carapace width from 5-20 mm were fed four size-groups of bivalves; 0-2, 2-4, 4-6 and 6-8 mm shell length. Predation rate was measured at two temperatures, 10 and 15 C and when bivalves were exposed in experimental dishes and buried.

Both crab species ate juvenile bivalves; *H. oregonensis* was a greater predator than *H. nudus*. Smaller size bivalves were consumed in larger numbers by all sizes of both crab species and larger crabs ate more bivalves than smaller crabs. *V. japonica* and *M. edulis* were eaten in greater numbers than *S. giganteus* by both crab species. Whether *S. giganteus* and *V. japonica* were buried in substrate or exposed in experimental dishes had little effect on predation rate of the two crab species, and predation was similar at the two temperatures.

ACUTE TOXICITY OF SPRUCE AND HEMLOCK BARK TO SOME ESTUARINE ORGANISMS IN SOUTHEASTERN ALASKA

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Newport, Oregon

The acute toxicity of Sitka spruce and western hemlock bark to pink salmon fry *(Oncorhynchus gorbuscha)*, pink shrimp adults and larvae *(Pandalus borealis)*, and Dungeness crab larvae *(Cancer magister)* was investigated.

For salmon fry, toxic effects were observed as soon as 3 hr after exposure to hemlock bark leachates. After a 96 hr exposure, a concentration of 56 mg/liter killed 50% of the test fry (96-hr EC_{50} for death). The 96 hr EC_{50} using death for spruce bark leachates was 100-120 mg/liter.

Although hemlock had little effect on the invertebrates tested, spruce bark leachates were consistently toxic to both vertebrates and invertebrates. The 96 hr EC_{50}'s for spruce bark leachates to larval shrimp, adult shrimp, and larval crabs, with death as the criterion, were 415, 205, and 530 mg/liter, respectively. Using loss of swimming as the criterion of toxic effect, the 96 hr EC_{50}'s for larval shrimp and larval crabs were 155 and 225 mg/liter, respectively. Spruce bark particles were found to be 2-6 times more toxic than leachates to shrimp larvae. The significance of these findings relative to log dumping and storage in southeastern Alaska is discussed.

IMPROVING PRODUCTIVITY BY USING TANNER CRAB MODELS

William F. Engesser and Daniel Cheung

Oregon State University, Corvallis, Oregon

A processor can conduct better long-range planning and quickly can get more, substantial short-range benefits by using pictorial and mathematical models. The authors demonstrated how motion-picture model-building techniques were used to define and measure current tanner-crab operations. In addition, simulated improved models were constructed from data of actual production runs and special equipment in-plant test runs. Preliminary potential economic benefits showed a direct labor savings of over 3 man hr./100 lbs. of extracted meat (plus other benefits in quality, sanitation and profitability).

Process charts and layout diagrams illustrated the difference between current and potential tanner-crab processing systems. The most important aim of our presentation was to seek both responses and future model building participation from NSA members and/or other interested people.

Lord Kelvin once said, "When you can measure what you are talking about, you begin to know something about it." Perhaps Lord Kelvin could have extended that truism to include: "and given this knowledge, you have the basis for improving and controlling the phenomenon in question."

For a written report on shrimp, crab and bottom-fish processing standards by Wm. Engesser write for Bulletin #47, Oregon State University Engineering Experiment Station, Corvallis, Oregon 97331.

IDENTIFICATION OF OYSTERS OF THE SOUTH PACIFIC ISLANDS

John B. Glude

National Marine Fisheries Service
Seattle, Washington

An assignment with the South Pacific Islands Fishery Development Agency in 1971 to investigate opportunities for shellfish aquaculture provided an opportunity for collection of oysters from Palau Islands, Yap Islands, Truk, New Caledonia, Ponape, Fiji Islands, Cook Islands, French Polynesia and American Samoa. In addition, oysters and oyster culture of Australia and New Zealand were observed. This report covers a comparative study of oysters from various parts of the Pacific, specimens at the U.S. National Museum and a study of the published literature on the taxonomy of oysters.

Oysters of the three genera *Ostrea*, *Crassostrea* and *Pycnodonte* described by Thomson (1954) were found in the Pacific Islands. Stenzel's (1971) description of genus *Lopha* was accepted instead of Thomson's genus *Ostrea* for the tropical cockscomb oyster.

The giant or black-rimmed oyster found in Palau, Truk, Fiji and New Caledonia was identified as *Crassostrea echinata* (Quoy and Gaimard), 1835, following Thomson's (1954) description. Thomson's use of spiny protuberances on the upper valve of juveniles as an identifying characteristic was questioned since my observations in Palau Islands and American Samoa indicate that similar spiny processes occur on the shells of *Crassostrea glomerata*. In Stenzel's (1971) classification system these oysters would fall in genus *Striostrea* Vyalov, 1936.

The most common oyster in the South Pacific Islands is the small mangrove oyster which was identified as *Crassostrea glomerata* (Gould), 1850. This species was found in Palau, Yap, Ponape, Fiji, New Caledonia, French Polynesia, American Samoa and New Zealand. The Sydney rock oyster of Australia, known as *Crassostrea commercialis* (Iredale and Roughley), 1933, is believed to be the same as *C. glomerata* (Gould), 1850, and the earlier name *glomerata* is preferred. Ecomorphic variations of these oysters among the islands may warrant designation as varieties, but further study is needed to decide this point. Stenzel (1971) would place these oysters in genus *Saccostrea* Dollfus and Dautzenberg, 1920.

The intertidal pink or coral rock oyster, which I collected in Palau Islands, Yap Islands, Fiji Islands and New Caledonia, is attached to the substrate by nearly all of the surface of the left valve which is thin in comparison with the upper or right valve. As a result it is difficult to remove these oysters from the substrate without cracking the shell. This oyster is triangular in cross section with crescent-shaped meats and usually with bright yellow mantle rim. Since the pink or coral oyster is a marine species occurring in full oceanic salinity it may have potential for culture in the low atolls. The pink or coral oyster was identified as *Crassostrea mordax* (Gould), 1850, although some authors have used the names *tuberculata*, *amasa* and *cucullata* for this species. Under Stenzel's (1971) classification system this oyster would be placed in genus *Saccostrea* Dollfus and Dautzenberg, 1920.

The green oyster occurs subtidally in various places in the South Pacific Islands and I have examined samples from Palau, Truk, American Samoa, French Polynesia and Cook Islands. These oysters, which are generally less than 50 mm in height, occur attached to coral or other substrate and are characterized by greenish interior, and by sharp crenulations which are apparent on both upper and lower valves. The green oyster of the tropics is identified as *Ostrea nomades* Iredale, 1939, although there is a possibility that further study will indicate that *O. crenulifera* Sowerby, 1871, may describe this same oyster, in which case that name would be preferred. Further study will be needed to determine if *O. plicatula*, *O. sandwichensis*, and *O. thaanumi* describe different species of oysters. Stenzel (1971) would place the green oysters in genus *Alectryonella* Sacco, 1897.

The hyotid, or subtidal rock oyster, is an extremely large subtidal oyster usually attached to coral heads or other hard substrate. I have observed specimens in Palau, Truk, Ponape, Fiji

Islands, American Samoa and New Caledonia. The most obvious characteristics of this oyster are its extremely large size, heavy shell and sharp crenulations along the lip. In some younger specimens the folds on the surface are more apparent and may be produced into tubular spines. This large oyster is identified as *Pycnodonte hyotis* (Linné), 1758, although Stenzel (1971) proposes a new genus *Hyotissa* for this oyster.

The cockscomb or bronze oyster is an unusual subtidal form of the South Pacific Islands, which has 6-12 deep, sharp radial folds extending to the margin. The exterior coloration is usually bronze-red, sometimes tinged with purple, and the interior is bronze or brown, as are the oyster meats. The name *Lopha cristagalli* (Linné), 1758, is used for the cockscomb oysters which I collected in the South Pacific Islands of Palau, Truk and Fiji, although Thomson (1954) considers this species as an ecomorph of a group including *Ostrea bresia* and *Ostrea folium* for which he uses the name *Ostrea folium* Linné, 1758. Stenzel (1971) places the cockscomb oyster in genus *Lopha* and subgenus *(Lopha).*

The paper included photographs and descriptions of these oysters.

DIVER OBSERVATIONS ON DISPOSAL OF DREDGE SPOIL AT DANA PASSAGE, WASHINGTON

C. Lynn Goodwin

Washington State Department of Fisheries Brinnon, Washington

Dredge spoil from Olympia Harbor navigation channel, composed primarily of soft mud, was barge-hauled to Dana Passage for disposal. This channel connects Case Inlet with various bays of southern Puget Sound and is about 2 miles long and ½ mile wide, with an average depth of 100 ft, and tidal currents up to 3 knots. Substrates in the disposal area before dumping were primarily firm sand with scattered shell and localized patches of gravel mixed with sand. The sand bottom supported populations of geoducks, *Panope generosa;* sea pens, *Ptilosarcus quadrangularis;* various species of sea anemonies, crabs, sculpins, and starfish. 21,000 cubic yards of clamshell dredged spoil was dumped on the disposal area. The majority of the barge loads were dumped within 100 ft of a marker buoy.

Observations made 3 days after disposal was finished, indicated the spoil was about 3 ft thick at the marker buoy, ½ ft 180 ft from the buoy, and beyond 245 ft only a trace of spoil could be found. Using 212 ft. as a radius of a circle upon which measureable quantities of spoil were found, an estimated 3.2 acres were affected. Assuming an average depth of 1½ ft, the volume of the spoil was estimated to be 7,880 cubic yards or 38% of the total disposed. Geoducks were buried and presumed killed within an 80 ft radius centered around the buoy, but were visible and appeared normal in areas where the spoil layer was 1 ft or less. Sea pens, sea anemonies, and other benthic organisms were buried and presumed killed under the thicker portions of the spoils.

Observations completed 4 months after disposal showed that the location of the spoil had not changed but the thickness had decreased due to scouring and settling. The volume of the spoil was estimated to be reduced to 25% of the total deposited. Geoducks were alive and apparently normal at every station where they were present before dumping, even at the marker buoy where the spoil was about 1¾ ft thick. Geoducks were able to reach the surface with their siphons. Large sea whips, *Stylata elongata,* sea pens, and sea anemonies were found in the spoil area. They were observed in the dredge site and may have been brought in with the spoil.

Large mounds of spoil were not observed as might be expected from barge dumping. These observations plus water turbidity measurements indicate that the barge loads remained intact as a discreet mass as they fell to the bottom. Upon contact with the bottom, the material spread out laterally resulting in a relatively flat layer. Thick deposits of spoil built up in the disposal zone, changing the substrate from a firm sandy bottom to a soft muddy one. Undoubtedly, benthic animals which could not escape the area were covered and killed by the thick layer of spoil. Some geoducks survived the disposal even where the spoil layer was thickest. Sea anemonies, and other organisms either survived the disposal operation or repopulated the disposal area after dumping was completed.

EPIZOOTIOLOGY OF *MARGARITIFERA MARGARITIFERA* (L.) (MOLLUSCA: MARGARITANIDAE) INFECTION IN SALMONID FISHES

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Environmental Protection Agency
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Glochidial development in freshwater mussels *(Margaritifera margaritifera)* located in the Siletz River, Oregon, was completed in 13 days at an average water temperature of 12.8 C. Glochidia were released by these mussels for 33 days, 13 May to 15 June, 1971.

The comparative susceptibility of four species of salmonid fishes, 30.5 to 87.0 mm in fork length, to glochidiosis was determined by examination of 343 caged and 177 free-swimming (native) fish for infection. Of the caged fish, 99% of the chinook salmon *(Oneorhynchus tshawytscha)*, 75% of the coho salmon *(O. kisutch)*, 88% of the cutthroat trout *(Salmo clarki)*, and 95% of the steelhead trout *(S. gairdneri)* were infected. There was a similar relationship in infection incidence in the native fish species. Mean infection intensities in the caged and native fish were: 446 and 399 for chinook salmon, 8 and 24 for coho salmon, and 72 and 88 for steelhead trout, respectively, and 212 for caged cutthroat trout (native trout were not captured).

This is the first detailed description of the metamorphosis of *M. margaritifera* glochidia in fish and the associated histopathology. Invading glochidia, 70 by 75 μ in size, increased in length by 500% during metamorphosis. Encysted glochidia occurred on the gill filaments, arches, rakers, and occasionally on the pseudobranchs of all fish species; however, most were on the lamellae of the filaments. Initially, the encysted glochidia have uneven walls approximately 15 μ in thickness, but as the parasite increases in size the outer wall, i.e., the part of the cyst that is not embedded in and surrounded by gill lamellae, becomes thinner. Also, approximately 15 gill lamellae may become fused to the wall. Except for the lamella grasped by the glochidium, blood apparently continues to flow through the capillaries of the fused lamellae. However, these lamellae apparently can no longer function in gas exchange, except for the outermost lamella. Parasites encysted on the side of the gill filament restrict blood flow by pinching the filamental arteries. Large cysts on the lamellae increase the physiological dead space in the water flow. Clubbing of the filaments results when large cysts are located near the distal end of the filament. These pathological changes in heavy infections may result in immediate death of the fish by asphyxiation. In less heavy infections, delayed mortality occurs due to secondary infection with fungi, probably *Saprolegnia* sp. The invading or exiting glochidia may provide portals of entry for the fungi.

WHY "FAT" OYSTERS DIE: A THEORY ON MORTALITY IN SOUTHERN PUGET SOUND

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Results from laboratory studies were compared with field data collected by the State of Washington, Department of Fisheries, to support the theory that oyster deaths in Puget Sound are a consequence of a bacterial disease. Water temperature, nutrient enrichment of the water, and the physiological condition of the oysters were shown to influence the outcome of the mortality.

AN OYSTER HATCHERY EVALUATION METHOD

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Oregon State University
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We evaluated the Oregon State University oyster hatchery for procedural efficiency and biological reliability. Efficiency was determined by recording man-hours expended on various tasks. Reliability records consisted of algal cell concentration, larval growth and survival and setting success. These records were maintained while the technician adhered to a strict routine of hatchery operation. Periodically the records were reviewed and the routine revised, if necessary, to alleviate problems of efficiency or reliability.

The evaluation revealed that the algal culture system was unreliable, indicated by a rise in man-hours expended in maintaining algal cultures, without a concurrent rise in demand for algae. This case points out the necessity of biological records to aid in interpreting man-hour/task data.

Importance of the evaluation lies in the fact that we have an effective evaluation method that is easily and inexpensively applied to a variety of situations. It is also significant that we have data to show how effort is expended in operating an oyster hatchery.

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[1] Supported in part by the National Oceanic and Atmospheric Administration, U.S. Department of Commerce; Institutional Sea Grant No. 04-3-158-4.

GRAYS HARBOR SHELLFISH INVESTIGATIONS

Louis Messmer and John M. Smith

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Aberdeen, Washington

Population density and size range sampling of *Mya arenaria* in Grays Harbor were carried out in the years 1971 and 1972. Commercially important quantities were not evident. A similar, less intensive investigation of Willapa Harbor yielded similar results.

Gonad samples were collected and evaluated to determine spawning times for *Mya*.

Ghost shrimp populations, *Callianassa gigas*, *C. californiensis*, and *Upogebia pugettensis*, were surveyed in Grays Harbor. High population densities were recorded in many parts of the bay. Extensive ghost shrimp beds are present and indications are that the *Callianassa* species have greatly extended their range in recent years.

Manila clam spat were planted at two gravelly locations in Grays Harbor in May 1973, to determine feasibility of more extensive clam culture. Preliminary sampling indicates good growth rates at both locations. Survival at two months ranged from a low of 3.2% to a high of 80%. The optimum planting density appeared to be 300 clams per m^2.

DISEASES OF SHELLFISH IN YAQUINA BAY, OREGON[1]

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Corvallis, Oregon

A disease with several potentially serious implications and ramifications — the so-called "neoplastic disease" of bivalve mollusks — has been reported in several species of economically important shellfish in Yaquina Bay. According to published and unpublished reports, the disease found in oysters *(Ostrea lurida, Crassostrea gigas)*, clams *(Macoma nasuta, M. iris)* and mussels *(Mytilus edulis)* exhibits several of the general criteria recognized as indications of neoplasia in vertebrates: proliferation of a single cell line, unrestricted infiltration, nuclear and mitotic abnormalities, and morbid, gross and histologic changes indicative of fatal outcome. However, it has not been universally accepted that these clearly abnormal conditions are, in fact, neoplastic or that it is even possible for molluscan cells or tissues to undergo malignant or neoplastic alterations.

A large sampling program is currently being conducted in an attempt to confirm the existence and authenticity of this disease. Information is also being accumulated which will hopefully provide clues relative to its etiology. Preliminary results, based on one year's sampling and tissue analysis of 700 oysters, were described and discussed.

As a result of this research program, a second disease was found in the native oyster, *O. lurida*, during the fall of 1972. The disease organism has been identified as a haplosporidan and constitutes the first report of a haplosporidan disease of *O. lurida* and perhaps of any *Ostrea* species.

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[1] Supported in part by the National Oceanic and Atmospheric Administration, U.S. Department of Commerce; Institutional Sea Grant 04-3-158-4.

RELATIONSHIP BETWEEN NUMBER AND SIZE OF PACIFIC OYSTER SPAT AND SUBSEQUENT GROWTH

Albert J. Scholz

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Brinnon, Washington

Fourteen different stocks from five areas of commercial seed production were grown at the North Bay Oyster Reserve. Plantings of two or more stocks were made each year from 1968 through 1972. Seed with high numbers of spat per shell grew more slowly than lower count seed for the first 6 months after planting. There was no difference in growth rate from 6-18 months after planting. There was no evidence of any difference in potential growth among the stocks tested.

A CONTINUOUS ALGAL CULTURE SYSTEM FOR FEEDING SHELLFISH

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University of Washington
College of Fisheries
Seattle, Washington

Our Sea Grant research has shown that continuous cultures are a practical way to produce large amounts of algae in small amounts of space. Given a 8′ x 6′ floor space, 2.0 x 10^{11} cells/day (equal to 1,000 l of 2 x 10^{5} cells/ml, in theory, enough to feed 200 cases of oyster larvae) can be produced on a daily basis with only a single line in operation. The space is adequate for a double line. The unit requires replenishment of pasteurized sea water and sterilized enrichment solution; a 2% CO_2-air source; and removal of the yield if it is stored. The unit may require cleaning at approximately monthly intervals due to wall growth, but this is highly variable and a unit may remain functional for three months or longer. Since there is space for two independent cultures, there is reasonable assurance of an uninterrupted supply of algae.

The approximate cost of our unit can range from $1,000 to $2,500 depending on the use of a shaker, the brand of pump, the type of gas-mixing apparatus and refrigeration. The cost and complexity of the unit can almost certainly be reduced for hatchery use.

The major labor requirement is for pasteurization of the sea water which we currently do in batches. If an in-line pasteurization procedure were used, the remaining labor needs would be small.

Proceedings of the National Shellfisheries Association
Volume 64 - 1974

LOBSTERS OF THE NORTHWEST COAST OF NEWFOUNDLAND, 1964-67

H. J. Squires[1], *G. P. Ennis and G. E. Tucker*
FISHERIES RESEARCH BOARD OF CANADA
BIOLOGICAL STATION, ST. JOHN'S, NEWFOUNDLAND

ABSTRACT

Lobsters of Northwest Newfoundland were located in a narrow (1-2 km) band along the largely unsheltered coast, among glacial boulder train or coarse talus and in potholes, joints and fractures in the limestone bedrock just offshore. The 400 km coastline studied gave an area of lobster grounds no more than 600 km², and from this area about 500 tons of lobsters were taken each year. Since the fishery took about 60% of the commercial-size lobsters annually, the total stock in any year was probably 800 tons, and average density on the grounds was therefore about 1 per 108 m². Temperatures were low: 0 C in winter, 5 C in early June and 16 C in late August, and salinities about 30% throughout the year. Females were first mature at 73 mm in carapace length at Sallys Cove and 67 mm at Port aux Choix. About 50% spawned annually and numbers of eggs carried to hatching were 8,000 to 16,000 at lengths of 74-97 mm. At these lengths the gain in weight after one moult was 47% and 101% after two moults. A forced reduction in fishing because of a storm in 1966 showed up in a reduced proportion of 1st year recruits in 1967.

INTRODUCTION

Detailed studies of lobster populations just south of our study area were done by Squires (1970) and Squires *et al.* (1971). Their data and those of the present study are comparable. Earlier data obtained by Templeman (1939) and Templeman and Tibbo (1945) are not. Although the latter used measurements of lobsters from the commercial catch to calculate proportions of small ones present in an area, they did not separate males from females in samples and emphasized average lengths for comparisons between areas. Also, although they recognized that different proportions of small animals could indicate different fishing rates between areas, they attributed the presence of large proportions of small ones to settlement of large numbers of larvae. Refutation of this thesis was proposed by Squires (1970) and Squires *et al.* (1971).

Objectives of the present study were: to examine the grounds for topographical features giving shelter or which might contribute to distribution and abundance of lobsters; to determine the temperature and salinity changes throughout the year which might affect size or maturity; to estimate rates of fishing on adjoining fishing grounds and estimate stock size and density; to identify possibilities of increasing production by changes in fishing rates or improving grounds, and to compare lobsters from different areas.

The following sections give an account of the predominant features of lobster grounds on this coast, the temperature regime, the fishery and fishing rates, an estimate of total stock and density, and some aspects of the biology of the lobsters.

DESCRIPTION OF THE LOBSTER GROUNDS

The following is modified from a description of

[1] Present address: Division of Fisheries, Box 358, Suva, Fiji Islands.

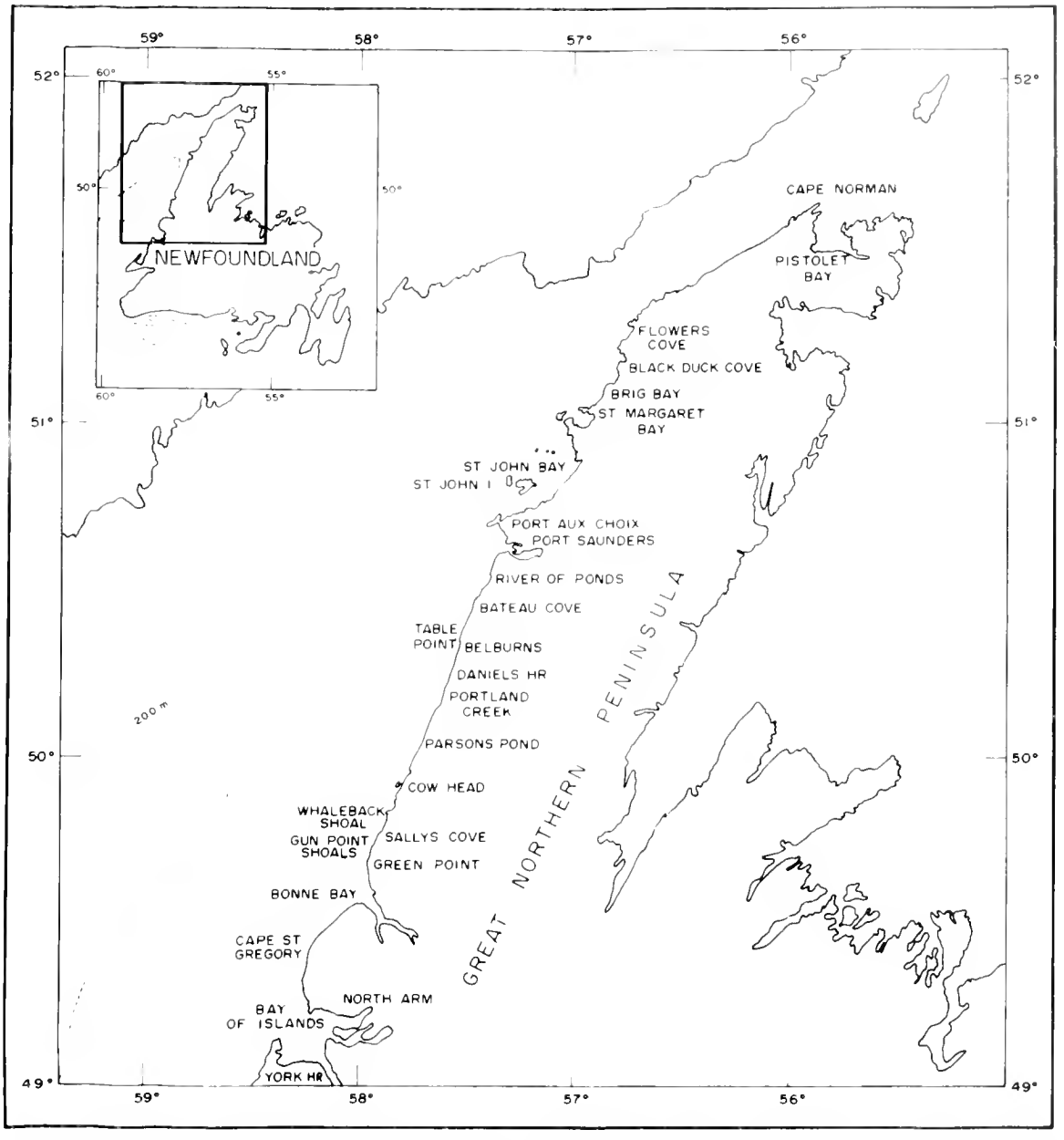

FIG. 1. *Map of place names used in the text. Lobster grounds are in a narrow band quite near the coast.*

the grounds by the late Hugh Lilly, a geologist from the Memorial University of Newfoundland. His observations from SCUBA diving surveys (Lilly, 1965, unpublished) are supplemented by ours in some of the areas.

Erosional processes which produced the landscape of western Newfoundland also provided the material which now rests upon the submarine shelf. These influence the distribution of benthic animals, including lobsters. The effects of Pleistocene glaciation are recognized everywhere, for the glaciers scooped out the fjords such as Bay of Islands and Bonne Bay and deposited much of the resulting debris as terminal

moraines at the mouths of the fjords or nearby. In many places the ice left the hills bare of residual soils and clays, adding this material to the shelf.

The coastal lowlands from Bonne Bay to Cape Norman include the raised postglacial terraces of the Great Northern Peninsula. Several terrace levels reflect the development of temporary shorelines during periods of interrupted uplift. The abundance of glacial material derived from the uplifted terraces nearby provide cover for lobsters. Much of the sand and silt of the scallop environment is reworked outwash of glacial origin, refurbished now by attrition of the present shoreline. Much of the other coastal areas are subject to vigorous wave action, and where the waters are shallow, cliff talus appears to become ground up or carried away as fast as it falls into the sea.

The coastal marine shelves in some areas do not exceed 55 m in depth within 18 km from the coast. They are gently rolling, averaging 45 m in depth with numerous shallow areas less than 37 m. Gun Point Shoal and Whaleback Shoal are examples. West of Sallys Cove, south of Whaleback Shoal and extending 16 km from the coast are the remains of an immense terminal moraine. Rock fragments representing the Precambrian crystalline complex of the northern Long Range Mountains, the St. Georges carbonate group and the Humber Arm group are found in this deposit.

On the Whaleback Shoal are exposures of bedrock and long reaches of sand where the action of waves produce effects to depths of at least 36 m between the shoals. Talus is not abundant everywhere in the exposed zone. The lobsters seem to frequent numerous potholes, joints and fractures in bedrock in these areas and near cliffs such as at Table Point, and the coarse talus is found well away from the shore, often at distances of several hundred metres. On some of the more sheltered lobster grounds, such as in St. John Bay, the bottom consists of shingle, talus derived from submarine and shore bedrock and glacial boulder train.

AREA OF THE LOBSTER GROUNDS FROM CAPE GREGORY TO FLOWERS COVE AND AN ESTIMATE OF THE POTENTIAL STOCK AND DENSITY OF LOBSTERS

The coastline from Cape Gregory to Flowers Cove is about 400 km long. Although lobsters were occasionally seen as deep as 35 m where diving was done along the coast, some areas just offshore such as the large shoal west of Sallys Cove were completely devoid of lobsters as shown by special fishing (Andrew Swim, personal communication). As a result of many years of fishing, trapping was done along the coast usually not more than 2 km from the shore and not deeper than 30 m. The lobster grounds, therefore, appear to be scarcely wider on the average than one and one-half km and the total area to be not more than 600 km^2 (Fig. 1).

Since the total catch from the lobster grounds has been about 500 tons annually over several years and the fishing rate observed in 1966 and 1967 was little more than an average of 60% in most areas, the potential stock of commercial sizes on the grounds would be about 800 tons annually.

From this available annual stock of approximately 1.8 million lbs the first year recruits, averaging close to 1 lb each, would equal about 1.1 million lobsters (60% of the total). The post-recruits, averaging slightly more than 1.5 lbs each, would equal about 0.4 million lobsters. The pre-recruit groups (two size groups smaller than the legal sizes are usually seen on the grounds) would both approximately equal in number the recruit group, or about 2.2 million lobsters. In total, therefore, there would be about 3.7 million lobsters of near-commercial size on these grounds at the beginning of the fishing season in any year. The density of lobsters on these grounds can be calculated from:

$$\frac{\text{area of the grounds}}{\text{total number of lobsters}} = \frac{600{,}000{,}000\ m^2}{3{,}700{,}000} = 108\ m^2 \text{ for each lobster.}$$

THE LOBSTER FISHERY FROM CAPE GREGORY TO FLOWERS COVE

During 1955-1969 the annual landings of lobsters in this area fluctuated between 320 and 510 tons (Fig. 2). They represented about one-quarter of the total Newfoundland landings each year. In the last five years the lowest catch in the area occurred in 1966, when about 370 tons were landed.

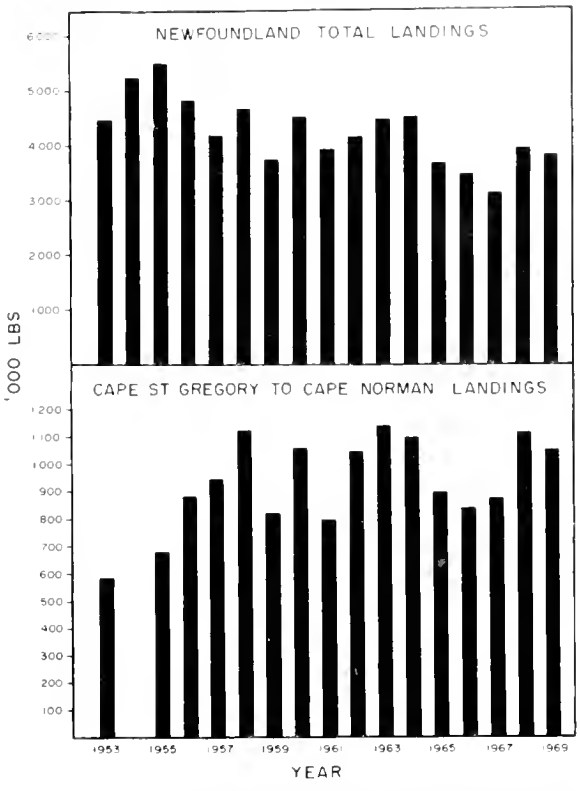

FIG. 2. *Lobster landings for the northwest coast (Cape St. Gregory to Cape Norman) and for all of Newfoundland in 1953-69.*

In that year, near the middle of the fishing season, a strong gale from the southwest destroyed a large proportion of the lobster traps along the coast. The fishermen recovered as many traps as they could to resume fishing after the storm, but their fishing effort during the season was appreciably reduced. In 1967 size-samplings from the commercial catch, the proportions of small lobsters were less than they had been in 1966. The reduced fishing rate in 1966 had left a considerable number of commercial-sized lobsters on the grounds and these contributed to the larger than usual proportion of large lobsters in the catch in 1967.

ESTIMATING FISHING RATES

Fishing rates may be estimated from the proportion of first year recruits appearing in histograms of length composition of the commercial catch of uninjured male lobsters (Squires, 1965; 1970; Squires *et al.*, 1971). The five populations from which length measurements had been obtained in both 1966 and 1967 (affected by the storm mentioned in the previous section) all had an appreciable decrease (4-10%) in the proportions of first year recruits present (Fig. 3). These comparisons within areas indicated that a change in fishing rate in one year would show up in the following year.

Comparisons between areas along the coast showed that fishing rates were higher, in most instances, from Bonne Bay to Port aux Choix than further north. Areas to the north had less than 60% first year recruits in samples while to the south, almost all had more than 60% (Figs. 3 and 4). Pistolet Bay, the area of lobster fishing

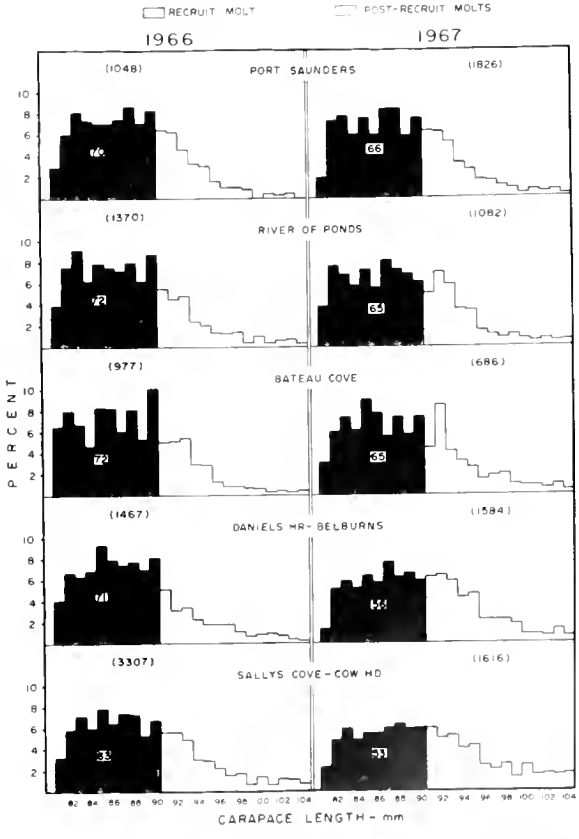

FIG. 3 *Percentage occurrence of first year recruits (first year at legal size) in histograms of length frequencies of commercial lobsters from the same areas in consecutive years (1966 and 67).*

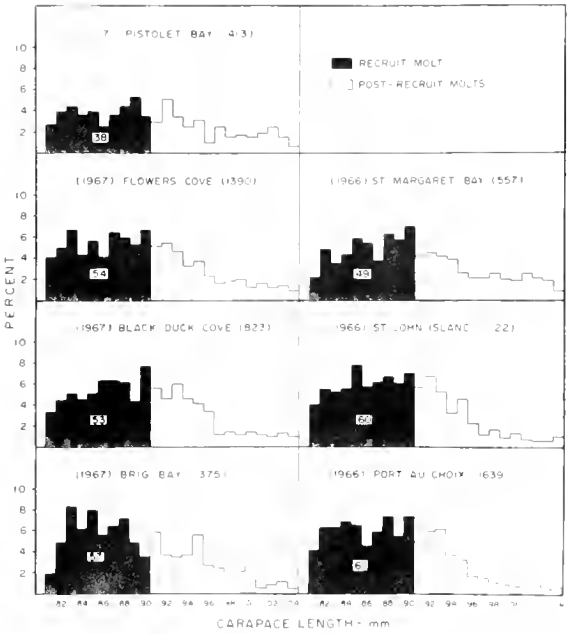

FIG. 4. *Percentage occurrence of first year recruits in length frequencies of commercial lobsters from the northwest coast of Newfoundland.*

farthest north in America, had only 38% because the fishery had started only recently in this bay and only a few traps were used.

COMPARATIVE BIOLOGY

Lobsters for biological studies were obtained from fishermen trapping about 150 specimens monthly on charter at Sallys Cove, Cow Head and Port aux Choix from June to October, 1966. Data on 460 males and 650 females from the three locations were combined. Details on each specimen included: total live weight and claw weight (weight of 1st pereiopods) on a Mettler precision balance; carapace length and length of claws with a vernier calipers; total lengths and tail widths on a mm measuring board, and egg and ova diameters under stereomicroscope on a mm scale (see also Squires, 1970). An experiment on growth of lobsters held and fed in metal cages was conducted at Port aux Choix in 1966 (Stewart and Squires, 1967). From the data on weights, *etc.*, the lobsters of the areas sampled were compared with those from Port au Port Bay, North Arm and York Harbour (Squires, 1970; Squires *et al.*, 1971).

Relative lengths and weights.

The graphs of chelae weight as a percent of total weight (Fig. 5) showed that in this respect the lobsters of the northwest coast were intermediate between North Arm and Port au Port Bay lobsters. Size-for-size they were more attractive market lobsters than those from Port au Port Bay because of their larger claws.

Regression equations of claw size in this paper (Table 1) and in others (Squires, 1970; Squires *et al.*, 1971) show that the crusher claw contributes most to the differences between lobsters from different areas. For example, in West Coast samples the slope of the cutter claw lengths varies only from 1.50-1.52 CL while the slope of the crusher claw lengths varies from 1.46-1.68 CL in male lobsters. (But in Bonavista Bay (Ennis, 1971) the slope in both cutter and crusher claws is the same at 1.41 CL. The weights of the crusher claw show even more distinct differences between lobsters from different areas than lengths (the range in slope of regression curves in the four West Coast areas was from 3.5566 log CL-4.3045 log CL). The comparative weights of cutter claws showed similar differences: greater in males than in females and different from area to area, but the range was less than in crusher claws. However, adding the weights of both claws together would make more distinct differences between areas, substantiating the use of this parameter as a percentage of total weight to show differences between areas (Fig. 5).

Growth increment.

Data from the experiment with lobsters moulting in cages at Port aux Choix indicated that increments in the carapace length of males varied rom 7-13 mm and averaged about 11 mm, increasing with the size of the lobster (Fig. 6). In females increments were 6-10 mm with an average of about 9 mm and decreased with the size of the lobster. The average increase in carapace length was 16.5% in males and 11.5% in females. Length increments of male lobsters from Port au Port Bay, North Arm, York Harbour and Bonavista Bay were estimated to be on the average 10, 9, 10 and 12 mm, respectively.

Hypothetical gain in weight on moulting.

The method of calculating hypothetical gain in weight from regression equations was described

TABLE 1. *Length and weight relationship equations for lobsters from the northwest coast of Newfoundland. TL = total length, CL = carapace length, Cut = cutter claw, Cru = crusher claw, Abd Wd = abdomen width, TW = total weight, W = weight. Logs are to base 10. Body weight = total weight less weight of 1st periopods.*

Relationship	Sex	Equation	Length range	C.c.	No. of Specimens
TL/CL	M	TL = 2.53 CL - 23.96	47-113	0.98	456
TL/CL	F	TL = 2.84 CL - 6.13	47-126	0.99	590
Cut L/CL	M	Cut L = 1.51 CL - 11.14	47-113	0.96	433
Cut L/CL	F	Cut L = 1.29 CL - 4.70	47-126	0.96	567
Cru L/CL	M	Cru L = 1.56 CL - 23.43	48-113	0.96	449
Cru L/CL	F	Cru L = 1.23 CL - 0.40	47-126	0.97	584
Abd Wd/CL	F	Abd Wd = 0.91 CL - 19.00	47-126	0.94	621
TW/CL	M	Log W = 3.1637 log CL - 3.4354	48-113	0.99	422
TW/CL	F	Log W = 2.9660 log CL - 3.0236	47-126	0.99	552
Body W/CL	M	Log Body W = 3.1358 log CL - 3.0764	48-113	0.99	422
Body W/CL	F	Log Body W = 3.0482 log CL - 3.3417	47-126	0.99	552
Cut W/CL	M	Log Cut W = 3.3567 log CL - 4.5857	47-113	0.97	437
Cut W/CL	F	Log Cut W = 2.6838 log CL - 3.3469	47-126	0.97	562
Cru W/CL	M	Log Cru W = 3.7597 log CL - 5.2340	48-113	0.97	446
Cru W/CL	F	Log Cru W = 2.8793 log CL - 3.6291	47-126	0.97	580
Cut W/Cut L	M	Log Cut W = 3.0188 log Cut L - 4.3521	61-166*	0.98	428
Cut W/Cut L	F	Log Cut W = 2.6700 log Cut L - 3.6584	63-175	0.97	557
Cru W/Cru L	M	Log Cru W = 3.1215 log Cru L - 4.3409	57-165*	0.99	442
Cru W/Cru L	F	Log Cru W = 2.8257 log Cru L - 3.7843	59-165	0.98	575

* Range of length of cutter or crusher claw

by Squires (1970). With a moult increment of 11 mm in males from this area, the length groups used were 70-80 mm, 81-91 mm and 92-102 mm. These groups were chosen to represent the pre-recruit, recruit and post-recruit sizes of lobsters. (The legal minimum size is 81 mm in carapace length.) From the data on weights at each carapace length the gain in weight on moulting was calculated to be 47% in one and 101% in two moults. This gain for northwest coast lobsters was less than for Port au Port Bay or York Harbour lobsters: 49% and 117%, and 59% and 119%, respectively. However it was somewhat larger than for North Arm lobsters: 45% and 100% in one and two moults (Squires *et al.*, 1971).

Maturity.

From the data for June and October (Table 4) the female lobsters of Sallys Cove and Cow Head were close to the expected 50% potentially ovigerous each year such as is found in these populations (Squires, 1970). The 70% ovigerous in the month of August was unusually large, but most of them had recently laid eggs (eggs were 100% yolky), and in this period when non-ovigerous females were moulting, the number of ovigerous animals entering traps to feed could be disproportionately high. The percentage potentially ovigerous at Port aux Choix in July (Table 2) was 44% substantially lower than the expected 50%. However, the data for one year might not be enough to indicate even in this nor-

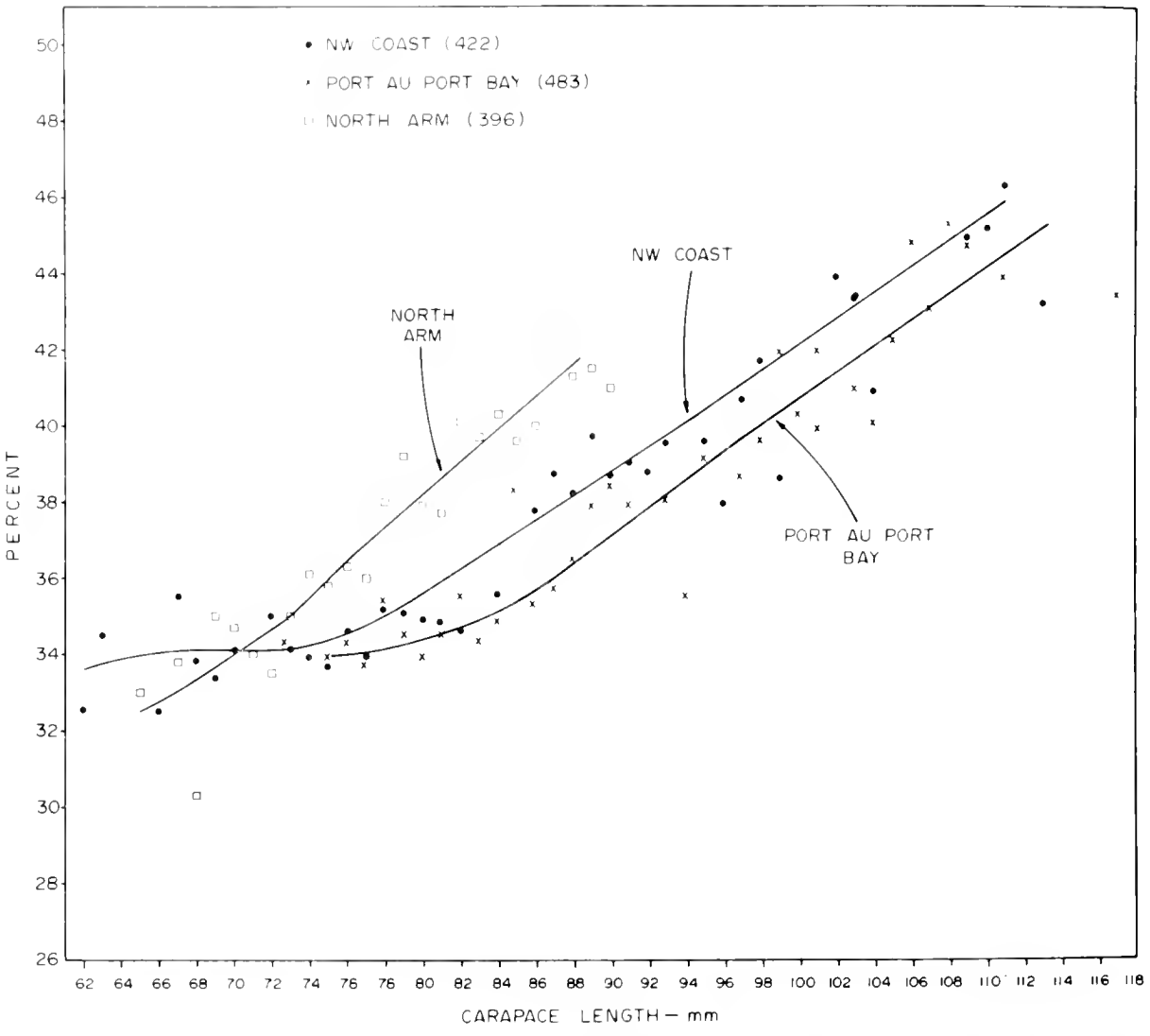

FIG. 5. *Claw (chelae) weight as a percentage of total weight in lobsters from North Arm, Port au Port Bay and the northwest coast of Newfoundland.*

therly area that the rate of egg-laying could be as low as was seen in lobsters at York Harbour, Bay of Islands (Squires *et al.*, 1971).

Size at first maturity.

Female lobsters from Sallys Cove and Cow Head were first mature at 73 mm in carapace length. This was the smallest size seen with large ova (Table 2). The smallest with large ova in Port aux Choix, however, was only 67 mm. These samples were obtained from the partly enclosed area of St. John Bay, resembling North Arm in this way, although the summer temperatures were lower. They were lower than off Cow Head and Sallys Cove so that temperatures could not account for the earlier maturity. Combined data from lobster sampling on the coast for abdomen width as a percentage of carapace length showed an acceleration in the rate of increase in abdomen width through the sizes of 64-76 mm (Fig. 7), representing the range of sizes when maturity first occurred in female lobsters from these areas. areas.

TABLE 2. *First mature sizes and ovigerous or potentially ovigerous female adult lobsters from the northwest coast of Newfoundland in 1966.*

Month	No. of adult specimens	Carapace length range of specimens mm	Percent ovigerous %	Percent potentially ovigerous %	Minimum size ovigerous mm	Minimum size with large ova mm	Maximum size juvenile mm	Area
June	170	52–107	15	52	75	73	71	Sallys Cove & Cow Head
August	119	67–116	70	23	–	–	–	" "
October	64	67–102	22	53	81	74	70	" "
July	50	61– 96	10	44	80	67	61	Port aux Choix
August	65	50–102	28	37	72	68	73	" "

Fecundity.

Lobsters from Sallys Cove and Cow Head had average counts of 8,200-16,300 eggs ready to hatch, according to carapace lengths of 74-97 mm (Fig. 8). These counts were higher than those from lobsters of the same length from Port au Port Bay: 6,200 – 15,000 (Squires, 1970). This finding supports the assumption that fecundities of lobsters from different areas may differ. Counts of recently laid eggs were higher than of eggs ready to be hatched in lobsters larger than 80 mm (Fig. 8) in agreement with findings of Saila *et al.* (1969).

DISCUSSION

Fishing conditions on the northwest coast.

In Lilly's (1965) report the reference to intense wave action suggests that the lobster grounds on this coast can be fished only with difficulty. Attrition of the shore and destruction of talus blocks, *etc.*, are evidence of occasional heavy winds and waves on this open coast. Occasionally, such as in 1966, a storm destroys almost all the lobster traps. There are few harbours and fewer villages where boats can be berthed without having to be hauled up on the beach, out of reach of the waves, or where floating lobster crates can be anchored in safety. Harbour improvement, such as breakwaters that might be built at some of the villages, would be of value if they were adequate for protecting the lobster-holding crates under storm conditions. However, the lobster fishery can be carried on with the small boats presently in use because the grounds are located close to shore. The use of plastic traps in addition to, or instead of, wooden ones would help to sustain fishing effort through stormy periods.

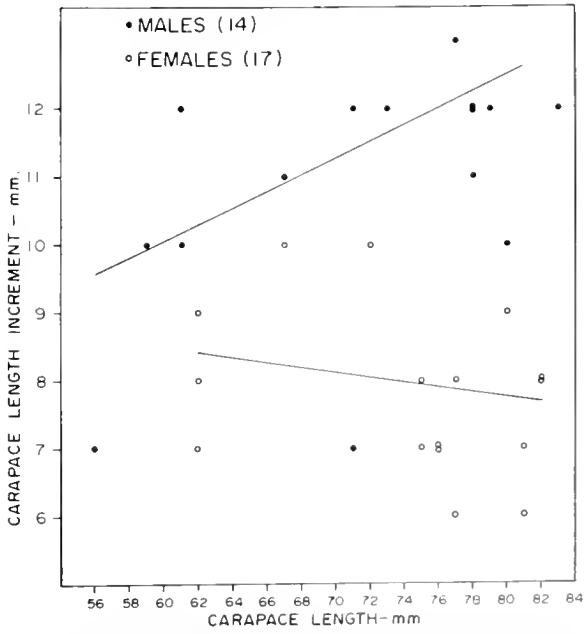

FIG. 6. *Moult increment in carapace length of male and female lobsters from the northwest coast of Newfoundland.*

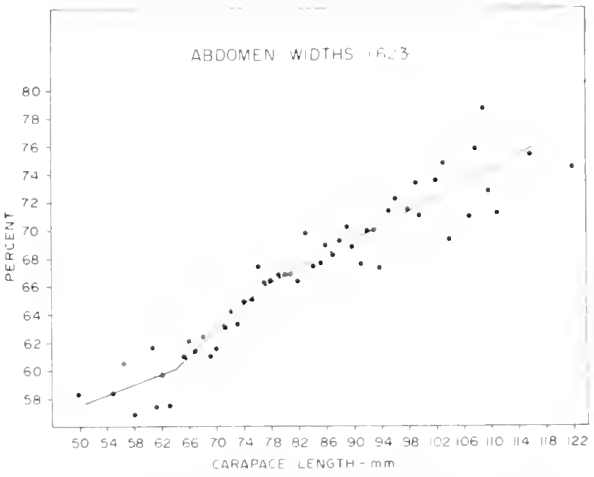

FIG. 7. *Abdomen widths as a percent of carapace lengths in female lobsters from the northwest coast of Newfoundland.*

The characteristics of the exposed shoreline, as described by Lilly, leave little possibility of improving the grounds by blasting cliffs, for example, to provide more sheltering rubble for lobsters. Only in partly enclosed areas, such as Port aux Choix, could this be effective (Lilly, 1965, and personal communication).

Fishing rates.

An estimate of a comparative fishing rate for lobsters based on the proportion of first year recruits in the commercial landings (Squires, 1965; 1970) may be more reliable than one based on fishing effort, which is difficult to define with accuracy. Fishing effort is not only the number of traps used or the number of times they are hauled; it is also the skill of fishing experience, which can put traps in advantageous positions on the grounds, and the frequent changes in position and replenishment of fresh bait each time a trap is hauled. Results can be dependant also on density of lobsters on the grounds, scarcity of natural food for the lobsters, *etc.*, all of which are difficult or impossible to measure. An estimate independant of these and based only on the composition of the commercial catch has a distinct advantage. A representative sample of the catch can be secured by sufficient measurements of specimens before the moulting period. About 1,000 obtained from each fishing ground appears adequate for comparisons between grounds.

With first year recruits comprising 60-70% of the lobster landings on the coast south of Port aux Choix, an increase in fishing could not be recommended. The fishing rate apparently made best use of the resource as shown by only slight fluctuations in annual landings. The accidental change in rate in 1966 was clearly apparent in 1967 indicating that the method of estimating fishing rates was applicable and also that its sensitivity might be due to a maximum level of fishing in most years. Low proportions of first year recruits, such as the 38% in Pistolet Bay, suggested that more fishing could be done to catch the large lobsters present but that these would soon decrease under fishing pressures.

Recovery from moulting and egg-laying.

The high percentage of ovigerous females captured in August (Table 2) indicated that they had laid eggs recently and were searching for food. Although the number of non-ovigerous females and males had been reduced on the grounds as a result of fishing, many of them could have been in the process of moulting and not feeding. This was shown in later months when the proportions of ovigerous ones were much less. The search for food by lobsters entering traps is an indication of the general paucity of natural lobster food on the grounds. The appropriateness of supplemental feeding is worth considering in the period after

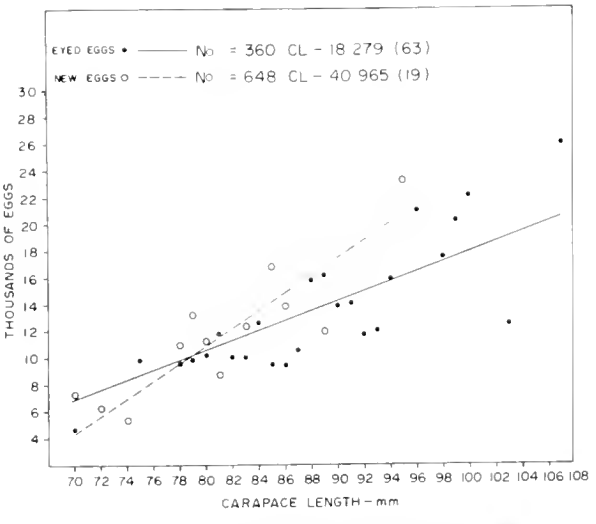

FIG. 8. *Numbers of eggs (recently laid or eyed and ready to hatch) at each carapace length in lobsters from the northwest coast of Newfoundland.*

moulting or egg-laying, to assist in the recovery of body proteins lost as a result of cessation of feeding (Squires *et al.*, 1971). Shore fisheries that leave offal or dead fish on the lobster grounds may be important to production. Dying-out of shore fisheries may be detrimental to lobster populations, and may have caused the decrease in lobster production in such areas as Conception Bay.

Comparing lobster populations.

Given sufficient data, comparisons may be made using the following characteristics: relative sizes of claws in males, percentage increase in weight as moulting in uninjured males, sizes of females when first mature, numbers of eggs carried, patterns of behaviour in nature, *etc.* Some of these may be attributed to conditions of the environment such as prevailing temperatures (phenotypical differences) or evolved attributes, as a result of long isolation (genotypical differences). Perhaps the most important, from the point of view of the fishery, are the patterns of behaviour such as those that keep the lobsters a relatively constant distance from the noise of surf at the shore. The lobsters of the northwest coast kept to a narrow band of seabed near the coast, not venturing any deeper than 35 m for the most part. Although there were extensive shallow banks not far from the coast with temperatures similar to the nearshore grounds, no lobsters could be trapped there. Yet, lobsters are found many miles from shore off southwestern Nova Scotia, for example (Wilder, 1947). If the lobsters of the northwest coast were genotypical in their behaviour pattern, it might be said that they would make ideal transplants to exposed coasts, where they would not be likely to wander into deep water nor to come too near the shore, but to build up a population within trapping distance.

Maturity and estimates of potential spawning.

Oogenesis, in these lobsters of northern low temperatures, was unmistakeably slow but accelerated during the summer, when temperatures became relatively high for a short period. The differences in sizes of ova would, therefore, have to be carefully defined in order to indicate whether they would be spawned in the current year. For example, ova less than 1.0 mm in diameter in June and July could not be expected to be spawned in August; and ova of 1.3 mm in diameter in September would not be spawned until the following year. The percentages of potentially ovigerous females observed by Ennis (1971) were based on a definition of "ova larger than the ova of ovigerous females" (about 0.5 mm or less) and were, therefore, unusually high. Generally, the potential spawners are about 50% or less in these areas.

Prevailing temperatures and salinities.

The general hydrography of the Gulf of St. Lawrence has been described by Lauzier *et al.* (1957) and Lauzier and Bailey (1957). With reference to the northwest coast of Newfoundland

TABLE 3. *Temperatures (C) at stations on the lobster grounds off the northwest coast of Newfoundland 1966.*

Station	JUNE					JULY		AUGUST				Depth
	4	10	18	25	28	19	27	9	17	23	31	
Green Point	7.3	–	–	–	10.6	–	14.0	–	–	16.8	–	Surface
	4.8	–	–	–	8.3	–	13.5	–	–	16.3	–	15 m
Cow Head	7.4	–	–	–	10.8	–	14.8	–	–	16.4	–	Surface
	5.0	–	–	–	8.6	–	14.5	–	–	15.8	–	15 m
Port aux Choix	—	7.1	8.6	8.1	—	11.8	14.3	15.4	16.2	15.8	15.0	Surface
	—	6.4	7.0	7.6	—	11.6	13.0	14.9	14.9	14.7	14.8	15 m

TABLE 4. *Salinities (‰) at stations on the lobster grounds off the northwest coast, 1966.*

Station	JUNE					JULY		AUGUST				Depth
	4	10	18	25	28	19	27	9	17	23	31	
Green Point	30.8	--	-	-	29.8	-	30.0	-	-	30.2	-	Surface
	30.6	-	-	-	30.4	-	30.3	-	-	29.8	-	15 m
Cow Head	27.8	-	-	-	30.3	-	29.6	-	-	29.8	-	Surface
	30.3	-	-	-	30.3	-	29.7	-	-	29.8	-	15 m
Port aux Choix	-	30.0	29.6	30.1	-	30.0	29.8	30.1	29.7	29.9	29.7	Surface
	-	30.5	30.1	30.1	-	30.3	29.9	30.1	29.7	29.9	29.9	15 m

they showed late spring and early autumn temperatures to be about 5 C at 20 m.

During the present investigations, temperatures on the lobster grounds (at about 15 m) increased from nearly 5 C to about 16 C from June to August. Surface temperatures were somewhat higher but began to decrease late in August when the peak had been passed. This peak was later off Green Point then off Port aux Choix, about 200 km farther north.

From previous work in Port au Port Bay and Bay of Islands (Squires, 1970; Squires, *et al.*, 1971) and personal experience of the senior author, temperatures were seen to decrease to somewhat lower than 0 C on the lobster grounds from January to March and there was ice cover at some time during the winter. Although the prevailing set of the current is toward the coast and northward, as described by various authors, offshore winds were seen to carry ice away from the coast, leaving clear water while they lasted. With the return of onshore winds, the ice soon re-appeared on the horizon and grounded again on the shore in less than a day. The ice cover often persisted into May but the prevailing currents brought warm water into the area and by early June the temperatures reached 5 C on the lobster grounds (Table 3).

Salinities did not vary much and were close to 30‰ throughout the year (Table 4). Some slight variations were a low reading of 27.8‰ off Cow Head in June, and a few high readings slightly over 32‰ in St. John Bay, also in June.

ACKNOWLEDGEMENTS

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DEPTH DISTRIBUTION AND SIZE OF SPOT SHRIMP, *PANDALUS PLATYCEROS*, TRAWLED IN DABOB BAY OF HOOD CANAL, WASHINGTON FROM 1966 to 1971 [1]

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ABSTRACT

The January size frequency distribution of male and female spot shrimp (Pandalus platyceros) *was presented and discussed. The females revealed more variability from year to year. There appeared to be two size groups of females in some years. The male size distribution was more consistent.*

The 1966, 1968, 1969, 1970 and 1971 spot shrimp length-weight lines were compared and the similarities and differences from year to year were shown. Further, it was shown that this species of shrimp may have a diel migration pattern. Trawl hauls for 1970 and 1971 revealed they were found to be greatest in numbers at shallower depths during the nighttime and greatest in numbers at deeper depths during the daytime.

INTRODUCTION

The spot shrimp *(Pandalus platyceros)* is caught commercially by pot gear in Dabob Bay and other parts of Hood Canal. Approximately 67,000 pounds were landed in 1972 (pers. comm. Mr. Dale Ward, Washington Department of Fisheries) and sold locally in Hood Canal and throughout the Puget Sound region. Small pot fisheries for this species also occur near Carmel in California; Cook Inlet and southeastern areas of Alaska; and off British Columbia.

Several biological studies of the spot shrimp have been conducted by various researchers; Berkeley (1930), Butler (1964, 1970), Hynes (1930) and Price and Chew (1972). This paper represents data collected in trawl-caught spot shrimp from 6 consecutive years (1966-1971) during the month of January in Dabob Bay in Hood Canal, Washington. The objective of the study was to determine the size distribution, length-weight relationship and daily migration pattern for the local population of spot shrimp in Dabob Bay.

MATERIALS AND METHODS

The shrimp samples were taken with a 40 ft. semi-balloon, Gulf of Mexico shrimp trawl using the University of Washington research vessel M/V *Commando*. The stretch mesh size of the cod end was ¼ inch and the outside linear ½ inch.

Day and nighttime tows averaging 20 minutes in duration, were made at the four depths as shown in Fig. 1. Sampling for the respective years were either the second or third week of January. The shrimp were sorted, weighed and placed in heavy plastic bags for freezing on the vessel. They were later thawed in the laboratory for processing. The sexes were separated, and the carapace length (mm) and corresponding individual weight (g) were measured. The sexes were separated by examination of the inner ramus of the first

[1] Contribution No. 399, College of Fisheries, University of Washington.

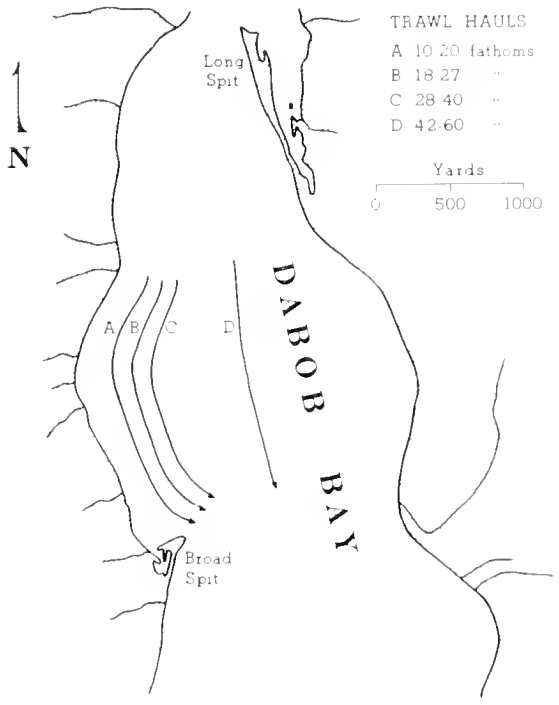

FIG. 1. *Location of trawl hauls taken in Dabob Bay.*

pleopod (Berkeley, 1930) or by the presence of eggs attached to the pleopods. The length was taken between the base of the eye socket to dorsoposterior point of the carapace. Individual weights were not taken if any part of the rostrum was broken off, or if the specimen was badly damaged. More than 8,000 individual measurements were obtained over the six years. Sub-sampling was necessary at times when large catches were made.

RESULTS AND DISCUSSION

Length Frequency Distribution

Fig. 2 presents the carapace length frequency distribution of male and female spot shrimp collected from 1966 through 1971. The distribution was expressed in percentages and discussed briefly as follows:

1966: Only eighteen females were measured and the correct distribution may not be as shown in Fig. 2. However, it was interesting to note that the males and females were of the same size range, reflecting an overlap. The

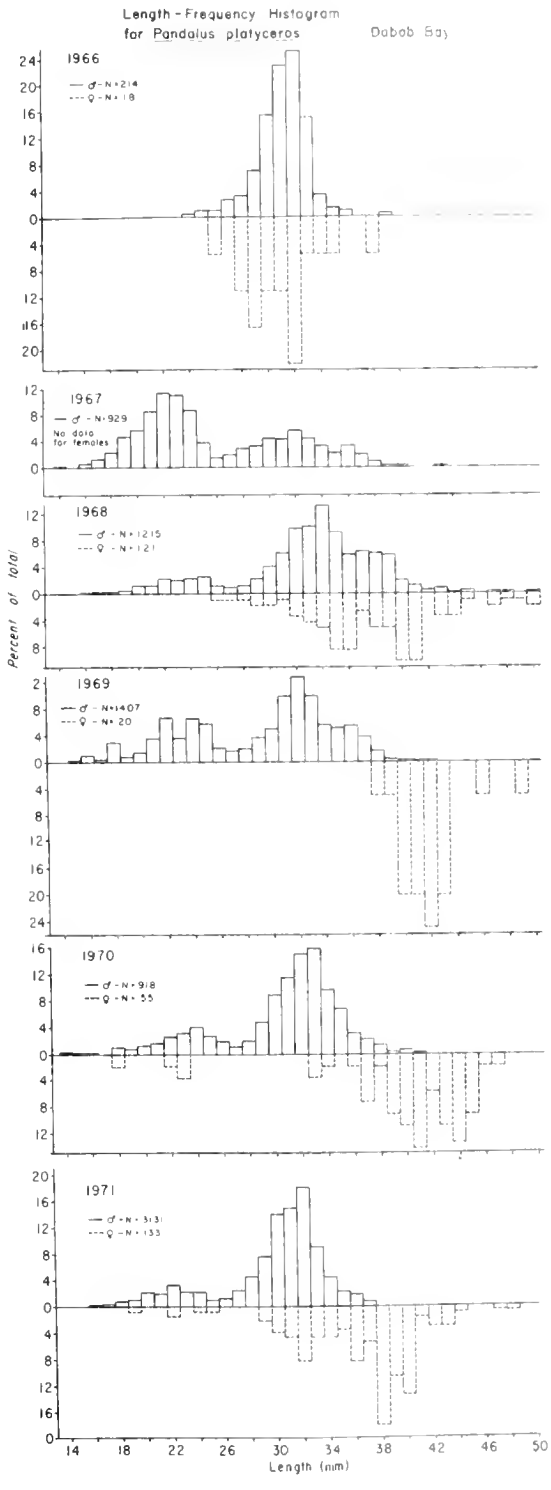

FIG. 2. *Carapace length-frequency distribution of spot shrimp from 1966 through 1971.*

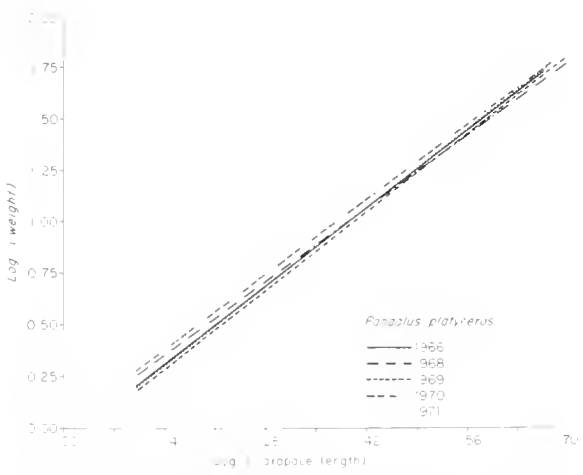

FIG. 3. *Length-weight relationship of Dabob Bay spot shrimp for five different years.*

reason for this overlap was unclear. Comparisons with subsequent years of male distribution would indicate we caught mostly 1+ or second year males. It has generally been recognized that spot shrimp in Dabob Bay can bear eggs as early as September-October through February and hatching could occur between December and February. Egg bearing females have been noted as late as March in some years.

1967: No female measurements were taken this year. Nine-hundred twenty-nine males were measured, indicating the presence of 0-group or first year and 1+ or second year males. There was a higher percentage of the former group caught.

1968: The 0-group or first year males and 1+ or second year male shrimp were again evident, with more of the latter group caught this year. The females were not much bigger and revealed some overlap with the larger 1+ or second year males. In fact, there appears to be two sizes of females, one group with a mode at 35-36 mm and another at 40-41 mm carapace length.

1969: Again, the two groups of males were revealed from the data. This time, the overall female sizes were larger. Similar to 1966, inadequate numbers of females measured may have given an inaccurate indication of its size distribution.

1970: The two age groups of males were

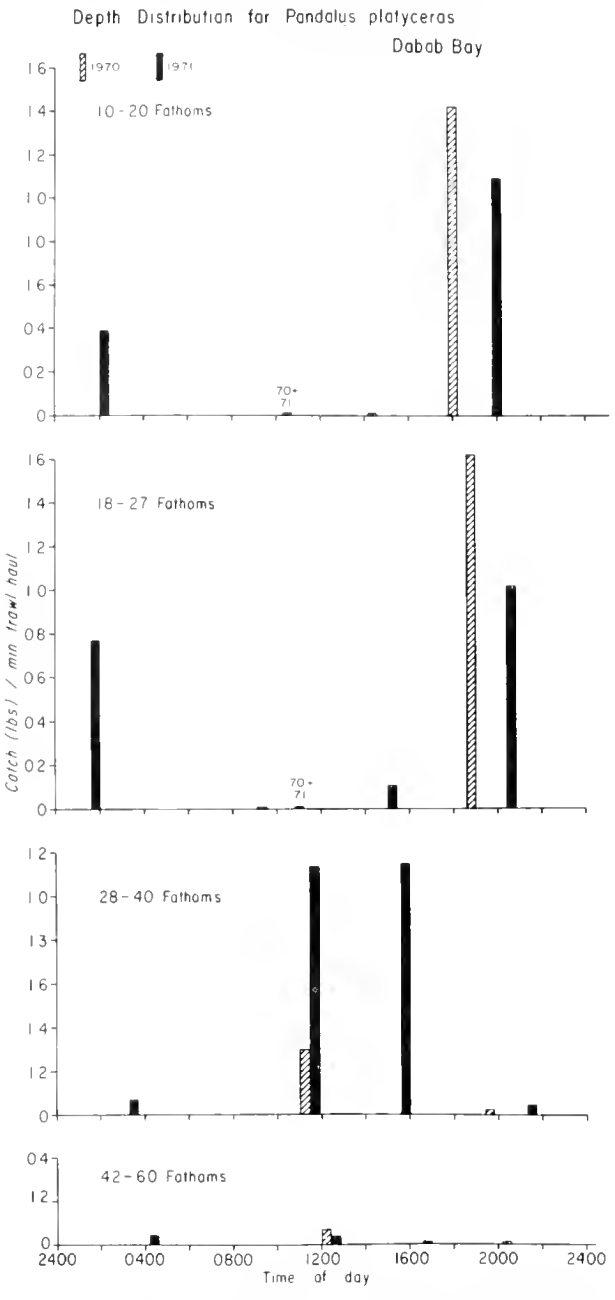

FIG. 4. *The 1970 and 1971 catch per minute haul during the day and night at four different depth ranges.*

again similar in size to those taken in previous years. Several small females (19-25 mm) were tabulated and included in Fig. 2, but it is assumed that this was an error in measurement or tabulation.

TABLE 1. *Analysis of Co-variance for Length-weight spot shrimp data.*

Source	Total d.f.	SS	Due to Regression d.f.	SS	Error d.f.	SS	F
1966	164	1.348	1	.773	163	.575	219 **
1968	626	26.077	1	19.609	625	6.468	1890 **
1969	539	31.220	1	26.918	538	4.302	3370 **
1970	686	32.716	1	26.310	685	6.406	2810 **
1971	624	31.735	1	21.975	623	9.760	1400 **
Within					2634	27.511	
Reg. Coef.					4	.358	8.57 **
Total	2643	131.376	1	103.317	2642	28.059	

** Significant at the .01 level.

1971: As in previous samples, two age groups of males were sampled. The distribution of females were similar to 1968 in that two size groups were indicated. The smaller group of females between 29-35 mm range was essentially the same size as the 1+ or second year males.

It should be recognized that sampling in a given month does not necessarily mean they should be the same size range each year. Depending on environmental conditions, they may moult earlier or later. Further, the availability of food and other conditions may cause the shrimp to increase in size more or less than the normal amount after each moult from one year to the next. It was interesting to note that some consistency was shown for the male size frequency distribution between 1966 and 1971. The general size range in carapace length for the 0-group or first year males, was comparable to British Columbia spot shrimp sampled during the same time of year (Butler, 1964). Although there were considerably fewer females measured they were more variable from year to year. As a matter of fact, there appears to be two size groups of females in some years such as 1968 and 1971, indicating the possibility of earlier transformation from males to females or little or no growth as they moult into the female stage.

Length — Weight Relationship

Fig. 3 shows the relationship between carapace length and weight of spot shrimp sampled for all years except 1967. Almost all data for the lines were taken from male shrimp and a few from females without eggs. Most females encountered were bearing eggs and thus were not weighed.

The length-weight data collected during the years 1966, 1968, 1969, 1970 and 1971 was fitted to the equation $W = B_0 L^{B_1}$, where W was weight, L was carapace length and B_0 and B_1 coefficients. The equation was linearized by transforming by common logarithms and fit by least squares techniques.

The analysis of variance Table 1 presents the results of the statistical analysis. The high

degree of fit to the data by the regression lines is reflected in the large F ratios. For example, testing the hypothesis that $B_1 = 0$ for the 1966 data results in an F ratio of 219, which is significant at the .01 level ($F_{1,164,.01} = 3.44$). Thus, we reject the hypothesis that $B_1 = 0$ and conclude that $B_1 \neq 0$.

Co-variance analysis of the data was performed to compare the means and slopes of the length weight regressions. The test of the hypothesis of equal means, $B_{(66)} = B_{(68)} = B_{(69)} = B_{(70)} = B_{(71)}$, results in an F value of 8.57 (Table 1) and was rejected at the .01 level ($F_{4,2634,.01} = 4.61$). Thus we conclude that the relationship between weight and length was not the same for the five years.

More detailed inspection indicated that the relationship during 1968 differed from the other years, in both B_0 and B_1 (Table 2). Excluding the 1968 data, the analysis of covariance for the other four years, 1966, 1969, 1970 and 1971 indicate that the four slopes (B_1) were the same, $F_{3,2009} = .16$, ($F_{3,2009,.01} = 3.79$). The test of the hypothesis of a common mean, i.e. common intercept (B_0) resulted in an $F_{3,2012} = 5.08$. Hence, we reject the hypothesis of a common mean at the .01 level. The four years in question (Fig. 3 — 1966, 1969, 1970 and 1971) can be represented by four parallel lines, but not by a single common line. This means that the 1966, 1969, 1970 and 1971 lines show similarity in rate of weight increase over length, but that the lines were operating at different levels (Fig. 3). Further, a common line could not represent the four years. The 1968 line was not similar in that the amount of growth in weight over increased carapace length was significantly lower than the other four years, even though all lines appear to be similar (Fig. 3).

Depth Distribution

Bathmetric distribution for spot shrimp was presented for the 1970 — 1971 catch data as shown in Fig. 4. Day and night tows at different depths and on the basis of catch per minute trawl haul reveal that the shrimp moved to shallow depths during the nighttime hours and returned to deeper depths when it was daylight. Very few spot shrimp were captured below 40 Fm for the area sampled. Although not presented, subsequent tows made in January, 1972 have shown similar results. These observations indicate a possible diel migration behavior of the spot shrimp.

ACKNOWLEDGEMENT

Special thanks go to the Washington Department of Fisheries for their support in this work by providing the necessary funding to have the data placed on computer cards and analyzed. Without such support, it would have been most difficult to analyze the data and give it proper treatment.

The senior author wishes to extend his gratitude and appreciation to the former students, who helped gather information for this paper.

The crew of the M/V *Commando*, Skipper Tom Oswold, Jr. and Engineer Olaf Rockness deserve special thanks for their patience and understanding.

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THE DISTRIBUTION OF MUD CRABS (XANTHIDAE) IN ALABAMA ESTUARIES

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ABSTRACT

Xanthid mud crabs are abundant associates of Alabama oyster reefs. Their distribution is affected by salinity, substrate and water quality. The crabs appear to function as commensals and scavengers rather than predators of oysters.

INTRODUCTION

Mud crabs of the family Xanthidae are among the most abundant macroscopic motile animals associated with oyster reefs in Alabama and probably elsewhere. The distribution of common species in estuaries is influenced by salinity and is largely restricted to hard substrates, especially oyster reefs. Little information is available on the density of mud crabs although they are important associates in oyster communities as predators, scavengers and as hosts for oyster parasites and diseases.

Data on the role of mud crabs in association with oyster reefs have been given by McDermott and Flower (1953), Menzel and Hopkins (1956), Menzel and Nichy (1958), McDermott (1960), Hoese (1964), Kenk (1967), Menzel, Hulings and Hathaway (1966), and others. Few of these studies considered geographical distribution in relation to salinity throughout an estuary. Menzel *et al.* (1966) observed that *Menippe mercenaria* was limited by salinities below 12-15 ppt and that *Neopanope texana texana* was more common at stations with highest salinity. Wass (1955) reported *N. t. texana* to prefer soft vegetated bottoms in shallows. Ryan (1956) found *Panopeus herbstii* rare on mud bottoms in Chesapeake Bay at a salinity range of 14-19 ppt. Schwartz and Cargo (1960) reported *P. herbstii* from 10-34 ppt in Chesapeake and Chincoteague bays but found them more abundant at higher salinities. The species was common on oyster reefs in central and lower Delaware Bay (McDermott and Flower, 1953) and on oyster reefs in North and South Carolina (Lunz, 1937). *Eurypanopeus depressus* was collected at salinities of about 4-20 ppt in Chesapeake Bay and its distribution was not thought to be limited by estuarine salinities above 4 ppt (Ryan, 1956). The species was abundant in lower Delaware Bay but not in the middle and upper portions (McDermott and Flower, 1953).

METHODS

Crabs reported in this study were collected from oyster reefs using scuba and random quadrats in May and June, 1969 and numerical data were reported by May (1971). Data on xanthids collected by trawling and seining in Alabama were reported by Swingle (1971). Samples were collected by picking up all material in square-yard grids by hand. The crabs make little effort to escape so even if a few were missed during sampling the relative abundance between different areas should be valid. No large stone crabs, *M. mercenaria*, which may have been in burrows were collected, however, the species is not abundant in Alabama and few burrows were seen. Small stone crabs apparently do not burrow (Powell and Gunter, 1968).

RESULTS AND DISCUSSION

Abundance per acre is reported by species in Table 1. Areas of collection are identified by numbers in Figure 1 where general salinity patterns are given during average river flows. On all reefs throughout the estuary, *E. depressus* (range 4-24 mm) was by far the most abundant species. Ryan (1956) found that this was the most abundant species of mud crabs on oyster reefs in Chesapeake Bay and showed a positive relationship between the presence of oysters or shells and this species. The majority of the specimens reported by Lunz (1937) from the Carolinas were collected from oyster reefs where they composed 25 percent of the population. *Panopeus herbstii* (15-29 mm) and *M. mercenaria* (15-29 mm) were collected in smaller numbers from higher salinity reefs in Alabama. There were more crabs on reefs which had larger amounts of boxes (empty, joined,

TABLE 1. - *May and June, 1969, abundance of mud crabs, per acre, in the Mobile Bay region stations shown in Fig. 1.*

Station	Eurypanopeus depressus	Panopeus herbstii	Menippe mercenaria
1	3,983	246	32
2	25,601	1,578	208
3	32,900	2,028	268
4	12,937	797	105
5	42,784	2,637	348
6	57,598	3,550	468
7	474	8	1
8	2,262	140	18
9	15,871	978	129
10	1,748	108	14
11	8,975	553	73
12	8,806	817	642
13	830	77	60
14	9,921	193	581
15	6,012	0	90
16	0	0	0
17	2,470	0	37
18	1,987	0	29
19	381	0	7
20	1,097	0	16
21	2,430	0	37
22	1,505	0	23
23	0	0	0
24	197	20	10
25	3,034	0	0
26	285	0	0
27	605	0	0
28	1,953	0	0
29	1,096	0	0

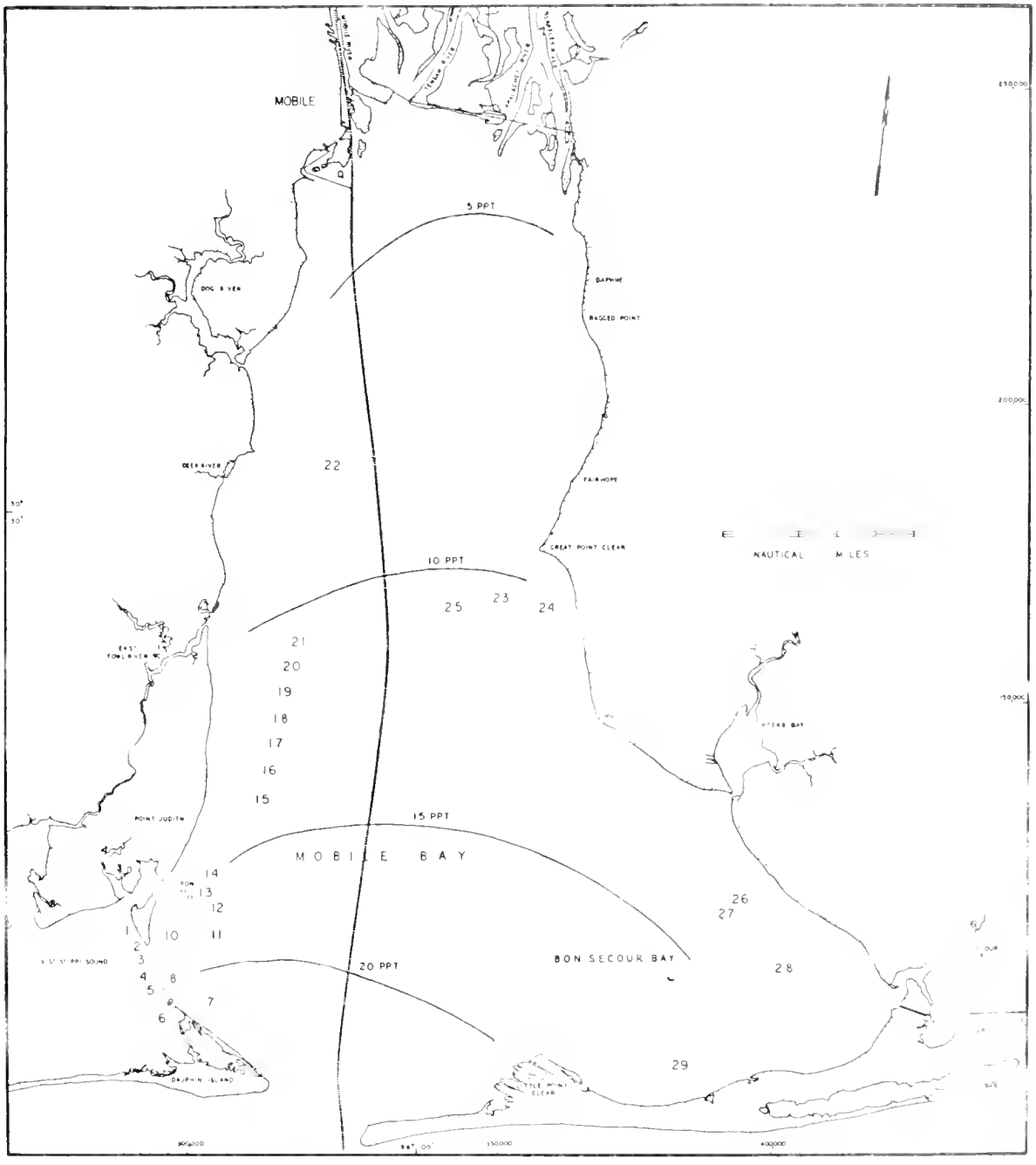

FIG. 1 *Locations where mud crabs were collected from oyster reefs and general salinity patterns during normal river flows.*

bivalve shells) and single shells indicating strong dependence on habitat provided by shells. All three species were more abundant in higher salinity areas and were lowest in abundance or absent in areas where salinity averages less than 15 ppt. Reefs in the upper bay where annual freshets are severe (May, 1972) had lower densities. *Eurypanopeus depressus* and to a

lesser extent *M. mercenaria* were less limited by lower salinity than *P. herbstii* which was found only on one reef where salinity normally averages below 15 ppt. Ryan (1956) collected none at salinities below 14 ppt. There is a salinity difference of about 5 ppt between area 1 (15 ppt) and area 6 (20 ppt) (May and Bland, 1970). The increase in numbers of crabs of all species proceeding toward the higher salinity area is evident.

No *Rhithropanopeus harrisii* were collected from the reefs in this study, but 49 specimens were collected in the Mobile River delta and from upper Mobile Bay by Swingle (1971). He found six specimens at salinities above 10 ppt but most were collected from freshwater. Similar distribution for this species is reported from Chesapeake Bay (Ryan, 1956) and upper Delaware Bay (McDermott and Flowers, 1953). Ryan (1956) collected this species from 3-19 ppt and stated salinity did not limit distribution. Costlow, Bookhout and Monroe (1966) found that larval *R. harrisii* developed at salinities from 1 to 40 ppt and no optimum salinity was detected. Swingle (1971) also reported one specimen of *Lobopilumnus agassizii* from Mobile Bay at 23 ppt, one *Eurytium limosum* from the usually highly saline Dauphin Island Bay, one *N. t. texana* from Perdido Bay at 32 ppt and four specimens tentatively identified as *P. occidentalis* from Mobile Bay and Mississippi Sound at salinities above 25 ppt.

With the possible exception of large stone crabs, which are not abundant here, xanthids appear to be mainly commensals or scavengers of oysters in Alabama rather than predators unless they prey on very small spat less than 5 mm. In the wild I have seldom observed spat or small oysters which were killed by small mud crabs even though spat are frequently numerous in joined bivalve boxes occupied by a crab. They more likely prey on other commensals on the reef since there is little direct relationship between the abundance of crabs and the abundance of oysters. Many reefs have more mud crabs than oysters and spat combined. Although there is no doubt these crabs will kill and eat oysters, Powell and Gunter (1968) believed that even stone crabs preferred barnacles to any other food item. Some of the field observations of xanthid predation on oysters could have been scavenging of moribund oysters killed or weakened by other causes. Although the destructiveness of mud crabs to very small spat is unknown, they apparently are not a serious predator of larger spat and oysters in Alabama.

Bottom water in upper Mobile Bay and Bon Secour Bay have extremely low dissolved oxygen levels in the summers (May, 1973). This condition affects the abundance and distribution of blue crabs and oysters and it is apparently true of xanthid crabs as well since high salinity reefs in Bon Secour Bay had few crabs. The severe flood in Mobile Bay from December 1972 through June 1973 either killed or caused migration of most of the mud crabs since very few crabs were collected from any reef in mid-May 1973 after the salinity had been below 1 ppt for about six weeks. Low salinity is common in late winter and spring throughout the estuary. Pearse (1929) found *P. herbstii* and *E. depressus* died after 4 to 5 hours in freshwater. Salinity below 12 ppt arrests the development of *P. herbstii* larvae (Costlow, Bookhout and Monroe, 1962). The rate of development of *M. mercenaria* is slightly slower at 20 ppt than at 30-40 ppt and no larvae survive at 10 ppt. Optimum salinity for larval development is in the range of 30-35 ppt at a temperature of 30 C (Ong and Costlow, 1970). Porter (1960) found that few stone crab larvae developed below 23 ppt at 27-30 C and felt that at 23-25 C larvae may not be able to survive below 27 ppt.

There is a positive correlation between the density of xanthids and the incidence of fungus disease *Labyrinthomyxa marina* (Beckert, Bland and May, 1972) but this is more likely due to the demonstrated response of both organisms to salinity variations rather than an actual interdependent relationship.

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THE PRESENT STATUS OF THE SOFT-SHELL CLAM IN MARYLAND

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ABSTRACT

The soft-shell clam, Mya arenaria, *has been a major commercial fishery in Maryland since the development and use of the escalator dredge in the early 1950's. Between 1960-70, annual landings have fluctuated between 4-8 million pounds of shucked meats, valued between 1-3 million dollars. As a result of tropical storm Agnes in June 1972, high mortalities occurred among the Chesapeake Bay soft-shell clams and all commercial fishing in the Bay was stopped.*

It was important to know the true extent of the mortalities in the Bay and to determine if those clams that survived the storm would spawn and establish new year-classes. Several extensive surveys were conducted throughout Maryland following Agnes and the results indicated that enough clams survived in certain areas so that the fishery could be reopened on 1 June 1973. Unfortunately, the greatest commercial numbers of clams were located in one county, Talbot, and unless careful surveillance is maintained these could be overfished.

From microscopic examinations, it was determined that the surviving clams had normal gonadal development and spawned around October of 1972. From this spawning, a set occurred in almost all major clam areas in Maryland, as indicated by special spat collectors used to monitor the Bay. Monitoring was done again in the spring of 1973 and setting was extremely light.

At this time, it is too early to know if the soft-shell clam will fully recover from the effects of tropical storm Agnes. Findings to date have been encouraging.

INTRODUCTION

The soft-shell clam, *Mya arenaria*, has been a major commercial species in the New England and Chesapeake Bay areas (Fig. 1). Prior to 1950, the majority of clams were harvested in two states, Maine and Massachusetts. The highest catch for this area was in 1940 when over 15 million pounds of meats were landed. Production declined (Hanks 1963) thereafter, reaching a low of 2.3 million pounds in 1959. Since then, production in New England has been increasing and, in 1971, 6.4 million pounds were harvested.

In 1950, the hydraulic escalator dredge was introduced into the Maryland portion of Chesapeake Bay. The dredge enabled the clam industry to use previously neglected subtidal stocks. Production increased rapidly and in 1964 8.1 million pounds were landed. Since 1969, when 7.9 million pounds were caught, production has

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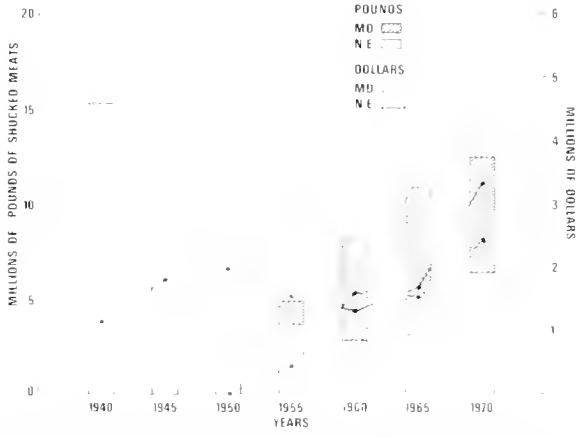

FIG. 1. *Soft-shell clam landings and values for Maryland and New England from 1935-1970.*

declined and in 1972 only 1.4 million pounds were landed. Part of the reason for the low 1972 figure was related to tropical storm Agnes and the closing of the fishery on 1 July 1972. The clam fishery was reopened on 1 June 1973, closed on 23 June, reopened for bait use only on 30 July, and reopened again on 27 August for human consumption.

TROPICAL STORM AGNES

On 22 June 1972, tropical storm Agnes hit Chesapeake Bay and brought as much as 11 inches of rain to some areas during the 3-day period, 21-23 June. Enormous amounts of fresh water entered the upper Bay, mainly from the Susquehanna River, and salinities dropped way below 5‰ in many areas where commercial numbers of clams were found. Shortly after the storm,

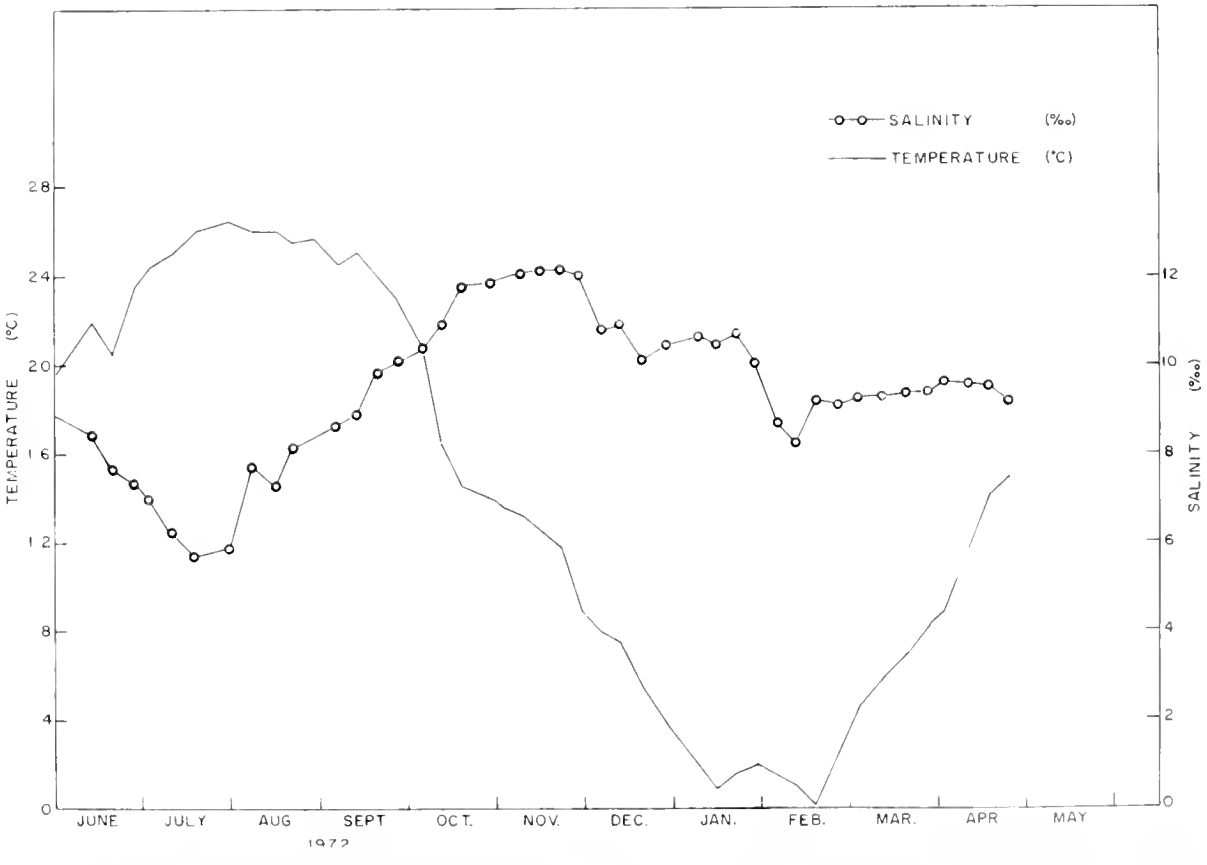

FIG. 2. *Weekly temperature and salinity data for the Tred Avon River, Oxford, Md. from June 1972 to May 1973.*

the Bay was subjected to a period of warm weather. The combination of warm water temperatures and low salinities caused extensive soft-shell clam kills. Especially hard hit were areas on the Western Shore and in the Chester River where mortalities were as high as 90%. In these areas, the salinities were 2% or lower and temperatures were in the high 20 C's. Mortality rates appeared much lower along Kent Island, Tilghman Island, Miles, Wye and Choptank Rivers. Here, temperatures were also in the high 20's but salinities never dropped below 5 ‰ (Fig. 2).

SURVEY OF CLAM POPULATIONS FOLLOWING AGNES

Federal Survey

Personnel from the National Marine Fisheries Service, Biological Laboratory, Oxford, Maryland, conducted a 12-day survey of soft-shell clam populations in the Choptank, Miles and Wye Rivers, and along Kent Island and Tilghman Island shores during a period from 29 August to 25 September 1972.

A commercial hydraulic escalator dredge was utilized during the survey. At each station, a 5-minute run was made. An anchor buoy was dropped at the beginning of each run and a line attached to the anchor was fed out until the 5-minute sampling period was completed. The area covered was estimated by multiplying the length of line fed out during the 5-minute period times the width of the dredge (36 inches). All clams on the escalator were removed, counted, and the length of each clam measured to the nearest millimeter with vernier calipers. An estimate of the number of clams per square meter was determined at each station (Fig. 3).

Clams were found in almost all areas sampled and recent mortalities were low. Clams from the 1971 year-class were greatest in number, which indicated that there had been a successful set of clams in the fall of 1971. In the area surveyed, it appeared that the majority of these clams had survived the effects of the storm. It was also apparent that if these clams continued to survive throughout the following winter and spring there would be enough to support a limited commercial fishery.

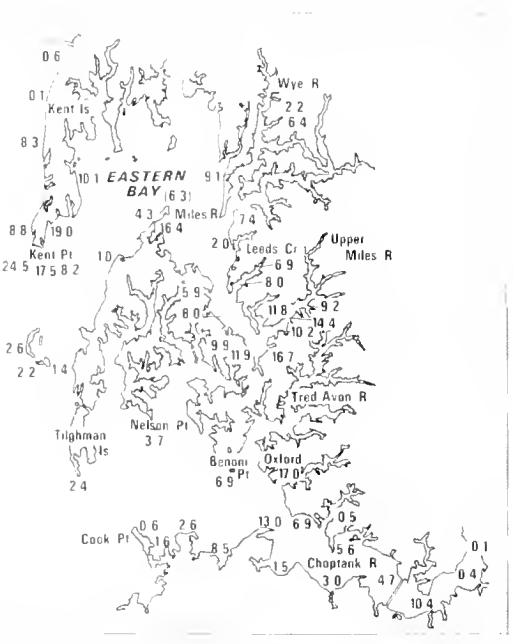

FIG. 3. *Total number of clams per m² found at each station during the 12-day NMFS survey.*

State Survey

As a result of tropical storm Agnes, special Federal funds were allocated to Maryland to determine the extent of the soft-shell clam kill throughout the state. Forty-eight commercial clammers, each with a helper, were hired to survey the clam beds. Sampling began on 8 January 1973 and was completed on 5 May. Each clammer collected 652 samples for a grand total of 31,296. Each sample equaled a 12-foot long distance times the width of the standard hydraulic escalator dredge. All market clams (2 1/4 inches or greater) were placed in a 5% measuring cup, and the fullness of this cup was recorded. A 5% measuring cup is a container that holds 5% of a bushel. Thus 4% or more means 4% of a bushel or more. When the sample was 4% or more, the clammers were required to mark the station with a buoy. This area was considered to have enough clams for commercial fishing.

Results of the survey are shown in Figure 4. The only areas on the Western Shore where commercial fishing would be profitable were a few locations in the Patuxent River and one 60-acre stretch opposite Horseshoe Point.

Populations of soft-shell clams on the Eastern

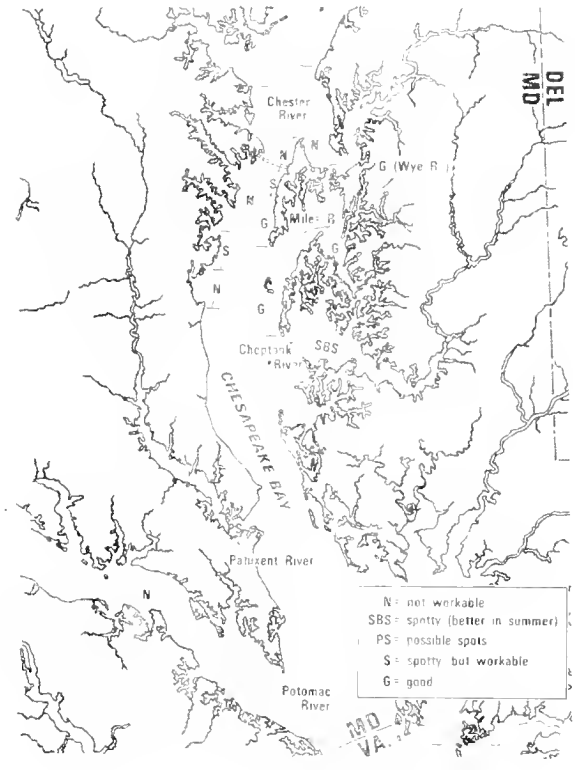

FIG. 4. *The results of the State of Maryland survey showing areas where clams were abundant enough to support commercial fishing.*

Shore were considerably greater. Although the Chester River was found to be commercially unworkable, most other areas south of the Bay Bridge had harvestable populations, especially in the Miles River, Wye River, along the southern end of Kent Island, and off Tilghman and Poplar Islands. In these areas there were 100 bushels or more of marketable clams per acre. The State's survey on the Eastern Shore, then, verified the findings of the 1972 Fall Federal survey.

MONITORING OF SOFT-SHELL CLAM SPAWNING AND SETTING, 1972-73

As stated earlier, soft-shell clam populations in many parts of the Bay were seriously depleted as a result of tropical storm Agnes. The Maryland Department of Natural Resources was concerned about the strength of the spawning stocks. In order for clams to fully recover from the effects of the storm, new year-classes would have to be established through successful spawnings and settings.

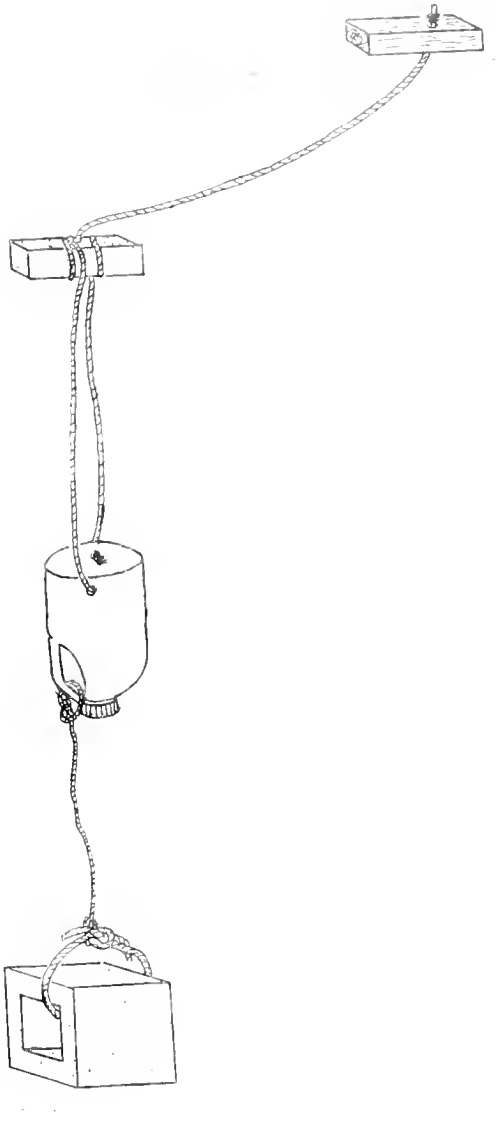

FIG. 5. *Bottle collector used to collect metamorphosing clams.*

One method of monitoring clam setting is to place special bottle collectors (Fig. 5) out at the time when spawning and setting occurs (Shaw, 1965a). With the cooperation of the Maryland Department of Natural Resources, collectors were placed throughout major clam areas in the Maryland portion of Chesapeake Bay. Since clams

are known to set during the fall and again in the spring (Shaw, 1965a, b; Pfitzenmeyer, 1965), bottles were set out from October to December 1972 and from March to June 1973.

Two collectors were placed at each station (Fig. 6) and were exchanged after about 30 days of exposure. The contents of each bottle were poured into a No. 80 (.0070 -inch opening) sieve and flushed with seawater to remove silt and detritus. The contents were then examined under a dissecting microscope and all metamorphosed bivalves were identified and counted.

In addition, soft-shell clams were collected weekly from shallow water in the Tred Avon River opposite the National Marine Fisheries Service Oxford Laboratory, during the above period and examined microscopically to determine if normal gonadal development occurred during fall and spring months. The gonad from each clam was fixed in Davidson's fluid, dehydrated in alcohol, cleared in xylene, and mounted in paraffin. The gonad was then sectioned at 7-10 μ with a standard rotary microtome. The sections were stained in Harris' hematoxylin and counterstained with eosin.

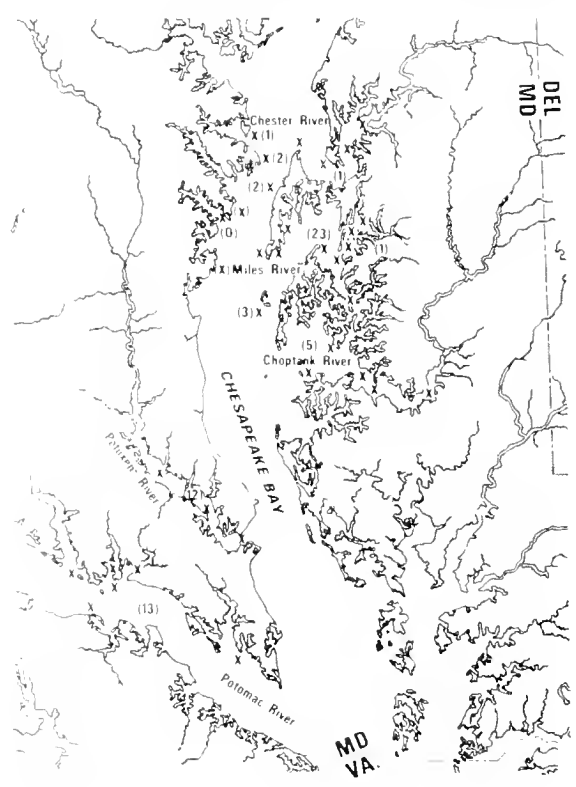

FIG. 6. *Stations where set collector bottles were located are designated by X's and the total numbers of setting* Mya *found in each area are enclosed in ( ).*

Fall Survey of Set Bottles

The contents of 52 bottles were examined during the fall survey and a total of 61 newly settled soft-shell clams were found. Clam sett[illegible] occurred in almost all major tributaries of the Maryland portion of Chesapeake Bay and on many clam flats of the Bay proper (Fig. 6). For example, 23 clams were found in bottles placed in the Miles River, 12 in the Patuxent River, 13 in the Potomac River and 5 in the Choptank River. Only one young clam was found in the Chester River, an area where mortalities were extremely high following Agnes. The highest number of newly settled clams (16) was found in bottles placed at Tilghman Point near the mouth of the Miles River. The clams had set between 3 October and 3 November. Unfortunately, the bottles placed at this site to monitor setting in November were lost.

On 22 May 1973 a survey was made at Tilghman Point to see if a set had actually taken place on the bottom. A standard hydraulic escalator clam dredge with a $\frac{1}{2}$-inch mesh belt was used to sample the clam flats. Three size groups were found, one of which, because of their size, had to be a 1972 fall set (Fig. 7); the most numerous size group at this site.

Spring Survey of Set Bottles

The contents of 77 bottles were examined during the spring survey. Only six newly settled soft-shell clams were found: three at Kentmoor in the Bay proper, one at Chlora's Point in the Choptank River and two at Cole's Creek in the Patuxent River. Based on these findings, it was apparent that the spring set was extremely light. Light spring sets have been found in the past by biologists at Chesapeake Biological Laboratory and at the National Marine Fisheries Service Oxford Laboratory (Shaw, 1965a,b; Pfitzenmeyer, 1965).

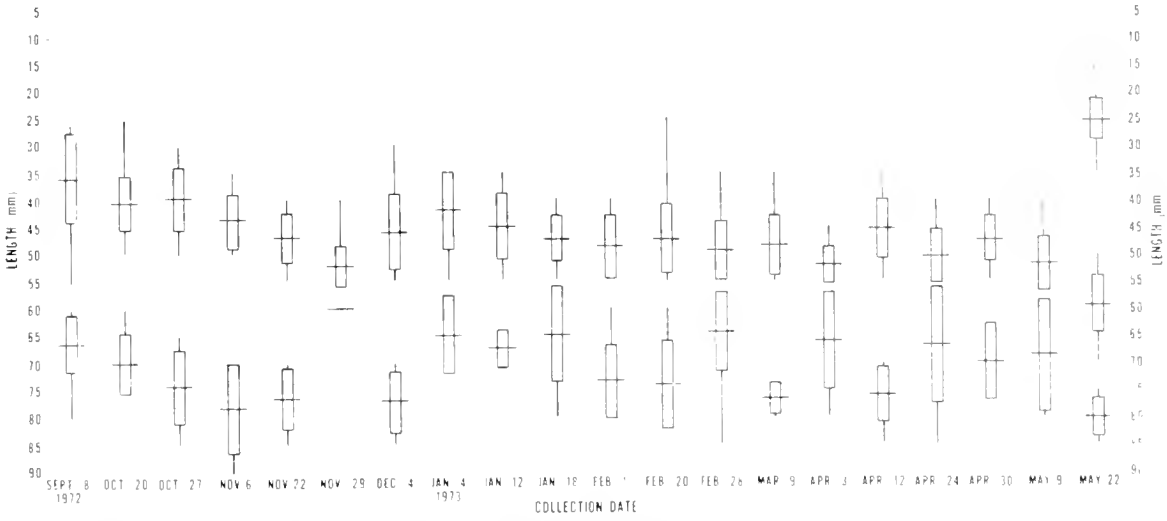

FIG. 7. *Mean length (horizontal line), standard deviations (white bar above and below horizontal line), and range (vertical line attached to bar), for year classes of soft-shell clams collected in the Tred Avon River adjacent to the Oxford Laboratory (the 29 November and 22 May samples were collected at Tilghman Point, Miles River and the 28 February sample was collected at Nelson Point, Choptank River). All clams 57 mm or less, were placed in the smaller year classes while all clams 58 mm or greater were placed in the larger year class.*

Microscopic Examinations of Histological Preparations

Gonadal development of some 394 soft-shell clams was examined microsopically from the Tred Avon River from 8 September 1972 to 30 April 1973 and the results were similar to those described by Shaw (1964, 1965b). In September, gametogenesis was in the early stage of development with small ovocytes at the base of the alveolar walls. By the third week in October, the clams were almost ripe to partially spawned out. By 6 November, the majority of clams examined were spawned out and some showed early gonadal stages similar to that found in September. The results of examinations indicated that major spawning occurred during October.

Gametogenesis began almost immediately after the fall spawning was over. During the winter months, gonadal development was slight. By March, some fully developed eggs were found but their numbers were far less than found during the previous fall. Similar conditions occurred in males. Only the centers of the alveoli contained mature sperm. By the end of April, soft-shell clams were in their summer stage (alveoli contained follicular cells and many inclusions), and the remaining ovocytes were undergoing cytolysis. No evidence of spawning during this cycle was observed. Because of the small numbers of clams examined during this period, it is the authors' opinion that some minor spawning had taken place since one newly settled clam was found in the set bottles placed in an area adjacent to the Tred Avon River.

Growth Rates of 1971 Set

Clams collected for microscopic examination in the Tred Avon River were all measured for total length to observe growth rate of the 1971 set (Fig. 7). This set would be the principal stock taken upon reopening the fishery. At the time of the first collection, 8 September 1972, the mean length was 35.8 mm and by 9 May, the last sample taken, the clams averaged 51.8 mm. (57 mm, 2¼ inches, is the minimum legal commercial size in Maryland.)

Two samples were also taken from a commercial clam bed in the Miles River, one on 29 November 1972 and the other on 22 May 1973, 9 days before the clam fishery was reopened. It is apparent from the mean sizes of these two samples, 52.2 mm and 59.2 mm, respectively, that clams grow faster in the sampled areas of Miles River than from the shallow flats in the Tred Avon River.

PRESENT AND FUTURE

After an analysis of the State survey, the clam fishery was opened for harvesting on 1 June 1973 but with the restriction of a 15-bushel daily limit. A total of 110 licenses were issued and most clammers easily caught the limits. The dockside value for clams averaged $10.00 per bushel, and the majority of clams caught were 57-63 mm (2 1/4 - 2 1/2 in.) in length and were from the 1971 set.

Marketing of Maryland soft-shell clams has some future problems. Landings in New England have increased since 1965 (Fig. 1). In the past the landing values of Maryland clams were considerably less than New England clams but in recent years value of Maryland clams has risen. In addition, many establishments, because of the past shortage of soft-shell clams, now use surf clams and may not rely on soft-shell clams again.

It can not be said with certainty that the present soft-shell clam populations in Maryland are great enough to withstand the fishing pressure. A 1972 set occurred in many areas and was especially heavy on the Western Shore in Anne Arundel County. After a year's growth these clams could reach market size and be available for harvesting. Many of the clammers will fish this area and take some of the pressure off the Eastern Shore.

The State plans to continue monitoring clam populations and to open or close areas, based on the results of such observations. Continued monitoring of fall and spring setting will be a useful tool to indicate future stocks for the fishery.

ACKNOWLEDGEMENTS

The authors wish to thank the following for their assistance in this study: Mr. Clyde Brown, Commercial Clammer; Mr. Carroll Smith, Maryland Department of Natural Resources; and Mr. Anthony Farmen and Mr. David McQuay, Fishery Aides, NMFS, Oxford, Maryland.

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AGE, GROWTH AND SIZE-WEIGHT RELATIONSHIPS OF THE SOFT-SHELL CLAM, *MYA ARENARIA*, IN PRINCE WILLIAM SOUND, ALASKA [1,2]

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ABSTRACT

Soft-shell clams, Mya arenaria, *from Simpson Bay, Prince William Sound, Alaska, were examined. A single sample of 178 specimens was used to determine the growth history of twelve year-classes by the annular method. In Prince William Sound soft-shell clams reach a harvestable size of 50 mm long in 6 or 7 years. Length-weight relationships are considered. Dry meat weight (solids) averaged 18.8%.*

INTRODUCTION

Mya arenaria, the soft-shell clam, is commonly encountered along the southeastern and south-central Alaska coast (Feder and Paul, 1973; Gross, 1967; Haven, 1971; Hubbard, 1971; Kirkwood and Gross, 1967). Preliminary observations by the authors indicate that some areas in Prince William Sound, an extensive embayment along the coast of southcentral Alaska, have populations of *M. arenaria* that may be dense enough to support limited commercial harvesting (Feder and Paul, 1973; Tussing *et al.*, 1972).

Soft-shell clams are highly esteemed for their distinctive flavor and are eagerly sought intertidally by commercial and sport diggers in eastern Canada and New England (Dow and Wallace, 1961; Hanks, 1963). Subtidal commercial harvesting of this species also occurs in Chesapeake Bay (Suttor *et al.*, 1968). The soft-shell clam was originally introduced into San Francisco Bay in the late 1800's (Hanks, 1963), and has since spread northward to southcentral Alaska (Gross, 1967; Feder and Paul, 1973; Tussing *et al.*, 1972). However, commercial harvesting of this species along the Pacific coast has only been reported for Oregon (Amos, 1966; Marriage, 1954).

In New England, *Mya* has proven to be a valuable renewable resource, and has been harvested continuously in Maine since the mid-19th Century (Hanks, 1963). However, the New England fishery is becoming less productive because of unmanaged fishing pressures and closure of many beaches polluted by industrial and domestic wastes (Dow and Wallace, 1961). The Chesapeake Bay fishery is currently the major source for soft-shell clams (Hanks, 1963).

Numerous papers deal with the basic biology and fishery potential of *M. arenaria* along the Atlantic coast of the United States: Dow and Wallace, 1961; Hanks, 1963; Newcombe, 1936; Suttor *et al.*, 1968; Turner, 1949; Wallace *et al.*, 1965. However, with the exception of the preliminary work of Porter (1972), no published work is available on the biology of the soft-shell clam from the Pacific coast of North America. The purpose of this investigation was to examine

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[2] This project was conducted with funds provided by the University of Alaska's Sea Grant Program (Grant No. 04-3-158-41), NOAA Office of Sea Grant, Department of Commerce.

the age, growth and size-weight relationships of a population of *M. arenaria* from Prince William Sound, Alaska.

METHODS

Specimens of *Mya* were collected from a mudflat in Simpson Bay, Prince William Sound (Fig. 1), May 18, 1973 by digging between the tidal heights of -0.43 m (-1.4 ft) and 0.0 m. All shells were examined under a 2x lens and shells with badly abraded surfaces were discarded (7% of the 192 clams collected). Age was determined for the 178 remaining clams by counting annuli; a series of closely-spaced concentric growth lines which are the result of slow winter shell growth (Newcombe, 1936; Paul and Feder, 1973; Weymouth, 1923). Growth history was determined by measuring the shell length at each annulus.

The size-weight relationships of 100 of the specimens were examined. The adductor muscles were severed, and the free water in the mantle cavities allowed to drain. The clams were then shucked, the shells weighed and the differences between the whole-live-weight (drained) and the shell-weight recorded as wet-meat-weight. Individual meats were dried to a constant weight at 80 C for dry-weight determinations.

Weights were obtained with a Mettler balance type P 120, and plots, regression lines, and regression equations were determined and plotted by an IBM 360 Computer. The Gauss-Jordan method was used in the solution of all normal equations (Cooley and Lohnes, 1962; Ostle, 1954).

RESULTS

Age and Growth

The specimens examined had recently-formed

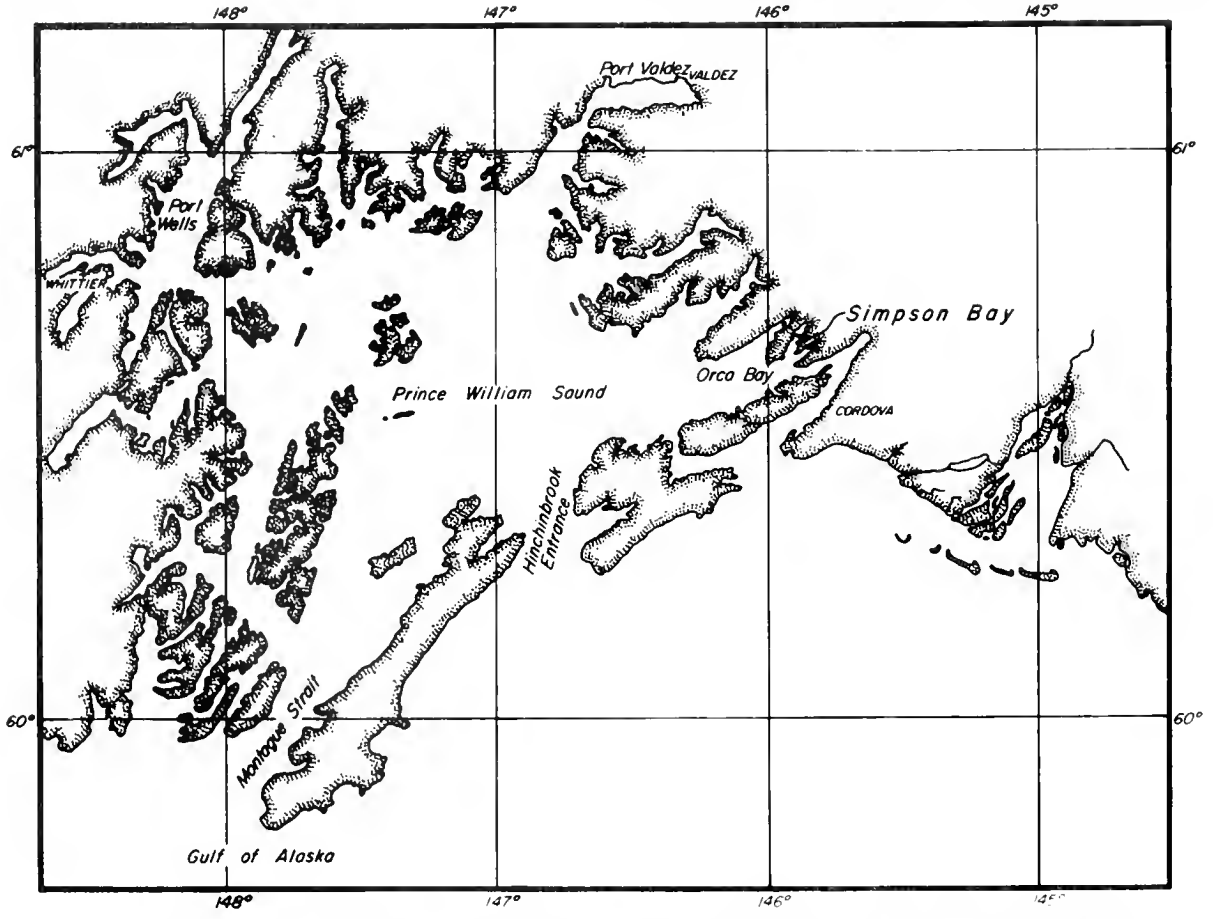

FIG. 1. *Map of Prince William Sound, Alaska; location of the Simpson Bay mudflat sampled for* Mya arenaria.

annuli and new growth was evident at the shell margins. The mean shell lengths for the various age classes of soft-shell clams from Simpson Bay are included in Table 1. The oldest clam examined was 12 years old and 87.0 mm long. A growth curve for *M. arenaria* from Simpson Bay is presented in Fig. 2.

From 1961 through 1972, the mean shell length at any given annular age, as determined from the examinations of the growth histories of individual shells, showed some variation (Table 2); however, the majority of the shell lengths at any given annular age falls within the standard deviations calculated for the various age classes in the collection (Tables 1,2).

TABLE 1. *Average size and age of 178* Mya arenaria *collected on a mudflat in Simpson Bay, Prince William Sound, Alaska on May 18, 1973. N = Number of clams. ML = Mean Length of Clams. SD = Standard Deviation. R = Range.*

Year Class (Age of Clams)	N	ML (mm)	SD (mm)	R (mm)
0*	4	9.87	1.76	8.4 - 10.9
1	33	13.41	1.10	12.0 - 15.2
2	13	17.73	2.83	16.0 - 21.0
3	9	26.04	2.38	21.0 - 30.0
4	13	30.87	2.39	25.1 - 33.2
5	24	39.01	3.22	33.8 - 49.2
6	21	48.15	3.78	40.1 - 55.3
7	7	57.50	4.68	50.5 - 60.1
8	16	64.96	3.38	58.0 - 70.0
9	19	73.42	3.11	69.0 - 81.0
10	11	77.95	4.37	70.0 - 84.0
11	5	76.88	3.31	73.2 - 80.0
12	3	85.07	1.90	83.2 - 87.0

* The 0 age group refers to those individuals of the settling year class that have undergone only one growing season (5 to 6 months) before forming their first winter annulus. Thus, individuals referred to as 1 year are actually 17 or 18 months old and have lived through two growing seasons.

Size-Weight Relationships

The equations describing the relationships between length and total weight (drained), length and wet-meat-weight, and length and dry-meat-weight are:

$$\text{Total Weight} = \left(\frac{\text{Length}}{23.40}\right)^{3.0278}$$

$$\text{Wet-Meat-Weight} = \left(\frac{\text{Length}}{27.75}\right)^{3.0370}$$ See Fig. 3

$$\text{Dry-Meat-Weight} = \left(\frac{\text{Length}}{48.51}\right)^{3.2524}$$ See Fig. 4

The equation of the line describing the relationship of dry-meat-weight (solids) to wet-meat-weight is:

$$\text{Dry-Meat-Weight (Solids)} = \left(\frac{\text{Wet Meat}}{5.15}\right)^{1.0676}$$

Dry-meat-weight (solids) was found to average 18.8% with a standard deviation of ± 1.5.

DISCUSSION

Intertidal beaches in Prince William Sound are subject to considerable environmental stress during January and February with observed water temperatures varying from -2.0 — + 1.0 C and air temperatures from -7 — +3 C. During this period 6 inches of ice were observed over the sampling area (Feder and Paul, unpublished). Under such conditions *Mya arenaria* forms a distinct winter annulus. (See Feder and Paul, 1973 and Paul and Feder, 1973 for data on winter annulus formation in the clam, *Protothaca staminea*, in Prince William Sound,) Newcombe (1936) also reported the formation of a single annulus for *M. arenaria* in the Bay of Fundy, and was able to use the annular method to age his specimens.

The time needed for *M. arenaria* to grow to harvestable size in Prince William Sound is slightly longer than that reported for other northern populations of soft-shell clams (Maine: Hanks, 1963; New Brunswick, Canada: Turner, 1949). In these areas the soft-shell clam requires 5 or 6 years to reach a length of 50 mm (2 in), an acceptable commercial size, as compared to 6 or 7 years for the clam in Prince William Sound (Fig. 2). In Massachusetts *M.*

arenaria reaches a harvestable length in only 3 years (Turner, 1949).

Examination of growth history of *M. arenaria* (Table 2) suggests that the growth rates for the various age classes have been relatively stable for an 11 year period. The Alaska earthquake of 1964, which destroyed approximately 36% of the clams in Prince William Sound (Baxter, 1971), had no apparent effect on the growth of the specimens examined.

The 18.8% solids determined for *M. arenaria* in Prince William Sound is close to the 18.6% reported for this species in Maine (Harriman, 1954). The solids value of the Alaska soft-shell clam is also similar to that reported for other commercially important north Atlantic bivalves: *Crassostrea virginica, Spisula solidissima* and *Arctica islanddica* with values of 17.0, 21.4 and 18.5%, respectively (Ropes, 1970).

The market for clam products in the United States is constantly expanding (Ropes, 1970; Suttor *et al.*, 1968). Simultaneously, an increasing

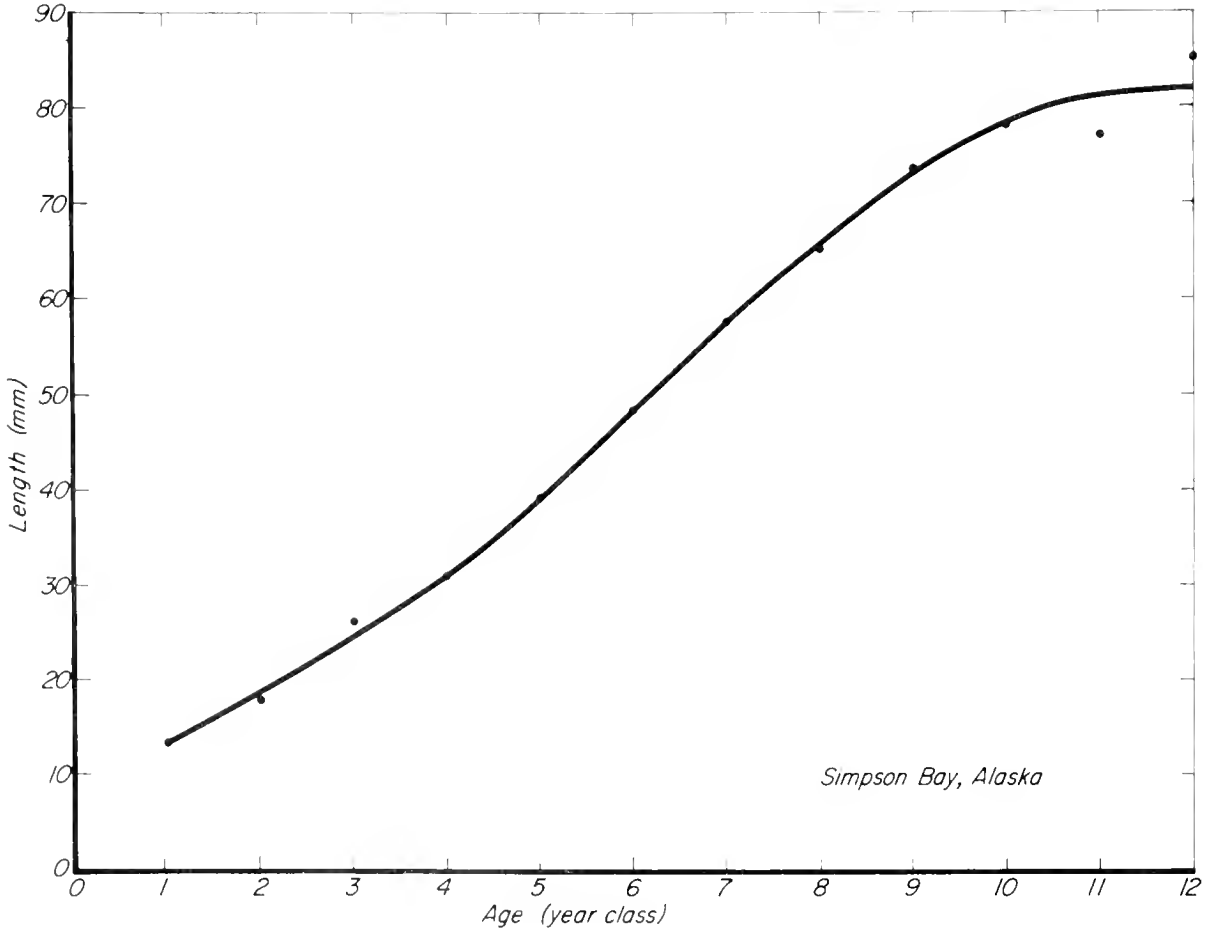

FIG. 2. *The relationship between shell length (mm) and age of* Mya arenaria *from a mudflat in Simpson Bay, Prince William Sound, Alaska.*

number of areas inhabited by these mollusks are becoming contaminated with domestic and industrial pollutants (Dow and Wallace, 1961; Hanks, 1963). Most of the coastline of Prince William Sound is uninhabited and not subjected to the pollution problems encountered elsewhere, and many of its shores contain populations of soft-shell clams as well as other species found intertidally, such as: *P. staminea, Saxidomus giganteа, Siliqua patula* and *Spisula polynema* (Feder and Paul, 1973; R. Nickerson, Alaska Department of Fish and Game, Cordova, unpublished manuscripts; Paul Feder, 1973; Tussing *et al.*, 1972).

Currently, the subtidal harvests of *M. arenaria* from Chesapeake Bay and the commercially important surf clam, *S. solidissima*, are supplying the bulk of the clam products consumed in this country (Dow and Wallace, 1961). However, in the future, intertidal soft-shell as well as hard-shell and razor clams from Prince William Sound and other areas in Alaska could become an

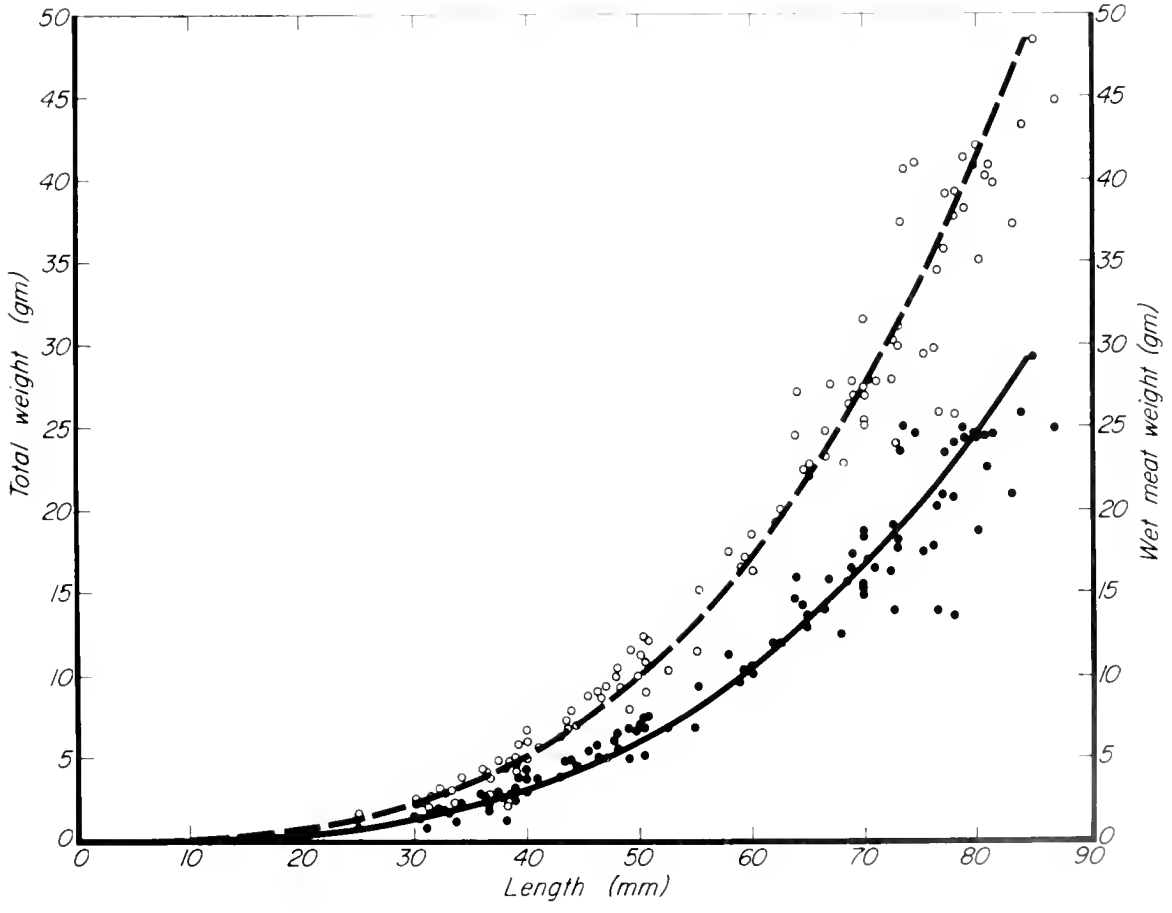

FIG. 3. *The relationship of clam length to total and wet-meat-weight for* Mya arenaria *collected from a mudflat in Simpson Bay, Prince William Sound.*

additional source of supply for clam products for commercial markets (Feder and Paul, 1973 and 1974; Kirkwood and Gross, 1967; R. Nickerson, personal communication; Nosho, 1972; Tussing *et al.*, 1972).

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We thank the following staff members of the University of Alaska: Emma R. Dieter for collection of material; Captain Ken Turner and the crew of the R/V Acona for support and general assistance; George J. Mueller, Marine Sorting Center, for taxonomic assistance in the final identification of *Mya arenaria* and for review of the manuscript; Rosemary Hobson for computer programming assistance, and Shirley Wilson for drafting all figures.

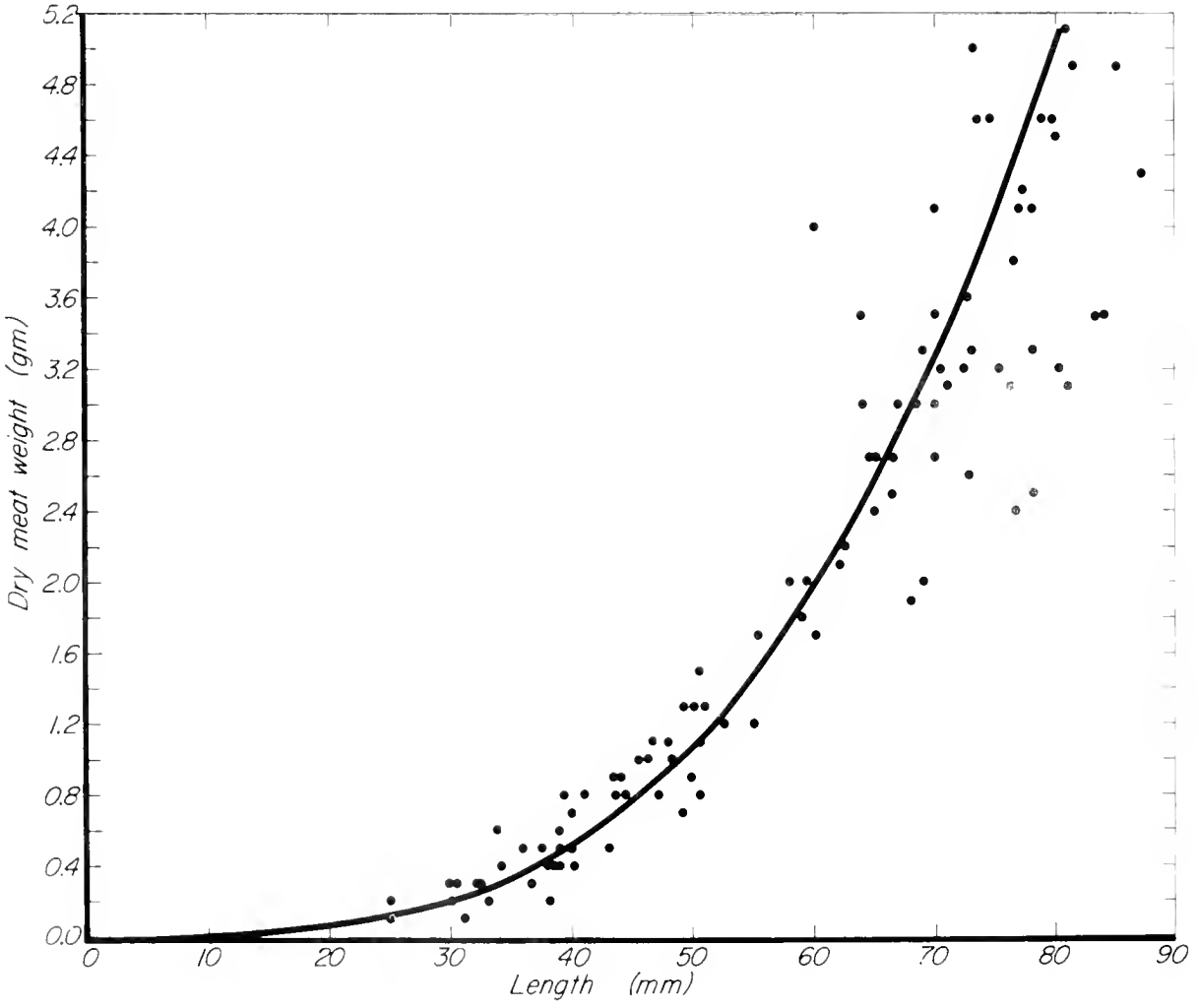
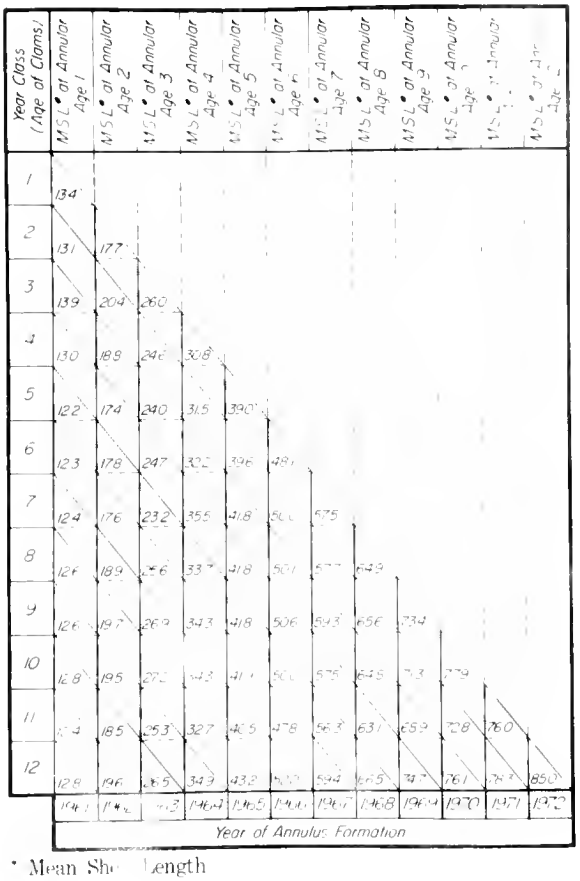


FIG. 4. *The relationship of clam length to dry weight for specimens of* Mya arenaria *collected on a mudflat in Simpson Bay, Prince William Sound.*

TABLE 2. *Growth histories of* Mya arenaria, *ages 1-12, collected from a mudflat in Simpson Bay, Prince William Sound, Alaska on May 18 1973. See Table 1 for the number of individuals included in each age class. Measurements are in millimeters.*



* Mean Shell Length

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REPRODUCTIVE CYCLE OF THE MANILA CLAM *(VENERUPIS JAPONICA)* FROM HOOD CANAL, WASHINGTON[1,2]

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ABSTRACT

Seasonal gonadal changes were observed histologically in samples of the Manila clam (Venerupis japonica, *Deshayes) collected from Misery Point and Big Beef Harbor in Hood Canal, Washington between October 1970 and November 1971. With few exceptions, ripe clams first appeared in May-June and most active spawning occurred in July. Spawning was nearly completed by October. Sexual maturation began at a shell length of 5 mm and spawning at 20 mm and over.*

INTRODUCTION

The Manila clam *(Venerupis japonica)* is one of the principal hardshell clam species commercially harvested in the State of Washington. The clam, introduced with Pacific oysters *(Crassostrea gigas)* from Japan, has also become one of the more important recreational-sports species along the Pacific coast.

The importance of the Manila clam in the commercial and sport fisheries has prompted investigations into the feasibility of developing new clam grounds or to re-establish previous commercial Manila clam producing areas in Puget Sound. The seed setting, growth rate and recruitment of select stocks of this species have been studied by Nosho and Chew (1972). This paper provides added information on the cyclical gonadal development and spawning of the Manila clam. These investigations were conducted prior to actual field transplantation studies of seed clams to select areas to determine its feasibility.

METHODS AND MATERIALS

Description of Experimental Areas: Two areas were chosen for this study, Big Beef Harbor (BBH) and Misery Point (MP) located on Hood Canal, Washington (Fig. 1). Temperatures at MP during the spring and summer were higher than those at BBH (Fig. 2), and the substratum at MP consists primarily of pea gravel whereas the substratum is unsorted at BBH.

The sampling site at MP is located in a lagoon which never empties completely. Water stands in the lagoon at low tide to a maximum depth of six inches. BBH clam beds are exposed at approximately the +2.5 feet tide level relative to mean low tide.

Sampling Scheme: Samples of 300 clams for each site were taken bimonthly during active gametogenesis, and monthly during the remainder of the year, and 40-50 examined histologically. Five to seven clams from each 5 mm length group (ranging from 5 to 65 mm) for both beaches were sampled for gonad tissue and fixed with Davidson's solution. Tissues were sectioned at 6-8μ and stained with Mayer's hematoxylin and eosin.

Definition of Gonadal Stages: Reproductive patterns and gonadal stages have been determined for clams and related bivalves by many

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[1] Contribution No. 398 College of Fisheries, University of Wash.

[2] This study was supported in part by the Sea Grant Program under the National Oceanic and Atmospheric Administration, U.S. Department of Commerce.

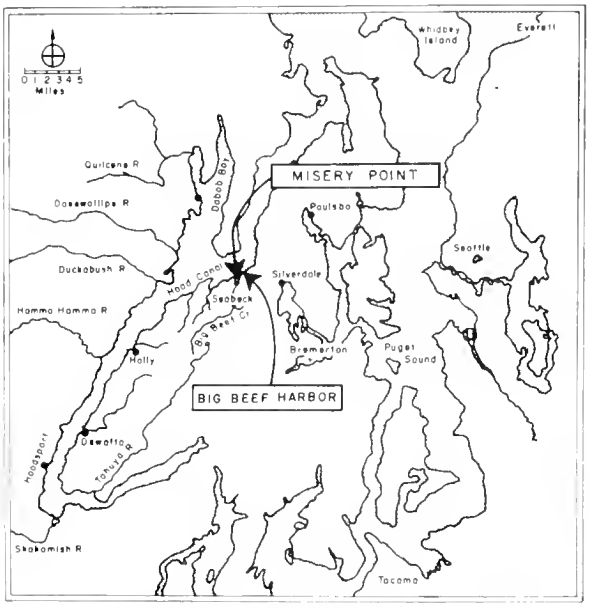


FIG. 1. *Location of study areas.*

researchers; Coe and Turner (1938), Quayle (1942), Allen (1953), Tranter (1958a,b,c,d,e), Turner and Hank (1960), Merrill and Burch (1960), Sastry (1963), Kennedy and Battle (1964), Porter (1964), Shaw (1964, 1965), Lammens (1967), Ropes (1968), and Holland's Master's thesis.[3]

Five arbitrary gonadal stages (early active, late active, ripe, partially spent and spent) were chosen to describe the reproductive cycle of the Manila clam. The stages were determined by the presence and degree of maturation of gametogenic cells in the follicles.

Developmental Stages of Male Gonad Follicles

Early Active: Many follicles with numerous follicle cells in foot tissue. Spermatogonia centripetal to follicle walls. Spermatocytes numerous and move toward center of lumen. Nutritive phagocytes, as described by Loosanoff (1937) for *Venus mercenaria*, abundant.

Late Active: Majority of follicle filled by spermatids and spermatozoa. Spermatocytes and spermatogonia located along inner periphery of follicle wall. Few follicle cells and nutritive phagocytes.

Ripe: Gonad composed of darkly staining spermatozoa with their tails pointing toward center of lumen forming concentric bands centripetal to spermatocytes. Spermatocytes, few in number, located along follicle wall. Follicles neat and orderly in appearance.

Stages of spawning and regression follow sexual maturity. These stages are characterized by the presence of phagocytes and residual gametes.

Partially Spent: Majority of follicles empty. Full follicles, if present, located near digestive gland. Follicles disorganized in appearance. Phagocytes abundant in center of shrunk follicles.

Spent: Gonad spent with few residual spermatozoa undergoing phagocytosis. Inner wall of follicle usually lined with spermatogonia.

Development Stages of Female Gonad Follicles

Early Active: Oogonia arise from stem cells (Tranter, 1958a) along follicle wall. Largest oocytes, attached to follicle wall, with nuclei darker than cytoplasm. Nutritive phagocytes abundant.

Late Active: Oocytes have nuclei lighter than cytoplasm. Free and attached oocytes equally abundant in follicle. Nutritive phagocytes few in number.

Ripe: Majority of oocytes lie free in lumen of follicle. Nutritive phagocytes rare.

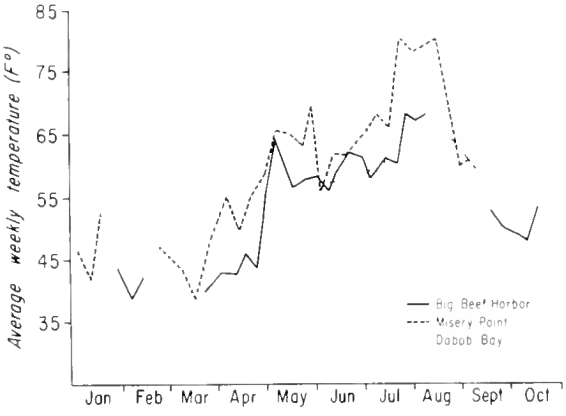


FIG. 2. *Temperature regimes at Big Beef Harbor, Misery Point, and Dabob Bay for 1971. Unconnected lines indicate a malfunction of the thermograph. Temperature at Dabob Bay was attained from May, 1971 to September, 1971 through the Washington State Department of Fisheries.*

[3] Holland, D.A. 1972. Various aspects of the reproductive cycle of the Manila clam *(Venerupis japonica)*. Master's Thesis, University of Washington, College of Fisheries, Seattle, Washington. 61p.

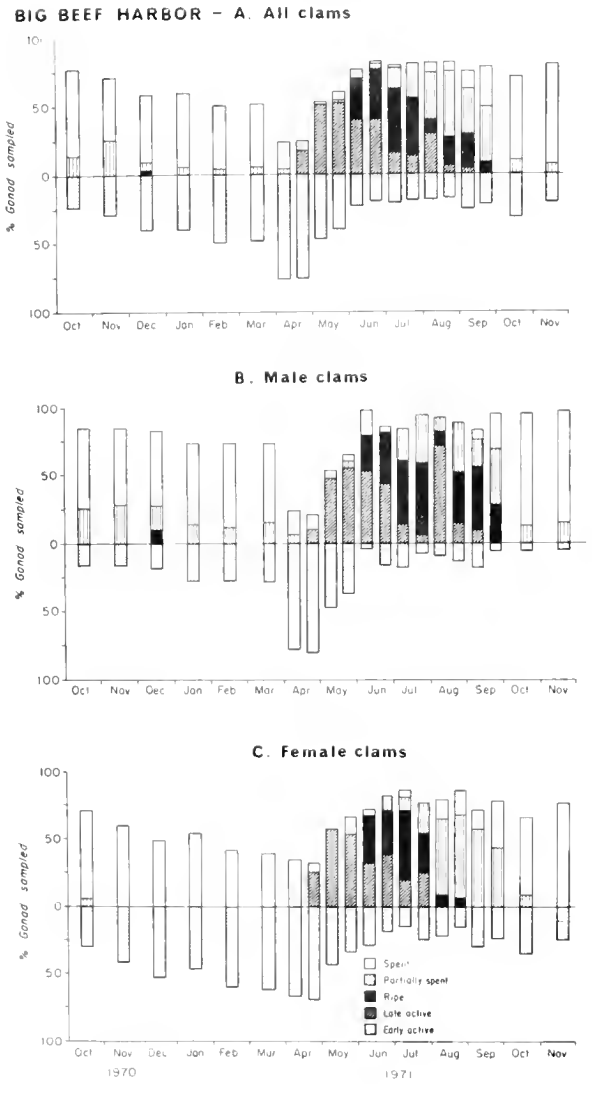


FIG. 3. *Gonadal stages of all* Venerupis Japonica *representing the breeding season (A) followed by the separation of males (B) and females (C) for Big Beef Harbor. The percentage of clams in each gonadal stage is represented by the length of each shaded area.*

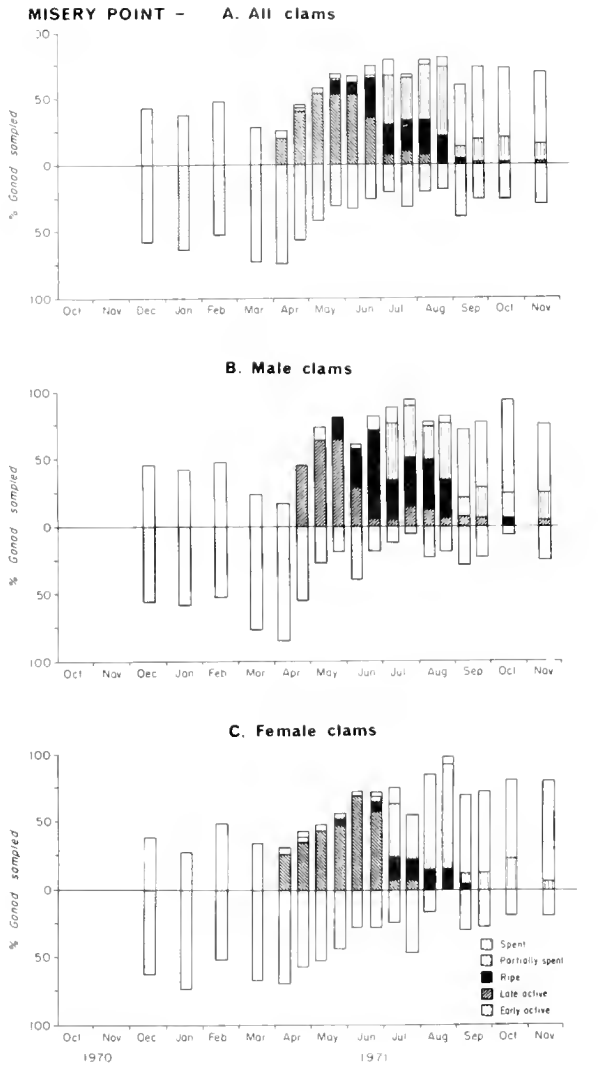


FIG. 4. *Gonadal stages of all* Venerupis Japonica *representing the breeding season (A), followed by the separation of males (B) and females (C) for Misery Point. The percentage of clams in each gonadal stage is represented by the length of each shaded area.*

Partially Spent: Few to over half of follicles empty. Many degenerate ova undergoing cytolysis. Follicle walls broken and disordered. Immature germ cells located on follicle walls.

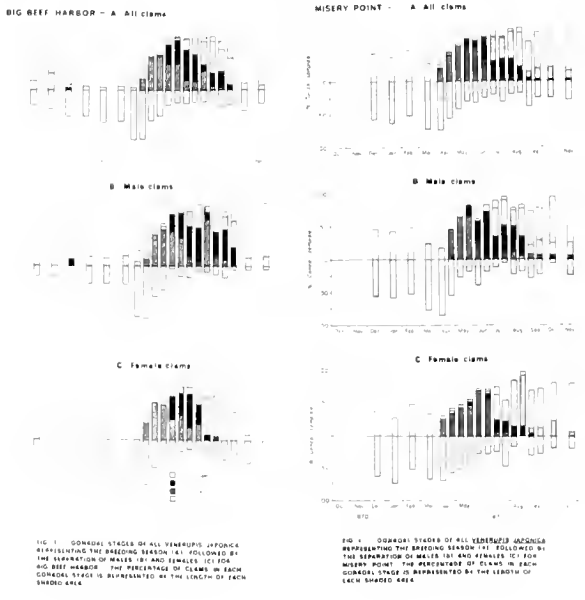
Spent: Few residual oocytes in lumen of follicle. Developing oogonia on follicle walls. Phagocytes numerous.

RESULTS AND DISCUSSION

All Clams

Early active gametogenic development for all sizes of clams occurred in every month of the year (Figs. 3A and 4A). The large percentage of clams in the early active stage during the summer months resulted from smaller clams, less than 20 mm in shell length, which developed sexual products but apparently failed to attain sexual maturity.

The majority of clams were either spent or showing early active gonadal development stage during the autumn and winter months. The absence of partially spent clams in MP during the winter months may have been due to the fact that they were exposed to a higher water temperature (Fig. 2) than those at BBH throughout the summer months and, as a result, spawning was complete. Further, clams at MP were continuously under water, which may have provided them more spawning time. Clams at BBH were exposed to air at least once a day during low tide. The partially spent clams found at BBH from October to April (Fig. 3A) failed to release all their gametes during the summer and early autumn and the sex products were being resorbed.



Late active clams from BBH began to appear in mid-April and ripe clams became prominent in mid-June (Fig. 3A). A small percentage of the clams spawned as early as the latter part of May whereas half the population of clams were spawning by early August.

The percentage of late active clams in late August at BBH was greater than during late July, perhaps indicating a second spawning. This increase in late active clams was actually due to partially spent male clams redeveloping and may have resulted from the sudden increase in temperature from late July to early August (Fig. 2). By the end of September the majority of BBH clams were spawning (39%), or spent (30%), and by October spawning was practically completed since 9% of all clams were partially spent and 61% spent (Fig. 3A)

Late active clams at MP first appeared in early April (18%) and by early May constituted 52% of the population (Fig. 4A). The first ripe clams occurred in late May and reached a peak (29%) in late June. Spawning began as early as April since one clam was found in a partially spent stage of spawning but the majority of the population was spawning by July. By early July 39% of all MP clams were partially spent and 11% spent.

Ripe clams from MP decreased from a maximum value of 30% near the end of June to a constant 21% during July and increased to 26% during the early part of August indicating a second maturation from partially spawned clams (Fig. 4A). A small percentage of partially spent clams occurred in late June (2%). By the end of August 54% of the MP population was spent as temperature began to decrease. Spawning was nearly complete by November (13% partially spent and 56% spent).

Spawning did not occur all at once. It appeared to be continuous throughout the summer and into the autumn months in Hood Canal, Washington. Quayle and Bourne (1972) indicated that this species spawns in late spring, although some spawning may continue throughout the summer in British Columbia. Yoshida (1935) and

Kinoshita (1939) believed spawning was continuous from spring until fall with one major peak for *V. japonica* in northern Japan and other Japanese researchers indicate that *V. japonica* has two major spawnings a year in southern Japan: Fujimori (1929), Tanaka (1954), Yasuda, Hamai and Hotta (1954), Ko (1957) and Ohba (1959).

Male vs Female Clams

Although the above discussions refer to the combined data of both sexes, percentages of male and female clams for each of the stages are also presented in Figs. 3 and 4 for BBH and MP. Trends at MP were similar to BBH for the corresponding sexes. The only difference was the timing of events or designated stages. As a result of this similarity, the reproductive stages of BBH male and female clams will be discussed with MP information presented only where appropriate.

One of the major differences between male and female clams at BBH (Figs. 3B, 3C) was the percentage of males remaining partially spent from October 1970 to April 1971, which were slowly resorbing large quantities of residual spermatozoa. A small percentage of ripe male Manila clams were found in December 1970 which were suspected to have been ripe in late summer or early autumn. Quayle (1942) states that mature males of a similar endemic species of clam, *Paphia (Venerupis) staminea* can be found year round in British Columbia waters.

Another major difference between male and female clams in BBH was the abundance of females in the early active stage throughout the autumn and winter months. When females were sexually mature, it was possible that inhibiting enzymes prevented the development or growth of young or new oocytes. Tranter (1958a) was able to demonstrate this for *Pinctada albina* in that there was arrest of growth of new oocytes until spawning took place, freeing space in the once-packed follicles. Once spawning commences, inhibiting enzymes apparently become inactivated and new oocytes proliferate, predominantly, around the digestive gland. The gonad then takes on the appearance of being early active in a large percentage of the female Manila clams.

During May clams from BBH were predominantly in a late active stage of gametogenesis. By mid-June, 26% of the males and 36% of the females were sexually mature. Active spawning began in late June and early July when 25% of the males and 14% of the females were in a partially spent or spent condition (Figs. 3B and 3C). Eighty-one percent of the females and 36% of the males were in a partially spent and spent condition near the end of August; 14% and 36% of the males in late August were in a late active and ripe stage of gametogenesis, respectively. Apparently, the males were able to release a portion of their sexual products and quickly redevelop to reattain maturity. By late September 67% of the BBH males were partially spent. Females spawned continuously at intermittent periods during summer and into autumn.

Female clams in early August to late September at BBH were dominated by the partially spent stage of oogenesis indicating continuous spawning over this period and occasionally we observed a spent clam (Fig. 3C). By October 1971 the majority of females were in the spent condition, which was similar to MP females (Fig. 4C).

Early active gametogenesis was seen in all months sampled during the year for both areas; this again partially resulted from clams under 20 mm in length which developed sex products but failed to attain maturity.

Size and Age at First Maturity

Three males collected at BBH during November and December 1971 in the length category of 5-10 mm had ripe sperm but were not suspected of spawning; the gonad was too tightly packed with no empty spaces. Lammens (1967) suggested that it was possible to determine which young clams have spawned by spaces in the gonad. The most advanced developmental stages attained by clams 5-10 mm in shell length were also in an early active stage of gametogenesis. Late active clams were observed histologically in early June and early July, 1971 for 15-20 mm clams. Clams ranging from 20-25 mm in length were ripe in early June and spawned in early August at BBH.

Three females in the 5-10 mm size range, two collected in the latter part of September, 1971, and one in October, 1971 had some ripe gametes. However, further histological study indicated that the clams had not spawned, but were resorbing their sexual products.

Similar results were found at MP. Size at maturity is much less confusing than age at maturity. Quayle (1952) states that if the breeding season were extended (as in *Venerupis japonica)* there would be a large range in size, and the animals of one year class may be confused with the smaller animals of a succeeding year class. Neave (1949) indicates also that the attainment of maturity for butter clams *(Saxidomus giganteus),* appears to depend upon size rather than age. Bivalves that spawn in late spring and early summer may produce young which will ripen and spawn later in the same season (Thorson, 1946).

Information from Japan reveals that Manila clams developed mature gonads at a shell length of 12 mm, and many individuals about 15 mm in shell length spawned (Ko, 1957). Clams at BBH and MP developed sex products at 5-10 mm and some matured at a shell length of 15 mm or greater and spawned at 20 mm. Nosho and Chew (1972) determined the size of a Manila clam at the end of the first year at BBH to be approximately 24 mm. Therefore, clams at BBH mature and spawn in their first year of life.

The sex ratios from BBH and MP were 48% males to 52% females and 44% males to 56% females, respectively. It was of interest to note that one clam out of 937 and one clam out of 768 were found to be hermaphroditic from BBH and MP, respectively. The specimen from BBH was sampled in September 1971 (50-55 mm) and the one from MP was sampled in August 1971 (40-45 mm).

Typically dioecious pelecypods produce few hermaphrodites. This is shown by several researchers such as the following: 3 out of 1000 soft-shell clams, *Mya arenaria* (Coe and Turner 1938); 0 out of 800 *M. arenaria* (Shaw, 1965); 0 out of 1400 *M. arenaria* (Ropes and Stickney, 1965); 2 out of several hundred quahogs, *Mercenaria mercenaria* (Loosanoff, 1936); one out of 2500 surf clams, *Spisula solidissima* (Ropes, 1967); 2 out of 3000 sea scallops, *Placopecten magellanicus* (Merrill and Burch, 1960).

SUMMARY

1. Specimens of Manila clams *(Venerupis japonica)* were removed periodically from Big Beef Harbor and Misery Point to determine the annual reproductive cycle.
2. Various gametogenic changes of development and regression are described.
3. Active gametogenesis began in April for both sampling sites and males matured earlier than females.
4. The majority of clams spawned in July; female spawning was continuous throughout the summer with no apparent second maturation of gametes but males released a large portion of their products and quickly redeveloped to maturity during the same season.
5. Active gametogenesis began with an increase in temperature as did spawning.
6. Clams developed sexual products at a shell length of 5-10 mm and reached maturity at 15-20 mm; all clams over 20 mm spawned and a small percentage of clams 15-20 mm also spawned.
7. Hermaphroditism for the Manila clam is described.

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FACTORS INFLUENCING THE SETTING BEHAVIOR OF LARVAL HARD CLAMS, *MERCENARIA MERCENARIA*[1]

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ABSTRACT

Laboratory setting experiments with larvae of the hard clam, Mercenaria mercenaria, *showed that chemical factors (pheromones) and physical factors influenced setting. Clams preferred to set in sand as compared to mud and in sediment treated with clam liquor rather than untreated substrate.*

Chemical factors masked physical selection of sediment by larvae. Clam liquor was a strong setting stimulus for clam larvae, indicating a gregarious setting behavior similar to that of oyster larvae.

INTRODUCTION

Survival of early stages of benthic organisms is a critical aspect in the establishment of benthic communities. Thorson (1966) postulated that benthic invertebrates are subjected to three major types of selection which determine the extent of survival and composition of a benthic community: 1) food, temperature, and predation during the planktonic stage, 2) effect of hydrography on the distribution of larvae, 3) substrate selection, competition among juveniles, and predation after metamorphosis.

A part of the third category forms the basis of this paper; that is, substratum (sediment) selection. Sanders (1958) and Bloom, Simon and Hunter (1972), in studies of benthic communities, showed that filter feeders and deposit feeders are dominant in coarse and fine sediments, respectively. Rhoads and Young (1970) related bottom stability and competition between similar species as determining factors in species distribution. For example, juveniles of suspension feeders, such as hard clams, are subject to environmental stress by deposit feeders that rework the surface, and survival and settlement are decreased by clogging of gills and the resuspension or burial of recently set larvae.

Wilson (1948, 1952, 1954) showed that survival of the larval polychaete, *Ophelia*, was dependent upon settlement on a favorable substratum. Thorson (1957) and Scheltema (1961) reported that larvae delayed metamorphosis and thereby increased the probability of encounter with a favorable substratum.

The mechanism of substratum selection is related to the physical, chemical and geological properties of the given sediment. Maurer (1969) found that sediment size limited the distribution of the tellinid pelecypods, *Tellina buttoni* and *Tellina salmonea*. Tenore, Horton and Duke (1968) showed the harmful effects of clay-silt sediments containing high levels of organic material on *Rangia cuneata*. Bader (1954)

[1] Contribution No. 87, College of Marine Studies.

reported that pelecypod density initially increased with an increase in organic level. However, at higher levels, products of decomposition produced an increase in bacteria and a decrease in oxygen levels which resulted in a decreased pelecypod population. Gurin and Carr (1971) suggested that larvae select substrata by means of sensitive chemoreceptor organs. Crisp (1967), Bayne (1969), Keck, Maurer, Kauer and Sheppard (1971), and Veitch and Hidu (1971) discussed the chemical basis of setting in the American oyster.

Oppenheimer (1961) and ZoBell (1963) discussed the importance of bacteria in aggregation, nutrient and mineral cycles in sediments. Crisp and Meadows (1962) and Meadows and Anderson (1968) postulated the importance of microorganisms and organic layers on the settlement of marine larvae.

Many investigations have been made of the hard clam, *Mercenaria mercenaria*, and its sediment preferences. Pratt (1953) reported a greater density of hard clams in fine sediments containing large particles, such as shell. Carriker (1961) indicated that juvenile clams favored fine sediments with appreciable amounts of organic detritus. He further stated that it was not possible to determine the setting preference of juvenile clams to a graded series of grain sizes. Wells (1957), in a study of hard clam distribution in Chincoteague Bay, found clams more prevalent in shell bottom than sand, sand-mud and mud. This distribution resulted from either selection of substratum or a pattern of survival. Planting experiments with shell aggregate by Castagna (1970) indicated that predation by crabs was a critical factor in natural distribution. Saila, Flowers and Cannario (1967) studied several environmental parameters in areas of high and low density. The difference in population abundance could not be explained in terms of sediment properties alone. They concluded that current, vegetation, predation and organic constituents all affect distribution.

The objective of our research was to determine the setting preference of hard clam larvae in the laboratory. The experiments were designed to study substratum selection based on particle size, chemical composition and treatment with pheromones. The interaction of the above factors was examined to determine which exerted the strongest influence on metamorphosing larvae.

METHODS

M. mercenaria larvae of setting size were obtained from the University of Delaware's Sea Grant Mariculture facility (Pruder, Epifanio, and Malouf, 1973). The larvae were raised by standard laboratory techniques (Loosanoff and Davis, 1963; Maurer and Price, 1967) and were released in a 284 l setting tank containing a grid with 36 randomly-placed sediment blocks and control blocks with no sediment (Fig. 1). Approximately 200,000 larval clams were used in each experiment to insure significant sets. Experimentation showed that the size and age of larvae are extremely critical variables. Carriker (1961) stated that pediveligers exhibit a searching behavior characterized by alternate periods of swimming and crawling. The capacity to swim widely extends the territory which the clam can examine, especially in the laboratory. Larvae must be released at smaller than setting size (150-170 μ) to assure that the clams are still highly mobile and capable of exhibiting a searching behavior. To select larvae, 9-12 day old stocks were poured through 149 μ and 125 μ screens. Larvae retained on the 125 μ screen were transferred to a large beaker, held for about two hours, and swimming larvae were poured off and used in the experiment; larvae that settled to the bottom were rejected. Larvae were released randomly throughout the setting tank.

After a 48-hour period, the experiments were terminated by draining the tank and exposing the grid. A plastic, one inch square, measuring device was placed on the bottom and all sediment and clams contained in this area were pipetted into small finger bowls. The samples were sieved and washed to separate juvenile clams from the sediment and to facilitate counting. The results were analyzed using analysis of variance, Kruskal-Wallis H test, or Mann-Whitney U test (Sokal and Rohlf, 1969).

Experiments

The experiments were conducted in the following series with the results presented in Tables 1 through 5.

TABLE 1. *Group I. Preliminary hard clam setting experiments with natural and incinerated sediments. Numbers represent sum of clams setting in 6 experimental blocks.*

	Experiment 1 Natural Sediments	Experiment 2 Natural Sediments	Experiment 3 Incinerated Sediments	Experiment 4 Incinerated Sediments
Sand (250 μ)	854	209	817	452
Sand Clam Liquor	1138	371	3369	447
Sand Mud	859	164	600	278
Mud (50 μ)	431	78	394	333
Mud Clam Liquor	140	23	1503	291
Control	1173	123	221	261
F Value	6.39 *	18.02 *	4.95 *	2.39

* Denotes Values Significant at 95% Confidence Level

TABLE 2. *Group II. Graded particle size experiments with natural sediments. Numbers represent sum of clams setting in 6 experimental blocks.*

	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5
1 mm	1844	2541	476	914	1406
707 μ	2724	2776	402	1409	1236
500 μ	4230	3125	629	1015	1663
250 μ	2696	5469	1621	866	1313
50 μ	3154	3060	405	470	1293
Control	2478	1483	332	341	1085
F Value	1.91	.642	1.17	1.07	2.73
H Value	8.05	6.86	4.77	4.66	1.43
U Value	27.5 *	28.0 *	26.0	27.5 *	21.0

* Denotes Values Significant at 95% Confidence Level

Group I. Preliminary experiments were set up with the following natural sediments and incinerated sediments: 250 μ sand treated with clam liquor, 250 μ sand, 50 μ sand-mud mixed in a 1:1 ratio, 50 μ mud, 50 μ mud treated with clam liquor and a control (no sediment). Clam liquor was obtained by shucking the clam over a beaker and retaining the liquid freed by the shucking process. Approximately 100 ml of clam liquor was used to treat 1 kg of sediment.

Group II. Larvae were exposed to natural sediment particles of different sizes: 1mm, 700 μ, 500 μ, 250 μ, 50 μ and control (no sediment).

Group III. Larvae were exposed to the same particle sizes as in Group II. However, the sediments were incinerated at 500 C for 1 hour prior to use.

Group IV. Larvae exposed to incinerated 500 μ

TABLE 3. *Group III. Graded particle size experiments with incinerated sediments. Numbers represent sum of clams setting in 6 experimental blocks.*

	Experiment 1	Experiment 2	Experiment 3	Experiment 4
1 mm	242	378	523	255
707 u	225	368	936	425
500 µ	364	724	355	193
250 u	412	752	301	345
50 u	248	322	345	259
Control	307	249	473	183
F Value	.546	4.98 *	3.89	.517
H Value	4.27	13.4 *	11.79	.828
U Value	24.0	31.5 *	32.0 *	19.0

* Denotes Values Significant at 95% Confidence Level

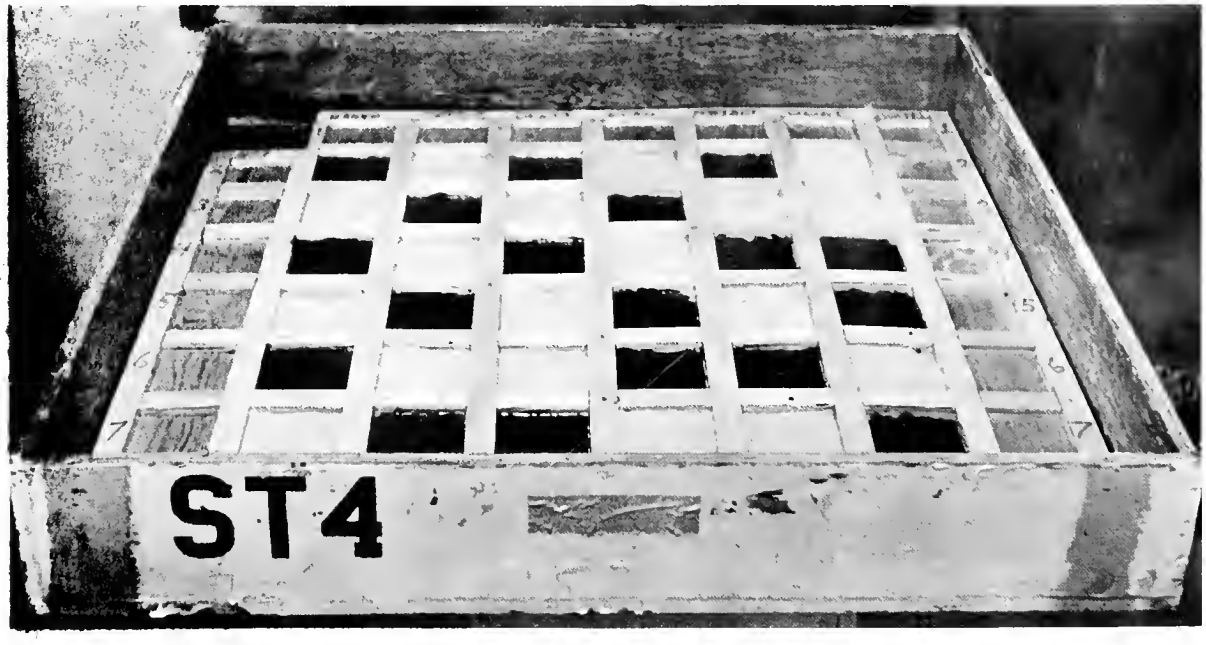

FIG. 1. *Setting tank with random arrangement of experiment blocks.*

TABLE 4. *Group IV. 500 μ sand vs. 50 μ mud ineinerated sediments. Experiment with numbers represents ranked pairing of observations.*

Sand	Mud
20	18
30	18
30	19
45	23
46	24
56	24
57	29
59	33
61	36
75	41
85	41
91	42
100	46
106	54
108	54
128	67
177	93
809	119
Total 2083	781
U = 254.5 *	

* Denotes Value Significant at 95% Confidence Level

sand and 50 μ mud.

Group V. Larvae exposed to 500 μ sand treated with clam liquor and untreated 500 μ sand.

RESULTS

Group I.

In Experiments 1 and 2 there was a statistically higher set of clam larvae in sand than in mud (Table 1). Sand treated with clam liquor also yielded higher setting than untreated sand. In both experiments, treated mud had the lowest set. These experiments showed that sediment was unnecessary for successful setting of hard clam larvae. Experiments with incinerated sediment showed the same association between high setting and treated sand; however, treated mud also yielded a high set. There was no statistical difference between setting in treated and untreated sand for Experiment 4. Nevertheless, setting was higher in sand than mud, sand-mud and treated mud.

Group II.

In general, results from Experiments 1 through 5 did not show any statistically significant associations between setting and a particular sediment size (Table 2). However, there was a definite trend between setting and 250 μ and 500 μ particle sizes. Moreover, for Experiments 1, 2 and 4 when the maximum set in sand sediment was compared to the maximum set in 50 μ sediment, there was a statistically significant difference (Mann-Whitney U test).

Group III.

Although there were higher sets in coarser sediments, results were not significantly different in three of four experiments (F Value, Table 3).

TABLE 5. *Group V. Treated and untreated (500 μ) sand. Numbers represent ranked assemblage of data.*

Experiment 1		Experiment 2		Experiment 3		Experiment 4	
Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
0	82+	17	38+	11	18+	5	17+
29	86+	17	48+	13	46+	7	29+
37	111+	26	49+	14	48+	8	40+
47	119+	36	60+	16	51+	12	44+
56	123+	43	74+	19	55+	12	47+
67	130+	45	75+	21	79+	14	48+
75	149+	49	80+	23	81+	18	54+
80	155+	50	84+	26	84+	28	59+
94	174+	52	94+	27	92+	40	67+
103	266+	54	98+	28	100+	45	79+
131	301+	55	102+	32	102+	48	84+
161	317+	60	104+	48	114+	61	85+
204	361+	69	118+	50	122+	98	104+
243	495+	70	250+	53	142+	119	116−
256	573+	89	289+	63	164+	139	204+
332	578+	94	381+	118	207+	148	349+
351	627+	97	585+	226	204−	287	365+
1523	645−	1643	2000+	247	227−	337	514+
Mann-Whitney U Test							
U = 237 *		U = 253.5 *		U = 255.5 *		U = 218 *	

* Denotes Values Significant at 95% Confidence Level

Any trends between set and sediment particle size included a range in the latter from 250 μ to 707 μ, which exceeded that recorded for Group II experiments. U values further compare maximum set in sand sediment with set in 50 μ mud.

Group IV.

Results of the single experiment performed showed that there was a statistically significant difference in setting between incinerated sand (500 μ) and incinerated mud (50 μ). In 18 pairs of plots, without exception, there were higher numbers of set in the sand compared to the mud (Table 4).

Group V.

Results from Experiments 1 through 4 showed that sand (500 μ) treated with clam liquor yielded higher sets than untreated sand (Table 5). Sixty-eight of 72 paired plots (94%) contained higher numbers of set in treated sand. Experiments 1, 3 and 4 had 1, 2 and 1 reversals, respectively, of the above pattern (Table 5). All experiments were statistically significant.

DISCUSSION

Gregarious setting among invertebrates and the oyster, *Crassostrea virginica*, in particular, has been well documented (Wilson, 1948, 1952, 1954; Thorson, 1966; Crisp, 1967; Bayne, 1969; Keck, *et al.*, 1971; Veitch and Hidu, 1971). The present research has expanded this phenomenon to include hard clams. Gregarious setting behavior was influenced by the presence of water soluble pheromones. We use the term pheromone in a broad sense as representing a biologically active substance, possibly protein or glycoprotein, emitted by adults or other juveniles by diffusion between water and shell liquor. Setting was statistically higher in sand than mud (Tables 2 and 4).

Treatment of sand with clam liquor dramatically enhanced the setting value of sand (Tables 1 and 5). However, treatment of non-incinerated mud reduced setting. High levels of organic materials and bacteria in the mud may have exceeded the threshold discussed by Bader (1954). We suggest that the addition of organic material may be responsible for reduced setting because of increased bacteria levels, reduced dissolved oxygen, and increased production of H_2S. Hard clam larvae also set on control plots with no initial sediment. Carriker (1961) reported that hard clam larvae set well on a hard surface covered with a thin layer of detritus. We observed that during the 48-hour testing period a thin layer of detritus settled on the control blocks.

There was a progression of setting from sand to mud in untreated incinerated sediments (Table 1). Nevertheless, sand and mud treated with clam liquor were the best substrata for settlement. Organic materials and bacteria normally occurring in natural sediment were destroyed in the incinerated sediments. The concentration of clam liquor acted mainly as an attractant for the larvae and did not increase organic levels or cause a bacterial bloom within 48 hours.

Even though metamorphosing hard clam larvae had higher sets in sand compared to mud (Tables 1, 2 and 4), which suggests a preference for the size range of sand particles, they apparently did not show a specificity of selection within that size range (Tables 2 and 3). Clam larvae did not discern among the particle sizes offered (Table 3). Regardless, the difference between maximum set in sand and mud further supported the conclusion that clams exhibited preferential setting behavior.

Experiments with incinerated sediments of the same particle size (Table 3) showed similar results to natural sediments (Table 2). Data from Group II (Table 2) showed statistically significant differences between setting and particle size and corresponding data from Group III (Table 3) were essentially insignificant for incinerated sediments, indicating that the natural chemical properties of sediments from Group II were the factors influencing setting behavior.

As reported earlier, sand treated with clam liquor yielded higher sets than untreated sand (Table 5). We suggest that a chemical isolate or prepared concentration of the pheromone would probably increase setting over that recorded with natural clam liquor. Carriker (1961) described clumped distribution of pediveligers in laboratory setting experiments. The clumping was particularly evident in pitted shells covered with detritus. This clumping may have represented gregarious setting caused by the release of pheromones by recently set larvae.

The gregarious setting factor caused by pheromones is probably adaptive. Evidence for this behavior is available (Keck, Maurer and Watling, 1972). On the other hand, distribution of juveniles has not been correlated with sediment size (Saila, *et al.*, 1967). However, our laboratory data are supported by field data (Wells, 1957; Pratt and Campbell, 1956; Sanders, 1958; and Bloom, *et al.*, 1972). Research should be designed to correlate clam distribution to chemical factors in the field. Raw clam liquor should be purified and setting factors that may be exploited in the mariculture of this species should be isolated.

We suggest that previous studies on the ability of larvae to metamorphose in various sediments (Scheltema, 1961; Wilson, 1948) are not analogous to this work on sediment selection by the hard clam because larval hard clams do not need sediment to metamorphose. The random block experiments reported here indicate that larval hard clams do actively engage in sediment selection.

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A HAPLOSPORIDAN INFECTION IN GAPER CLAMS, *TRESUS CAPAX* (GOULD), FROM YAQUINA BAY, OREGON [1]

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ABSTRACT

A haplosporidan parasite was found in 43% of 226 gaper clams, Tresus capax, *from Yaquina Bay, Oregon. Of these infections, 80% were rated as light and caused no apparent damage to the clams. Heavily infected clams were emaciated, sluggish in response to prodding, were dark in color and had little gonad development even during the spawning season. Grossly, the most apparent sign of infection was numerous white cysts in the tissues. Cysts ranged up to 2.0 mm in diameter and were present predominantly in the mantle overlying the viscera and under the epithelium in the siphon. In heavy infections cysts also occurred in the gills, the digestive gland, gonadal tissue and the musculature of the body wall and foot.*

Histological examination of cyst-bearing tissue revealed stages of multinucleate plasmodia 10-35 μ long, which contained spherical nuclei 1.4-3.0 μ in diameter with light nuclear membranes. Many necrotic plasmodia were observed and some were phagocytized.

Cysts were formed by host cellular response to the parasite and were composed of massive aggregations of hemocytes circumscribed by fibroblast-like cells and light connective tissue depositions.

INTRODUCTION

Haplosporidans have been reported in several species of bivalves including oysters from the East coast of the United States (Sprague, 1970). Serious oyster epizootics have been attributed to haplosporidans of the genus *Minchinia*. *Minchinia nelsoni* was found to be the etiological agent in mass mortalities of *Crassostrea virginica* populations on the Atlantic coast (Andrews and Wood, 1967; Sprague, 1970), and *M. costalis* has caused heavy losses of *C. virginica* in the seaside bays of Virginia and Maryland (Wood and Andrews, 1962; Andrews *et al.*, 1962). Subsequently *M. costalis* has been reported in *C. virginica* held in California bays (Katkansky and Warner, 1970a; Krantz *et al.*, 1972), and a single incidence of a haplosporidan in the Pacific oyster *(C. gigas)* was reported by Katkansky and Warner (1970b). Recently, Mix and Sprague (in press) reported plasmodial stages of a haplosporidan in *Ostrea lurida* from Yaquina Bay, Oregon. Taylor (1966) described *Haplosporidium tumefacientis* from the California sea mussel *(Mytilus californianus)*, but found no associated mortalities.

This paper reports a haplosporidan infection in gaper clams, *Tresus capax*, in Yaquina Bay, Oregon, and also discusses physiological trauma to infected animals.

METHODS AND MATERIALS

Clams were collected from Yaquina Bay, Oregon, predominantly from the Sally's Bend

[1] Technical Paper No. 3684, Oregon Agricultural Experiment Station.

area on the north side of the bay. Collections were made sporadically from May through November 1972, and no clams were taken again until June 1973. Whole clams were removed from their shells, blotted with towels and weighed to the nearest g. The mean shell length was 112 mm (range: 76-135 mm) representing an average age ofabout 4 yr. (Marriage, 1954). The animals were then examined macroscopically for cysts and were dissected to inspect internal tissues and organs.

Preliminary histological examination of infected tissues revealed that all parasites were present within host-formed cysts and no life stages were observed outside these areas of host cellular reaction. Therefore, haplosporidan infections were diagnosed solely by the presence or absence of such cysts. Infection was categorized as N, I (less than 30 cysts per clam) or II, denoting no infection, light and heavy infections respectively. Regression line anlysis of shell dimensions versus body weight were run on the three groups, and analysis of co-variance was performed to determine if there were differences in weight due to infection.

Tissues were fixed 12-18 hr in either seawater-Bouin's solution or 10% formalin, dehydrated, embedded in Paraplast and sectioned at 7 μ. Stains used were Mayer's hematoxylin and eosin, Geimsa, or Zeihl-Nielson acid fast, counter stained with Harris' hematoxylin and eosin (Farley, 1965). Sections of the mantle, siphon, gill, kidney, digestive gland and foot musculature were studied.

RESULTS

Gross signs of haplosporidan infection were white, spherical cysts ranging in diameter from 0.25-2.0 mm. In heavy infections, cysts were often so numerous that they appeared to fuse into larger masses with diameters up to 5 mm. Of 226 clams examined, 43% contained cysts and 80% of these infections were rated as light. Cysts were primarily located in the mantle overlying the viscera (Fig. 1) and in the siphon under the epithelium bordering the inhalent and exhalent channels. In more heavily infected clams, cysts were also present in the gills (Fig. 2), digestive gland, Leydig tissue and throughout the musculature of the body walls and foot.

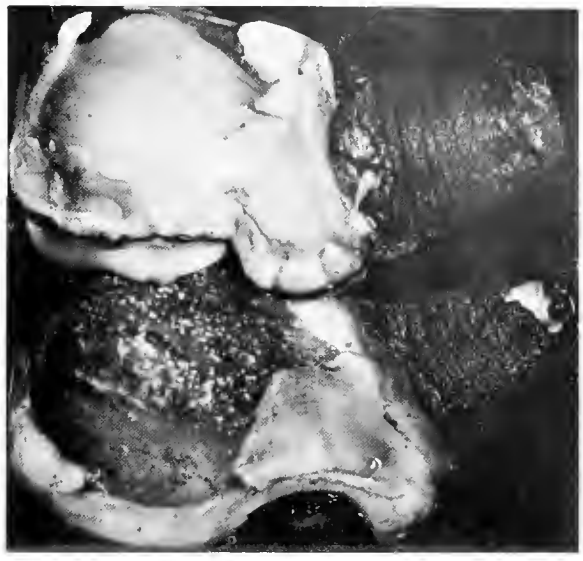

FIG. 1. *An uninfected clam (above) and heavily infected clam (below). Note the transparent quality of the infected mantle revealing dark silt accumulations in the mantle cavity.*

Lightly infected clams appeared to be in good condition and were qualitatively indistinguishable from uninfected animals. Clams with heavy infections were moribund. They appeared emaciated and were sluggish in response to prodding. The siphon was often flaccid and not retracted into the shell. The mantle was abnormally transparent and watery in texture. The body hue was dark and contrasted markedly with the creamy white appearance of healthy clams (Fig. 1). This color difference was partially attributable to sparse gonad development in heavily infected clams, even in mid-spring when healthy animals contained profuse gonads. Silt had accumulated on the gills of several clams indicating impaired ciliary movement on this organ (Fig. 2).

Histological examination of cyst-bearing tissue revealed the etiological agent to be a haplosporidan parasite (V. Sprague, personal communication). Only plasmodial stages were observed and these had none of the acid-fast qualities described by Farley (1965) for mature spores. Within a cyst there were numerous plasmodia, often more than one hundred. Plasmodia were spherical to oval in shape and ranged in length from 10-35 μ (Fig. 3). These organisms were often isolated in lacunae (Fig. 3)

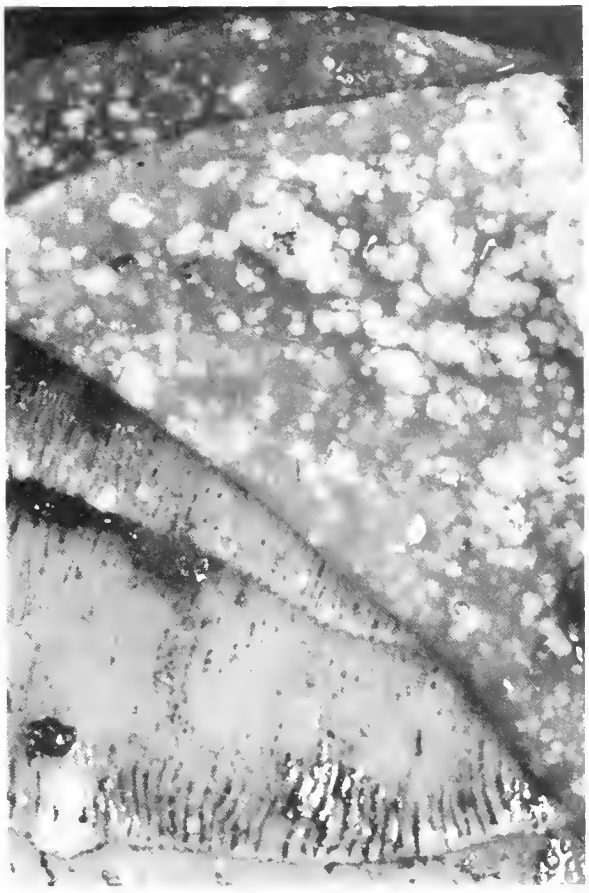

FIG. 2. *View of a heavily infected gaper clam. The mantle has been cut and pulled back to show the cysts in the gills and the accumulated silt.*

but sometimes had hemocytes directly in contact with the plasmodial membrane. Plasmotomy of enlarged plasmodia, as described by Farley (1967) and depicted by Couch *et al.* (1966), was not observed. The supposition, based on our preliminary histological examination of clams, that stages of the haplosporidan were not found outside cysts was substantiated by this more extensive survey.

There were variable numbers (2-60+) of spherical nuclei that ranged in diameter from 1.4-3.0 μ within plasmodia. Two stages of nuclear development, in what were judged to be viable plasmodia, were observed. Nuclei were either small (1.5 μ) with large amounts of chromatin (Fig. 3) or were about 3.0 μ in diameter with little chromatin aggregated against the nuclear membrane, sometimes with a thin rod transecting the nucleus (Fig. 4). In several sections the larger nuclei appeared to be exiting from the plasmodium into the lacuna and surrounding cellular material. Necrotic plasmodia were frequently observed. Their nuclei were pyknotic and the cytoplasm was more acidophilic than the cytoplasm of viable plasmodia. Many necrotic plasmodia were in stages of dissolution and were being phagocytized. In several cases small plasmodia were entirely within the membrane of a single phagocytic cell.

Cysts were formed by host reaction to parasitic invasion of tissues and were composed of massive aggregrations of hemocytes circumscribed by fibroblast-like cells and connective tissue deposition (Fig. 5). Dr. Sprague noted that he had never seen a similar enclosure by cells resembling fibroblasts in sections of oysters bearing haplosporidans. Formation of cysts was accomplished by displacement and destruction of normal tissues (Fig. 5, 6).

Regression lines of weight *vs* shell length for groups N, I and II had r^2 values of 75%, 53% and 61%, respectively. Analysis of co-variance showed the only significant difference ($P = 0.05$) was between the elevations of lines for groups N and

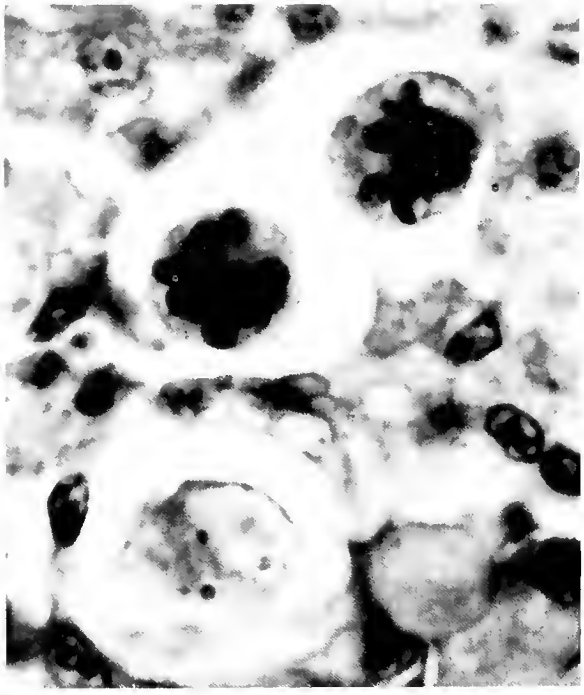

FIG. 3. *Plasmodial stages of haplosporidans in lacunae of a cyst in the mantle. 1600 X.*

II. The lower elevation value for group II represented a mean weight reduction of 14% from group N.

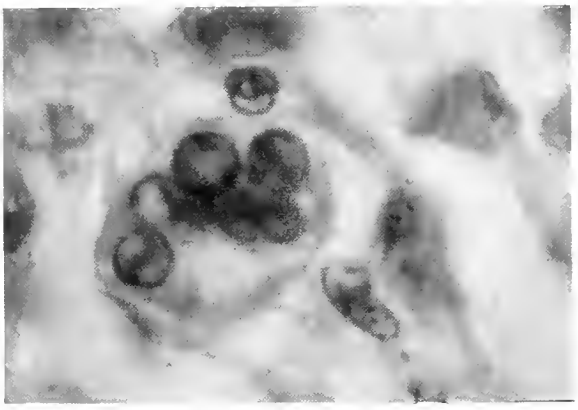

FIG. 4. *Plasmodium in a cyst in the mantle. Note less chromatin in these nuclei than in Fig. 3, and thin rod transecting them. 1000 X.*

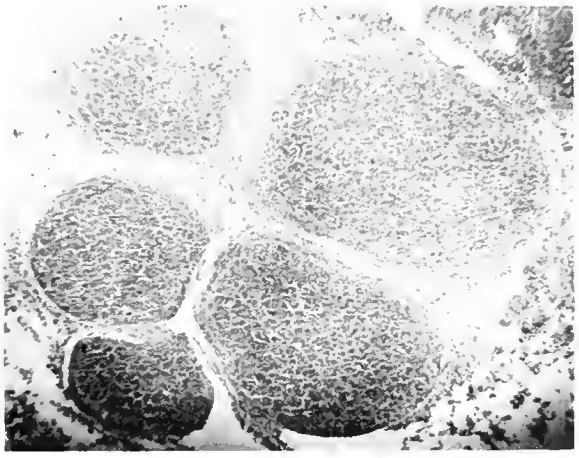

FIG. 5. *Section of a mantle showing several cysts about 1-2 mm in diameter. Note the displacement and necrosis of mantle tissue and the connective tissue deposition around each cyst. 50 X.*

DISCUSSION

This is the first report of a haplosporidan infection in clams on the Pacific coast. Specific identification of this organism was not possible without observing more developmental stages and mature spores. If this haplosporidan is not a new species peculiar to *T. capax*, it may have been introduced into Yaquina Bay with eastern oysters, where it was able to survive and infect other bivalves. *C. virginica* was cultivated in Yaquina Bay for about 50 years, sometimes with heavy mortalities of stocks (Sweetser, 1907; Dimick *et al.*, 1941). Recent reports of haplosporidan infections in *C. virginica* held in California bays (Katkansky and Warner, 1970a; Krantz *et al.*, 1972) and in indigenous oysters in bays where *C. virginica* have been reared (Katkansky and Warner, 1970b; Mix and Sprague, in press) support the possibility of transmission in this case.

Presently, the existence of this parasite in *T. capax* is known only from Yaquina Bay. Infections as described here were noted in *T. capax* taken from this bay as long ago as 15 years, but they have not been seen in clams taken from other bays in the state (Dale Snow, Oregon Fish Commission, personal communication). This infection has not been seen in large samples of *T. capax* taken in British Columbia (Neil Bourne, Fisheries Research Board of Canada, personal communication) and northern California (John De Martini, Humboldt State University, personal communication) for growth and reproductive studies.

The incidence of infection in our samples (43%) must be qualified by two observations. First, infection was diagnosed by macroscopic observations of cysts. Undoubtedly, there is some period following the initial stages of infection in which cysts have not been formed. Also, non-resistant animals may not form cysts in response to the parasite, thus making our estimates low. Second, our samples were

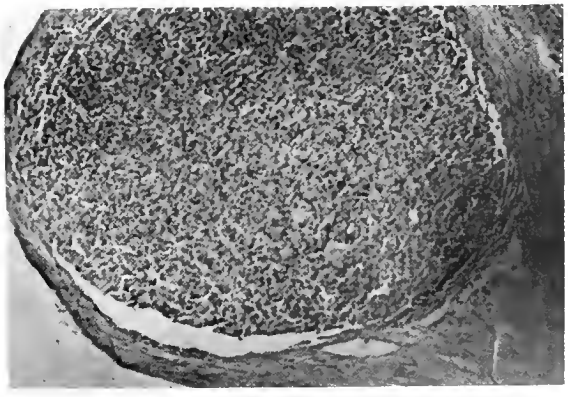

FIG. 6. *Cyst, 1.5 mm in diameter, under the epithelium of a siphon. 100 X.*

primarily composed of older clams. About 55% were 110-130 mm in shell length (4-5 years old, Marriage, 1954) and accounted for 70% of all infections, which precludes an accurate estimate of the incidence of infection based on all age groups of *T. capax* present in the bay.

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We thank Dr. Victor Sprague who kindly examined slides of this material and shared his observations with us. Appreciation is also expressed to Drs. Raymond Millemann, Michael Mix and Robert Olson who discussed this topic with us and reviewed the manuscript.

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Volume 64 - 1974

A RE-EVALUATION OF THE COMBINED EFFECTS OF TEMPERATURE AND SALINITY ON SURVIVAL AND GROWTH OF *MYTILUS EDULIS* LARVAE USING RESPONSE SURFACE TECHNIQUES

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ABSTRACT

Response surface techniques were used to critically examine the combined effects of temperature and salinity on late larval survival and growth of Mytilus edulis *using experimental data reported in the literature. The range of conditions estimated for maximum survival was found to be significantly different than those for maximum growth. Temperature exerted a strong effect on both larval survival and growth, while a temperature-salinity interaction effect was not significant.*

INTRODUCTION

Since Lough and Gonor (1973a, b) have critically examined the combined effects of temperature and salinity on the larval life of *Adula californiensis* by multiple regression analysis and the fitting of response surfaces to survival, growth, and respiration, Lough (1974) has undertaken further studies of bivalve larvae reported in the literature to re-evaluate their tolerances. Response surface techniques permit the estimation of an organisms response to a wide range of untested conditions and allows a visual interpretation of any change in its response at various stages of development. This relatively new and rigorous approach to the field of marine ecology has been reviewed by Alderdice (1972). The purpose of this paper is to re-evaluate the combined effects of temperature and salinity on the late larvae of *M. edulis* using the experimental data reported by Brenko and Calabrese (1969).

METHODS

Brenko and Calabrese (1969) reared the larvae of *M. edulis* at normal seawater conditions (18 ± 1 C and 27 ± 1 ppt salinity) to the straight-hinge stage and then transferred them to six levels each of temperature and salinity to determine the effects of these factors on larval development. Survival and growth were determined when the first cultures reached setting size (16-17 days).

The mathematical model used in the analysis was of the form:

$$Y = b_0 + b_1(T) + b_2(S) + b_3(T^2) + b_4(S^2) + b_5(T \times S)$$

where Y = percentage survival or growth, b_0 = a constant, T = linear effect of temperature, S = linear effect of salinity, T^2 = quadratic effect of temperature, S^2 = quadratic effect of salinity, T x S = interaction effect between temperature and salinity.

Table 1 - *Multiple regression analyses of* Mytilus edulis *larvae*

Regression Step Number	Variable	R^2	F-Level	D.F.	Level of Significance	Coefficients	T-Value	Level of Significance
				16 - 17 day survival				
1	T^2	.828	164.135	(1,34)	1%	-.2430	10.339	1%
2	T	.914	32.838	(2,33)	1%	5.3634	5.331	1%
3	S_2	.931	7.583	(3,32)	1%	2.8254	2.092	5%
4	S^2	.937	3.429	(4,31)	5%	-.0428	1.82	N.S.
5	T x S	.937	.002	(5,30)	N.S.	.0007	.035	N.S.
	Constant					17.9229		
				16 - 17 day growth				
1	T^2	.051	1.844	(1,34)	N.S.	-.4308	.136	N.S.
2	T	.745	89.681	(2,33)	1%	15.1655	11.151	1%
3	S_2	.751	.833	(3,32)	N.S.	10.2691	5.623	1%
4	S^2	.878	32.321	(4,31)	1%	-.1781	5.606	1%
5	T x S	.879	.139	(5,30)	N.S.	-.0101	.373	N.S.
	Constant					-192.5717		
				16 - 17 day survival and growth				
1	T^2	.291	28.701	(1,70)	1%	-.3369	8.217	1%
2	T	.567	43.995	(2,69)	1%	10.2644	5.850	1%
3	S_2	.577	1.559	(3,68)	N.S.	6.5472	2.779	1%
4	S^2	.619	7.369	(4,67)	1%	-.1105	2.695	1%
5	T x S	.619	.018	(5,66)	N.S.	-.0047	.135	N.S.
	Constant					-87.3244		

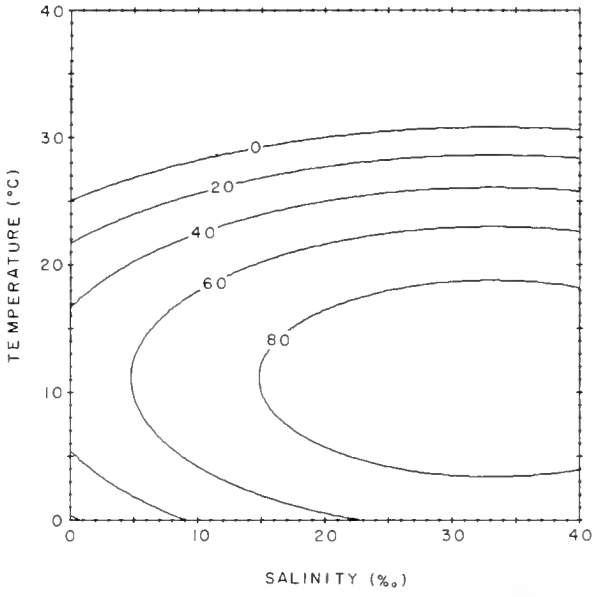

FIG. 1. *Response surface estimation of percent survival of* Mytilus edulis *veliger larvae after 16-17 days of development at experimental temperature and salinity combinations given in Brenko and Calabrese (1969).*

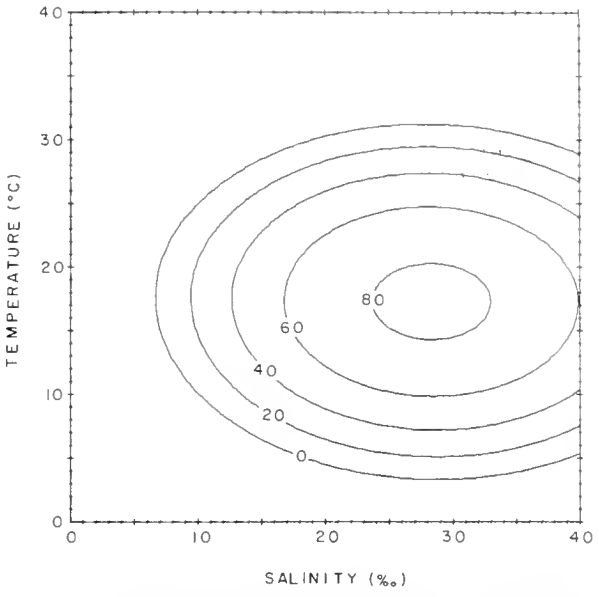

FIG. 2. *Response surface estimation of percent growth of* Mytilus edulis *veliger larvae after 16-17 days of development at experimental temperature and salinity combinations given in Brenko and Calabrese (1969).*

The coefficients in the model (b's) were estimated by the Oregon State University Statistical Program Library, *STEP, a stepwise multiple regression computer program. F-levels were set equal to zero to enter and remove variables. This allowed all variables to come into the equation by a forward selection process, their order of insertion determined by using the partial correlation coefficient as a measure of their importance. The contribution a variable makes in reducing the variance of the equation can also be considered by looking at the various values given as the program proceeds. One of the more useful is the square of the multiple correlation coefficient, R^2, defined as the sum of squares due to regression, b_0/ total sum of squares, corrected for the mean. It is often stated as a percentage, $100R^2$. The larger R^2 is, the better the fitted equation explains the variation in the data. Values of R^2 can be compared at each stage of the regression program. A t-test is also made indicating the equality of the individual regression coefficients of zero and their level of significance.

The calculated regression coefficients from a particular equation were fitted by computer to a full quadratic equation in temperature and salinity in order to print a contour diagram of the response surface. The computer program was instructed to print 20 percent contour intervals, wide enough to exclude the approximate ± 10 percent experimental error reported by the authors.

Analysis of covariance methods, as used in Lough and Gonor (1973a, b), were used to test the significance of the difference between the estimated polynomials for larval survival and growth.

RESULTS

Multiple regression analyses and response surfaces for the percentage survival and growth of *M. edulis* larvae at 16 to 17 days are given in Table 1 and Figures 1 and 2. Larval survival after 16-17 days of rearing was most affected by the quadratic and linear effects of temperature (T^2, T). The orientation of the survival response contours clearly shows this effect. Maximum survival to 16-17 days (80% contour) was estimated to occur between temperatures of

3.5 to 19 C and salinities above 14.5 ppt.

Growth of the larvae after 16-17 days was most affected by the linear effect of temperature (T) followed by the quadratic effect of salinity (S^2). Maximum growth (80% contour) was estimated to occur between temperatures and salinities of 14.5 to 20.5 C and 24 to 33 ppt. Analysis of co-variance showed a significant difference (1% level) between the polynomials estimated for survival and growth indicating that the narrower temperature and salinity range for maximum growth is significantly different than the range for maximum survival.

Analysis of the combined survival and growth at 16-17 days indicated that the linear and quadratic effects of temperature (T, T^2) were the two most important factors explaining the data. Optimum temperature and salinity conditions (80% contour) for maximizing both larval survival and growth was estimated to occur at 11 to 14 C and 22.5 to 36.5 ppt.

DISCUSSION

Bayne (1965) found that successful development of Talyfoel larvae from fertilization to trochophore stage occurred only in the range of 30 to 40 ppt salinity. The early embryos and larvae of *M. edulis* require a narrow range of salinity, near oceanic, despite the fact that the adults and late larvae (16-17 days of age) are euryhaline. Survival of the late larvae is delimited by temperature as noted for several other species of bivalve larvae studied (Lough and Gonor, 1973a, b; Lough, 1974). Maximum growth of late larvae required a much narrower range of temperature-salinity conditions than maximum survival. A true temperature-salinity interaction effect was not found for this species. The analysis of growth for all other species of bivalve larvae showed a pronounced interaction effect of temperature and salinity. This would indicate that within the suitable range of salinity, growth of *M. edulis* larvae is dependent only upon temperature.

Natural seawater conditions (18 ± 1 C, 27 ± 1 ppt) are found on the upper temperature limit of the 80% survival contour and within the area for maximum growth. These are the conditions which were used for rearing the early larvae. Conditions under which the early larvae develop may influence the center and range of the contours for maximum survival and growth of late larvae.

ACKNOWLEDGEMENT

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TRENDS IN PESTICIDE RESIDUES IN SHELLFISH

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ABSTRACT

The National Estuarine Monitoring Program, a cooperative effort between the State and Federal Governments, collected and analyzed shellfish samples for persistent synthetic pesticides at monthly intervals during the years 1965-1972 in 15 coastal states. The recently completed study of the 8000-plus analyses demonstrates that: (1) the residues found, primarily DDT and its metabolites, were universally too low to have human health significance, (2) areas of both high and low residues were clearly defined geographically, (3) in some areas there has been a trend towards a wider distribution of smaller residues, and (4) there has been a marked decline generally in DDT residues since 1968 when peak levels in molluscs were detected.

INTRODUCTION

During the period 1965-1972, samples of oysters and other bivalve molluscs were collected at monthly intervals at about 180 estuarine locations to determine the incidence and magnitude of pesticide residues along the Atlantic, Pacific and Gulf of Mexico coasts. More than 8000 samples were screened for the presence of 12 of the more persistent chlorinated pesticides. In the later years, chlorinated pesticides. In the later years, chlorinated biphenyls or PCB's were included in the analytical procedures. This report briefly summarizes the implications of some of the principal findings. A detailed report of the sample collections and analyses has been published recently (Butler, 1973).

BACKGROUND

Oysters exposed to varying concentrations of pesticides under controlled conditions in the laboratory demonstrate their sensitivity to these pollutants. In aquaria with flowing unfiltered seawater, for example, as little as 1.0μg/kg (ppb) of DDT inhibits oyster shell growth by about 20 percent in a 4-day period. One μg/g (ppm) inhibits shell deposition completely at water temperatures of about 17-20 C (62-68 F) (Butler, 1966).

Concentrations as high as these were not anticipated in the natural environment and so it was of importance in the development of a proposed monitoring program to discover that oysters were sensitive to the presence of DDT in ambient water at levels as low as 10×10^{-12}(10 parts per trillion). Exposure of oysters for 7 days to this extremely low concentration led to the formation of DDT residues in the tissues of about 70 μg/kg, a biological magnification of 70,000x. DDT levels of this magnitude might be anticipated in the marine environment since it is less than the solubility of DDT in water. Further laboratory experiments demonstrated that oysters and other molluscs would be reliable as biological tools to monitor estuarine ecosystems because of this tendency to concentrate persistent chemicals (Table 1).

Additional experimentation showed that contaminated oysters cleansed themselves of resi-

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TABLE 1. *Uptake of DDT by eastern oysters maintained in flowing seawater. Exposure period 7-15 days in different tests. (Butler, 1968).*

Concentration in water	Residue in oyster	Biological magnification
(µg/kg) or (ppb)	(µg/g) or (ppm)	(x1000)
10.0	150.0	15
1.0	30.0	30
0.1	7.0	70
0.01	0.72	70
0.0001	0.07	0
control	0.06	

dues when returned to clean water. The disappearance time or biological half-life of the residues in molluscs was short; a matter of days as compared to months or years in fish and other vertebrates. Consequently, when oysters were sampled at about 30-day intervals, it was possible to estimate when pollution entered the estuary and thus gain some insight as to its source.

FINDINGS

Analyses of monthly collections of oysters in an estuarine complex near Pensacola, Florida revealed a seasonal pattern of DDT residues later found to be typical of estuaries in many coastal areas. In the period February through May there was a gradual increase in residue magnitude to a seasonal high in late spring. This was followed by a decline to 'background' levels typical of the remainder of the year. It seems reasonble to assume that this picture results primarily from the occurrence of seasonal rains and surface water run-off which carry soil eroded from agricultural lands through the river basin and into the estuary. In contrast to this picture, there was a second seasonal peak of DDT residues during the winter months in samples from the South Texas coast. This bimodal cycle probably reflected the double cropping of farm lands and the associated multiple applications of pesticides in this sub-tropical area.

A more obvious result of the seasonal agricultural use of DDT was indicated by residues in oysters monitored in the Caloosahatchee River Basin in southwest Florida. Here, peaks in DDT residues in oysters appeared soon after the seasonal application of DDT to maturing crops of sweetcorn and sugarcane. In 1967-68, the early spring residues were nearly ten times the level of residues found during the other months of the two-year monitor period (Fig. 1). In some instances, seasonal and annual patterns of pesticide accumulation in estuarine oysters could be associated with the dumping of industrial effluents or with the control of noxious insect populations. The declining use of DDT in stable-fly control in northeast Florida, for example, was clearly indicated by annual decreases in DDT residues in local oysters in the period 1965-1968. DDT residues were no longer

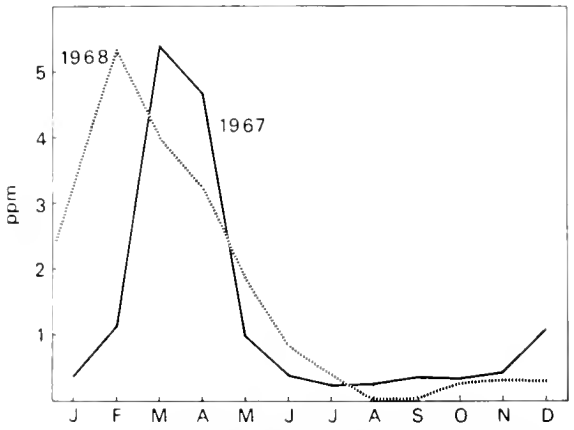

FIG. 1. *DDT residues in the eastern oyster from the Caloosahatchee River Basin, Lee County, Fla., by month of collection (Butler, 1973).*

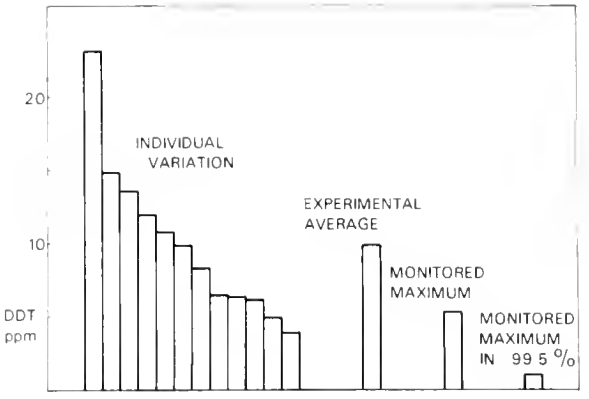

FIG. 2. *DDT residues in experimental and field-collected oysters in the period 1965-1972. See text for explanation.*

identified after the substitution of methoxychlor, a less persistent compound, for fly control in 1969. More importantly, methoxychlor was not detected in the monitor samples in succeeding years.

The significance of DDT residues in field samples may be judged to some extent by the magnitude of DDT residues observed in laboratory experiments. Market-size eastern oysters were exposed to 1.0 μ g/kg of DDT in flowing seawater for a 10-day period and then 12 were individually analyzed. The sum of DDT and its metabolites found as residues ranged from a low of 3.9 to a high of 23.2 μg/g with an arithmetic average of 10.1 μg/g (ppm) for the group. This value is about twice the largest DDT residue observed (5.39 ppm) in all of the molluscan samples collected in the 7-year monitoring period. It should be noted further that DDT residues were less than 1.0 μg/g in 99.5 percent of the 8000+ monitoring samples analyzed. It appears that despite the build-up of large residues in higher carnivores DDT pollution of estuarine waters generally has been at levels below 1.0 μg/kg (Fig. 2).

It must be emphasized that the observed levels of DDT residues in molluscs were too low to have human health significance or to have demonstrable effects on the oysters themselves. Only in isolated area were DDT residues high enough to indicate that some elements of the estuarine fauna might have been damaged by the magnification and accumulation of DDT residues in the food web.

With these observations in mind, the overall findings of the monitoring data may be summarized geographically. The lowest average incidences of DDT positive samples were found, in order, in Washington, Georgia and Maine. Highest incidence rates were observed in New Jersey, Alabama, North Carolina and California. However, the *largest* residues of DDT and its metabolites were found in samples collected in the estuaries of Florida, California and Texas.

There has been a well-defined but gradual decline in both the incidence and magnitude of DDT residues in oysters during the monitoring period in most areas. In some coastal estuaries this trend is obscured by the lack of uniformity in the timing of sample collections or by variations in the kind of mulluscs collected. Despite erratic fluctuations in magnitude and the fact that individual residues were never very high, it is clear that DDT pollution in estuaries was at peak levels in 1966-1967 and gradually declined thereafter. This 1966 peaking in the magnitude of residue data parallels, not unexpectedly, the findings of peak DDT levels in fresh water monitoring samples in 1966 followed by sharp declines in 1967 and 1968 (Lichtenberg, et al., 1970).

Data demonstrating the overall decline in the magnitude of DDT residues in estuarine molluscs are summarized in Fig. 3. This dia-

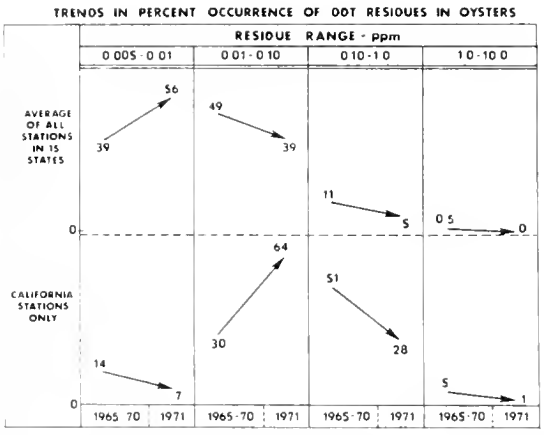

FIG. 3. *Percentage occurrence of DDT residues in estuarine bivalves in the period 1965-1970 as compared to 1971. Data summarize about 7000 analyses of more than 75,000 animals. See text for explanation.*

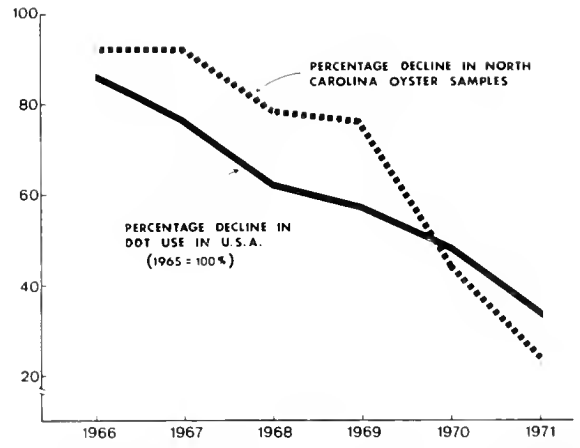

FIG. 4. *Percentage decline in DDT residues of more than 10 μ g/kg in North Carolina oysters as compared to the decline in the consumption of DDT in the entire United States in the period 1965-1971.*

gram shows that, in the period 1965-1970, 39% of all samples contained negligible DDT residues, less than 0.01 ppm. while in 1971 this value increased to 56%. Conversely, in these same years the percentage of samples containing larger residues declined sharply. In California and a few other isolated locations there was an exception to this generalized picture in that the number of samples with DDT residues in the 0.01-0.10 ppm range increased during the monitoring period but the percentage of samples with high residues decreased sharply as in other coastal areas. Apparently in these drainage basins, there was an increased cycling of DDT in the trophic web accompanied by a diminution of the amount present in individual animals. In other words, DDT residues were distributed more thinly among more members of the biota.

At ten monitoring stations in North Carolina, where the continuity of sample collections was especially good, the data provide a clear picture of annual trends in DDT pollution levels. Fig. 4 shows the decline in the percentage of samples having measurable DDT residues as compared with the approximate percentage decline in the domestic use of DDT throughout the United States after 1965. DDT supplies in that year have been arbitarily designated as 100% for the basis of this comparison (USDA, 1967-72). These data demonstrate the progressive loss of residual DDT from at least one segment of an estuarine ecosystem following the generalized curtailment in the agricultural use of DDT, and controvert the widespread belief that environmental problems with DDT would be longlasting regardless of how soon its use was terminated.

SUMMARY

These monitoring data show that the domestic use of DDT resulted in only nominal residues in estuarine molluscs in the United States in the period 1965-1972. By extrapolation from laboratory data, we may infer that these residues were too small to have a deleterious effect on the growth and productivity of estuarine bivalves. Despite the chemical stability of DDT, curtailment in its use was almost immediately reflected by declines in the magnitude of residues in estuarine molluscs. The data establish a baseline for levels of DDT pollution in estuaries during the monitored period, and suggest that despite the stability biologically unavailable soon after its widespread use is discontinued.

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BACTERIAL PATHOGENICITY IN LABORATORY-INDUCED MORTALITY OF THE PACIFIC OYSTER *(CRASSOSTREA GIGAS*, THUNBERG)

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ABSTRACT

Mortality of Pacific oysters (Crassostrea gigas) *was monitored in four trials with low-temperature control (10 C), high-temperature control (20 C), high-temperature + UV (20 C), high-temperature +* Vibrio *spp. or* Vibrio anguillarum *(20 C), and high-temperature + oxytetracycline (TM-50) (20 C) treatment groups. Mortality was highest in the bacteria-inoculated treatments and lowest in low-temperature control troughs. Computer analysis, using contingency table analysis, substantiated observed mortality results by testing the independence of trial number, treatment, time, and temperature. High temperature was a substantial contributing factor to mortalities. TM-50 successfully decreased bacterial counts of water and oysters, as well as decreasing mortality. UV treatment decreased water counts but not mortality. Moribund or dead oysters previously held at elevated temperatures, compared with normal low-temperature control, Puget Sound (Eld Inlet), and Humboldt Bay oysters, consistently had higher bacterial counts in all media tested. Bacteria associated with normal and moribund or dead oysters were isolated and identified as* V. anguillarum, Vibrio alginolyticus, Vibrio parahaemolyticus, Vibrio *spp.*, Pseudomonas *spp., and* Aeromonas *spp.* V. anguillarum *and* V. alginolyticus *were implicated as facultative pathogens for Pacific oysters at elevated temperatures.*

INTRODUCTION

Mass mortalities of *Crassostrea gigas* have occurred annually in recent years in Pacific waters, including Japan, Washington, and California. In studies at the University of Washington, apparently "similar" mortalities have been induced in the laboratory. In the laboratory systems, bacteria, apparently vibrios, were involved. A comprehensive review of this subject was presented by Grischkowsky (1973).

The etiology of these mortalities has not been determined despite the many theories proposed. Lipovsky and Chew (1972) produced mortalities of Pacific oysters under laboratory conditions at 14 and 21 C. They implicated unknown bacteria as the causative agents under enriched environmental conditions. Breese (1971), working with *C. gigas* spat at 12 and 25 C, found significantly higher mortalities at the elevated temperature.

Colwell and Liston (1962) reported the normal microflora of *C. gigas* to be mainly *Pseudomonas*, *Flavobacterium*, *Micrococcus* spp. and gut group vibrios. Baross and Liston (1968, 1970) and Liston and Baross (1973) found *V. alginolyticus*, *V. anguillarum*, and *V. parahaemolyticus* in healthy

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Puget Sound oysters directly correlated with temperature in environmental ranges. *V. parahaemolyticus* and related halophilic vibrios were found by Thomson and Trenholm (1971) in unidentified species of clams, oysters (probably *Crassostrea virginica*), periwinkles, mussels (probably *Mytilus edulis*), and snails.

Various species of bacteria, including vibrios, have been implicated as pathogens for pelecypod mollusks. Tubiash (1971) found the soft-shell clam *(Mya arenaria)* susceptible to *V. alginolyticus*, *V. anguillarum*, and *Vibrio* spp. when inoculated actively into heart, incurrent or excurrent siphon tissue at 20 and 22 C. The same two species of *Vibrio* were isolated from dead and moribund larvae and juveniles of the hard clam *(Mercenaria mercenaria)* and oyster *(C. virginica)*. They were identified as the etiologic agents of a disease called bacillary necrosis (Tubiash *et. al.*, 1970). These authors speculated that during midsummer, conditions in Chesapeake Bay and Long Island Sound that favor both molluscan spawning and bacterial proliferation may be responsible for natural epizootics of bacillary necrosis in commercially valuable bivalve mollusks.

Colwell and Sparks (1967) found the natural microflora of *C. gigas* to be composed of organisms representing the genera *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Vibrio*, and *Micrococcus*. The flora of dead or dying oysters included a greater incidence of *Pseudomonas* (particularly *Pseudomonas enalia*). *P. enalia* was thought to be pathogenic to *C. gigas* held in laboratory tanks when the body tissues were injected with viable suspensions. Histological examination suggested a bacterial invasion. The laboratory mortality was variable and sample sizes were small.

Vibrios, including *V. parahaemolyticus*, have been shown by Colwell *et al.*, (1973) and Kaneko and Colwell (1973) to increase rapidly during summer temperatures of 14 to 19 C in Chesapeake Bay water and zooplankton. The authors determined that *V. parahaemolyticus* overwinters in sediment and shellfish or scavenger fish such as gobies, which occur on the bottom.

Since little was known about bacterial diseases of oysters, a laboratory model system was developed for a study of oyster-bacterial interaction. An investigation of mortalities induced in the model was conducted and an attempt made to relate findings to the natural conditions of oysters in the field.

METHODS AND MATERIALS

Oysters were held in containers supplied with water at high (20 C) or low (10 C) temperature, which was recycled through a filter and aeration system to maintain quality. Mortalities of the oysters in tanks held under various conditions with and without bacterial challenge were monitored, together with bacteriological factors.

Seawater

Seawater used in the experiments was collected from Seabeck Bay, Washington and held in 55-gallon poly-drums with plastic inner barrels.

Oysters

Specimens of *C. gigas* (2+ years) collected from commercial beds in Eld Inlet (Mud Bay), Puget Sound near Olympia, Washington were used in all experiments. Eld Inlet is historically a high mortality area (Scholz *et al.*, 1971). Water temperature and salinity were monitored monthly at the collection site during 1972. Oysters were held in tanks with recycled, filtered seawater cooled to 12 C prior to use. Little or no natural mortality was experienced under these conditions for as long as six weeks. Before use, oysters were scrubbed under tap water and adherent large barnacles and mussels removed.

Bacteria

Strains of bacteria used in the mortality experiments were isolated by culture of dead or moribund oysters. The organisms were grown in a seawater (50%), peptone (2%), yeast extract (1%) and glucose (0.5%) broth (SWPYG) and the bacteria and medium were added directly to the water in the tanks. No indications of sterile broth toxicity to oysters was noted and the toxicity of bacterial metabolites introduced with the broth was not tested. Identification of the strains was made following the general characteristics described by Sakazaki *et al.*, (1963); Sakazaki (1969 and 1971) and confirmed in the case of *V. anguillarum*, *V. alginolyticus* and *V. parahaemolyticus* by DNA homology analysis (performed by Dr. E. J. Ordal,

Microbiology Department, University of Washington), using procedures described by Kiehn and Pacha (1969) and Anderson and Ordal (1972).

Oyster Food

Stock oysters were fed *Monochrysis lutheri* at the rate of 20 ml of a culture containing 10^6-10^7 cells/ml per oyster per day. The *Monochrysis* was supplied by Dr. F. B. Taub from her continuous culture unit (Taub, 1971).

Temperature Control and Aeration

In general, seawater was added to the containers (and to the reservoir system in the case of chilled water) and allowed to stabilize to the desired temperature. Scrubbed oysters were then added and permitted to acclimate in the tank for three days. Bacteria were added or other treatments initiated at this time.

Cold water at 10 C was supplied to low-temperature group containers through a recycling unit. The reservoir consisted of a 30 gallon polyethylene cylinder fitted with refrigeration coils which were linked to a compressor (one ¼ HP for trial 1 and two ¼ HP for trials 2-4).

Containers varied in different experiments, as indicated below. However, where tanks were used they were fitted with glass-wool/charcoal filters, aerators, and air pumps, following the general concept of Spotte (1970). This system maintained aerobic conditions and prevented accumulation of toxic metabolites in the tanks. High-temperature groups were maintained at approximately 20 C.

Statistical Analysis

Data from the four major experiments were analyzed using Biomedical Computer Program contingency table analysis, BMDO2S (Dixon, 1968) on a Control Data 6400 computer. The variables tested at the 0.95 1-α significance level for independence were: trial number, treatment group, mortality, temperature, and time. These tests were first grouped to include all trials, then separately by trial number and by specific comparisons of treatment groups. The null hypothesis, H_0, for all of these tests was that there was independence between the selected variables.

Preliminary Experiment

A preliminary experiment, trial 1, was conducted with low-temperature control (LTC), high-temperature control (HTC), and high-temperature + *Vibrio* sp. (HT + *Vibrio* sp.) Three, 20 gal. glass aquaria containing 29, 22, and 22 oysters were used. The HT + *Vibrio* sp. tank received 10^5/ml unidentified vibrio isolated from pericardial fluid from a moribund oyster, in SWPYG Broth.

Large Scale Experiments

Three additional trials (2-4) utilized four salmonid rearing troughs, each containing 37 gal. of seawater from Seabeck Bay. Each trough was recirculated by a Teel chemical magnetic drive pump. Treatment groups included the previous three (LTC, HTC, and HT + *V. anguillarum*), plus a high-temperature trough (HT + UV) treated with a 10GPM AquaNomics ultraviolet sterilization unit and a high-temperature group in a 50 gal. fiberglass tank to which TM-50 (50 g oxytetracycline/lb of inert powder) was added at regular intervals (HT + TM-50). Troughs held 100 oysters each from the same source as before, while the fiberglass tank contained 50 oysters. All containers were aerated, filtered through sterilized glass wool/charcoal filters, and received 2l./day of 10 cells/ml *M. lutheri*.

To determine bacterial counts of normal and moribund experimental oysters and to recover introduced organisms, samples of oysters were washed and shucked by procedures recommended by the American Public Health Association (1970), and an equal weight by volume (g/ml) mixture of sample and dilution fluid (1.5% NaCl, 0.5% peptone in distilled water) was homogenized in a Waring blender for 60 sec. The mixture was serially diluted and plated on various media, including 5% blood agar (BA) at 25 C, bromothymol-blue Teepol agar (BTB) at 25 and 37 C, salt-starch agar (VPS) in an anaerobic jar (BBL gas pack) at 43 C and seawater starch agar (SWS) at 25 C.

Suspensions of *V. anguillarum* (culture number 728), isolated from moribund oyster pericardial fluid, was added to troughs for a final concentration of 10^4-10^6 bacteria/ml. The TM-50 treatment groups received approximately 20 mg

of the compound/day. To test the effectiveness of oxytetracycline, Terramycin sensitivity discs (10 mcg), millipore filtered water from the HT + TM-50, and TM-50 powder were placed on blood agar plates previously streaked with *V. anguillarum* or *V. alginolyticus* and incubated at 25 C. At the end of one of the trials, surviving low-temperature control oysters were transferred into high-temperature control waters, and in another LTC oysters were inoculated with *V. anguillarum* at low temperatures.

During all trials, close comparisons were made between microflora of treatment group oysters and regular monthly sample or LTC oysters from Puget Sound (Eld Inlet), Humboldt Bay, and the appropriate trials.

RESULTS

Field Data

Temperature and salinity of the sampling area water for 1972 are depicted in Figure 1. The wide littoral temperature range from 1 to 27.5 C must resultantly place a large stress on resident oyster populations and may affect bacterial invasiveness. Field studies included total heterotrophic bacterial counts at 25 C, mesophilic vibrio counts for water sediment, and monthly samples of oysters (Figure 2). Sediment samples had the highest counts, followed by oysters, and water. Total counts and mesophilic vibrio counts of oysters responded directly to seasonal temperature increases (Fig. 1 and 2).

Preliminary Investigations

During preliminary investigations, various tissues and fluids of healthy oysters from monthly samples and LTC treatments and heat-treated moribund oysters were examined under phase-contrast. Oyster fluids exhibited only amoebocytes, fat globules, and gonadal material in normal oysters. Moribund oyster heart and pericardial fluids consistently contained many actively motile curved rods with rounded ends easily visible under phase microscopy. Bacteria were not enumerated or isolated during the preliminary investigation. Mantle and gill tissue and shell liquor usually contained a wide range of mixed organisms.

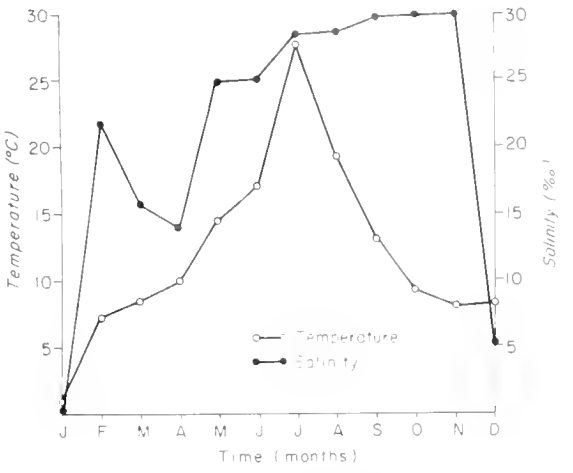

FIG. 1. *Temperature (C) and salinity (‰) for Eld Inlet, Puget Sound (Mud Bay) during 1972.*

Mortality Results

Trial 1, utilizing 20 gal. tanks and taking place in January, showed no mortality in the LTC, 46% for HTC, and 86% for HT + *Vibrio* sp. after 37 days (see Figure 3). The combination of an elevated temperature and vibrio inoculum significantly increased mortality. Dramatic mortality increases (of 46 and 86% respectively in HTC and HT + *Vibrio* sp. tanks) at the high temperature level occurred here and in successive trials.

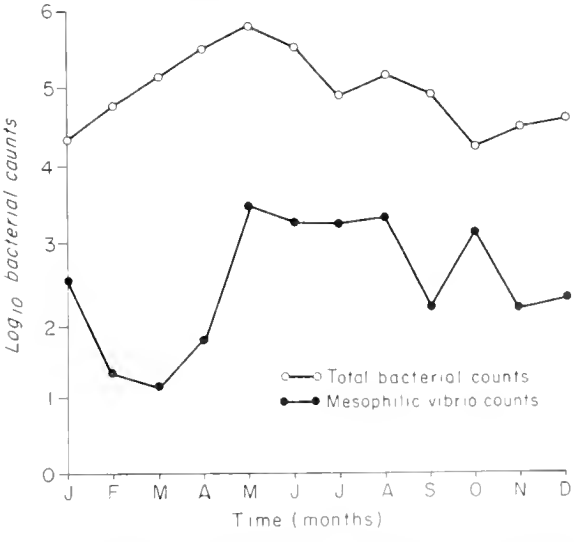

FIG. 2. *Total bacterial counts and mesophilic vibrio counts per gram of oyster meats from Eld Inlet, Puget Sound (Mud Bay) during 1972.*

Trial 2, utilizing salmonid rearing troughs and taking place during May, represented the beginning of large scale experiments using 100 oyster sample sizes. A mortality of 2% occurred in the LTC, 100% in HT + UV and HT + *V. anguillarum* after 15 days, and 100% in HTC after 12 days (see Figure 4). The bacterial inoculation was apparently not virulent on this occasion. The mortality rate was greatly accelerated in this and subsequent trials during warmer months over that which took place during the winter. This phenomenon might be attributed to increased total counts, mesophilic vibrio counts, seawater temperatures, or ambient high temperature levels. Surviving LTC oysters, when transferred to the HTC tank, exhibited a 94% mortality after 10 days as compared with 2% in the LTC tank.

Mortality for trial 3 was 1% for LTC, 43% for HTC, 61% for HT + UV after 15 days, 100% for HT + *V. anguillarum* after 12 days, and 4% for HT + TM-50 (oxytetracycline) after 15 days (Figure 4). High temperatures and *V. anguillarum* increased oyster mortality, while the addition of oxytetracycline but not UV light decreased mortality significantly.

Trial 4 caused the most rapid mortality. The usual 2% occurred in the LTC after 13 days, while the HTC mortality was 98% after 9 days,

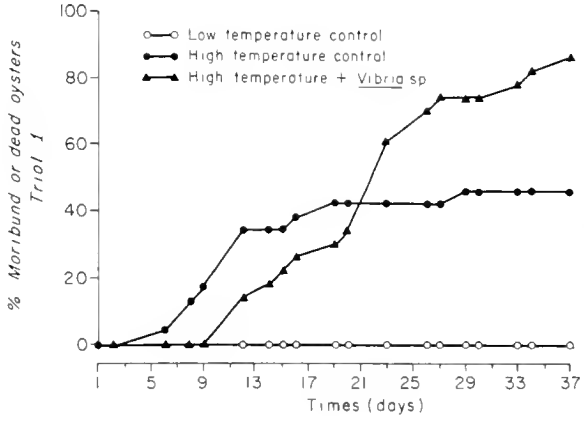

FIG. 3. *Rate of mortality, preliminary experiment (trial 1), for low temperature control, high temperature control, and high temperature* + Vibrio *sp. treatment groups.*

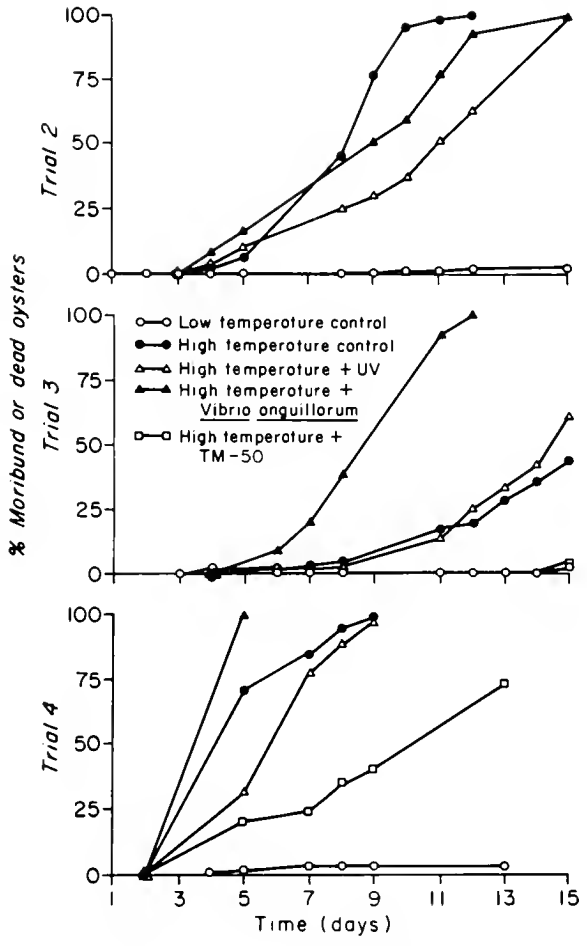

FIG. 4. *Rate of mortality, large scale experiments (trials 2-4), for low temperature control, high temperature control, high temperature* + *UV, high temperature* + Vibrio anguillarum, *and high temperature* + *TM-50 treatment groups.*

HT + UV 97% after 9 days, HT + *V. anguillarum* 99% after 5 days, and HT + TM-50 72% after 13 days (Figure 4). Of the high temperature treatment groups, the inoculated one showed the highest mortality, while the TM-50 treatment displayed the lowest. UV apparently was ineffective in reducing mortality. Surviving LTC oysters were inoculated with *V. anguillarum* and maintained at the low temperature for 15 days with 7.5% mortality.

Bacteriological Analysis

In trial 2 the number of bacteria as measured by MPN in SWPYG increased in all

troughs over the baseline, with a range of 1-log increase in the LTC and HT + UV to a 5-log increase in the HTC. Bacterial isolates were all gram-negative, motile, oxidase (Kovacs) positive, slightly curved rods, with rounded ends. Normal low-temperature oysters contained *V. alginolyticus*, *Pseudomonas* spp., and *Aeromonas* spp. The high-temperature moribund oysters contained large numbers of *V. alginolyticus*, some *Vibrio* spp., and few *Pseudomonas* and *Aeromonas* spp. Recovery of *V. anguillarum* from the inoculated tank oysters was frequent; of the 16 *V. anguillarum* isolates, 10 were recovered from the HT + *V. anguillarum* trough. Homogenate from moribund or dead oysters, when streaked on BA, yielded relatively pure cultures of *V. anguillarum* compared with mixed cultures usually obtained from low-temperature oysters.

For trial 3, several MPN counts were made. The baseline seawater counts were 2 logs higher for trial 3 than trial 2. The LTC trough yielded surprisingly high numbers, 10^6/ml, or 1-log higher than the inoculated trough and 2-logs higher than the HTC trough after 8 days. After 12 days, the highest counts were 10^7, 10^6, and 10^5/ml respectively in the UV, *V. anguillarym*, and the TM-50 (oxytetracycline) treatment groups. The bacterial counts of oysters, using homogenate of grouped oyster samples for moribund or dead oysters, yielded in VPS, 10^4-10^5; SWS, 10^7-10^8; BTB, 10^4-10^7/g. For healthy oysters results were VPS, 0; SWS, 10^5; and BTB, 10^3-10^4/g. Recovery of *V. anguillarum* from moribund or dead oysters was excellent in the inoculated trough. Of the 15 *V. anguillarum* isolates, 9 were from the HT + *V. anguillarum* trough. Most *V. alginolyticus* isolates were from the HTC and HT + UV troughs. Normal oysters contained low levels of all listed bacteria.

MPN counts in SWPYG for trial 4 of treatment waters, indicated a 2-4 log increase after 5 days, from a basal level of 10^3/ml. Bacterial counts of normal oysters held for 13 days in the LTC trough show the same approximate levels as those sampled on the collection day. Although water counts increased 2-4 times, oyster counts in the LTC trough remained constant. UV treatment usually reduced water counts but failed significantly to decrease mortality. TM-50 (oxytetracycline) reduced water counts, oyster counts, and mortality. Counts of moribund or dead HT + *V. anguillarum* oysters increased by 4-logs in VPS 43 C plates, probably denoting increased numbers of vibrios. High-temperature oyster counts increased 1-4 logs over normal oyster counts. Recovery of *V. anguillarum* from moribund HT + *V. anguillarum* oysters was poor. Of the 13 *V. anguillarum* isolates, only one was recovered from the HT + *V. anguillarum tank*. *V. parahaemolyticus* was isolated from a moribund oyster once in this tank. However, *V. anguillarum* was isolated from every oyster which died in the LT + *V. anguillarum* trough. In this case, 11 of the 13 *V. anguillarum* isolates were from the LT + *V. anguillarum* trough. Recovery of *V. anguillarum* from mixed oyster microflora, including *V. alginolyticus*, is made more difficult by spreading characteristics of the latter vibrio. *V. alginolyticus* and *Pseudomonas* spp. were well-represented in most trial 4 groups.

Bacterial Identity

The identification of 8 vibrios was confirmed by Dr. E. J. Ordal, using % homology and change of Tm. The organisms used for inoculation, culture no. 728, and bacteria recovered from the inoculated troughs of trials 2 and 3 (culture nos. 1445, 1491, 1800, and 1808), were 99.2, 100, 100 and 100% homologous (change of Tm = 0-0.2 C) with *V. anguillarum*, V-2911. Cultures 1432, 1439, and 1823, isolated from HTC troughs of trials 2 and 3, were determined to be 94.5, 100 and 96.2% homologous (change of Tm = 0-1.3 C), with *V. alginolyticus*, ATCC 17749 and 65.9-68.0% homologous (change of Tm = 7.5-8.0 C) with *V. parahaemolyticus* ATCC 17802. Culture 4167 isolated from trial 4, HT + *V. anguillarum* was 100% homologous with ATCC 17802 (change of Tm = 0 C). G + C content of the DNA was 44.9-45.6 mole % for all of the tested isolates.

All vibrios tested were found sensitive to oxytetracycline sensitivity discs, TM-50 powder and millipore-filtered water from HT + TM-50 treatment groups.

Computer Analysis

Computer analysis substantiated qualitative

differences already apparent. Results of contingency table analysis on mortality data are indicated by the Chi-Square/degrees of freedom figures provided in parentheses. In the test grouping all trials, mortality was significantly related to the trial number (186.53/33), treatment (396.02/44), time (greater mortality with increased time (213.13/99), and temperature (142.01/121). Considering trials 1, 2, and 3 each alone, mortality was associated with treatment, time, and temperature. For trial 4 alone, with all treatment groups combined, mortality was allied with treatment and time.

Comparing LTC and HTC, the high-temperature group had higher mortalities in all trials grouped (135.23/11) and individually for trials 1-4. In the test comparing mortality and trial number for LTC and HTC (130.66/33), all treatments and trials support the observation that mortality was more rapid in the summer than in the winter. Specifically comparing the treatments of HTC and HT + UV, the mortality of the two groups for all trials together (4.22/11) and individually is not significantly different. This result confirms observations that UV generally did not decrease mortality. Comparing the treatments HTC and HT + *V. anguillarum*, mortality was higher in the inoculated groups with all trials grouped (40.60/11) and with trials 1, 3, and 4 individually.

Regarding the treatments HTC and HT + TM-50, the addition of the antibiotic significantly decreased mortality in the two trials, 3 (6.92/1) and 4 (7.87/2), in which it was employed. ployed.

DISCUSSION

These data implicate *V. anguillarum* and *V. alginolyticus* as principal causes of heat-induced laboratory mortality, because they are most frequently isolated from heart blood and pericardial fluid of diseased oysters. Other organisms, e.g. *Aeromonas*, may also be involved. Mortality monitoring and computer analysis indicated an increased mortality rate among vibrio-exposed oysters. *V. anguillarum* and *V. alginolyticus* were both associated with moribund or dead oysters. We think they are facultative pathogens, since *V. alginolyticus* and *V. anguillarum* also occur in healthy oysters. Oysters held at high temperature consistently died at a higher rate than oysters at low temperature. This finding substantiates the reports of Lipovsky and Chew (1971, 1972, and 1973). These authors found 100% mortality in Pacific oysters at 20 C, with low mortalities at 10 C, and described *V. parahaemolyticus* as a "suspected marine molluscan pathogenic bacteria." *V. parahaemolyticus* identification at that time was made by Dr. John Baross, on the basis of biochemical tests then available. New techniques for bacterial identification, including DNA homology, have indicated that some earlier identifications of *V. parahaemolyticus* may be in doubt, (Lipovsky and Chew, 1971; Anderson and Ordal, 1972; Vanderzant, 1973). Lipovsky and Chew later (1972) suggested these bacteria might more appropriately be called mesophilic vibrios.

V. parahaemolyticus has been implicated as a pathogen of brown shrimp, *Penaeus aztecus*, in pond cultivation in the Gulf of Mexico (Vanderzant and Nickelson, 1970; Vanderzant *et al.* 1971; and Vanderzant, 1973) and of blue crabs, *Callinectes sapidus*, in Chesapeake Bay (Krantz *et al*, 1969). We isolated *V. parahaemolyticus* by direct plating at 25 C from one moribund oyster of the HT + *V. anguillarum* trough from trial 4, with confirmation by DNA homology. We used only direct plating techniques in this investigation. Perhaps the use of enrichment techniques (Fishbein and Wentz, 1973) might have significantly increased the recovery of *V. parahaemolyticus*.

Lipovsky and Chew (1972 and 1973) found 18 C to be the apparently critical temperature for significant laboratory oyster mortality. Temperature, nutrient level, stress, and bacterial infestation were indicated by the authors as decisive factors in laboratory oyster mortality. Exposure to elevated temperature for extended times increased bacterial numbers (total counts and mesophilic vibrio counts). This process may effectively stress oysters, enhance the virulence of vibrios in the laboratory and perhaps in the field under crowded summer conditions.

Katkansky and Warner (1969, 1971) in Humboldt and Tamales Bays reported *Crassostrea*

gigas mortality was highest during June through August in 1968 and 1969. Scholz *et al.* (1971) substantiated the seasonal occurrence of Pacific oyster mortality in Case, Eld, and Totten Inlets (Puget Sound) and indicated August through October, 1967, was the highest mortality period. They also estimated September mortality of commercial oyster stocks as the highest for the same year in Eld Inlet.

Wedemeyer (1970) determined that stress caused by any number of environmental conditions can allow potential (facultative) fish pathogens to increase infectious processes. Perhaps a similar situation exists with oysters in the laboratory and in the environment when increased temperature may permit invasion of host tissue by vibrios.

Detailed pathology of the oyster mortalities in the experimental trials is not known. It was obvious from examination of recently dead and moribund oysters that nearly all showed high enough counts of living bacteria in the heart blood and pericardial fluid to be observed easily in phase-contrast slide preparations. Healthy oysters frequently contained very low levels of bacteria in the heart blood, but never enough to be seen by direct microscopy. It might be postulated that a near-terminal event in the disease process is invasion of the blood by bacteria. Certainly the observation of large numbers of bacteria (hundreds to thousands per field) in microscopic preparations of heart blood can be considered indicative of disease situation. This method provides a relatively rapid test to assay the disease potential in an oyster population.

Addressing the treatment of bacteria-related oyster mortalities, this study has determined that oxytetracycline (Terramycin) in the form of TM-50 is effective in reducing heat-induced oyster mortality. Ultra-violet treatment of the water did not reduce heat-induced oyster mortality, although bacteria counts were decreased. Vasconcelos and Lee (1972), in determining the ability of Pacific oysters to purge themselves of microbial contaminants, found only coliform counts reduced by ultraviolet irradiation of seawater, while *Vibrio/Pseudomonas* bacteria were not reduced in oysters by UV treatment. Sindermann and Rosenfield (1967) proposed the control of mass invertebrate mortalities by maintaining production in artificial environments where disease could be controlled by UV treatment of filtered seawater, antibiotic treatment of water, maintainence of general sanitation, control of contaminants in phytoplankton cultures, and elimination of shellfish associates that act as intermediate hosts of disease agents. Fryer *et al* (1971) considered manipulation of the environment in ways which would favor the host and work to the disadvantage of the pathogen. TM-50 has been found effective for control of vibrio diseases by Umbreit and Ordal (1972) in goldfish, *Carassius auratus* and in salmonids by Anderson and Conroy (1970). It could be used in combination with other methods adequately to treat heat-induced bacterial-associated oyster mortality.

The results presented in this paper should be expanded by related studies to identify agents causing mortalities under field conditions.

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Contribution No. 375, College of Fisheries, University of Washington.

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TISSUE GRAFTS IN THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA*

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ABSTRACT

Tissue implantation was one of several techniques used in a study of the transmission of the oyster disease agent Minchinia nelsoni. *The fate of several tissue types, plus response to inert materials, was followed for up to 35 days by periodic sampling and histological examination. Both normal and infected gill and mantle tissue were capable of fusing with the recipient and persisting as recognizable entities for at least the period of observation. The response of the recipient appeared to be influenced by the nature of the implanted material. Viable gill and mantle tissue and paraffin shims elicited a minimal host response. There was some leucocytic infiltration of the immediate area and the appearance of fusiform cells along exposed surfaces followed by re-establishment of epithelia. In the case of moribund tissues or implants of digestive gland, there was a more intense response in the form of leucocytic infiltration followed by a rapid dissociation of the implant. In this case the fusiform cells were also found oriented around the implant. There was no evidence for transfer of* M. nelsoni *to the recipient oysters.*

INTRODUCTION

With the exception of the rather extensive studies on pearl formation by implanted mantle tissue in pearl oysters, few studies on the fate of tissue transplants in bivalve molluscs have been recorded. Pearl formation is reviewed by Alverdes (1913) and Tsujii (1960). Drew and deMorgan (1910), Butcher (1930), Cushing (1957), Tripp (1961) and Chernin (1966) have described various aspects of host response to a variety of implanted tissues in gastropods and bivalves. Canzonier (1963) reported the results of initial attempts to implant tissues in *Crassostrea virginica*. However, the only detailed histological description of the fate of implants in bivalves is that of DesVoigne and Sparks (1969) concerning the reaction of *Crassostrea gigas* to homologous mantle tissue. Recently, Cheng and Galloway (1970) have reported in detail the response of the freshwater snail *Helisoma duryi normale* to both allografts and xenografts from three species. Cheng and Rifkin (1970), in their review of cellular responses of marine molluscs to helminth parasites, have also summarized and discussed the reported instances of the observed responses elicited by a variety of materials that have in some way come in contact with the internal tissues of molluscs.

As part of a series of experiments designed to transmit *Minchinia nelsoni* (Haskin *et al.*, 1966), a haplosporidan parasite causing extensive mortalities of *Crassostrea virginica*, both infected and normal tissues were successfully transplanted in the American oyster. Though the primary objective of transmitting infections of this pathogen

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in the recipient oysters was never realized, several aspects of host response, tissue repair, and implant incorporation are clearly demonstrated in some of the preserved material. In this series, sufficient material was available to illustrate some of the mechanisms of tissue repair and host-implant interaction which are the result of deliberate manipulations performed under relatively well-controlled conditions. Though there still remains some question as to the suitability of the design of such experiments, it was felt that the observations correspond sufficiently with those occurring under normal conditions to be of value in interpreting some aspects of molluscan tissue responses.

MATERIALS AND METHODS

Recipients were normal *C. virginica* (8-12 cm long) tonged in the Navesink River, New Jersey. They were maintained in a flow-through seawater system at Pierces Point, Cape May on the eastern shore of Delaware Bay. Temperatures ranged from 14 - 20°C with salinities from 18-24 ‰ . Normal donors were of the same origin as the recipients, maintained in the aquarium system until used. Infected donors were oysters from Delaware Bay or James River, Virginia, transplanted to the tidal flats of Cape May for infection studies. On some occasions, tissues of infected gapers (moribund oysters) were used in lieu of living materials.

Various procedures for exposing soft parts had been developed and tested but the most successful involved forcing the hinge ligament until it parted and propping the valves in an open position with a wooden shim at the anterior end. Oysters maintained in this condition were flushed frequently with a stream of water to remove accumulated feces and mucus. Other procedures such as the grinding of windows, though successful, caused difficulty in interpretation of tissue response because of local trauma by contact of the soft parts with the rough periphery of the window. There was also a tendency for the exposed surface to secrete new periostracum with subsequent shell formation, an undesirable complication.

Tissues were removed from the mantle or gill, cut into approximately 3 x 5 mm pieces and placed in filtered seawater. The implants were pushed beneath the lateral surface of the visceral mass in proximity to the overlying mantle. In a few cases, the tissues were inserted between the bases of the demibranchs or in the free areas of the mantle. Earlier data have indicated that deep penetration of the visceral mass resulted in excessive rejection of implants.

The instrument used to insert the implant was a blunt lancet-like probe. It was constructed by flattening the end of a No. 16 gauge soft iron wire and sharpening the flattened portion to resemble an arrow head.

In a series of 10 experiments conducted over a 4 year period, 207 oysters received implants of infected tissue and 159 received normal tissue. A previous series of 3 experiments using infected tissue from gapers had not been successful in achieving fusion of implants to recipients, though they provided useful material for the evaluation of response mechanisms, as well as leads for the development of suitable procedures.

Parallel implants of paraffin and agar slivers were performed to evaluate response to this type of injury.

Samples of oysters were removed at intervals of 1-35 days after implantation, opened, examined grossly, and fixed in either Zenker's Acetic (5%) Fixative or in Davidson's Fixative (modification of Shaw & Battle, 1957) and prepared for histological examination according to routine procedures.

Although a certain percentage of the oysters were dead or moribund at the time of sampling, only active oysters (pumping and healthy in gross appearance) were considered representative of normal responses.

Mortality

Oysters implanted with normal living tissues sustained a 21% overall mortality for the series of 10 experiments. Overall mortality for those receiving infected tissues was essentially the same, 23%. There was no overt time pattern for the mortalities. They appeared to be more closely correlated with oyster condition and environmental parameters than with the implant procedure *per se*. Of the several factors noted as influencing survival, the most significant were water temperature (optimum 14 - 16 C), turbidity (it was occasionally necessary to use sand filtered water) and maintenance of adequate circulation in the aquaria. Mortality in the in-

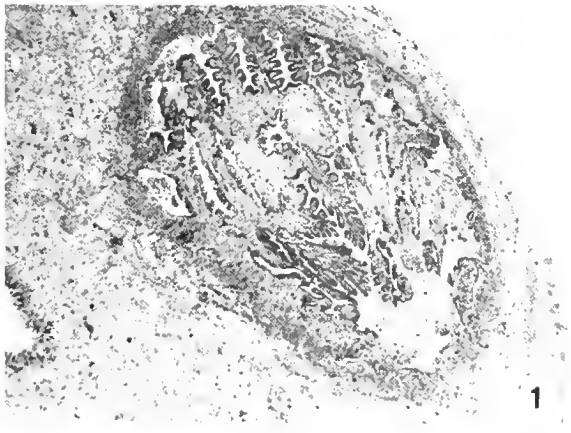


FIG. 1. *Gill implant, 3 days post-transplantation (P-T). Notice minimal infiltration and initiation of fusion. 10* X obj.

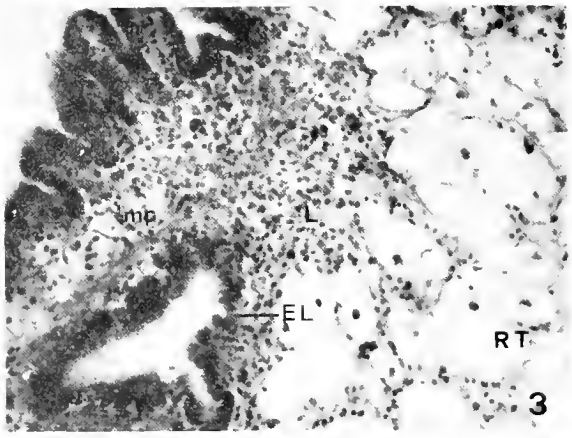


FIG. 3. *Gill implant, 25 days P-T. Notice integrity of epithelial lining and juncture of implant with recipient. 40* X obj.

dividual experiments ranged from 0 - 60%, reflecting variations in these factors.

Rejection of Implants

Mechanical Rejection. Rejection of some implants occurred within the first 24 hours in all experiments. This appeared to be primarily mechanical in nature; involving a squeezing out of the implanted tissue accompanied by an oozing of aggregated leucocytes. This response was associated with the site of implantation, occurring most frequently where there was a minimum of firm tissue surrounding the lesion and a maximal potential for compression of the tissues by mechanical or hydraulic forces. Implants in the dorsal portion of the visceral mass anterior to the adductor muscle experienced almost 100% rejection. In other sites the percent loss of at least 1 piece of tissue per oyster ranged from 0 - 50%.

Chronic Rejection. There was no evidence in the material studied of a chronic response and subsequent rejection or resorbtion of an implant that had become successfully established. However, it should be pointed out that these experiments were not primarily designed to study such phenomena and were of rather short duration (max. 35 days).

Recipient Response

Infiltration. Leucocytic infiltration in the case of living implants and inert materials was usually minimal, though always present to a limited degree (Figs. 1-4). However, when gaper

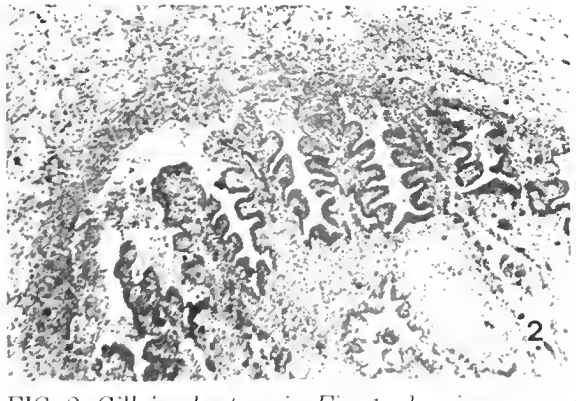


FIG. 2. *Gill implant as in Fig. 1, showing area of fusion. 25* X obj.

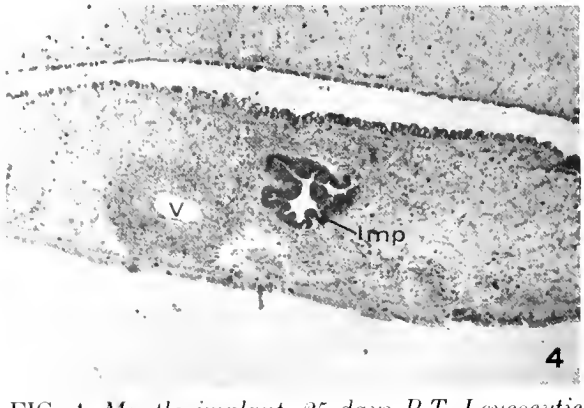
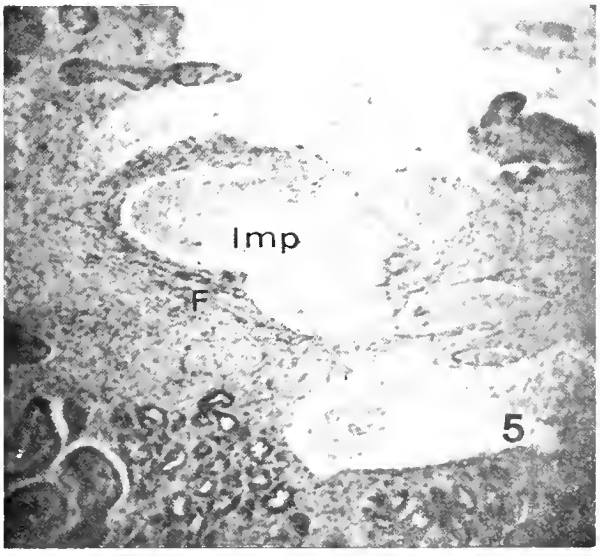


FIG. 4. *Mantle implant, 25 days P-T. Leucocytic cuffing of blood vessel occurs frequently in normal oysters at this location and is probably not associated with implant. 20* X obj.



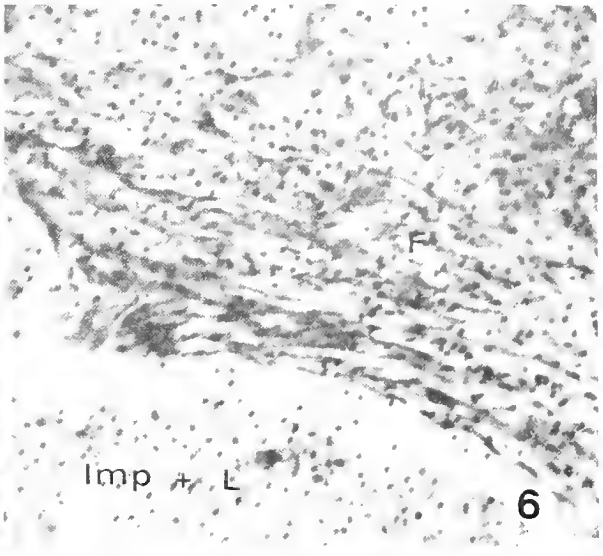
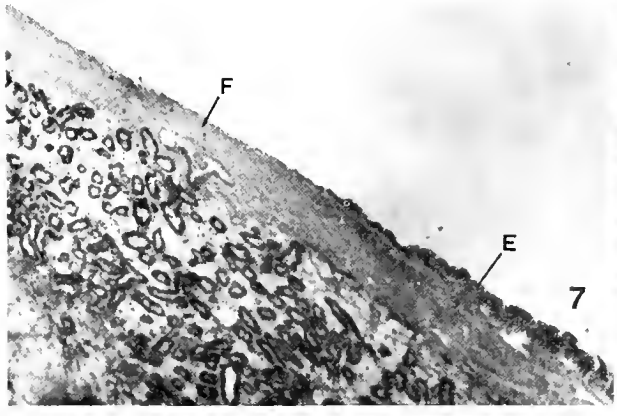


FIG. 5. *Moribund mantle tissue implant three days P-T. Note complete infiltration of implant by recipient leucocytes. 10 X* obj.

FIG. 6. *Same section as in Fig. 5 showing nature of response to moribund tissues. Note fusiform cells peripheral to the implant. 40 X* obj.

tissues were implanted, mobilization of leucocytes, beginning in the first 24 hours, was often very intense and eventually resulted in the complete infiltration of the implant (Figs. 5-6). This resulted in rapid loss of implant integrity and after 3 days cell boundaries were no longer recognizable and nuclear staining was diffuse. The plasmodia of *M. nelsoni* degenerated in these situations, becoming eosinophilic, agranular and losing nuclear detail within 2-3 days.

Tissue Repair. Within 1 day, the injured surfaces showed evidence of repair in the form of fibrocyte-like cells aligned on all exposed tissue surfaces not in direct contact with subepithelial portions of the implant or adjacent tissues of the recipient. These cells increased in number to several layers deep (Figs. 7-8). These cells were fusiform, 20-30 μm in length and 2.5 μm maximum diameter. The nuclei were elongate, 3.5-5.5 x 1.5-2.5 μm and densely stained with hematoxylin. After 3 days, there was often



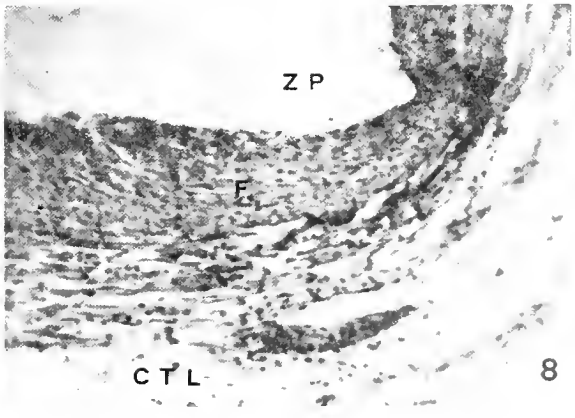


FIG. 7. *Tissue repair on outer surface of visceral mass, 10 days P-T. 10X* obj.

FIG. 8. *Tissue repair in deep connective tissue of visceral mass, 15 days P-T. Note density of layer of fusiform cells. 40X* obj.

evidence of an accumulation of fine granular yellow material (refractile to both hematoxylin and eosine in and around these cells). Eventually the cavity or exposed outer surface became covered by small epithelial cells. The origin of these cells is difficult to ascertain from the material at hand. The fusiform cells were possibly the descendents of leucocytes that had migrated to the area. It appears that the epithelial cells may migrate or grow outward from the intact epithelium that surrounds the lesion (Fig. 7). In the case of implants, the epithelial cells of the grafted tissue appeared to contribute to the re-establishment of the epithelial layer. Indeed, in the case of the lining of cavities surrounding implanted mantle, the cells were more characteristic of the graft epithelium than the cells of the epithelium that had been penetrated (Fig. 4). Even in the case of gill implants, it was often impossible to determine where the epithelium of the graft terminated and that of the recipient began (Figs. 3 and 9).

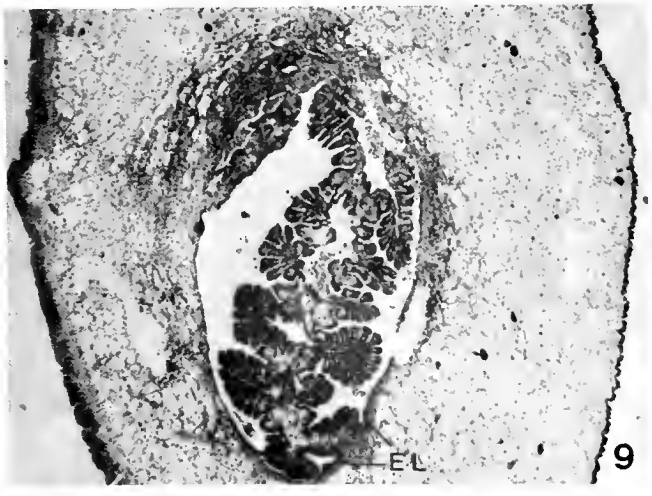
Fusion of Implants

Unsuccessful. In the case of gaper tissue or pieces of digestive gland we never observed fusion of implant to recipient. These tissues always disintegrated and an abcess filled with leucocytes and cellular debris was all that remained after 10-15 days (Fig. 5).

Successful. Living gill and mantle tissues, both normal and infected with *M. nelsoni*, fused with the host tissues and remained viable and identifiable for up to 35 days. After the initial period of mechanical rejection, during which as many as 50% of the oysters lost at least 1 implant, the retained tissues showed evidence of successful fusion in at least 85% of the oysters surviving to the time of sampling. Survival of the implant was apparently not significantly influenced by the presence of *M. nelsoni* plasmodia, since success with infected tissue was equal to that with normal tissues, provided the implant was living and otherwise healthy.

Gill tissue retained its integrity for the entire period of observation, even when completely contained within a closed abcess without access to external surfaces (Figs. 9, 10 and 11). The only major change observed was a swelling and delamination of the chitinous rods (Figs. 10, 11 and 13).

Mantle tissue, being composed of only 2 major cell types, Leidig cells and epithelium, assumed the appearance of an extension of the tissues at the site of the implant. However, as mentioned above, the nature of the epithelium that lines the



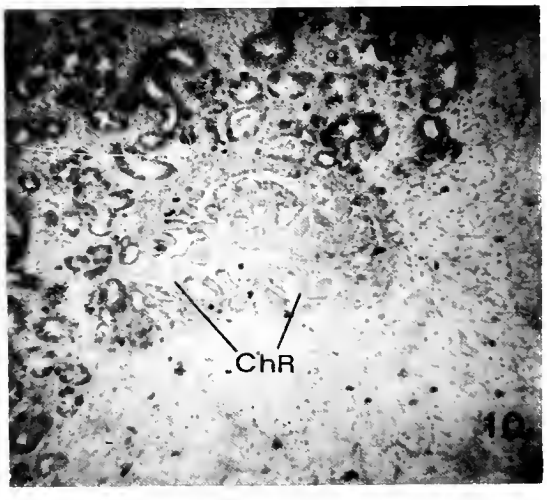
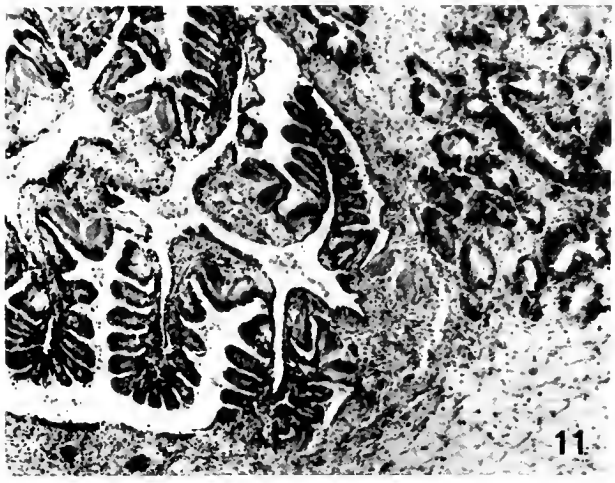


FIG. 9. *Gill implant in free portion of mantle, 25 days P-T. The cavity is lined with a low epithelium and is relatively free of leucocytes and debris. 10* X obj.

FIG. 10. *Gill implant in connective tissue of visceral mass, 35 days P-T. Note there is no indication of incapsulation and only a slight increase in the density of the tissues surrounding the implant. 10 X* obj.



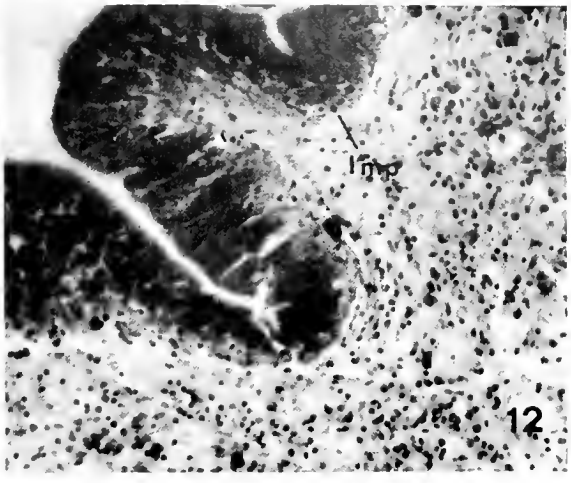
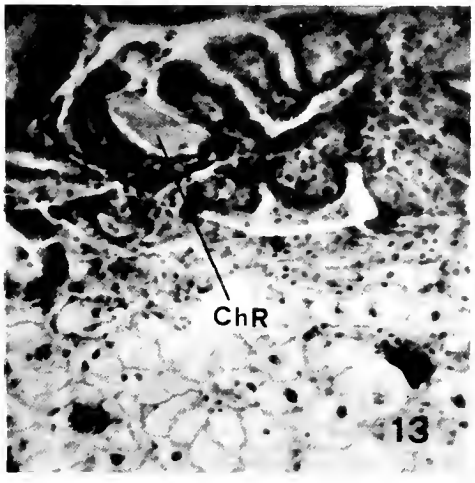


FIG. 11. *Zone of fusion of gill implant 35 days. P-T. Other than swelling of chitinous rods there is little change in the composition of tissues. 25* X obj.
FIG. 12. *Zone of junction of implanted mantle shown in Fig. 4, 25 days P-T. It is not possible to determine the boundary between implant and recipient. 40* X obj.

site of the implant more closely resembles the higher columnar cells of the free mantle border than the epithelium covering the central visceral mass (Fig. 12).

In the case of tissues infected with *M. nelsoni*, the parasite appeared normal in a few implants after 25 days. In no case was there evidence for transfer of the infection to the recipient oysters (Fig. 14). In a few oysters the parasites appeared to mingle with the tissues of the recipient but it was not possible to determine whether this was the result of active migration, or if these sites merely represented an area of fusion where it was impossible to distinguish reliably between the tissues of the recipient and implant.

Occasionally, other organisms were associated with the lesions created by the disintegration of tissues in the area of unsuccessful implants. In three cases, the flagellate *Hexamita inflata* infested the debris-filled ulcers. In two cases, a fungal mycelium (Mackin, 1962) had become established in the zone between implant and host tissue.



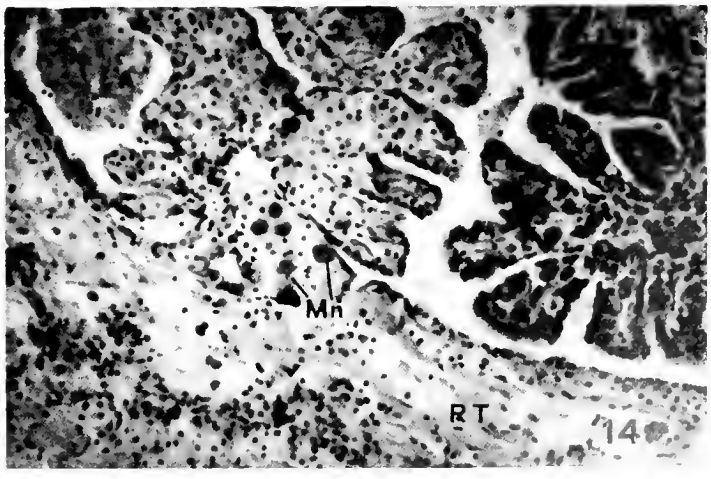


FIG. 13. *Gill implant 35 days P-T, showing the swelling of chitinous rods. 40* X obj.
FIG. 14. *Gill tissue infected with* Minchinia nelsoni, *25 days P-T. 40* X obj.

DISCUSSION

Reports of tissue transplantation in molluscs range from successful fusion of the implant with the recipient to rejection or encapsulation. In the case of implants of mantle tissue to form pearl sacs in *Pteria martensii*, there is apparently consistent success using techniques perfected over many years by the Japanese pearl industry (Tsujii, 1960). The site of implantation, the physiological state of the implanted tissue and its origin are all important factors determining both graft acceptance and pearl formation. Successful fusion of implant to host has been reported by DesVoigne and Sparks (1969) for *C. gigas*. These authors state that rejection of mantle tissue implants was common, "but considerably less than 50%." They do not state exactly the frequency of successful implant fusion, mentioning only "one specimen, after 280 hr," showing incomplete fusion with the host and "an implant which had fused completely with the host tissue in the palp region was observed at 448 hr." The latter case closely resembles the histological picture of successful fusion in *C. virginica*. They specifically note that in this case the "fusiform cells typical of healing were not present." We have observed that such fusiform cells are generally confined to the repair of exposed surfaces hence their absence in an established implant is not surprising. DesVoigne and Sparks also indicate some of the more critical factors that affect implant acceptance and fusion, including the size and site of incision in the host, the importance of mechanical factors and nature of the implanted tissue.

In other bivalves, successful fusion has been reported in the case of implanted autologous gill tissues in the scallop *Gibbus borealis* (Butcher, 1930). Partial success in transplantation of mantle tissue in another scallop, *Pecten irradians*, has been reported by Cushing (1957). This report (abstract — no details) implies that the site of implantation or operative procedure may influence survival of the graft. Apparently sites deep in the visceral mass are not suitable for maintenance of transplanted tissues.

The failure of Drew and deMorgan (1910) to achieve successful grafting of gill tissue in *Pecten maximus* is possibly due to a combination of factors. The adductor muscle, chosen by them as a site for implantation, is an environment not mechanically compatible with the much softer gill tissues. We are also in agreement with Feng (1967) in his criticism of their technique which involved forcing the implant through the bore of a hypodermic needle, probably causing excessive traumatization.

The report by Tripp (1961) of the successful transplantation of foot tissues in *Australorbis glabratus* illustrates the degree of success possible when certain selected tissues are transplanted to a suitable site (in this case cephalopedal sinus) with a minimum of tauma to implant and recipient. The failure of Cheng and Galloway (1970) to have implants of digestive gland persist in *H. durgi normale*, using procedures similar to those of Tripp, could possibly be due to an excessive release of lytic metabolites by the cells of the transplant. Early experiments using digestive gland tissue in *C. virginica* always resulted in disintegration of the implants and large areas of necrosis in the recipient oysters. Drew and deMorgan (1910) reported similar results when they used digestive gland implants in scallops. The fact that the intensity of response varied when different donor species were used by Cheng and Galloway (1970), indicates that there was some degree of compatibility for tissues originating from the same species, the failure to survive and fuse with the recipient being related to some factor common to all the tissues used.

The accumulated observations of this series of experiments with *C. virginica*, in addition to results of similar investigations with other species, indicate some factors of primary importance in successful tissue transplantation in molluscs. The type and physiological state of the implanted tissue must be considered. Tissues, such as digestive gland, with a potential for producing large quantities of extracellular enzymes, are likely to create an unsuitable environment at the site of implantation. Likewise, excessive injury to, or disruption of such tissues in the recipient is not desirable. Provided there have been no irreversible changes due to toxic or other factors, it is also possible to transplant tissues infected with at least 1 quite virulent parasite, *(M. nelsoni)*. Though this procedure did not prove to be a successful means of transmitting the disease, it might be further investigated as a means of studying the fate of this

and other parasites in hosts of varying susceptibility.

The response of the recipient to implanted material depends on the nature and subsequent fate of the implant. If the implant is viable, acceptable to the recipient, and becomes established with sufficient contact with the recipient to maintain essential metabolic functions, there is likely to be minimal response *(e.g.,* leucocytic infiltration or encapsulation), at least for the duration of the experiments reported. In the case of inert material (paraffin), no excessive infiltration into the area was noted but the normal responses to an injured surface proceed to produce an "encapsulation" of the insert. In these cases there seems to be some displacement of the connective tissue surrounding the implant. We would agree with Cheng and Rifkin (1970) that some of the flattened cells that form the "encapsulation" are merely flattened Leydig cells. However, it is evident that distinct fusiform cells ("fibroblastic" cells of Cheng) are also involved in such encapsulation since they can be traced to outer portions of the lesion where the compressive factor is not present. It is not possible to determine the origin of these cells from the material described but there is an indication that they are the descendents of leucocytes that have migrated to the area in response to the injury. Some degree of leucocytic infiltration, commencing soon after injury, always precedes the appearance of fusiform cells. Infiltration by hemocytes has been noted as a response to both mechanical injury in *C. gigas* (DesVoigne and Sparks, 1968, 1969) and tissue destruction by ionizing radiation in the same species (Mix and Sparks, 1971). The latter authors indicate that some of these cells at first form aggregates or cell "nests" on the surface and that subsequent migration and transformation of these cells re-establish the integrity of the tissue surface. Armstrong *et al.* (1971), reporting on wound healing in the abalone, *Haliotis cracherodii*, noted a sequence of leucocytic infiltration, appearance of "fibroblast cells" and re-establishment of epithelium by migration or extension of cells from intact areas. The overall histological picture was slightly different from that observed in *C. virginica* because of the rather muscular nature of the abalone tissues. Similar responses were noted for the encapsulation of the cestode *Tylocephalum* in *C. virginica* (Rifkin and Cheng, 1968), though we have not observed the fibrous elements apparently characteristic of capsule formation.

A somewhat different response resulted when the tissues were not viable. There was a marked influx of leucocytes into the area surrounding the implant and then into the implant itself. Even gross examination of a stained section through such a site will reveal an area of intense leucocytic aggregation ("inflammation"). Fusiform ("fibroblastic") cells appear within one day in the area adjacent to the implanted material. This sequence of events appears to be identical to the response to non-viable implants in *Pecten* described by Drew and deMorgan (1910). Tsujii (1960) also implied a similar response in the case of "death of the graft" or "inflamed pearl sac." We have occasionally observed a corresponding phenomenon in cases of infection with the larval trematode *Bucephalus cuculus* in oysters. Normally sporocysts of this parasite, even though displacing and disrupting large areas of host tissue, elicit no overt response. However, when moribund or parasitized, the sporocysts elicit an intense influx of leucocytes into the surrounding tissues (unpublished observations). It seems that this type of response is primarily dependent on a chemical stimulus from the site of the lesion or invading foreign materials and less dependent on mere physical disruption of the tissues.

In the case of successful implants, "inflammation", infiltration by leucocytes, is usually minimal. Variation in the intensity and extent of initial response appears to be common to other bivalves and dependent on several factors including site of injury, nature of the foreign material introduced and the species involved (Mikhailova and Prazdnikov, 1961; Pauley and Sparks 1966, 1967; Pauley and Heaton 1969; Prazdnikov and Mikhailova, 1965).

One aspect of response to tissue implants has scarcely been touched upon: the maintenance of the implant over extended periods and the subsequent long-term response of the recipient. In the series reported here, 35 days was the maximum period of observation. The persistence of the pearl sac in the Japanese culture

procedure is perhaps the best evidence available for long term compatibility. In that particular case there is some indication of a change in the cellular composition of the implant (Tsujii, 1960). This change is probably due to a shift in prominence of some particular cell type. Mantle implants in *C. virginica* appeared to be composed of only one major cell type, perhaps the one most favored by the environment at the site of implantation. The deterioration of the chitinous rods in gill implants might indicate a reduction of the cells responsible for the maintenance of these components.

It is evident that interpretation of histocompatibility and recipient response to tissue grafts in bivalve molluscs must take into account several factors that influence the potential for successful fusion and maintenance of the implant. Some of these factors are suggested in the various studies cited. However, these factors are still poorly understood while other aspects of preliminary host response, tissue repair and implant fusion mechanics remain unexplored areas for investigation.

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THERMAL TOLERANCE OF OYSTER LARVAE, *CRASSOSTREA VIRGINICA* GMELIN, AS RELATED TO POWER PLANT OPERATION

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ABSTRACT

The upper thermal tolerance of Chesapeake oyster larvae (fertilized eggs, ciliated gastrulae and 2-day veliger larvae) was determined for 10-second to 16-hour exposures. A biphasic temperature tolerance curve was noted with time of exposure, the inflection point coming at 2 hours. Considering all life stages tested, at 2 hours significant mortality (LD_{10}) came at just over 30 C, with slight increases in effect noted up to 16 hours of exposure. Tolerance levels increased with decreasing exposures less than 2 hours, until at 10 seconds significant reduction came at approximately 40 C. The relevance of these findings to power plant design and operation on estuaries is discussed.

INTRODUCTION

The increasing use of estuarine waters by electric power generating stations for condenser cooling has necessitated a more detailed understanding of the tolerances of shellfish larvae and other entrained organisms to high temperatures. Mihursky and Kennedy (1967) state that the doubling time for electricity needs in the United States is now 6 to 10 years. From 1960 to 2010, for example, there is anticipated a 30-to 250-fold increase in electricity demand. Thus, more and larger coal-fired or less efficient atomic electric plants will be constructed in the near future. Since most choice sites in fresh water have been used, and fresh water volumes, in many cases, are inadequate for the large capacity plants planned, this expansion may take place in estuarine and marine waters.

Vast quantities of cooling water, up to 2.7 million gallons per minute in larger plants, may be drawn from the environment, heated at condensers to engineering optima and released back to the environment. Entrained species may be subjected to high temperatures for periods of one minute to several hours depending on the design of effluent canals and provision for cooling by mixing with "tempering water". ("Tempering water" is an engineering innovation which adds ambient temperature water to the cooling water just after condenser passage to allow conformance with certain state and federal regulations which only stipulate maximum temperatures at the point of release to the environment.)

The temperature tolerance of estuarine

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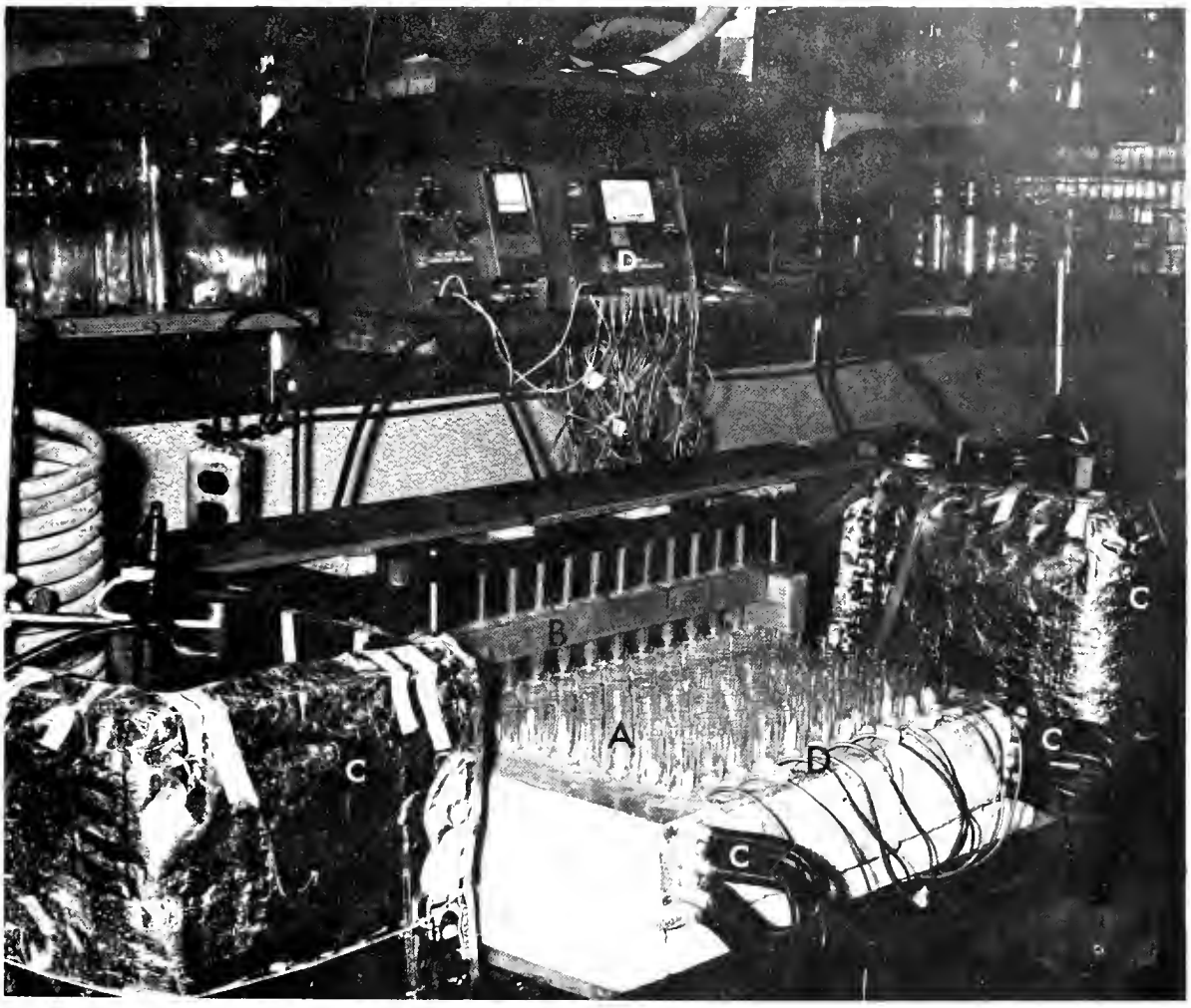

FIG. 1. *Experimental thermal gradient block showing: A-test tube environment with 11 tubes on the thermal axis; B-syringes to allow simultaneous injection of experimental animals on the 11 tube axis; C-hot and cold water sources and block circulation arrangement; D-block temperature sensors and recording apparatus.*

molluscan larvae has drawn considerable research interest. Loosanoff, *et al.* (1951) found that if the fertilized eggs of the hard-shelled clam, *Mercenaria mercenaria*, were placed at 33 C, development to abnormal veliger larvae resulted. However, if fertilized eggs which had been raised to normal veligers at 24 were placed at 33, then normal rapid veliger growth ensued. The authors stated that this observation coincided with the view of Pelseneer (1901), that young cleavage stages of molluscan eggs exhibit a narrower temperature tolerance than the later life history stages. Davis and Calabrese (1964) investigated the interaction of temperature and salinity on development of fertilized eggs and growth and survival of veliger larvae of *M. mercenaria* and oysters, *Crassostrea virginica*. With clam larvae, they found that development, growth, and survival were not affected generally between 17 and 30. However, at non-optimal salinities, temperature tolerances were much reduced. Oysters showed a similar pattern, with fertilized eggs not tolerating 32.5. The fully developed veligers survived and grew optimally at 32.5 but not below 22.5 C. Stickney (1964) investigated the temperature tolerance of New England and Chesapeake Bay stocks of the soft-shelled clam,

Mya arenaria. He found that optimal survival of fertilized eggs to veligers occurred at 15 and 18 C but was reduced drastically at 22.5 and 28 C. Similarly, veliger larvae grew well at 18 and 22, but all died at 28.4 C. Ambient conditioning temperatures for adults was between 2 and 13 C. Stickney suggested that there were differences in temperature tolerances between the New England and Chesapeake stocks, possibly genetically controlled, although his experimental results appeared not to be decisive.

The role of temperature of acclimation of adult spawning stock of bivalve mollusks in the temperature tolerance of their pelagic embryos and larvae appears to be nonrelevant since gonad production and spawning are largely temperature dependent. Numerous studies have demonstrated that for a given species and race, spawning and thus larval occurrence takes place within a rather narrow temperature range, probably no greater than ± 5 C. [See for example, Loosanoff (1937, 1942) and Pfitzenmeyer (1962).]

Our experiments were designed to determine the short-term (10 seconds to 16 hours) upper thermal tolerance of oyster embryos and 2-day veliger larvae. The experimental design was dictated, in part, by a consideration of engineering specifications for 2 plants newly constructed in the Chesapeake Bay, in close proximity to oyster producing areas (Morgantown on the Potomac and Calvert Cliffs Nuclear on the Bay proper). Both of these plants employ tempering water and are required to meet the 1967 Maryland and Federal temperature regulations, which specify seasonal differentials and effluent maxima. In contrast to older designs, such as the Chalk Point plant on the Patuxent Estuary, total transit times for cooling water at condenser temperature is measured in minutes rather than hours. These experiments attempt to simulate thermal change which will be experienced by entrained larvae in the two types of plant design.

Appreciation is expressed to Drs. L. Eugene Cronin, T. S. Y. Koo and Mr. Elgin A. Dunnington for helpful advice throughout the project. Summer students, Miss Judith L. Baab, Patricia E. Albert and Sandra Wrenn aided in experimental work.

MATERIALS AND METHODS

Six experiments determined the short-term temperature tolerance of oyster larvae as follows:

For these experiments, a multivariate temperature block (Keller, *et al.*, 1968) was used. The aluminum block (Fig. 1) is 4" x 24" x 15", with 8 rows of 11 one-inch diameter holes into which test tubes were placed. On the left side of the block, cold water was circulated; the right side was heated by circulating warm

Experiment	Temperature Range	Duration of Exposure	Larval Stage
1	17–37 C	2–16 hours	fertilized eggs
2	27–37 C	1– 8 hours	fertilized eggs
3	26–44 C	10–sec. –1 hour	6–hour ciliated gastrulae
4*	29–49 C	10–sec. –1 hour	fertilized eggs
5*	29–50 C	10–sec. –1 hour	6–hour ciliated gastrulae
6*	29–49 C	10–sec. –1 hour	2–day veliger larvae

* utilized the same brood of larvae

water. A linear temperature gradient was developed along the 11-tube axis which was regulated by controlling the temperatures of the circulating waters. Eight rows of tubes permitted replication of treatment and allowed investigation of the effect of an additional factor, in this case, time of exposure.

Adult Chesapeake Bay oysters were first conditioned and spawned in the shellfish hatchery at Solomons by the techniques described by Hidu, *et al.*, (1969). Conditioning temperatures ranged between 20 and 25 C for several weeks. Spawning stimuli included 1 to 4 hours of fluctuating temperatures ranging from 25 to 30 C. Naturally-spawned eggs then were brought back to 24 C in stock cultures with egg densities at approximately 60/ml. Salinities for all experiments ranged between 10 and 15 ‰ .

Stock cultures of fertilized eggs were then transported 25 miles to the Hallowing Point Field Station where the temperature block had been brought to equilibrium at the desired temperature range. Test tubes in the block contained 30 ml of salt water collected at Solomons and filtered to 10 μ. Larvae were then simultaneously introduced into a full row of 11 test tubes from 5 cc syringes mounted in a rack (Fig. 1). This resulted in densities of about 300 larvae per test tube or approximately 10/ml. The larvae were left in the test environments for the allotted time, after which they were simultaneously removed and poured into large test tubes containing about 300 ml of Solomons sea water at 24 C. Larvae were incubated for 48 hours at 24 C to enable the eggs or gastrulae adequate time to develop to normal veligers, and in the case of the veligers, to decompose and leave only the empty shell, if they suffered mortality during the temperature exposure. At 48 hours, larvae were poured into a disposable beaker containing an adequate amount of formalin, so that they were concentrated on the bottom of the beaker. The volume of water in the beaker was reduced to about 30 ml by careful siphoning, after which all the larvae were washed into vials and preserved with buffered formalin for counting.

Counts of larvae were made by first decanting the vial and placing the entire sample on a Sedgwick-Rafter cell. In the case of experiments using the fertilized eggs and gastrulae, counts were made of resulting normal 2-day veliger larvae. Normal is defined as a larva between 70 and 80 μ, measured parallel to the hingeline, and with a straight-line hinge. A marginally stressful treatment will produce all types and degrees of abnormalities (Loosanoff and Davis, 1963). In the case of the veliger experiment, mortality was measured by counting live and dead larvae. A dead larva, after 48 hours, only left an empty shell. Within each experiment, two replicate runs (of 11 environments) were made at a specific time of exposure.

To determine whether embryos suffered mortality due to exposure to the block and associated manipulations, separate control larvae were incubated in the 300 ml test tubes for 48 hours at 24 C. There were no significant differences in survival between these and those held in the block at known optimal temperatures in the first experiment, so the practice was discontinued. Further, high percentage retrieval rates of the innoculated larvae at optimal temperatures in all remaining experiments attested to the suitability of the block environment for the experiments.

Temperature in the block was monitored by spot checking (stem thermometer) and continuously by thermistor probes (YSI — Telethermometer Model 47) accurate to .2 and .5 C, respectively. Because of stem lag and delay in cycling time, temperature values for the short-term experiments (10 sec and 1 min) had to be corrected. This was done in a separate study in which temperatures were simultaneously determined by telethermometer and stem thermometer. Regression analysis was used to compare the apparent (YSI) and actual (stem thermometer) values. These were highly correlated ($r = .994$) and yielded the following equation: actual temperature (C) = .794 apparent temperature + 5.7. This equation was used to correct temperature data for short-term runs. Analysis of actual and apparent temperatures for times greater than 1 minute showed no appreciable differences and the YSI values were used directly.

Analysis of Data

From a given experiment, the relative per-

centage survival for each pair of vials in a replicate was computed using maximum survival pair in the entire experiment as the base (Fig. 2). This technique has been used by Davis (1958, 1964) and others. These data were plotted as a response surface and lines of 80, 60, 40, 20 and 0 percent survival interpolated by inspection. There is some danger in slightly overestimating percentage effects by the procedure, however, the proximity of survival contour lines indicates critical areas of thermal effect.

Data were further analyzed by calculating LD10, 50, and 90% values for all experiments combined LD50 values for fertilized eggs, ciliated gastrulae and veliger larvae were considered separately. This was accomplished by probit analysis (Finney, 1962). To validly use the probit analysis, it was necessary to eliminate natural (background) or nontreatment mortality from being confounded with that due to experimental treatment (See Davis and Calabrese, 1964; Tables 4-6). This was done as follows:

Each time-group (10 sec, 1 min, etc.) within an experiment was considered a homogeneous treatment group and background mortality was estimated from inspection of the raw data for that particular group. In most cases, a marked change in percentage mortality occurred as higher test temperatures were encountered. This inflection, from about 10% mortality to values of 30-50% or greater, could be determined by inspection and was used to estimate background mortality. Background mortality was estimated by averaging individual percentage mortalities below the inflection point. The number of individual values used to estimate background mortality ranged from 2 to 7. The computed average mortality was substituted in Abbott's formula (1925) and each individual raw score corrected. Probits (Finney, 1962) of these percentages were then taken. In most cases, the raw data were considerably smoothed by this practice and probit lines could be easily fitted to the data points. Probit lines were fitted by inspection to data points and LD10, 50 and 90 points estimated from the line by determining the antilog of temperature for a particular probit. No attempt was made to calculate confidence intervals for a particular LD value. Past experience with such calculation has produced unrealistically small intervals (± .01 C) that are not justified by the experimental procedures employed. A more realistic estimate of confidence interval is probably on the order of ± .5 C. However, in short-term experiments (4 hrs) there was variation between comparable life history stages run at different times. Although some slight, between-run variation existed, all short-term experiments yielded high LD points.

RESULTS

Data are expressed first as percentage survivals in all experiments (Fig. 2). Further, trends and relationships are clarified in plotting LD10, 50 and 90 values with exposure time derived by combining data from all experiments (Fig. 3). The LD50 values obtained with the three life stages (fertilized eggs, ciliated gastrulae, and 2-day veliger larvae) are plotted in Fig. 4.

Oyster larvae exhibited a direct relationship of mortality to duration of exposure to elevated temperature and the relationship appears to be biphasic. For exposure times of 10 sec to 2 hrs (Fig. 3), all data points were fitted by a line having considerable slope. Thus, in short duration exposures, LD50's, decreased rapidly up to 2 hrs exposure. From 2 to 16 hrs, the slope changed and LD50 values were close to 33 C. This second phase of tolerance was more in keeping with published data on the upper tolerances of bivalve mollusca.

Exposure to temperatures below ambient for 1 to 16 hours was also detrimental to larval survival (Fig. 3). LD10 values came at approximately 20 C and showed little trend with time of exposure.

The stage of larval development appeared to be a significant factor in thermal tolerance as illustrated by plotted LD50 values obtained for fertilized eggs, ciliated gastrulae and 2-day veliger larvae (Fig. 4). Fertilized eggs were least tolerant with LD values falling approximately 3 C below those obtained for ciliated gastrulae. Veliger larvae were the most tolerant with LD values occurring 8 to 12 degrees above those obtained for fertilized eggs.

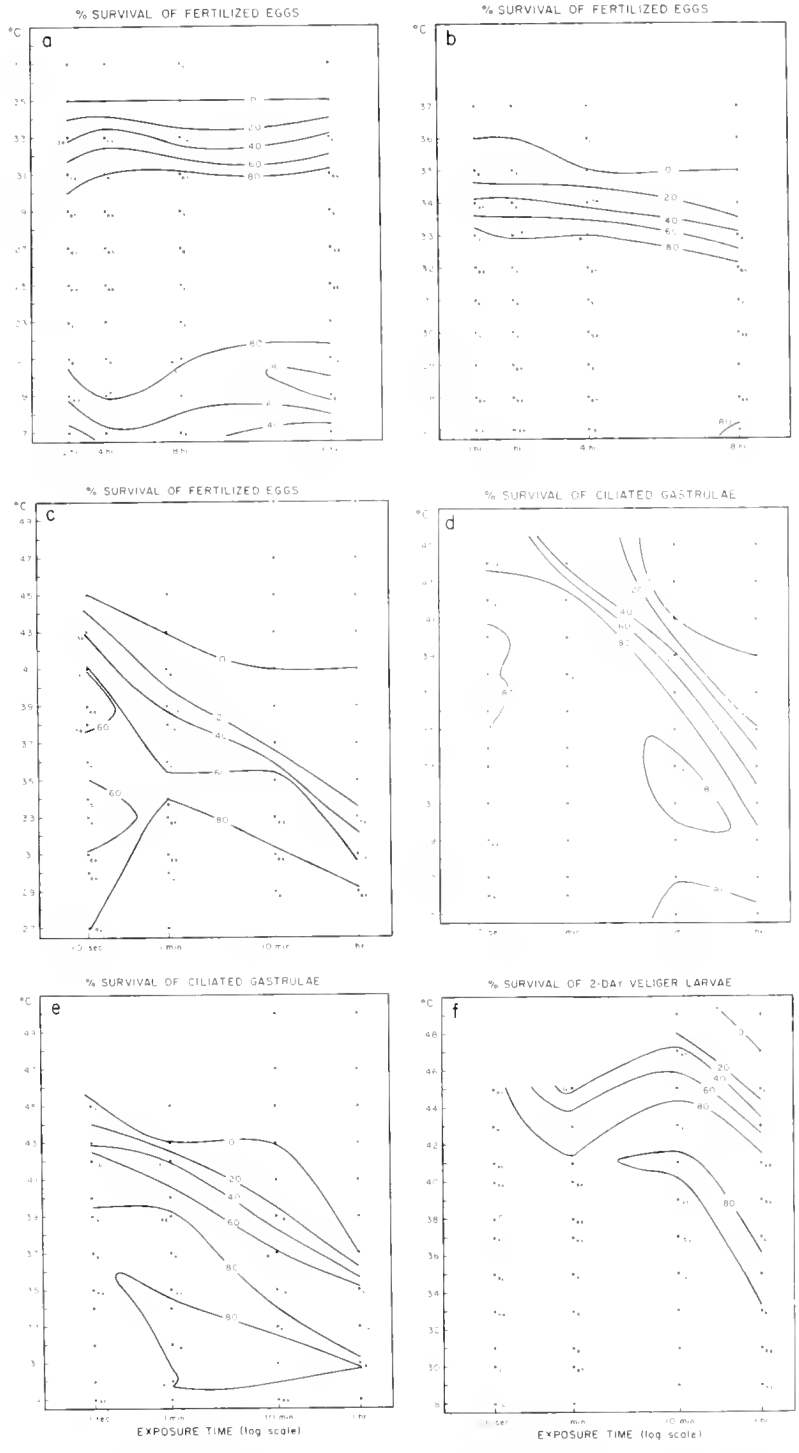

FIG. 2. *Calculated percentage survivals for all experiments setting the maximum survival pair within a treatment at 100%. Percentage survival contours were extrapolated visually between data points.*

DISCUSSION

These experiments indicated that the early cleavage stages, the fertilized egg and ciliated gastrula, are considerably more temperature sensitive than later stages of development. This adds weight to the contention of other workers who have noted a similar effect with a variety of species. Pelseneer (1901), noted that the early cleavage stages of molluscan eggs are limited to a narrower temperature range than more advanced stages. Loosanoff and Davis (1963) confirmed these observations through extensive trials with oysters and the hard-shell clam, *Mercenaria mercenaria*. Brett (1960) found that fertilized embryos of salmon are also the most thermally sensitive life stage even though this is a short-lived stage.

The sensitive fertilized egg and gastrula stage in oysters exists for 6 to 8 hours and the more tolerant veliger for two weeks. However, the argument that thermal regulations could discard consideration of the early stages because their short duration would make power plant predation insignificant is not valid. It should be realized that any subsequent stages evolve from the early stages and loss may have great later effect on recruitment.

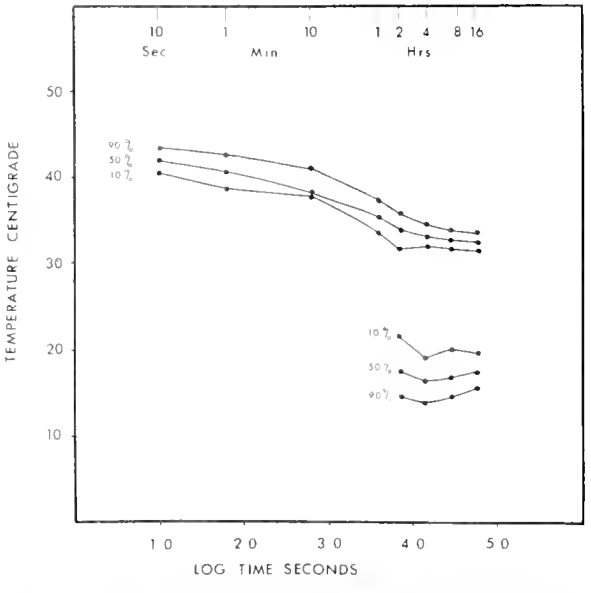

FIG. 3. *Upper and lower temperature tolerance combining all data for all oyster larval stages expressed as* LD_{10}, $_{50}$, *and* $_{90}$ *values.*

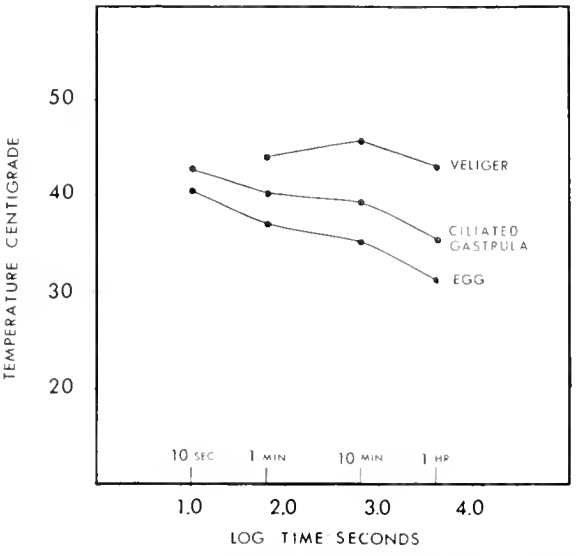

FIG. 4. *Upper temperature tolerance of oyster fertilized eggs, 6-hour ciliated gastrula and 2-day veliger larvae expressed as* LD_{50} *values.*

The time of exposure, as stated, greatly affects the temperature tolerance of larvae and this may have considerable bearing on future power plant design. For example, the Chalk Point Power Plant on the Patuxent estuary (Fig. 5), in its original design, took in cooling water at ambient temperatures, passed it over condensers where it was heated 6.5 C, after which it flowed back to the estuary in a long effluent canal. The excursion in the effluent canal to final discharge in the river took 2.7 hours with an approximate temperature loss of 0.5 C in transit. These experimental results indicate that during critical summer temperatures, effluent canals may become long killing chambers for entrained forms. At river temperatures of 24 C, which is optimal for oyster larvae, the effluent temperature of 30.5 C, no doubt, had detrimental effects on early oyster larval stages during 2.7 hours of transit.

A more recent development (Morgantown Power Plant on the Potomac and recently, Chalk Point), one designed to allow a plant to conform to discharge regulations and yet obtain higher temperatures within the condensers, is the innovation of using "tempering water" in the system just after the condensers (Fig. 6).

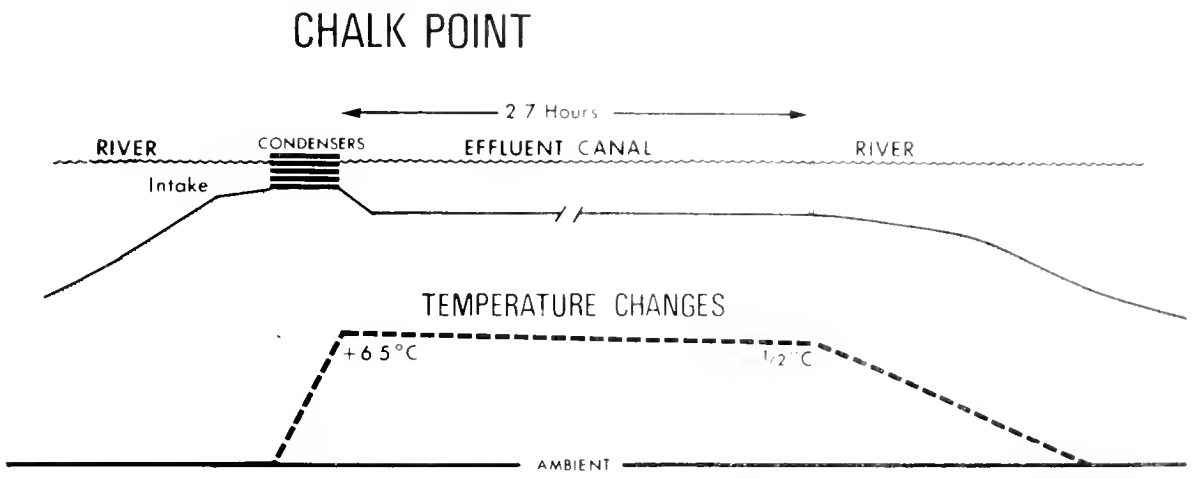

FIG. 5. *Schematic diagram of cooling water transit time and temperature elevation at the Chalk Power Plant (in its original design) on the Patuxent estuary on Maryland Chesapeake Bay.*

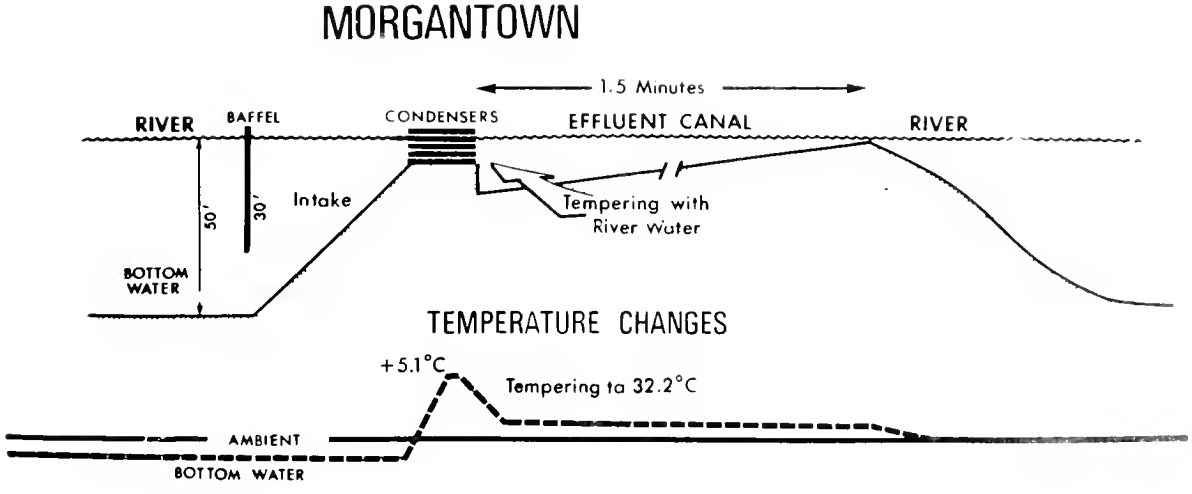

FIG. 6. *Schematic diagram of cooling water transit time and temperature at the Morgantown Power Plant on the Potomac estuary on Chesapeake Bay.*

Here, entrained forms will be exposed to very high temperatures within the condensers for about 1 minute and then cooled immediately to meet effluent temperature regulations. The excursion in the effluent canal, before release again to the estuary, is expected to be less than 3 minutes. A shortening of exposure time, from hours to minutes, increases the temperature tolerance of oyster larvae by as much as 5-7 C.

Even though the water temperatures may, by themselves, provide an adequate margin of safety, there could be other factors which may heighten temperature sensitivity of species. To establish a basis for proper regulations which will protect entrained forms will require the study of many aspects of the problem. These laboratory experiments should be augmented by depletion rate studies at specific power plant sites, effects of power plant biocides, the influence of metals, turbulence from pumps, and rapid pressure changes. Laboratory experiments should test the most sensitive stages of various entrained forms, from planktonic primary and secondary producers to egg and larval stages of marine fishes. The wide differences between thermal tolerances of *Mya* larvae obtained from northern New England (Stickney, 1964), and those shown in this study in the Chesapeake area, as well as those of Loosanoff and Davis (1963) point out that studies and regulations should pertain to a specific geographical area.

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A PROPOSED METHOD OF WASTE MANAGEMENT IN CLOSED-CYCLE MARICULTURE SYSTEMS THROUGH FOAM-FRACTIONATION AND CHLORINATION[1]

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ABSTRACT

A scheme of waste management independent of bacterial filters in closed cycle mariculture systems was presented. It recommended foam-fractionation for organic removal to prevent build-up of high ammonia levels in the system. Breakpoint chlorination was recommended for the removal of remaining low levels of ammonia. Dechlorination was achieved through carbon filtration. An algal production system can be easily coupled in the system if mollusks are to be cultured.

The non-bacterial filters remove contaminant more rapidly and completely and are not limited by disadvantages associated with the bacterial filters, such as excessive space requirements and build up of high nitrate in the system.

INTRODUCTION

Biological filters have generally been considered a satisfactory method of maintaining water quality in closed system mariculture. However, they have some serious drawbacks. One is excessive space requirements due to overdesigning for emergency situations. The second is the possibility of the bacterial population being destroyed and the need for subsequent time interval to re-establish the bacteria. Third, biological filters convert ammonia to nitrate which builds up in the water. High nitrate concentrations in the water may be unfavorable to organisms and also may promote undesirable algal growth.

Any incidence of high mortality of marine life will tend to overload a biological filter, resulting in poor quality and further loss of marine life. Decaying animal tissues have two adverse effects on closed system mariculture: the reduction of oxygen and the production of toxic ammonia. An alternative to larger biological filters is to extract the tissues directly through the use of a foam-fractionating device, commonly known as a protein-skimmer, to prevent the build up of ammonia.

Previous work (Dwivedy, 1973) established that the foam-fractionation process can be employed effectively to remove dissolved and suspended organics from the water and thus to prevent build up of ammonia. However, foam-fractionation of water does not remove ammonia from the system. An alternative to biological filtration to remove ammonia from the system would be to chlorinate the water. It is well documented that ammonia is removed from the water upon addition of a sufficient quantity of chlorine (Baummer *et al.*, 1969; Griffin, 1944; Harvill *et al.*, 1942; Streeter, 1943; Tchobanoglous, 1970).

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The purpose of this study was to work out a scheme of waste management through the use of non-bacterial filters in closed cycle mariculture systems.

CHARACTERISTICS OF WASTE IN A MARICULTURE SYSTEM

Organics are the primary components of waste in a mariculture system. The organics give rise to toxic ammonia and bacteria and also their decomposition produces substances that lower the pH of the water. Ammonia is also directly excreted by some marine organisms. Oxygen depletion is another serious problem if organics are allowed to decompose in the system. To illustrate the characteristics of waste in a typical mariculture system, various sources and types of waste that may be expected in an oyster culture system are: decomposed meat from dead oysters, feed residue (dead or live algae) and excretion products.

Typical values for the constituents of oyster meat as computed for 100 gms of meat are: 9.8 g of protein, 5.6 g of carbohydrate, 2.1 g of fat and 80.5 g of water (Galtsoff, 1964). Parsons, *et al.* (1961) reported the chemical ratios of several species of algal cells. The values for *Dunaliella salina*, for example, are 1.43 protein/carbon, 0.8 carbohydrate/carbon and 0.15 fat/carbon. These values are ratios of components to carbon present in the cells. Although data on the chemical composition of oyster fecal material are not available, it is reasonable to assume that it contains large proportions of proteins and other nitrogenous compounds.

NON-BACTERIAL FILTERS

Foam-Fractionation Process for Waste Removal

The foregoing explanation of the characteristics of waste indicates that one would expect large amounts of proteins and other nitrogenous compounds in the system. It is well known that proteins and some other nitrogenous compounds are excellent foam-producing agents (Gaudin, 1957). The presence of fat in the water provides high viscosity and high surface activity. Large amounts of heterogenous ions, naturally present in salt water, aid in foam formation. The author has evaluated the foam-fractionation process in detail for contaminant removal in a closed-cycle mariculture system (Dwivedy, 1973). It was found that the process removed organic matter, bacteria, dust particles, algae and some weak acids. The removal of organic matter resulted in the prevention of ammonia build up in the system (Fig. 1). The foam-fractionation process helped maintain the pH of the system, since probably some weak acids were being removed with the foam. Since ammonia build-up from decay of organic matter is controlled by the use of a foam-fractionation unit, the removal of the remainder of ammonia by chlorination is a possibility.

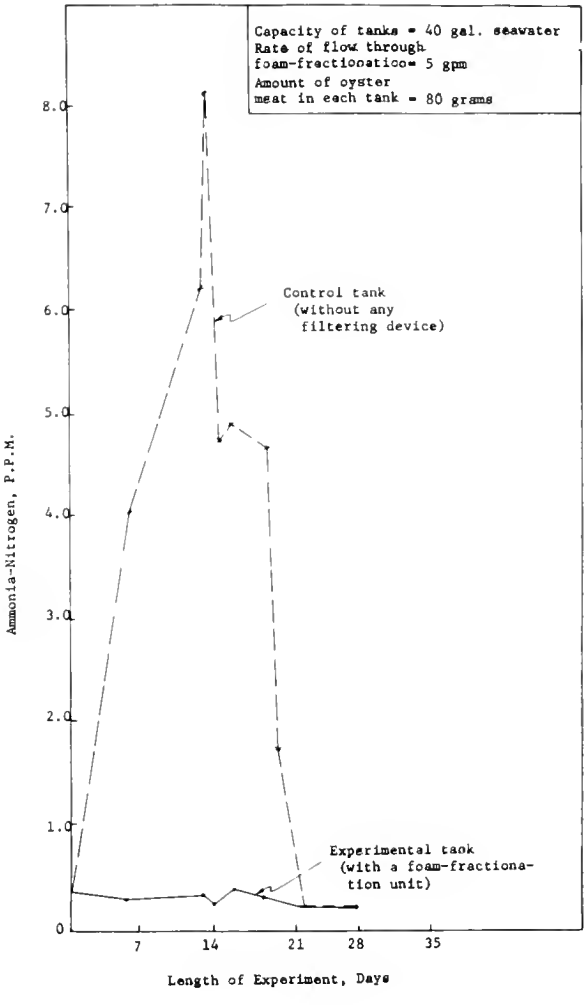

FIG. 1. *Control on ammonia through foam-fractionation. (Redrawn from Dwivedy, 1973).*

Theory of Ammonia Removal with Chlorine

Ammonia could be removed from water chemically by adding chlorine to form monochloramine and dichloramine as intermediate products and nitrogen gas and hydrochloric acid as end products. According to Sawyer and McCarty (1967), when elemental chlorine is dissolved in water, the following equilibrium equation takes place:

$$Cl_2 + H_2O = HOCl + H^+ + Cl^- \quad (1)$$

Hypochlorous acid (HOCl) reacts with ammonia to form chloramines. This is a stepwise reaction:

$$NH_3 + HOCl = NH_2Cl + H_2O \quad (2)$$

$$NH_2Cl + HOCl = NHCl_2 + H_2O \quad (3)$$

$$NHCl_2 + HOCl = NCl_3 + H_2O \quad (4)$$

In the presence of excess ammonia (NH_3), nitrogen trichloride (NCl_3) reacts with ammonia to form nitrogen gas and hydrochloric acid as end products; as shown by the following equation:

$$NCl_3 + NH_3 = N_2 + 3\ HCl \quad (5)$$

According to Griffin (1944) the process can be summarized and the equations be written in terms of NH_3 and Cl_2, as presented below:

$$2NH_3 + 2\ Cl_2 = 2NH_2Cl + 2HCl \quad (6)$$

$$NH_2Cl + Cl_2 = NHCl_2 + HCl \quad (7)$$

$$NH_2Cl + NHCl = N_2 + 3\ HCl \quad (8)$$

$$2NH_3 + 3\ Cl_2 = N_2 + HCl \quad (9)$$

From equation (9) the theoretical amount of chlorine required per mg/l of ammonia is about 6.3 mg/l. In practice, however, Griffin (1944) and Baummer *et al.* (1969) found that a slight excess of 10 mg/l of chlorine was required for each mg/l of ammonia.

Breakpoint Chlorination for Ammonia Removal

When chlorine is added to water containing ammonia, the oxidation of ammonia and reduction of chlorine take place provided the molar ratio of chlorine to ammonia is greater than 1.0. A substantially complete oxidation-reduction process occurs at about 2:1 and, if allowed sufficient time, this reaction leads to the disappearance from water of all the ammonia and oxidizing chlorine. This effect is called the breakpoint phenomenon (Sawyer and McCarty, 1967).

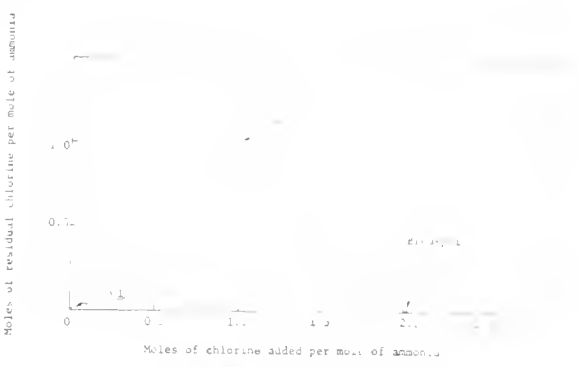

FIG. 2. *An idealized schematic diagram of breakpoint chlorination (Redrawn from Sawyer and McCarty, 1967).*

Fig. 2 shows that the molar ratios of chlorine to ammonia are less than 1.0 between points 1 and 2 and chlorine is all in the form of chloramine in this region. Beyond point 2, a dechlorinating action apparently takes place until complete oxidation-reduction occurs at the breakpoint.

Thus at breakpoint, theoretically all ammonia and all chlorine are removed from the water. However, Griffin (1944), pointed out that this is not the case in practice (Fig. 3). Although ammonia is completely removed, some residual chlorine is left in the water beyond the breakpoint. Therefore, some dechlorinating device

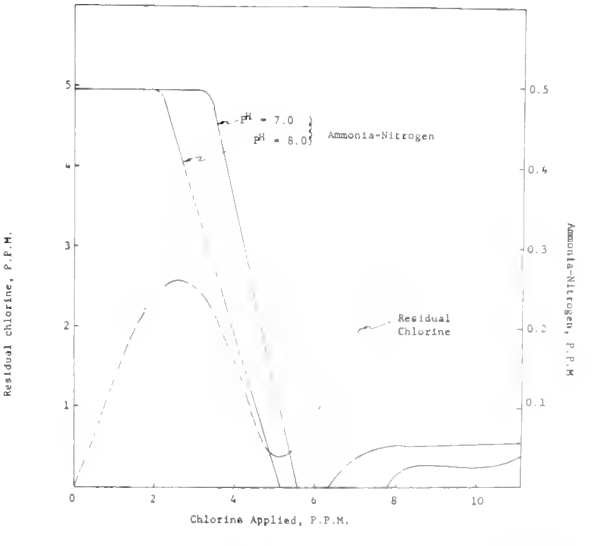

FIG. 3. *Ammonia removal through breakpoint chlorination (Redrawn from Griffin, 1944).*

must be used to reduce chlorine to a safe level before chlorine-treated water can be introduced into the culture tanks.

A definite time interval is required for the reaction between chlorine and ammonia to reach completion. Harvill *et al.*, (1942) found this time to be 15-20 minutes at pH 7.7 at 85 F. Baummer *et al.*, (1969) reaffirmed this time as 15 minutes with salt water of pH 7.4 at 18 C. It must be noted that the speed of reaction between ammonia and chlorine is influenced by the pH and temperature of the water. It also is interesting to note that Griffin's (1944) conclusion that the optimum pH for maximum removal of ammonia and chlorine lies between pH 7.0 and 8.0. This characteristic of the reaction is favorable since in any mariculture system, the pH lies in this range.

Bactericidal Action of Breakpoint Chlorination

A number of investigators have noted that the presence of ammonia in the water greatly increased the bactericidal action of chlorine. This effect has been attributed to the formation of chloramines. (Equations (6) and (7). The greater killing effect of chloramine upon bacteria has been accounted for on the assumption that it is more soluble than chlorine and penetrates the bacterial cell more easily (Gerstein, 1931; Coventry *et al.*, 1935; Sawyer and McCarty, 1961).

Gerstein (1931) reported water temperature had a marked effect on bactericidal efficiency; efficiency decreasing with the lowering of temperature within the range of experimental work, 20 C - 0 C.

Conditioning of Water After Chlorine Treatment

Since chloramines are highly bactericidal, it becomes very important that the water be processed for dechlorination to bring chlorine residuals within safe limits before its introduction to the culture tanks. Coventry *et al.*, (1935) ran a series of experiments to test and compare several treatments for dechlorination of water. Dechlorination treatments used were: (1) chemicals; (2) prolonged boiling; (3) aeration by (a) an atomizer spray and (b) porous artificial stone blocks using compressed air; and (4) adsorption of the chloramine upon activated carbon. Their conclusions were that aeration or boiling of such water, for biological purposes, is ineffective, and that some chemicals may be used but there are some associated disadvantages. Activated carbon was found to be the most effective and simple for chlorine removal. The authors were able to remove chlorine to as

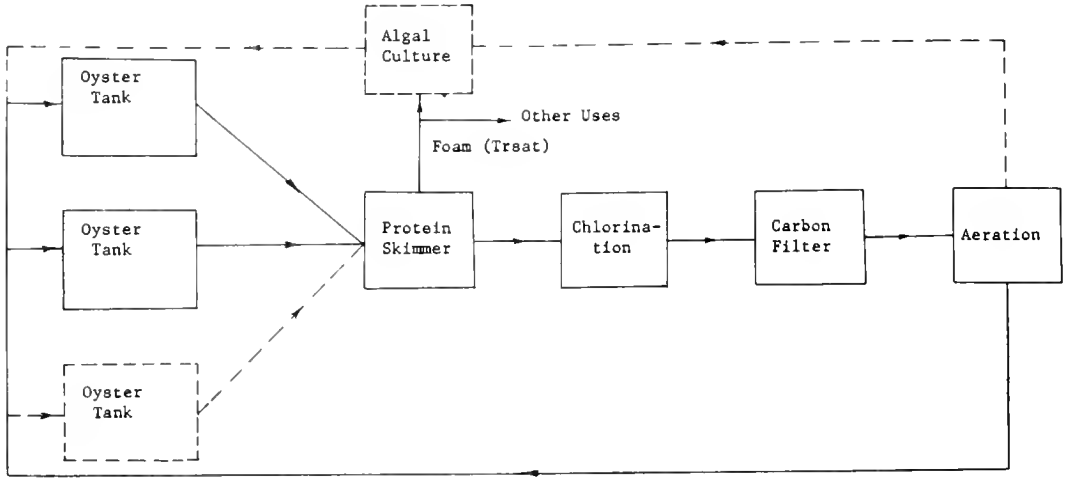

FIG. 4. *Proposed closed-cycle system using non-bacterial filters. Dashed lines show optional components. Number of oyster tanks in a system will depend upon the size of filtration units.*

low as 0.01 ppm with the use of activated carbon. However, they pointed out that oxygen is also removed by this method, and water must be re-oxygenated before its use in the culture tanks.

Baummer *et al.*, (1969) used activated carbon successfully to dechlorinate salt water for use in aquariums that held *Fundulus* sp.

THE EXPERIMENTS

Materials and Methods

Preliminary experiments were run to evaluate the merit of non-bacterial filters for waste removal from sea water in a closed-cycle oyster culture system. A foam-fractionation unit as described elsewhere was used for organic removal (Dwivedy, 1973). Calcium hypochlorite (CaOCl) was used as a chlorinating agent for ammonia removal.

The tank used for these experiments held forty gallons of sea water that contained oyster waste. The flow rate through the foam-fractionation unit was 5 gpm. The water was foam-fractioned for two hours to provide the maximum possible removal of the organics (Dwivedy, 1973). After organic removal, ammonia in the water was measured. The amount of CaOCl needed was determined on the basis that 10 mg/l of chlorine is required to remove 1 mg/l of ammonia (Baummer *et al.*, 1969). Fifteen minutes reaction time was allowed after adding calcium hypochlorite to the water. At the end of this time, the foam-fractionation unit was replaced by a carbon filter for dechlorination of the water. The carbon filter was cylindrical, in shape with 18 in long and 4 in in diameter. The water was circulated through the filter at a rate of about 5 gpm.

The water in the tank was sampled at regular intervals to measure the following parameters; COD, BOD, total-N, pH, ammonia-nitrogen, total available chlorine, dissolved oxygen (D.O.), temperature and salinity. COD and BOD were determined by Standard Methods (1971). Total-nitrogen was determined through Kjeldahl digestion with an ammonia electrode (Model 95-10, Orion Research, Inc.). Total available chlorine was determined by the orthotolidine procedure (Standard Methods, 1971). The temperature and salinity of the sea water were about 68 F and 26 ppt, respectively.

Results

The results of a typical experiment are given in Table 1. Ammonia from the sea water was successfully removed by chlorination. Dechlorination of the water was effectively achieved when the water was passed through the carbon filter (Table 1).

PROPOSED CLOSED-CYCLE MARICULTURE SYSTEM

A diagram of the proposed system is shown in Fig. 4. The scheme is mainly directed toward shellfish culture and specifically toward oyster culture. It must be noted that oysters are filter feeders, and therefore feeding and filtering operations in the system cannot be performed simultaneously. The procedure in this case would be to feed a given number of hours and then to pump the water to the treatment tank. The water in the treatment tank would be foam-fractionated until the removal of organic matter has been essentially achieved. The water could then be chlorinated for ammonia removal and circulated through the carbon filter for dechlorination. (Common commercial chlorinating agents are hypochlorites such as calcium hypochlorite). After dechlorination, the water is ready for the oyster culture tanks. A portion of this treated water might be directed into the algae culture tanks. Algae would be fed to the oysters, thus completing the cycle. Foam obtained by foam-fractionation is very rich in organic matter, since it concentrates organic materials from the water (Dwivedy, 1973). Therefore, the foam might be used, after some processing, as nutrient to the algae culture, or for some other purpose, as a source of high-nitrogen compounds. One of the several possible uses of the foam would be in compost for agricultural fertilizers.

The number of culture tanks that can be connected to a treatment tank would depend upon the size of foam-fractionation unit.

Two points must be borne in mind. One is that the water must be foam-fractionated before chlorination to reduce "chlorine demand" and second, the water must be re-oxygenated before return into the culture tanks because dissolved oxygen also is removed through adsorption onto

TABLE 1. *Waste removal from sea water through non-bacterial filters*

Sampling time	Water quality parameters						
	pH	COD (ppm)	BOD (ppm)	Total-N (ppm)	NH_3-N (ppm)	Total available chlorine (ppm)	D.O. (ppm)
9:30 AM+	7.6	30-35	25-30	6-8	2.2-2.6	0.00	7-8
11:30 AM*$_T$	7.8	10-12	8-10	3-5	2.2-2.6	0.00	8.5
11:45 AM	7.8	below 10	5-7	2-3	below 0.02	0.13	8-8.5
12:45 PM#	7.8	below 10	below 5	below 2	below 0.02	0.01	7-8

+ Foam-fractionation was started.
* Just before adding CaOCl to the water.
T Fifteen minutes after adding CaOCl but just before putting carbon filter.
After one hour of carbon filtration.

the activated carbon (Coventry *et al.* 1935). Reoxygenation can be readily and cheaply accomplished by allowing the filtrate to trickle slowly down a trough with a corrugated bottom.

SUGGESTIONS FOR FUTURE DEVELOPMENTS

Although the proposed scheme of waste management in a closed cycle mariculture system appears to be feasible, some aspects must be investigated before it can be freely recommended. It is suggested that tolerance levels to residual chlorine, monochloramine and dichloramine be determined for any organism to be cultured. Any adverse effect of chlorination, such as possible depletion of some essential elements, should be determined. If such losses should occur, they can be computed and compensated by replacement in the make-up water.

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DESIGN CONSTRAINTS ON AN OYSTER SHUCKING MACHINE[1]

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ABSTRACT

An oyster shucking machine designer must combine biological and mechanical characteristics into a practical system of hardware. Oyster biological properties which must be considered include meat, shell and hinge characteristics, type of final product desired and effects of fouling organisms. Machine parameters of reliability, capacity, wear, capital and operating costs, labor requirements, corrosion, sanitation and continuous or batch process must also be considered. Biological constraints and machine characteristics are then incorporated to produce a machine, the characteristics of which should approximate as close as possible those of an ideal shucking machine.

INTRODUCTION

Severe labor shortages and rising labor costs in the United States oyster industry are limiting the supply of fresh shucked oysters. This results in higher retail prices thus placing oysters in a specialty item market which limits market volume. Unfamiliarity of the consumer with oysters further limits markets.

In spite of the problems, the oyster industry is of considerable importance to many of the coastal states. The 1972 United States catch of 52,546,000 pounds of oyster meats was valued at $33,819,000 (Fisheries of the United States, 1972). In the same year Maryland, the leading oyster producing state, accounted for 16,276,421 pounds of oyster meats valued at $9,898,678 (Maryland Landings, 1972).

Survival of the present commercial Maryland oyster industry depends to a large degree on the ability of the industry to shuck (remove the meats from the shell) the available oysters. Commercial shucking is hard work, dangerous, seasonal in nature and carries limited prestige as a career. The rising average age of oyster shuckers, now somewhat over 50 in Maryland (Wheaton, 1970), is mute evidence that young people are not becoming professional oyster shuckers. Mechanization of the shucking operation, the production limiting operation in oyster processing (Wheaton, 1970), is the industry's best alternative. This paper discusses the constraints placed on design of a shucking machine by the oyster's biological properties, and attempts to outline desirable features of an ideal oyster shucking machine.

BIOLOGICAL PROPERTIES AS MACHINE DESIGN CONSTRAINTS

The physical properties and relative location of the three major components of an oyster, the meat, shell and hinge, are important in design of a shucking machine. The oyster gills, very thin and delicate structures, are located directly beneath the thinnest shell areas. The oyster muscle consists of two sections: semitranslucent

[1] This work was supported by the National Marine Fisheries Services and the Maryland Department of Fish and Wildlife under PL 88-309 as Subproject 3-129-D.

and opaque. The semitranslucent part, located nearest the hinge, detaches at a lower temperature than the opaque section. The oyster's mouth is in contact with the inner surface of the hinge. Since the oyster's mantle is in contact with the inner surface of both valves when the oyster is closed, access to the muscle-shell attachments is limited. The meat has very little strength and will not withstand cutting, tearing or abrasion. If an oyster meat is placed in fresh water, it will absorb water to help equalize the osmotic pressure across its exterior surface. The meat is attached to the shell at three points: each end of the adductor muscle and at one point beneath the gonadal area (Quenstedt's muscle). The adductor muscle-shell attachments are stronger than the muscle tissue, while the attachment of Quenstedt's muscle is very weak and, therefore, of little significance in shucking machine design.

The oyster's adductor muscle-shell attachment can be broken by heating the attachment layer. Unfortunately, the attachment layer does not break down until temperatures exceed 160 F. The oyster meat will start to cook at 160 F and prolonged exposure at temperatures of about 130 F may cause some flavor changes in the meat. For those reasons heating oysters is not favored by processors selling raw shucked oysters. The requirement for a raw product severly limits use of this principle in the design of a shucking machine unless some means is developed to eliminate cooking of the meats.

The characteristics of the oyster meat limit the amount of acceleration which an oyster can withstand, particularly after the oyster has been removed from the shell. Thus, the vibration an oyster can withstand and the distance an oyster can be allowed to drop within a shucking machine is limited by its biological structure.

The oyster shell is composed of calcium carbonate crystals laid down in a protein matrix (Galtsoff, 1964). This structure makes the shell hard, brittle and layered. Fracture of the shell results in sharp jagged edges which easily damage the meat on contact. Shell strength varies with bar location, bottom type on which they grow, severity of boring organism infestation, and shell geometry. Some shells will collapse when squeezed by hand while others will withstand compressive forces exceeding 100 pounds.

Shell shape is even more important than shell strength in shucking machine design since no two oysters have the same shape. In Maryland harvestable oysters will vary in length from 76 to 200 mm (3-8) in.), and in shape from nearly round to long and narrow. In width they vary from about 38-152 mm (1½-6 in.), while maximum thickness varies from about 25-64 mm (1-2½ in.). Fouling organisms attached to the shell exterior extends the shape variation to nearly any shape.

The hinge is the third part of an oyster important in shucking machine design. Fig. 1 shows a cross section of a typical hinge. The two hinge areas "A" are strong in tension while area "B" is strong in compression (Galtsoff, 1964). The hinge consists of an elastic organic nonliving material. The elastic property prevents efficient use of impact forces to sever the hinge. The tensile strength of the hinge can be appreciated by measuring the force necessary to sever it with a conical point. Up to 100 pounds is required to drive a 9.5 mm (3/8-inch) diameter conical point with a 70° apex angle into the hinge and sever it. However, the hinge can be weakened by dehydration. Since free water inside the shell is in contact with the inner hinge surface, it is nearly impossible to dehydrate the hinge of a living oyster.

MACHINE CHARACTERISTICS

Two overriding needs will largely determine shucking machine design characteristics: (1) the final product must be a raw oyster, and (2) cut-

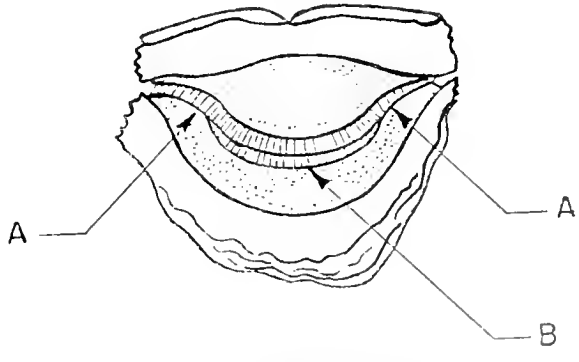

FIG. 1. *Cross section of an oyster hinge.*
A. Strong in tension.
B. Strong in compression.

ting and tearing of the oyster meat must be eliminated or reduced to an absolute minimum. The irregular shape of the oyster will also affect the design.

At present, a shucking machine must be able to handle wide variations in shell shape since oyster culture methods can not change rapidly enough to meet the demand for uniformly shaped oysters in the next five to ten years. There are two possible methods of dealing with this problem: (1) to handle oysters individually, or (2) in a bulk lot. The first requires all devices to handle almost any possible shape; a difficult design constraint to meet. The bulk lot approach requires subjecting each oyster to the energy necessary to shuck it while the oyster is in a bulk lot. The handling method will depend on the handling procedures used, energy form used to shuck the oysters, overall system design, and oyster culture methods.

Fouling organisms such as barnacles, mussels, water weeds, sponges and other organisms find the exterior of an oyster shell an ideal home. Fouling changes the shape of the shell and often changes the physical properties of the shell. Washing the oysters with a mechanical scrubbing device will remove most of this fouling but some barnacles and other organisms will remain on the oyster despite severe scrubbing.

Any shucking machine will be exposed to the corrosive effects of salt water and the abrasive effects of oyster shells. Most structural materials corrode in salt water and materials which do not are expensive. Stainless steel withstands salt water quite well, as do many plastics. Abrasive wear on conveyors and parts which touch the oyster shells will be high. Thus, it is necessary to keep the cost of these parts as low as possible to reduce machine maintenance costs. Since stainless steel is expensive, it would be desirable to use less costly materials in areas where there is no contact with the oyster meat and where abrasive wear is high.

A large number of oysters must be handled by a shucking machine. Two desirable characteristics of such a machine would be a continuous flow process and a high capacity. A continuous flow process is desirable because velocity changes are minimized, capacity is usually higher and machine time is better utilized. Indexing devices tend to require the product and various machine components to undergo velocity changes during operation which usually increases stress on machine components. Thus, heavier machine components, necessary to withstand the increased stress, will increase machine weight and cost of construction.

Product quality must be maintained or improved by any shucking machine introduced into the industry. The product must be raw, show a minimum of cuts and other physical damage and be as free as possible of shell chips and grit. No one likes to bite down on an oyster and end up chewing on a piece of shell or grinding sand between his teeth. The present health regulations allow raw fresh oysters only a ten day shelf life after packing. Any quality reduction caused by a shucking machine would shorten the shelf life and increase loss of oysters at the retail level.

Wheaton (1970) has shown that an oyster processor can pay about $30,000 for a shucking machine which will maintain product quality without increasing his shucking costs. Such a machine will shuck 60 oysters per minute, operate 720 hr per year at an operating cost of $5.00 per hr and have a 5 yr. life. The operating costs include any labor needed to operate the shucking machine as well as maintenance and repair costs. If the operating costs exceed five dollars per hour, the initial cost of the machine must be less than $30,000.

Reliability of any shucking machine must be high. The oyster season runs from about mid October to mid April; with the greatest demand during the holiday season in November and December. A machine breakdown during this peak period could place a processor in a difficult financial position. His inability to supply regular customers could have long range effects on his markets.

A shucking machine must also meet all sanitation regulations and requirements. Surfaces must be impervious to dirt and water and easily cleaned and sanitized. Sharp corners must be minimized and disassembly for cleaning must be quick and convenient. All surfaces in contact with the oyster meats must be stainless steel, plastic or other noncorrosive materials.

SUMMARY

Any shucking machine of value to the oyster industry must consider several product and machine characteristics in its design. Product characteristics must include such things as the physical properties of the oyster meat, shell and hinge and the type of final product desired. Machine characteristics must include capacity, reliability, product fouling, corrosion and abrasive wear, capital and operating costs of the machine, continuous or batch flow through the machine and sanitation. All of these machine and product characteristics will influence design of a shucking machine, and will determine the success or failure of the machine in performing the task it is designed to do.

The ideal machine would have a new cost of less than $30,000 and produce a raw shucked oyster of better quality than the handshucked product. It would be of continuous flow design with a minimum capacity of 60 oysters per minute, and would handle single or clustered oysters of any shape. The machine would also have enough design flexibility to at least double this capacity by relatively minor additions or changes. While a machine possessing all the necessary characteristics would be an ideal machine, a practical machine should have as many as possible. The better a real shucking machine approximates this ideal the more acceptable it will be to the oyster industry.

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BASIC STUDIES ON OYSTER CULTURE I. HOW DO SINGLE OYSTERS LAND ON THE BOTTOM WHEN PLANTED?

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ABSTRACT

It has been assumed that the non-incubatory oysters, genus Crassostrea, *when planted in singles fall to the bottom with the left (cupped) valve down, predominantly, or right side up. A thousand oysters ranging from small to large, when thrown into eight feet of water, fell with the right side up only 41% of the time. This may be very important in oyster planting if oysters with the right valve (wrong side) down die faster than the others on planted beds.*

The bottom or the left valve of the oysters of the genus *Crassostrea* is usually fairly deeply cupped and the oyster lies within this part of the shell, covered by the right valve. This is especially true of the non-incubatory oysters with the elongate, heavy, thick shells of the genus *Crassostrea*, in contrast to the so-called European or flat oyster, which lacks the promyal chamber, is rounder and incubates eggs and larvae (genus *Ostrea*).

The recovery rate of planted oysters is a very important matter in oyster culture. Without going into the vagaries of this proposition, such as oysters landing in the mud, being transferred to much different salinities, *etc.*, we feel that little factual information exists. Some Louisiana oystermen are reputed to make money in transplanting seed if they get a one-to-one volume return. This means, of course, that sometimes there is a very high mortality rate between transplanting seed and harvesting adults. There are actually few figures published on the survival rates of any kind of transplanted oysters.

Not all the differences between planting and harvesting are due to mortality, because the oysterman can never recover everything he plants by ordinary commercial methods. Recently, oysters from polluted beds in Lake Pontchartrain were planted in Louisiana waters and harvested some 9 days later. The return to the fisherman who planted and fished the oysters was about 65-75% (Tarver and Dugas, 1973). This was done with ordinary oyster dredges and, supposedly, this is a fair indication of the return to dredgers over oyster beds. In short, one-fourth or more oysters may be lost after planting simply because of fishing inefficiency.

Our interest in this question began with the assertion of fishermen in Mississippi that oysters placed wrong side (left valve) up on the bottom would eventually die. We can see how such oysters falling into the mud and lying with the flat shell down would have the shell opening in the mud. However, there is no evidence that oysters caught on the underside of a boat or turned upside down in trays would suffer any harmful effects. We undertook to make a study of this by placing some oysters in a tray under the wharf at the Gulf Coast Research Laboratory. However, some thief decided he wanted the oysters and took them tray and all. We have not repeated the experiment.

The question also arose about how many oysters fall right side up when they are planted. Therefore, we took a thousand oysters, approximately a barrel and a half, culled them out

in singles and threw them overboard into a 7-8 ft. deep, fresh water swimming pool. They were distributed with a shovel, using the sweeping motion that the oystermen generally use for scattering oysters. The actual specific gravity difference between fresh and brackish water is so small that we believe it had no influence on the sinking gyrations of the oysters. After this planting, Miss McGraw and Mr. James Franks dove overboard with scuba gear and recorded the position of the oysters on the bottom of the pool.

The senior author has always held forth at length that the cupped shape of the left valve would naturally cause oysters to sink with the bottom side down. Contrary to expectations, 41% of the oysters landed right-side-up and 59% had the right or upper valve turned down. It means, among other things, that if the ideas about the harm caused by the wrong position of oysters are valid, the more careful handwork used in some parts of the world in cultivating oysters has a great advantage over the crude strewing methods used on our Atlantic and Gulf coasts.

Emery (1968) has studied the positions of empty pelecypod valves on the continental shelf. He found that in areas of strong currents and heavy wave action most shells were in concave-down positions, as they appear on the sea beaches. On the offshore shelf, where weak currents are prevalent, many shells remain in the concave-up position.

While we have raised many more questions than we have answered, it is clear that these simple matters are of importance in oyster cultivation and deserve more attention than they have received in the past.

LITERATURE CITED

Emery, K. O. 1968. Positions of empty pelecypod valves on the Continental Shelf. J. Sediment. Petrol. **38:** 1264-1269.

Tarver, J. W. and R. J. Dugas. 1973. Experimental oyster transplanting in Louisiana. La. Wildlife Fish. Comm. Tech. Bull. No. 7, 10 p.

ASSOCIATION AFFAIRS
ANNUAL CONVENTION

The 65th annual meeting of the National Shellfisheries Association and the Shellfish Institute of North America was held jointly 24-28 June, 1973 at the Jung Hotel, New Orleans, Louisiana.

Officers and executive committee members elected were:

President . . . . . Ronald Westley
Vice-President . . . . . Dexter Haven
Secretary-Treasurer . . . . . Michael Castagna
Members-at-Large . . . . . Robert Hillman
Herbert Hidu
Neil Bourne
Editors of the Proceedings . . . . . Sara V. Otto
Haskell S. Tubiash

Custodian is Janet B. Hammed, National Marine Fisheries Service, Biological Laboratory, Oxford, Maryland 21654.

Respository for back issues of the Proceedings is National Marine Fisheries Service, Biological Laboratory, Oxford, Maryland 21654.

A resolution was passed to honor Dr. Les Stauber (deceased) for his great contribution to the field of shellfish biology. It was further moved that a volume of the Proceedings be dedicated to him.

Dr. Daniel Quayle, Dr. J. C. Medcof, Dr. Gordon Gunter, Dr. Lyle St. Amant, and Dr. Dennis Crisp were made honorary members of the organization.

It was moved that page charges be assessed starting with Vol. 64.

Fifty new members were accepted by the organization.

A proposed revision of the constitution was read and will be voted on by mail prior to the next meeting.

The PCSNSA and the PCOGA met September 7-8, 1973 at Olympia, Washington. The new officers are:

Chairman . . . . . Neil Bourne
Vice-Chairman. . . . . Al Scholz
Secretary-Treasurer . . . . . Terry Y. Nosho

ERRATA

Vol. 63: Abundance of the Low Salinity Clam, *Rangia cuneata*, in Southwestern Louisiana. H. Dickson Hoese.

p. 103: col. 1: under SIZE, line 2: " . . . shallow water samples" (not stations)

p. 103: col. 2: last line: " . . . 14% of the means" (not clams)

p. 104: col. 1: line three: " . . . 84% of the means" (not clams)