# 1968 PROCEEDINGS

# NATIONAL SHELLFISHERIES ASSOCIATION

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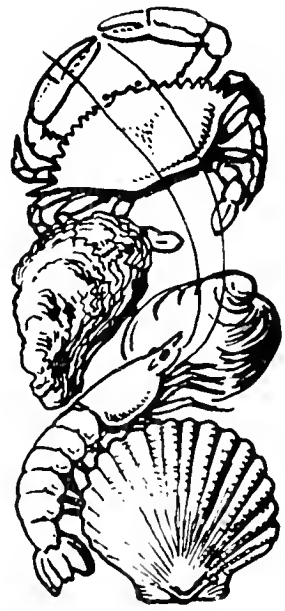

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# ABSTRACTS OF TECHNICAL PAPERS PRESENTED AT THE 1968 NSA CONVENTION

## REMOVAL OF PEA CRABS FROM LIVE OYSTERS BY USING SEVIN

Jay D. Andrews,
Donna Turgeon and
Marian Hreha

*Virginia Institute of Marine Science*
*Gloucester Point, Virginia*

Pea crabs *(Pinnotheres ostreum)* living in the mantle cavity of oysters *(Crassostrea virginica)* were killed by exposure to 10 mg/1 Sevin ® for 24 hours. Crabs were ejected by oysters in winter and summer but elapsed time from first treatment varied with size of crabs and oysters. Oysters pumped freely in concentrations of technical 95% Sevin up to 100 mg/1 without apparent injury. Low solubility of powdered Sevin presented no problems, therefore organic solvents were avoided. Data on crab prevalences in oysters are given.

## RECENT STUDIES OF HARD CLAM ACTIVITY AND DEPURATION[1]

Walter J. Canzonier

*Department of Zoology and Department of Oyster Culture*
*N. J. Agricultural Experiment Station*
*Rutgers University, New Jersey*

A study of viral depuration of *Mercenaria mercenaria* using an *Escherichia coli* bacteriophage in a model system has been continued with some modification to approximate more closely conditions that might be encountered when clams are polluted in natural waters. In the current series of experiments the sampling sensitivity was increased by assay of individual clams (10-20/sample) in contrast to pooled samples used previously and by refinement of preparation techniques which allow recovery of better than 60% of the viral particles present in the clam tissues. Exposure to phage particles (pollution) was in running sea water for prolonged periods (7-18 days) at levels of 5-9 particles per ml. When exposed at this level, the clams concentrated the particles. Means for pooled samples were 10-20 times those of the seawater levels, and some individuals assayed at 50 times the concentration of the water in which they were exposed. When exposed at higher levels ($10^3$ —$10^4$ particles/ml), titre in the clams did not exceed seawater titre. Clams exposed for short periods (24 hrs) exhibited no essential differences in patterns or rates of depuration when compared with clams exposed for longer periods. Clams exposed to low levels (90 to 140 particles/ml of clam tissue) rid themselves of 90% or more of their viral load; however 10-80% of the clams sampled remained positive for as long as 12 days after depuration was initiated. Companion experiments using *E. coli* as a pollutant had excellent depuration in 12 — 24 hours. Possibly some of the viral particles are in some way sequestered and protected outside the lumen of the digestive tract either by adsorption or migration between cells or by phagocytosis by the clam leucocytes.

[1] Supported under contract with the U. S. Public Health Service (UI-00155)

## UPTAKE AND ELIMINATION OF *GYMNODINIUM BREVE* TOXIN(S) BY THE OYSTER, *CRASSOSTREA VIRGINICA*[1]

Joseph M. Cummins and
Abner C. Jones

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*Dauphin Island, Alabama*

The marine dinoflagellate, *Gymnodinium breve*, has been strongly incriminated as the primary source of toxin(s) present in edible oysters and clams exposed to *G. breve* "red tides". The public health importance of this relationship prompted the investigation into intoxication and detoxifica-

tion of shellfish after exposure to *G. breve*-rich and *G. breve*-free environments.

One hundred twenty nontoxic oysters, *Crassostrea virginica*, accumulated 88.7 to 95.1% of the initial numbers of *G. breve* (2400 — 3900 organisms/ml) present in each of three, 50-liter, unialgal cultures in 2 — 4 hrs. Culture salinities ranged from 27.2 — 28.8 ppt; culture temperatures ranged from 23.2 — 25.5°C. The relative toxicity of the oysters after 9 hrs exposure to *G. breve* cultures was 40.5 mouse units toxin/100 g oyster meats. Following 36 hrs of exposure to *G. breve*-free, aquatic environments having salinities of 6.8 ppt and 28.5 ppt, the toxic oysters displayed a 40.7% and 60.7% reduction in toxicity, respectively. For control purposes, toxic oysters were subjected to a moist air environment for 36 hrs. During this period no marked change in the toxicity of the oysters was observed. Thus, the uptake and elimination of *G. breve* toxin(s) by oysters appeared to be primarily a function of the feeding and elimination activity of the oysters themselves.

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[1] Contribution No. 49. Gulf Coast Marine Health Sciences Laboratory, P. O. BOX 158, Dauphin Island, Alabama 36528. A part of the National Center for Urban and Industrial Health, Cincinnati, Ohio 45202.

## PRELIMINARY OBSERVATIONS ON THE UPTAKE, DEPURATION AND EFFECT OF IRRADIATION ON VIRUSES IN PACIFIC AND OLYMPIA OYSTERS

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*University of Washington*
*Seattle, Washington*

Pacific oysters *(Crassostrea gigas)* and Olympia oysters *(Ostrea lurida)* were exposed to seawater contaminated with $10^{40}$ P.F.U./ml infective particles of Poliovirus LSC-2ab in 5 gallon stainless steel aquaria. Virus titre was determined by subculture of oyster extracts on Primary African Green Monkey cell cultures. Depuration following virus exposure was determined by analyzing tissue from the oysters that were held in clean seawater replaced at 12-hr intervals.

Significant uptake of virus was noted; maximum counts were reached at 24 — 48 hrs after exposure at which time total counts in the oyster were about 90% of water counts. Maximum recovery of virus was from digestive diverticulum. Depuration was slow; even after 120 hrs detectable counts of viruses were still present. Because depuration was so slow, tests were made to determine the effectiveness of gamma radiation to eliminate virus from shucked and unshucked samples. Significant numbers of virus particles were still detectable in all samples after irradiation, minimum and maximum recovery was approximately 40% and 90%, respectively.

It was concluded that West Coast oysters accumulate viruses as readily as East Coast shellfish, and in about the same quantities. The irradiation method used does not show much promise at this time for removing virus from shellfish.

## THE EFFECT OF EARLY FOULING OF SHELL SURFACES ON OYSTER SPATFALL

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*Oyster Unit, Ellerslie*
*Prince Edward Island, Canada*

The attractiveness of cultch materials to setting oysters *(Crassostrea virginica)* is an important factor in both field and hatchery spat collection. In the fouling and silting environment at Ellerslie, field observations show that sea scallop shells of *Placopecten magellanicus* become more attractive to oyster larvae after exposure to sea water over at least a 2 week period. The same phenomenon has been demonstrated under hatchery conditions. Results indicate a maximum effect after 6 days. It is suggested that microbiological fouling is the basis of the attractiveness of the shell surface.

## OYSTER LEUCOCYTES IN TISSUE CULTURE: A FUNCTIONAL STUDY[1]

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M. R. Tripp

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*Mercer County Community College*
*Trenton, New Jersey and Department of Biology*
*University of Delaware, Newark, Delaware*

Yeast cells were boiled, washed and suspended in Kreb's Basal Salt Solution (KBSS) with oyster *(Crassostrea virginica)* leucocytes for 3 hr at 20°C. Non-ingested yeast cells were then removed and oyster leucocytes were maintained in the balanced medium. Samples of leucocytes were removed daily over a period of 8 days and tested for the presence of acid phosphatase and nonspecific esterase. These enzymes are enclosed within lysosomes and, as such, serve as indicators for intracellular digestion.

Yeast cells were quickly phagocytosed by oyster leucocytes but no association was noted be-

tween lysosomes and phagosomes within the cells even after 50 hr. By the fourth day lysosomes were observed in the vicinity of the phagosomes. After 5-8 days a progressive increase of enzyme activity within the phagosomes was observed suggesting a release of lysosomal enzymes into the phagosome. At the end of 8 days of intracellular residence, the large majority of phagosomes showed evidence of lysosomal enzyme activity. This relatively long period preliminary to intracellular digestion in the oyster leucocyte is consistent with observations at other laboratories. It is in marked contrast to the rapid lysosomal activity observed in mammalian phagocytes.

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

[2] Present address: Department of Biology, Trenton State College, Trenton, New Jersey.

## THE RESPIRATORY PHYSIOLOGY OF *MERCENARIA MERCENARIA*[1]

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A direct method was used in the study of pumping and respiratory rates of the hard clam, *Mercenaria mercenaria*, under a variety of combinations of temperature, salinity and oxygen tension. The clams were kept in running sea water. Pumping rates were measured by the method of Ansel and Coughlan in which the incurrent flow is replaced by a metered stream of sea water colored by a soluble non-toxic dye. The respiratory rates were measured by the polarographic method, and the quantity of oxygen used was calculated from the difference in oxygen concentrations between incurrent and excurrent water.

The results indicate:

1. A linear relationship exists between pumping rate and respiratory rate, suggesting regulation of water transport in relation to rate of metabolism.
2. The rate of oxygen uptake is a power function of the tissue weight with values of the exponent of 0.3561 for wet weight and 0.3556 for the dry weight suggesting that the respiration is less than proportional to surface.
3. The weight specific respiration ($QO_2$) is generally higher than values previously reported for bivalves. It is a function of pumping rate and decreases with increasing size of animals.
4. When the clam is deprived of oxygen, an oxygen debt is incurred which is paid during the first hours of the subsequent aerobic period in which the utilization coefficient is high and decreases gradually until a more or less constant level is reached.
5. With decreasing external oxygen concentration reduced to a critical value of about 5 mg $O_2$/liter, *M. mercenaria* is able to regulate its oxygen consumption by increasing its efficiency of withdrawal of oxygen from the water. Below this value oxygen consumption declines continuously.
6. Oxygen consumption increases with decreasing salinity indicating a euryhaline response.
7. Oxygen consumption increases with increasing temperature up to about 26°C beyond which it drops rapidly.

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[1] Supported under contract with the U. S. Public Health Service (UI-00155).

## POPULATION DYNAMICS OF OYSTER DRILLS ON A DELAWARE BAY OYSTER GROUND[1]

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*Rutgers University, New Jersey*

Since 1964 populations of oyster drills *(Urosalpinx cinerea)* on two adjoining Delaware Bay oyster *(Crassostrea virginica)* grounds have been under study. One of the grounds has been treated annually with the "drillicide" *Polystream*. Of particular interest are the changes in the drill population on the untreated or control ground. Annual recruitment to the population has ranged from 40 to 56% of the total population. Population fluctuations have not been closely related to food supply as represented by available young oysters. High drill mortality has occurred even with abundant food supply and it now appears that most of the populations do not live beyond 1 — 2 yr.

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[1] Supported under PL 88-309 contract 3-3-R-3 with the U.S. Bureau of Commercial Fisheries.

## RECENT EVIDENCE OF MSX-RESISTANCE IN VARIOUS STOCKS OF OYSTERS[1]

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*N. J. Agricultural Experiment Station*
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In past reports, evidence has been presented for increasing resistance to MSX *(Minchinia nelsoni)* mortalities in native Delaware Bay oyster *(Crassostrea virginica)* stocks. Since 1960 similar evidence has also been accumulating from laboratory-reared stocks of known parentage. After 3 years of exposure in disease enzootic waters, only 8% of a susceptible 1964-year-class oyster stock of Connecticut parentage survived in contrast to 40% survival in a stock of "resistant" Delaware Bay parentage. More recently the 1966-year-class stocks, first exposed to MSX in that same fall, are providing additional evidence. The offspring of parent stocks, all selected against MSX in Delaware Bay, from Delaware Bay, Virginia or Long Island, had disease mortalities of 22 to 36% in 1967; unselected Navesink River stock offspring had significantly higher mortalities of 42 — 47% and unselected Sheepscot River stock offspring had a mortality over the same period of 83%.

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[1] Supported under PL 88-309 contract 3-3-R-3 with the U.S. Bureau of Commercial Fisheries.

## VITAL MARKING THE SHELLS OF MOLLUSKS WITH ALIZARIN[1] — ADDITIONAL EXPERIMENTS

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James E. Hanks

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*University of Maryland, Solomons, Maryland and*
*U.S. Bureau of Commercial Fisheries*
*Biological Laboratory, Milford, Connecticut*

Trials are continuing in the development of the alizarin shell marking method for more general applicability in molluscan research. Thus far, the most easily marked are juvenile *Mya arenaria* which are less than 2 cm in length. Half-year old *M. arenaria* from the Chesapeake grew 0.2 cm wide red shell bands when subjected to 8 ppm alizarin for 2 weeks. Standing culture water was changed daily with no addition of supplementary food. Larger, earlier year class *M. arenaria* produced a shell check mark in alizarin but deposited little new, colored shell. Success in colored shell production appears to be dependent on small rapidly growing animals subjected to proper alizarin concentration under favorable nutritive conditions.

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[1] Alizarin Sodium Monosulfonate. Fisher Scientific Company, Cat. No. A-427.

## KELLY-PURDY UV SEAWATER TREATMENT UNIT: KINETICS OF POLIOVIRUS INACTIVATION[1]

William F. Hill, Jr.,
Frederick E. Hamblet and
William H. Benton

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*Dauphin Island, Alabama*

The effectiveness of ultra violet radiation (UV) to inactivate poliovirus type 1 (LSc2ab) suspended in continuously flowing seawater has been investigated. These studies were implemented to satisfy the requirement for seawater treatment for application to shellfish depuration systems. The UV treatment unit developed for depuration systems and shown to effectively destroy coliform organisms is commonly referred to as the Kelly-Purdy UV Seawater Treatment Unit. This unit is considered to be of a design and capacity for application to commercial use (Kelly, Amer. Jour. Pub. Health, **51**(11):1670-1680,1961). Its effectiveness to inactivate viruses under operational conditions has not been previously reported. The fixed parameters coincident to the experimental design were as follows: (1) seawater salinity, 25.6 ppt, pH 7.7; (2) average UV intensity of the 13 lamps, 68.8 $\mu w/cm^2$; (3) seawater flow rate, 144 liters/min; (4) seawater turbidity, 42 ppm. Five replicate samples were assayed for surviving virus at each sampling point within the unit. The virus was assayed by the plaque technique in Hep-2 cells. The survival ratio of the virus and the exposure times were then calculated, plotted and analyzed. The slope of the inactivation curve and its standard deviation were determined. Statistical inference indicated that the curve obeyed first-order kinetics. The analytic findings indicated that the reduction of virus was greater than 99.97% in 15.7 sec. No virus was detectable in 20.6 sec. The calculated half-life ($T^1_2$) value for poliovirus type 1 was 1.29 sec. The data indicated that the Kelly-Purdy UV treatment unit effectively inactivated poliovirus in flowing seawater.

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[1] Contribution No. 47: Gulf Coast Marine Health Sciences Laboratory, P. O. Box 158, Dauphin Island, Alabama, 36528. A part of the National Center for Urban and Industrial Health, Cincinnati, Ohio 45202.

## HISTOCHEMISTRY OF MUCOSUBSTANCES IN THE SECOND FOLD OF THE MANTLE OF THE QUAHOG.

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Work on histochemical localization and characterization of mucosubstances in the mantle of the quahog, *Mercenaria mercenaria*, is in progress to understand the physiological role of the various complex mucopolysaccharides and glycoproteins present in the restricted confines of the mantle. Results of a variety of histochemical reactions on mucosubstances in neutral formalin-fixed tissues indicate that in the second fold of the mantle edge there are two mucous cell types in juxtaposition with one another, one secreting a sulfated acid mucopolysaccharide and the other forming a neutral mucosubstance.

The neutral mucosubstance is PAS-positive prior to and following diastase digestion. It also exhibits a positive PAS reaction following staining with alcian blue at pH 2.5, both prior to and following hyaluronidase digestion.

The acid mucopolysaccharide generally stains intensely with alcian blue at pH 2.5 and somewhat less at pH 1.0. It retains its alcianophilia in solutions of magnesium chloride up to 1.0 M, and after testicular hyaluronidase digestion. The material also reacts strongly with iron diamine and aldehyde fuchsin when these reagents are followed by alcian blue. Portions of the acid material stain with less intensity than maximum; this material may be a precursor to the final product which appears to be a strongly sulfated acid mucopolysaccharide.

The results suggest that acid mucopolysaccharide and neutral mucosubstance are related in function and that both may be involved in calcium metabolism within the mantle.

## ASPECTS OF TEMPERATURE STRESS AND RESPIRATORY METABOLISM IN SOFT-SHELL CLAMS, *MYA ARENARIA*

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*Natural Resources Institute*
*University of Maryland*

The respiratory rate of young-of-the-year soft-shell clams was measured at 1°, 10°, 20° and 30°C for clams acclimated to these temperatures. Results confirm the familiar inverse relationship between body weight and rate of oxygen consumption. In general, weight-specific respiration increased at high temperature "stress" and decreased under low temperature "stress." An exception was noted for acclimated animals at 1°C whose respiration rate at 30°C dropped abruptly from the level noted at 20°C.

Young clams in the natural environment subject to a non-lethal heated discharge would probably respire at such a rate that their food requirements would be greatly increased. Lack of an adequate food supply could therefore result in decreased or negative growth and possible death from starvation.

## THE OCCURRENCE OF TRACE METALS IN OYSTERS *(CRASSOSTREA VIRGINICA)* FROM THE SOUTH ATLANTIC AND GULF OF MEXICO[1]

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Oysters possess the ability to extract and retain certain elements present in relatively low concentrations in the surrounding water. This phenomenon is of importance since oysters in polluted estuaries can accumulate elements harmful to human health.

In a study initiated in 1964, it was found that the copper and zinc content of oysters could be conveniently determined by a polarographic method. In a total of 136 oyster samples from several locations of the South Atlantic and Gulf Coast analyzed for these elements, the concentration of both elements varied with the geographical location from which the samples were obtained. The average copper content of the 136 samples was 19 mg/kg of wet oyster tissue with a range of 1 to 55 mg/kg; the average zinc content was 230 mg/kg with a range of 24 to 820 mg/kg.

Results of a more recent investigation demonstrated that a wide variation existed in both copper and zinc content of individual oysters harvested from a particular site. Statistical analysis of the data indicated that both copper and zinc were normally distributed in the population studied, and the coefficient of variation for both elements was found to be essentially the same, indicating that the variability of the occurrence of both copper and zinc in oysters was comparable.

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[1] Contribution No. 48. Gulf Coast Marine Health Sciences Laboratory, P. O. Box 158, Dauphin Island, Alabama, 36528. A part of the National Center for Urban and Industrial Health, Cincinnati, Ohio, 45202

## SHELL ORIENTATION IN RECENT AND FOSSIL OYSTER COMMUNITIES[1]

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Little is known about shell orientation in communities dominated by *Crassostrea virginica* or its precursors. Shell orientation may be analyzed by standard geologic surveying techniques.

These techniques have been applied to intertidal *C. virginica* communities in a tidal flat-channel complex in Charleston County, South Carolina. Computer analysis of orientation of over 1800 individuals, drawn from 11 localities, indicates that the compass orientation of adult shells is not random. Rather, the anteroposterior direction in shells of crowded communities is preferentially aligned with the direction of current flow. Apparently, some factor correlative with the hydrodynamic regime strongly influences shell orientation in these Recent communities.

Compass orientation has also been studied in 3 samples of an *in situ* fossil oyster community from the Coastal Plain of North Carolina. The community is dominated by *C. gigantissima*, the Mid-Tertiary precursor of the Recent Atlantic oyster. The community occurs in a channel setting; anteroposterior orientation parallels the axis of the channel and the inferred direction of current flow.

Vertical orientation (uprightness) has been analyzed in these large and massive fossil oysters; comparable data are now being gathered in the Recent. Additional work on shell orientation might yield data useful in a spectrum of problems, ranging from (1) increasing the viability of relayed Recent oysters, to (2) using shell orientation in fossil oyster communities to infer current patterns in the geologic past.

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[1] Recent work has been supported by a Grant-in-aid of research from The Society of the Sigma Xi.

## FACTORS CAUSING MORTALITIES OF OYSTERS IN LONG ISLAND SOUND: A QUANTITATIVE STUDY

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*Milford, Connecticut*

The causes and seasonality of mortalities of oysters, *Crassostrea virginica*, were determined by regular inspections and sampling of about 30 commercial oyster beds by SCUBA divers for more than 2 years. Predation by starfish, *Asterias forbesi* (mostly in May, June, October, November and early December), and oyster drills, *Euplcura caudata and Urosalpinx cinerea* (May to November), smothering in deposits of silt (mostly in late April and May), overgrowth by *Crepidula* sp. (within a few weeks after setting) and by larger oysters, and damage from dredging operations were responsible for most mortalities. Smothering in the bottom also killed a portion of oysters raised in commercial hatcheries and planted on oyster beds. No mortalities could be attributed to disease or to "winter kill". The percentage of mortality resulting from each cause varied widely from bed to bed. Historically, oyster companies lost over 99% of their zero-year-class seed and over 96% of their 1-year-old seed before it attained market size. Recently, through more progressive management, mortalities resulting from predation have been sharply reduced, and spring silting losses have been avoided by early transplanting. As a result, yields from high count seed are expected to increase, in some instances, from 1 for 1 to from 10 to 20 for 1. The prospects are bright for even greater yields through improved techniques in oyster culture.

## QUANTITATIVE STUDIES OF FEEDING IN THE OYSTER, *CRASSOSTREA VIRGINICA*[1]

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During June — August 1966 and 1967 natural phytoplankton from Delaware Bay littoral waters was used for quantitative studies of feeding by laboratory-reared oyster larvae. The larvae used in various experimental series ranged from straight-hinge to eyed stages.

Generally, oyster larvae were found to selectively remove phytoplankters 1 — 30 $\mu$ in size. The feeding pattern changed in relation to larval stage. While size of food organisms apparently influenced selection, there appeared to be a qualitative selection with respect to phytoplankters of comparable size. This may be summarized as follows.

Straight-hinge oyster larvae selected phytoplankters 1 — 10 $\mu$ in size including small green flagellates, *Chromulina* sp. and *Chlamydomonas* sp. Early and late umbo larvae extended the size range to 18$\mu$ and in addition to the above selected *Navicula* sp., *Prorocentrum triangulatum, Gymnodinium punctatum, Euglena* sp. and *Amphidinium fusiforme*. Eyed larvae select food

species up to 30 $\mu$, additionally including *Prorocentrum micans, Peridiniopsis rotunda* and *Exuviella compressa.*

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[1] Supported under PL 88-309 contract 3-3-R-3 with the U.S. Bureau of Commercial Fisheries.

## HISTOPATHOLOGICAL EFFECTS OF IONIZING RADIATION ON THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*

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Oysters were irradiated at the following levels: 0, 1,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000 and 400,000 rads using gamma radiation from a cobalt source. Mortalities began to appear 4 days after irradiation in the 200 and 400 Krad groups and, with the exception of one animal, all oysters in these 2 groups were dead after 11 days. One animal in the 200 Krad group lived for 30 days, at which time it was fixed and sectioned. There were 3 deaths in the 100 Krad group and one each in the 1, 10 and 20 Krad groups.

Grossly, the irradiated oysters began to appear watery by the 16th day and, depending on the dose, this condition persisted or became more evident in time. Animals in the highest dosage groups had darkened inner surfaces (subnacreous layer) of their shells. Pale digestive glands were noted in the higher groups throughout the experiment. Control oysters and those irradiated at 1, 5 and 10 Krads became "fatter" as the experiment progressed, while those at the higher levels remained watery. Abscesses were observed in the muscle, near the heart and in the mantle area in three different oysters irradiated at 100 Krads. No abscesses were observed in any other group.

Histologically, the digestive epithelial cells (stomach, gut, etc.) were first affected. Fragmentation, karyolysis, hemocytic infiltration and sloughing characterized these changes. All tissues are apparently affected and changes in these are also described.

## MSX-PREVALENCE IN VARIOUS STOCKS OF LABORATORY-REARED OYSTER SPAT[1]

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*N. J. Agricultural Experiment Station*
*Rutgers University, New Jersey*

*Minchinia nelsoni* (MSX) prevalence and intensity were determined in various 1966 and 1967 year class laboratory-reared stocks of oyster spat *(Crassostrea virginica).* Frontal sections made every 200 $\mu$ through the gills and palps were examined microscopically for the presence of the disease organism.

In July and August 1967, after one year's exposure to disease enzootic waters, 1966 year class spat from presumed-resistant parents had 0 — 40% prevalences of *M. nelsoni,* while spat from presumed-susceptible parents had 70 — 90% prevalences.

Three presumed-resistant and 4 presumed-susceptible stocks of 1967 year class laboratory-reared spat were exposed to disease enzootic waters in September 1967 and examined for MSX-prevalence and intensity during January and February 1968. Fifty-nine percent of all resistant and 56% of all susceptible spat were infected. A linear relationship between MSX-prevalence and body weight was found: almost none of the smallest and 100% of the largest spat were infected. Infections in susceptible stocks were significantly ($P<.05$) more intense than in resistant stocks, and infection intensity was not related to size of spat.

The small size of these spat and the high prevalence levels indicate a vast number of infective particles in the water during the infection period. All large oysters were almost certainly exposed to infection at this time. As previously reported for large oysters MSX infections in spat apparently start from a single focal point in gill epithelium, regardless of the number of infective particles present in the water.

Following first exposure to MSX, striking differences in prevalence levels develop among susceptible and resistant stocks.

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[1] Supported under PL 88-309 contract 3-3-R-3 with the U. S. Bureau of Commercial Fisheries.

## A SYSTEMS APPROACH TO OYSTER PROPAGATION OFF THE BOTTOM — OPTIMIZATION OF YIELD ESTIMATES SUBJECT TO THE CONSTRAINT OF PUMPING COST ESTIMATES

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Curtis A. Johnson and
J. W. Zahradnik

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*Amherst, Massachusetts*

Through the use of a digital computer, a family

of curves was developed to estimate the economic break even point in system pumping costs as a function of market price and various oyster *(Crassostrea virginica)* system characteristics. These calculated estimates were based on data available in the literature pertaining to oyster age, size and water requirements. A relationship between oyster size and water requirements was derived for a closed environmental control system designed to utilize the siphon effect of the system's discharge waters. The implications of this analysis on the direction of future research were discussed.

## POST-EMBRYONIC DEVELOPMENT OF LABORATORY-REARED SPOT SHRIMP

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Kenneth K. Chew

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*University of Washington*
*Seattle, Washington*

Spot shrimp *(Pandalus platyceros)* were reared in the laboratory from eggs stripped from females caught in Hood Canal, Washington. Eggs were incubated in tanks in a recirculating salt-water system. Hatching was dependent on temperature. Larvae were cultured in beakers in a water-bath and were fed newly hatched brine shrimp. Five stages were observed before development of the post-larval stage. Larvae were kept at 10.6°, 11.7° and 12.8°C. For the early stages, survival was best at low temperatures but later stages had better survival at higher temperatures.

Coon stripe *(Pandalus danae)* larvae were also raised from egg through post-larvae and survived best at higher temperatures.

## GREENING, COPPER UPTAKE AND CONDITION IN OYSTERS IN RELATION TO A STEAM ELECTRIC GENERATING STATION

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*Natural Resources Institute*
*University of Maryland*

The Chalk Point coal-fired electric generating station uses Patuxent River water for condenser cooling. This non-consumptive use is 500,000 gal/min at full pumping rate with a temperature elevation over the condensers of 7°C.

A study of the effects of heated effluent on oysters *(Crassostrea virginica)* has been conducted since 1963. Trayed and natural bar oysters near the station's outfall displayed green color soon after the plant initiated operation. The condition spread from the effluent region to stations farther removed and increased in intensity with time. Chemical analysis of dried oyster samples indicated that the green color was caused by copper uptake. Stations located near the effluent displayed the highest copper levels; correspondingly lower copper levels were encountered with increasing distance from the plant effluent. Oyster copper concentrations at each station displayed seasonal fluctuation which was inversely related to oyster condition factors. The inverse relationship was most intense with high copper levels. Oysters accumulated additional copper for at least 2 years in the apparently modified environment. Additional experiments should define the role of power plant condenser copper loss, thermal loading, plant biocides and their interactions in oyster heavy metal accumulation.

## DISTRIBUTION OF SOME MICROPARASITES IN OYSTERS FROM CHESAPEAKE BAY, 1963-1968

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Oysters *(Crassostrea virginica)* from beds located in the Maryland section of Chesapeake Bay were sampled regularly from 1963 to the present and only occasionally before this. Fresh squash examinations of oyster tissues from these samples have given some indication of the distribution and prevalence of some oyster microparasites.

Over the 6 year study period an incursion of *Minchinia nelsoni* was observed further into Chesapeake Bay to as far north as Eastern Bay on the Eastern shore and Governor's Run on the Western Shore from its previous highest latitudes, the Honga River and the Potomac River respectively. Highest prevalences of *M. nelsoni* were recorded in the Manokin River, Tangier Sound, and the Honga River during 1965. Extensive oyster mortalities were observed in these areas at the same time but were relatively light before then. In the fall of 1966 there began what appeared to be a southward retreat of the organism and by the spring of 1968 the organism was found only in Holland Straits and the Potomac River and then in prevalences of less than 10%. Oyster mortalities were few after the peak period in 1965. The northward intrusion followed by the southward regression in distribution of the parasite appeared to follow the fluctuation in salinity

that existed in the Bay during the period of study. No implication is made here that salinity is the only factor that controls the distribution of *M. nelsoni*.

*Dermocystidium marinum* (=*Labyrinthomixa marina*) was found in oysters from several sample areas. Areas of highest prevalence were the St. Mary's River and the Tar Bay area of Punch Island. These areas are generally considered to be seed areas and are heavily populated with oyster beds.

Spore stages of the gregarine, *Nematopsis ostrearum*, were found to be particularly prevalent during the summer of 1967 in oysters from Punch Island, Holland Straits, Big Annemessex River, and the Honga River but were found in oysters from several other of the study areas. As expected, *Hexamita* sp. trophozoites were observed particularly in the winter months in oysters from all sampling stations.

Larval stages of the trematode, *Bucephalus* sp., were found but only rarely in oysters from the Honga River, Tangier Sound, and the Manokin River.

## SEDIMENT — BIOTA RELATIONSHIPS IN A PRINCE EDWARD ISLAND ESTUARY

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Bideford River, a Prince Edward Island estuary, has been the site of a combined investigation of sediments and benthic biota. It was found that the distribution of many species and benthic communities was closely related to the physical properties of sediments and that the trophic relationships of benthic fauna were correlated with the organic content of their substrata. Coring studies have given evidence of temporal changes in sediments and in their associated molluscan fauna. Laboratory and field experiments have shown that silt deposition may be accelerated by some fouling organisms, particularly colonial diatoms. Experiments have also shown that some members of the benthic fauna are capable of reducing natural sediment accumulation on the bottom, while other benthos have the opposite effect.

## A SYSTEMS APPROACH TO OYSTER PROPAGATION OFF THE BOTTOM — UNIT REACTOR CONCEPTS APPLIED TO EXPERIMENTAL DETERMINATION OF WATER REQUIREMENTS FOR JUVENILE OYSTERS — A PROGRESS REPORT

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A closed system, comprised of 6 separate 1 $ft^3$ metal-free chambers, with Wareham River water being pumped at the rate of 1, 3, and 5 lb/min in each of two replicate chambers, was used to study the effect of flow rate on juvenile oyster (*Crassostrea virginica*) growth. Natural river conditions were used as controls.

The flow rate was related to the population in the chambers, the lower flows were associated with the larger numbers of oysters. Population increases in all the chambers were greater than those occurring in the river controls, due in part, to the absence of predators in the experimental systems.

Cumulative growth rates during the early part of the growth period increased with increasing flow rates and were higher than those of the river controls. However, by the end of the season, the highest cumulative growth rates were achieved by the river controls.

The flow rates studied were based on estimated water requirements using a derived relationship between oyster size and water requirements. The results from the early part of the season indicated the higher flow requirements estimated were in reasonable agreement with actual oyster demands.

# NSA PACIFIC COAST SECTION

## MODIFICATION OF REFRIGERATED SEAWATER FOR THE PRESERVATION OF FISHERY PRODUCTS

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Experimental studies were conducted at the laboratory to determine if carbon dioxide gas, dissolved in refrigerated seawater, can effectively be used as a microbial inhibitor to extend the storage life of fish. Results from 5 experiments show that the storage life and quality of black rockfish *(Sebastodes melanops)* and Pacific pink shrimp *(Pandalus jordani)* are significantly improved when stored in carbon dioxide-treated refrigerated seawater.

## CLAM RESEARCH IN BRITISH COLUMBIA

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Only 4 species of clams are used commercially in British Columbia: butter clam, *Saxidomus giganteus*; little neck clam, *Protothaca staminea*; Manila clam, *Tapes japonica* and razor clam, *Siliqua patula*. The 4 species also comprise almost the entire sports fishery catch.

Commercial landings of the 4 species increased steadily from about 1 million lbs at the turn of the century to an all time peak of about 8 million lbs in 1938. Since then landings have fluctuated widely with the last major peak slightly under 7 million lbs in 1952. Since 1952 landings have decreased markedly and recently have been under 3 million lbs. The landed value has never been high and has fluctuated with the landings: recently it has been around $150,000.

The recent decline in clam landings is due partly to closure of more than half the coast line because of paralytic shellfish poison and also to important social changes in the province. It has become increasingly difficult to find qualified clam diggers since better paying jobs are available. An urgent need in the industry is the development of a mechanical shellfish harvester which will make clam digging financially more attractive to fishermen. Experiments with a small hydraulic clam rake indicate it may be the best type of mechanical digger for British Columbia where the clam flats are usually small, very rocky, widely separated and rather isolated.

The present clam research program comprises 2 main fields of study: ecological and laboratory. Ecological studies on hard clams include a general survey of clam flats throughout the province to assess populations, measure recruitment and determine growth and mortality rates. At present a project is underway to accurately measure the growth and mortality rates of butter clams on selected beaches separated at intervals of roughly 1 degree of latitude throughout the province. Other field work involves a detailed ecological study of a major clam flat in the southern part of the province. This work includes a study of fluctuations in the clam population during the last 30 years, distribution of clams in relation to water currents and particle size, growth and mortailty rates, recruitment, and predation by flounders and scoter ducks. Studies on razor clams have been confined to estimating the adult populations and measuring recruitment on the major clam beach at Masset in the Queen Charlotte Islands. At present good commercial quantities of clams exist on these beaches but landings are very low because of the difficulty in obtaining diggers.

Laboratory studies have been devoted mostly to studying the larval development of butter and little neck and the factors affecting this development. Difficulty has been experienced in spawning butter clams but a method has been devised to obtain larvae by stripping adult clams.

## PROPOSED PILOT OYSTER HATCHERY IN OREGON

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The production of seed oysters in laboratories has been demonstrated by many workers. What is needed is a pilot oyster seed hatchery to demonstrate the economic feasibility of seed production and to investigate whether techniques have been developed to the point where oyster seed hatcheries can be profit making businesses.

Some of the advantages of hatchery seed are production stability, seed oysters set on cultch tailored for various culture methods, and seed for breeding experiments.

Methods of algal food production, oyster spawning, rearing and setting are discussed in relation to oyster hatcheries. Seed planting and cultural methods for different cultch materials may change some present cultural practices.

## AN UNUSUAL HISTOPATHOLOGICAL CONDITION IN *OSTREA LURIDA* FROM YAQUINA BAY, OREGON

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During the early winter months unusual cells of unknown origin appear in the Leydig tissue of some native oysters *(Ostrea lurida)* from Yaquina Bay, Oregon coinciding with mortalities in this area. These cells are about the size of large hemocytes and possess granular cytoplasm and large, dense clumped nuclei. The pathological changes are as follows: First, the nuclei of epithelial cells become karyolytic, that is, rounded and lightly stained. Then, during the midwinter period, the unusual cells disappear, but the changes in the epithelium become marked. Karyolysis continues with a breakdown of the walls between the epithelial cells in the stomach and those in the gut, resulting in a disorganized appearance. Finally, large numbers of inclusion cells appear in the stomach and gut lumina. These pathological conditions are not fully understood, nor is it known whether the early-appearing cells are microorganisms or abnormal hemocytes.

## RESULTS OF RECENT SURVEYS ON SUBTIDAL GEODUCK POPULATIONS IN WASHINGTON STATE

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A continuing survey of the subtidal hardshell clam populations of Puget Sound was initiated in 1967 by the Washington State Department of Fisheries[1]. A phase of this study initiated in May 1968 includes surveys to determine distribution and abundance of the geoduck, *Panope generosa.* Previous random observations by Department of Fisheries and U. S. Navy divers disclosed some abundance of subtidal geoducks at several locations in Central Puget Sound and Hood Canal. Surveys by SCUBA divers are now being conducted during warm months of the year when geoduck siphons are most visible above the substrate and are available to counting by observers.

Objectives are to develop practical methods for enumerating geoducks, determine general abundance and bounds of geoduck beds, establish the early life history and recruitment rates in different ecological areas, obtain data on geoduck ecology and growth, and develop estimates of harvest schedules. Initial studies have been completed on typical beds and a sampling technique was developed, based on the expectation that for any given transect, 58% of geoducks will have their siphons exposed and clearly visible at any given time. Survey sampling techniques also have been developed and validated on known geoduck areas through repeated re-surveys.

General abundance surveys are being conducted throughout Puget Sound to delineate areas where geoducks are present and absent. Thus far extensive beds have been located in Hood Canal, Port Townsend harbor, Agate Passage, Port Orchard, Squaxin and McNeil Island areas, Liberty Bay, Useless Bay, Port Madison, Port Gamble and several other areas. Abundance up to 1.0 geoducks /ft$^2$ have been observed; average size has been 3 lbs. Gonad samples are being collected to determine spawning season. Limited efforts have been made to naturally spawn and culture geoduck larvae. Successful spawnings of males and females have been achieved; the resulting larvae have been reared to the trochophore stage. The program will continue until the needed management information is gathered.

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[1] Federal matching funds under P. L. 88-309, U.S. Bureau of Commercial Fisheries.

## BEHAVIOR OF OYSTERS OF DIFFERENT GEOGRAPHICAL AREAS UNDER COMPARATIVELY LOW TEMPERATURES

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Large groups of adult oysters *(Crassostrea virginica)* were collected in Long Island Sound, New Jersey, Virginia, North Carolina, and Florida, and kept suspended in Milford Harbor, Connecticut for a period of about 3 months. In the middle of January all groups were brought to the laboratory to be conditioned at temperatures of 12°, 15° and 18°C for the observation of their gonad development and the degrees of ripeness. Some of the oysters that originated in Long Island Sound were able to ripen at even 12°C, while New Jersey and more southern groups of oysters were unable to carry on active gametogenesis at this temperature. At 15°C the Long Island Sound oysters gave approximately 60% spawners after a conditioning period of 45 days, while New Jersey oysters, even after 72 days of conditioning, showed only 20% of individuals with recognizable but, nevertheless, very unripe gonads. The more southern groups were even less developed. The radical difference between

Long Island Sound and other groups was again demonstrated at 18°C. Several examples are cited when large shipments of oysters from more southern waters were planted by the industry in Long Island Sound but were unable to release their spawn even after being in the new environment for about 3 years. It is concluded that there are distinct races, or populations, of *C. virginica* that require different temperature regimes for completion of gametogenesis and spawning, and that the old assumption that the breeding temperature for this oyster is the same over all parts of its range is incorrect.

## EXPLORATORY SCALLOP *(PATINOPECTEN CAURINUS)* SURVEYS OFF THE OREGON AND WASHINGTON COAST

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Since 1963 exploratory scallop surveys have been conducted off the Oregon and Washington coast between Cape Argo, Oregon and Cape Alava, Washington and in Puget Sound, Washington. The primary fishing gear was an 8-ft, New Bedford-type, scallop dredge. The greatest concentrations of scallops off the Oregon coast occurred between Tillamook Head and Cape Falcon, Oregon, in 45 to 55 fm where catch rates reached 753 scallops (5 bu)/1/2-hr tow. On the Washington coast the highest availability occurred off Breakers, Washington, where catches reached 257 scallops/1/2-hr tow. Availability of scallops in coastal waters fluctuated widely from year to year. The average height of scallops in coastal waters ranged from 4.2 to 4.6 in, whereas in Puget Sound they averaged 5.4 in. Meat yield of scallops ranged from 7 to 10%. There is a strong indication that heavy infestations by the shell-boring worm, *Polydora* sp., may be the cause of reduced growth and heightened mortality in offshore stocks.

## LARVAL REARING OF BIVALVE MOLLUSKS

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Larvae of the following Pacific coast bivalves were reared in the laboratory to metamorphosis: Pacific and Kumamoto oysters *(Crassostrea gigas)*, the native oyster *(Ostrea lurida)*, the European oyster *(O. edulis)*, the cockle clam *(Clinocardium nuttali)*, the butter clam *(Saxidomus giganteus)*, the gaper clam *(Tresus capax)* and the razor clam *(Siliqua patula)*.

Adult spawning was induced by thermal and chemical stimulation. Preliminary studies were conducted to determine optimum temperatures, salinities and larval densities for laboratory rearing. Larvae were fed mixed and unialgal diets of *Monochrysis lutheri* and *Isochrysis galbana*.

Various synthetic clutch materials were tested for setting oyster larvae. Future studies are being planned to monitor post-larval growth in the field.

## STUDIES OF DIET AND DIET CONCENTRATION EFFECTS ON DUNGENESS CRAB ZOEAE

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Studies were conducted to determine the effects of selected diet types and selected food concentrations on dungeness crab *(Cancer magister)* zoeae. Five single organism diets and 6 combination diets were tried. Diet organisms used were large Utah brine shrimp *(Artemia salina)*, small Utah brine shrimp, barnacle nauplii *(Balanus glandula)*, mussel larvae *(Mytilus edulis)* and a prepared diet of ground cockle *(Clinocardium nutalli)*. A diet of small Utah brine shrimp resulted in 100% zoeal survival after 3 weeks of rearing and any combination diet containing this organism resulted in high zoeal survival (64 — 91%). Small Utah brine shrimp were thus considered a potential laboratory diet for dungeness crab zoeae.

Biologists have reported inconsistent results for other animals reared with Utah brine shrimp. Therefore, Utah and San Francisco brine shrimp were tested at uniform food concentrations of 1/ml of rearing water as food for zoeae. San Francisco brine shrimp were also tested at concentrations of 10 and 20/ml. The best survival and growth occurred when zoeae were fed S. F. brine shrimp at a concentrtion of 10 per ml. Survival of zoeae fed S. F. brine shrimp was better (46%) than survival of zoeae fed Utah brine shrimp (7%) at identical food concentrations (1 shrimp/ml). Zoeae fed Utah brine shrimp did not grow as well as zoeae fed S. F. brine shrimp.

The effect or presence of an ecto-commensal protozoan of the genus *Vortecella* in all rearing flasks for the diet concentration study can not be appraised.

## PRELIMINARY STUDIES ON UTILIZATION OF PACIFIC SCALLOPS

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Preliminary studies were conducted on developing a mechanized procedure for shucking Pacific scallops (*Patinopecten caurinus*), which were taken in the Strait of Georgia. A method for shucking scallops and removing the viscera from the muscle using a conveyor belt and the application of heat and vacuum was demonstrated in our laboratory experiments. Some of the concepts demonstrated were incorporated in a production-scale model, which was constructed and installed aboard a scallop processing vessel. Movies taken of the trial run aboard the vessel were shown.

## PROGRESS REPORT ON THE USE OF CARBON DIOXIDE IN PROCESSING DUNGENESS CRAB (*CANCER MAGISTER*) MEAT

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Treating crab meat with carbon dioxide at high pressure causes a temporary lowering of the specific gravity of the meat. This, and the fact that the specific gravity of the crab shell and tendon is not changed by the carbon dioxide treatment, are the basis of research designed toward improving the separation of the meat from the shell and the tendon. Carbon dioxide at pressures of 250-300 p.s.i. and a rapid release of the pressure are important factors affecting the change in specific gravity. The lower specific gravity permits flotation-separation of the crab meat in a weak brine; however, separation must be accomplished within a minute after the pressure release.

## GROWTH AND SURVIVAL OF KOREAN OYSTER SEED IN WATERS OF WASHINGTON STATE

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During the spring of 1966, the Washington State Department of Fisheries received 7 cases of oyster seed (assumed to be *Crassostrea gigas*) from the Pusan area of Korea. This was a test shipment arranged by Pacific Coast Oyster Growers Association to determine suitability of Korean seed for the Washington Oyster Industry.

Initially the seed was held in quarantine at the Point Whitney Laboratory pending histological examination by the Oxford Laboratory of the U. S. Bureau of Commercial Fisheries. In May, 1966 this oyster seed from Korea was placed out at 2 areas for observations of growth and survival. For control purposes, identical plantings were made of 1966 Japanese oyster seed from Miyagi Prefecture.

The experimental plantings were made in 1 area of known good oyster growth (Keyport), and 1 area of known poor oyster growth (Point Whitney). For the first year little difference in growth or survival was noted at either Keyport or Point Whitney between the Korean and Miyagi oysters. However, starting in the second summer, the Miyagi oysters began growing consistently at about twice the rate of the oyster seed from Korea. Survival of oysters in the 2 test areas seems comparable between the Korean and Miyagi oysters.

Visual examination of the shells of the 2 strains indicates some differences. The Korean oysters seem to be somewhat more fluted than the Miyagi oyster. At this point we assume that the oyster seed from Korea is a different race of *Crassostrea gigas* than the oysters from Miyagi Prefecture of Japan.

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# THE CHALLENGE OF THE ESTUARY [1]

*Russell E. Train*
THE CONSERVATION FOUNDATION
WASHINGTON, D. C.

The Conservation Foundation is concerned about the future of our estuaries — one of our most misunderstood and under-rated national resources. A major concern of The Conservation Foundation is that the nation treat its coastal resources with reason, that we respect their multiple values — as habitat for fish and wildlife, as critically needed food-producing areas, as places that provide unusual opportunities for vitally important scientific research and for education, as areas of broad recreational significance, and finally, but not necessarily least, as places whose beauty and diversity enrich our human surroundings.

Over the past year the Foundation has begun a series of regional demonstration projects designed to encourage civic interest in protecting these values, and to indicate ways in which protection may be accomplished. There seems no question but that the estuary constitutes the single most important biological, ecological and economic element of the shoreline complex, and yet is at the same time the least understood and the most threatened. The estuaries of our nation face a difficult future. I would like, therefore, to discuss why . . . and what we can and must do to conserve the estuarine environment.

In the last few years we have made some progress in this regard. It was not long ago that the states and federal government followed what was virtually a laissezfaire attitude toward the estuary. Now the federal government and a number of states have begun to take effective measures to protect this resource from such specific threats as pollution, dredging and filling. Led by Massachusetts, most of the New England coastal states have recently passed statutes prohibiting dredging and filling without state approval.

On the Pacific coast, California has taken measures to protect its primary estuary, San Francisco Bay, after its citizens suddenly awoke to the fact that the Bay had diminished from 700 to 400 thousand acres in the last century, that its wetlands had shrunk from 300 to 75 square miles. In 1965 the state legislature created the San Francisco Bay Conservation and Development Commission, and gave it the authority to issue or deny dredging or filling permits. The Commission will soon recommend an overall conservation and development plan for the Bay — the kind of comprehensive approach desperately needed in *all* shoreline areas.

Another example of state action has been set by Florida. After long neglect of its estuaries, the state last year passed an act designed to prohibit the destruction of state-owned submerged and tidal land of biological and ecological value.

The federal government has also taken important steps. The Federal Water Pollution Control Administration's comprehensive estuarine study has already had significant local and regional results through its series of public meetings. Last year, the Interior Department and the Corps of Engineers reached an agreement designed to protect estuaries from damaging dredging and filling in navigable waters. Finally, early in 1968 the House of Representatives passed H.R. 25, a modest bill authorizing the Secretary of the Interior to take inventory of the nation's estuaries and to enter into agreements with states or local governments to manage and protect these areas.

These positive actions are evidence that we are, at the least, no longer ignoring the value of the estuary. They do not, however, assure proper protection for those values tomorrow. One statistic tells why. In thirty years 300 million or more people will live in the United States, and more than half of them will live within 50 miles of the Great Lakes or ocean coasts. As a result, future demands on the estuary will be almost overwhelming in their magnitude.

We will certainly hear a lot more about thermal pollution. By the year 2000, the demand for

[1] Remarks of Judge Russel E. Train, President of the Conservation Foundation, before a meeting of the 60th Joint Annual Convention of the Oyster Institute of North America and the National Shellfisheries Association at Arlington, Virginia on 15 July 1968. Judge Train was recently named Under Secretary of the Interior.

electricity will multiply seven times. Electric companies will want all the facilities and power sources they can muster. A host of new generating plants along our coasts will be needed to supply power to the interstate strip cities of the future. It is estimated that by 1980 the amount of electricity generated by nuclear fuel will be more than ten times the present level. But whether conventional or nuclear, steam generating plants will require immense amounts of cooling water. The thermal effects can be significant from either kind, and while these effects may benefit the cold waters of Maine or be quickly dissipated in the deep waters of some Great Lakes, in the relatively shallow, confined waters of most estuaries, the unnaturally warm water may have serious effects on marine life.

At this moment, the Atomic Energy Commission, which licenses nuclear generating plants, does not take into account the thermal effects of a proposed plant. The agency may be understaffed as it is, and might be severely overworked if charged with this responsibility. But someone must undertake the responsibility. When an agency possesses and exercises the power to license, I believe it likewise possesses an obligation to exercise that power in a fully responsible manner. The present practice of ignoring thermal pollution effects in the licensing of nuclear power plants is contrary to the public welfare.

Conventional steam plants are not licensed by any Federal agency. The Federal Power Commission has no jurisdiction over such operations, although it has thoroughly considered fish and wildlife in its licensing of hydroelectric plants. One possible solution, proposed by one Congressional bill, is for the Secretary of the Interior to assess and approve thermal pollution aspects of all electric generating plants.

To illustrate some of the dangers to estuarine habitat posed by the demand for electricity we can look at a conventional generating plant planned just north of Galveston and Trinity Bays. I might add that oyster fishermen in that area are already unhappily familiar with the shell dredging that threatens to destroy the Galveston Bay oyster reefs. But that is another story. In the case of the proposed power plant the "traditional" problems of dredging, spoil deposits, turbidity and changes of current will, of course, be created. And because it will obtain cooling water from upper Galveston Bay through an intake canal, and will discharge the water into upper Trinity Bay, the plant will, by the mere transfer, introduce polluted water into a relatively unpolluted and less saline area.

But the thermal effects of the plant are significant also. The water leaving the plant will be between 7 to 12 degrees warmer than at the point of intake. Approved state water quality standards require that the temperature within a "reasonable mixing zone" be heated no more than 1½° F. The extent of this zone, and the definition of "reasonable" have not been clarified.

So this business of supplying sufficient electric power to our cities is requiring increasingly tough choices on the part of our public officials. We can help them by offering alternative plant locations, and by offering what we believe are reasonable policy definitions, reasonable in terms of environmental quality.

On another environmental front, our nation of 200 million, going on 300 million, seems to want bigger ships, as does the rest of the world. With bigger ships come other problems for the estuaries.

The Suez Canal has been closed for over a year, with far fewer economic consequences than resulted from the 1956 shutdown. The reason is that many ships were already too large for the canal. Now oil tankers of 500,000 ton capacity and more are being planned. The number of ships will also increase. Average daily traffic through the Panama Canal, for example, has more than doubled since 1950. Therefore it seems we must have deeper channels and larger ports, with all the destruction these developments imply. Likewise we must prepare ourselves for oil-pollution disasters that could dwarf the wreck of the Torry Canyon in the English Channel — when a mere 123,000 ton vessel spewed out 30 million gallons of oil.

Though the likelihood of extensive oil damage certainly calls for strong national and international oil-pollution control measures, there are no real guarantees against the disasters. Should we then think about limiting vessel size? And what about the ports and channels? How well do our present local, state and federal planning mechanisms operate to discourage them when the advantages are purely local and the disadvantages, in terms of lost resources, are national?

Then there is the problem of solid wastes. Three hundred million Americans with greater material wealth per person will mean more solid waste for disposal.

Let's look for a moment at figures for the New York metropolitan region. That region is expected to increase from 19 to 30 million people by the end of the century — a 60% increase. Solid waste — half of it in paper products — already amounted to 17 million tons in 1965. The Regional Plan Association of New York estimates that the solid waste may more than triple by the year 2000. What are we going to do with all of it?

In the past we have dumped waste in marshes and estuaries. This has resulted in major losses of water-fowl production, migration and winter-

ing habitat, spawning and nursery habitat for fish and shellfish. Some dumps along our coastal wetlands and other shorelines contain waste products that leach into adjacent waters killing aquatic organisms or making them unfit for human consumption. This method of disposal has been identified by the Department of the Interior's office of estuarine studies as one of the greatest destroyers of estuarine values.

In the San Francisco metropolitan region alone, the Association of Bay Area Governments has reported that some 3 million tons of refuse a year are dumped by 83 collection agencies at 77 sites. Of these 77 sites, no fewer than two-thirds are along the shoreline of San Francisco Bay.

In metropolitan Washington a solid waste disposal study prepared last fall proposed five new major land-fill sites for the region. Significantly, of the five, four are in or along the Potomac River estuary.

New York City last year learned how enforcement of the city's air pollution control ordinance threatened to destroy a beautiful wetland area along an outlying city park in the Split Rock section of Pelham Bay. The incident illustrates the interactions among the three principal forms of environmental pollution — of air, water, and land.

When New York's ordinance went into effect, many apartment house owners balked at installing new control devices in their incinerators. Instead, they said, they would put all their trash in garbage cans and the city's sanitation trucks could haul it away. City sanitation officials objected; they said they were running out of waste-disposal sites. The trash from the apartment houses, they said, meant they would have to dump it at Pelham Bay.

Conservationists pointed out that the estuary was a nursery and sanctuary for wildlife and fish — and one of the last reaches of unspoiled shoreline in the region. Turning it into a dump would not only destroy this irreplaceable resource, they said, but also could pollute Long Island Sound.

So, one spring evening last year, New York City planning, park and sanitation officials, and Mayor John Lindsay gathered on the spot to see for themselves what was at stake. The gratifying result was a decision that Split Rock should be saved as a natural area. The Mayor told the sanitation department to find another site for its garbage dump, and decided that Pelham Bay would be the site of a nature center for school children. I am sure that those involved in the chain reaction at Pelham Bay must have wished that the conflict could have been settled in advance and in a more orderly way.

But this kind of decision will become harder, not easier, for harrassed public officials to make. Alternatives to shoreline and estuarine destruction will be less easily found. In the meantime the appetite of sprawling cities for new, dry land continues — new land that can only come from estuaries and marshes.

Resently the Senate subcommittee on air and water pollution held hearings on solid waste management programs. At that hearing it was clear that solid waste management is being recognized as a problem requiring the work of our best scientific and technological talent. Massive federal aid programs to states and municipalities are already required. The Conservation Foundation suggested to the committee that use of federal funds by states and local governments be contingent upon criteria and procedures for landfill, incinerator sites, and other activities that give full consideration to environmental resources and values, including fish and wildlife, recreation, historic, scientific and aesthetic values.

There is still another challenge to the estuary that we ought to recognize now. What are we going to do when a sizeable proportion of 300 million Americans want to live on, or otherwise enjoy the shoreline? Marinas, motels and large scale subdivisions along the shoreline are the present result of this desire. They are made possible and profitable because of increased individual leisure, mobility and income.

There seems to be a national trend toward the possession of second homes. It is a trend being acutely felt along our coast line, whether in Puget Sound, the Florida west coast, or the coast of Maine. It threatens to make prospects less likely for sufficient public shoreline to meet future recreational needs.

And of course significant biological values may be lost as a result. A recent situation in Florida, involving a trailer court extension illustrates this point. The case challenges the right of the Corps of Engineers to deny a dredging and filling permit on the grounds of its damage to fish and wildlife values. It is the case of *Zabel & Russell v. Tabb.*

For ten years the owners of a trailer court sought to expand their operation into Boca Ciega Bay, near Tampa. They planned to do so by filling in about ten acres of submerged land that they had bought from the state. Finally, after state court order, they obtained local permission, then state permission. But last year the Corps of Engineers refused a federal permit, citing the significant estuarine resources that would be destroyed. The owners contested the Corps' right to deny the permit on this ground. They went to court and won the first round. But the case is still far from a final decision.

It is clear that everyone who wishes to cannot have his little lot on the estuary because our

shoreline is finite — it will not increase with the population. And even if far greater development is biologically reasonable along our shores, there are significant aesthetic values to protect. The natural beauty of the unspoiled shoreline provides an important dimension to the life of the resident as well as a new sensory experience to the visitor. These are not simple values. They are not to be lightly disregarded.

The aesthetic factor is a powerful force, but we have as yet no way to measure its importance against quantifiable economic values. It is interesting to note that the judge recognized this fact in the Boca Ciega trailer court case, though he decided the preliminary motion in favor of the developers. In spite of his decision, he said he did not like what was happening to the Bay.

So this is yet another challenge to the estuary. How are we to buttress its aesthetic values against development; how are we to express effectively the feeling that a certain place simply ought to be left alone? And how are we to win this battle once and not have to worry about fighting it again and again in the same place?

I have mentioned only a few threats to the estuary. There are, as you are well aware, many more. They represent all of our most severe environmental challenges. I believe there are ways to meet these challenges to protect the estuary and all its values.

Many of the failures of conservation in the past have resulted from the failure of various interests to work together to accomplish common goals. I suspect that in many communities the most strategic, most effective tool for the convervation of the estuarine resource may be the creation of regional coalitions — coalitions of groups sharing a common concern for the estuary. I say this for three reasons:

(1) The estuary is usually an identifiable manageable ecological unit, although there are also larger and more complex systems such as Chesapeake Bay and its tributary estuaries.

(2) Along with federal planning a large measure of local and regional planning is required if our estuaries are to be protected — for example, through appropriate zoning, subdivision restrictions and real estate tax policies.

(3) Within the estuarine environment, proper conservation and planning cannot depend wholly upon public programs and action. The private sector — citizen organizations and commercial interests — must be involved. And that includes people like you.

Let me cite some examples of the kind of regional conservation activity I think is required. One of many examples of regional citizen associations throughout the country is the Chesapeake Bay Foundation, of which I am a new trustee. Its primary goal is to stimulate private and governmental activity to promote rational use of the Chesapeake Bay resource.

Another is the Potomac Basin Center — an organization dedicated "to citizen involvement in decisions determining the destiny of the Potomac River Basin". The Center, which is presently a part of The Conservation Foundation, is sponsoring a series of workshops on land-use controls together with 20 or more nongovernmental groups — including the Farm Bureau and AFL-CIO units, as well as a host of other conservation organizations. I might just add that a past president of the Oyster Institute, Mr. G. I. Rupert Lore, is a member of the Center's board of trustees.

Quite clearly the interest you people have in preserving the estuary is akin to the interest of these citizen organizations. They will need your support as a part of their effort. Yours is an interest that must be represented effectively before local, state and federal public bodies as they attempt to wrestle with the location of a pulp mill, a channel, an electric generating plant, or a sanitary dump. Your long term economic stake in the estuary will buttress, with quantifiable data, other arguments of citizen and conservation groups. I believe the climate is right at local and state levels for this kind of complementary, but coordinated effort.

The record of the Oyster Institute — its historic concern for pollution, dredging and filling, and other threats to the estuary — is superb. And, of course, its conservation activities will continue. But all of us must broaden and quicken our efforts to respond to the complex environmental challenges that we face right now, as well as the increasingly critical challenges in the years ahead.

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# THE POTENTIAL OF THE ESTUARY FOR SHELLFISH PRODUCTION[1]

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## ABSTRACT

*Shellfish production, if dependent upon the food produced in the water in which the shellfish grow, can average no more than about 150 kilograms (meat weight) per hectare per year, a figure which is representative of the yields from large estuarine regions. Intensive cultivation in small areas can produce annual yields from 5,000 to 500,000 kilograms per hectare depending upon the method of cultivation. These high yields result from the concentration into a small area of the organic matter produced over a much larger region. Yields depend upon the concentration of food in the water and the rate at which it is brought to the shellfish by tides or current, of which the latter is more variable and hence more critical in estuaries*

*Raft culture is the most efficent system now in use for shellfish culture, potentially 1,000 times or more effective in presenting food to the animals per unit of area than conventional bottom culture techniques.*

*Since high yields of shellfish depend upon the concentration of food produced over a large area, both intensive and extensive culture cannot be practiced in the same estuary, and yields from a small area of intensive culture cannot be extrapolated to the estuarine environment in general.*

## INTRODUCTION

Although shellfish do not represent a major source of food in the world today, their production in certain restricted estuarine areas is, without much question, greater per unit of area than that of any other form of animal protein on earth. Average yields within large estuarine systems are not overly impressive. Chesapeake Bay produces annually some 15,000 metric tons of oysters and clams (McHugh, 1967), Japan's Inland Sea about 25,000 tons of oysters (Ryther, 1968), and the Wadden Sea of Netherlands, as much as 75,000 tons of mussels (Korringa, 1967) (all figures, meat weight without shell). These numbers are large, but if they are divided by the areas of the respective estuaries, the annual yield per hectare of bottom is in each case roughly 100 kilograms (ca. 100 pounds/acre.)

On the other hand, when specific areas of high shellfish production are considered on the basis of yield per hectare, the statistics are astounding. The best oyster production in the United States and Europe, using conventional bottom culture techniques, are of the order of 5,000 kilograms/hectare/year. The hanging culture method of growing oysters in the Inland Sea of Japan may produce an annual crop of 6,000 kilograms of oyster meat from a single bamboo raft measuring 24 x 18 meters. The same technique used for mussel culture in the Galacian Bays of Spain produces roughly ten times the quantity of mussels per raft (Ryther, 1968). Translating these figures (which are maximum yields, and should not be considered average values) to an areal basis by assuming that there are eight rafts per hectare of intensive cultivation (a conservative estimate) gives figures of nearly 50,000 kilograms of Japanese oysters and 500,000 kilograms of Spanish mussels per hectare per year (meat weights). The latter figure (in English units,

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about 250 tons/acre) is roughly 1,000 times greater than yields from any other form of husbandry or culture in which animals are grown naturally, with no supplemental or artifical feeding. What is the explanation for these tremendous yields of shellfish? It is principally this question that will be addressed in the following discussion.

## ORGANIC PRODUCTIVITY OF ESTUARIES

In considering the primary (photosynthetic) production of organic matter and the environmental conditions which influence and control the process in an estuary, as compared with the open sea, it is clear that the estuary has many advantages. The shallow depths prevent the unicellular algae from sinking or being mixed downward into non-productive, aphotic depths which comprise most of the volume of the open sea. The same feature prevents strong thermal stratification in summer months, which is common in deeper water and which provides an effective barrier to mixing and the vertical transport of essential nutrients into the surface, euphotic layers. Shallow depths insure that the nutrients contained in organic and inorganic particulate matter are not lost to the system through sedimentation, but are readily stirred up and periodically recirculated. Land drainage may provide an additional source of enrichment.

As a result of these favorable environmental conditions, the average estuary is capable of producing organic matter at an annual rate of about three metric tons (dry weight) per hectare, 10-100 times greater than that of a typical offshore area. Large as this figure may seem, it does not, in itself lead to an equally impressive yield of shellfish. Photosynthetically-produced organic matter, in the form of unicellular algae, is consumed by filter-feeding herbivores, such as bivalve molluscs, and converted to animal tissue with an overall efficiency which is variable but probably averages about 10%. On that basis, an annual rate of primary production of three tons per hectare could theoretically lead to the production of 300 kilograms dry weight or roughly 1.5 tons wet weight of shellfish (equivalent to some 500-600 bushels of whole oysters). In actuality, the expected yields of a given shellfish crop would be much less than this, since the organic matter becomes channeled into a vastly complicated and largely unknown "food web" of competing organisms (zooplankton, other benthic species, herbivorous fishes or fish larvae, etc.), most of which cannot be controlled or eliminated from the system. A bottom-dwelling population of oysters or other shellfish would do well to obtain as much as one-tenth of the organic matter produced in the overlying waters, leading to an annual yield of the order of 30 kilograms or less dry weight (150 kilograms wet weight) per hectare (equivalent to about 25 bushels of whole oysters per acre per year). There is no way to calculate or even estimate this figure with any accuracy, but it is probably correct within an order of magnitude.

Yields are therefore not very large if the cultured organisms must depend upon the basic productivity of the same waters in which they grow. For this reason, shellfish culture in enclosed saltwater ponds or embayments is not particularly attractive. True, such enclosures can be fertilized artificially, a practice which theoretically could increase the rate of primary organic production and the resulting yield of food organisms by as much as tenfold.

There are, however, inherent dangers in fertilization, particularly at the level implied above. There can be no guarantee, for example, that the microorganisms which would result from such a treatment would be the same as those which occur naturally, or that they would be suitable food organisms for the species being cultivated. We know far too little, at this stage, of how to control qualitatively, the growth of unicellular algae and how to encourage desirable and discourage unfavorable species. A greater hazard would be that of loading the environment with organic matter beyond its carrying capacity. This term, strictly speaking, refers to the amount of organic matter which, if it all were decomposed, would exhaust the oxygen in the water and turn the system anoxic. While such complete oxidation is unlikely, heavily enriched ecosystems can exceed the carrying capacity sufficiently that respiration may exceed photosynthesis for sufficiently long periods of time to utilize all of the dissolved oxygen, with disastrous results. The fine distinction between enrichment and pollution quickly disappears in such situations.

Finally, there is the cost factor in fertilization. Increasing the primary production by tenfold would require the annual addition of some 7.5 tons per hectare of nitrogen and corresponding amounts of other essential nutrients — a prohibitive cost if furnished in the form of commercial fertilizer. Perhaps the day will come when the controlled use of processed sewage will be possible in such aquacultural ventures, but we first need much more knowledge of both the technological problems involved and their ecological consequences.

How much food is available to a salt water pond which is opened up to the adjacent estuarine environment so that its waters can be renewed regularly by tidal exchange? If the volume of water in the pond is exchanged completely as often as once a day, will this make more food available to a shellfish population living in the

pond? The answer is probably no if the basic productivity of the contiguous waters is roughly the same as that of the pond. At the rate of primary productivity assumed above, and taking the average population of plankton normally found in estuarine waters, a turn-over time or renewal rate of the population of about once a day is implied. Thus a 24-hour flushing rate would bring in new organisms at just about the same rate that they would be produced in a completely enclosed pond. Tidal action may be desirable for other reasons — to remove metabolites, renew nutrients or oxygen, better distribute the food microorganisms, etc. — but it would appear not capable of providing a greater source of food *per se* to the animals living within an impoundment than could be produced in the pond itself.

## THE CONCENTRATION FACTOR

Where, then, lies the food potential of the estuary? What is the mechanism by means of which a production of tens or hundreds of thousands of kilograms of shellfish per hectare is possible? The secret lies in the ability of sessile, filter-feeding animals to ulitize the food produced over a much larger region than that which they occupy and thereby concentrate it in a small area. Growth under these circumstances is a function of both the concentration of food particles in the water and the rate with which the water is brought to the animals. Both of these interdependent factors are equally important, but the former (food concentration) tends to be the more constant in most estuarine situations, seldom varying by more than a factor of tenfold. The flow of water caused by tides, currents, winds or a combination of the three, can vary from an almost completely static situation to speeds of several knots. It is primarily the variability in water movement rather than food concentration that is responsible for the wide range in shellfish production which occurs in different estuaries or in different parts of the same estuary. An example will illustrate this point.

We have already seen that the amount of food produced in an estuary will average about three tons per hectare per year. This is equivalent to (and was derived from) an assumed average production rate of 1 gram (dry weight) of organic matter per square meter per day. Under these conditions, the mean concentration of particulate organic matter suspended in the water will be about 1.0 milligram (dry weight) per liter.

It was estimated above that a bed of oysters on the bottom in a non-flowing system might obtain one-tenth of the organic matter produced above it, or about 0.1 gram/meter$^2$/day. On the other hand, if the same oyster bed is located in an open estuary, is 2 centimeters thick (the depth of the oysters), and if a tidal current of 1 knot (51.5 centimeters/second) is flowing over the bed, each linear meter of oysters will be able to intercept 1,000 grams of organic matter per day from water containing the same concentration of food — ten thousand times more than can be provided in the stagnant situation.

It is impossible to predict, or even to estimate, how efficiently shellfish can remove the particles from water flowing past them, which presumably is a function of both rate of flow and food concentration. There is also no way of knowing from what depth of water above the bottom the oysters can effectively filter the food organisms. There is the further complication that in any linear flow, the animals on the upstream side will have an advantage over those below them and may leave the latter with less food. Finally, it is difficult, if not impossible, to predict how effective vertical turbulence may be in exchanging the water near the bottom, thereby continually bringing a new source of food to the shellfish. This again will vary according to strength of the tide, depth of water, wind action and roughness of the bottom, among other factors.

These are some of the factors which cause shellfish production to vary from one place to another in the same estuary with the same production and concentration of food. As noted, they can be evaluated only roughly and qualitatively, and cannot be used to predict quantitatively the productive potential of a given region. Clearly, hydrologists must join forces with biologists to obtain answers to some of these questions.

## ARTIFICIAL CONCENTRATING TECHNIQUES

We have seen that high levels of shellfish production in an estuary are achieved by concentrating in a restricted area the organic matter which is produced over a much larger region and, further, that the concentration of food depends upon the rate of water movement and the efficiency with which the animals can remove the food from the water as it flows by them. In spite of the fact that bottom culture is the prevailing method of shellfish production in this and many other countries, clearly this is one of the most inefficient ways of utilizing food which is evenly distributed at all depths in an estuary. The Japanese have increased production with the raft culture technique, whereby strings of oysters are suspended evenly throughout the vertical water column, literally from top to bottom. It is this method, now successfully adopted in many other places, which results in the astronomical yields referred to at the beginning of this discussion.

Another simple calculation will illustrate this point.

Above, it was assumed that in a 1 knot tidal current of water containing 1.0 milligram (dry weight) of particulate organic matter per liter, an oyster bed one meter across and 2 centimeters deep would intercept about 1,000 grams of food per day. A hanging raft culture one meter across and 10 meters deep, in the same environment, would intercept nearly 500,000 grams of food. The rafts used for Japanese oyster and Spanish mussel culture average about 20 meters across (i.e., perpendicular to the current axis) and the hanging strings, about 10 meters deep. This 200 meters$^2$ cross section is capable of intercepting nearly 10 million grams of food per day under the conditions stipulated above.

The example is exaggerated to the extent that even a dense culture could not be solid shellfish. On the other hand, if the raft is sufficiently wide in the dimension parallel to the flow of water, the succeeding ranks of suspended shellfish should be able to utilize the food which escapes the front line. Theoretically, it should be possible in this way for a hanging culture to remove virtually all of the food passing through it. That this situation may be approached is suggested by the fact that growth on the upstream side of a raft (i.e., that facing the opening of an embayment or a prevailing current) is considerably greater than at the rear, often giving a decided list to the structure and requiring additional buoyancy at the leading edge.

Another method of increasing shellfish production over that which is possible by conventional bottom culture, and one which is receiving much attention of late, is that of pumping water through a tank, flume, artificial pond, or other type of enclosure. Again it should be possible in such an artificial system to so arrange the shellfish, horizontally and vertically that all of the water passes over and around the animals and that the food can thereby be completely utilized. Under these conditions, as in a well-designed hanging culture, the growth of shellfish will be a function of food concentration and water flow.

There has been a considerable amount of speculation concerning the possible use of the cooling water effluents from large nuclear power plants for growing shellfish. Most of this has centered around the beneficial effects of the warm water (usually 20-25°F above the intake temperature), particularly in temperate climates where low winter temperatures normally arrest the feeding and growth of molluscs. Aside from the temperature effects, however, it is of interest to consider the food imput and hence the growth potential of such a system. The larger power plants now under construction pump cooling water at a rate of some 1,000 cubic feet per second or 2.4 x $10^9$ liters per day. Assuming the same concentration of food in the water as used in the earlier calculations (1.0 milligram dry weight/liter), the total amount of food passed through the system would therefore be 2,400,000 grams per day.

While this figure is large, it is not particularly so in relation to the potential food availability in the example of raft culture given above. However, the actual utilization of food in such a system, resulting in part from the beneficial effects of the warm water and in part by the possibilities of better presenting the food to the shellfish in an artificial configuration, could be greatly improved over any system of natural culture.

## POTENTIAL PRODUCTION OF SHELLFISH

An attempt has been made above to show the relative amounts of food which will be available to shellfish under different culture conditions and techniques. The actual quantities will be highly variable under different conditions of productivity, water circulation, etc. and the numbers are therefore useful only for comparative purposes. To go beyond this and estimate shellfish production from food availability would involve so many additional uncertainties and ill-founded assumtions that the exercise would be meaningless. It is perhaps worthwhile, however, to make one additional comparison.

It was calculated above that 10 tons of dry weight per day of food would be available to the shellfish suspended from a Japanese or Spanish raft, under "normal" estuarine conditions of productivity and current flow. The highest known yield of shellfish is that reported above for the raft culture of mussels in Spain, 60 tons fresh weight or roughly 10 tons dry weight per raft per year. If the above calculations concerning food availability are quantitatively correct even within an order of magnitude and applicable to the Galacian Bays of Spain, they suggest that, in spite of the high shellfish yields, the system is extremely inefficient in capturing and utilizing the organic matter suspended in the water flowing through the system. There are many possible reasons for such inefficiency. Probably the most important of these, in the author's opinion, are, first, that the hanging raft culture does not, in fact, intercept a very large fraction of the particulate organic matter which passes through it. This would be particularly true during the early stages of the annual culture cycle when the animals are very small. It also undoubtedly results from the fact that the shellfish do not feed continuously on either a seasonal or diurnal basis. The

other probable major cause of the inefficiency is that a large fraction of the particulate organic matter consists of microorganisms or particles of detritus which are too large or otherwise unacceptable by or unuseful to the shellfish.

Thus the inefficiency of shellfish in utilizing the available particulate organic matter in estuaries is not particularly surprising. This fact does not negate the preceding argument, which simply illustrates the relative efficiency of different culture methods. It does however, leave the impression that improved technology and new concepts of shellfish culture could, perhaps, lead to more efficient food utilization and even greater yields than have yet been achieved.

There is one further, most important point in considering the potential of the estuary for shellfish production. It was pointed out early in the discussion that if shellfish were dependent upon the organic matter produced in the same water in which they live, an annual yield of no more than about 150 kilograms of meat per hectare would be possible. The higher yields which result from intensive culture of various kinds all depend upon the concentration of the organic matter produced over a much larger area than that involved in the culture. Thus the yields from successful bottom culture utilize the organic production from nearly 50 times, Japanese raft culture of oysters 500 times, and Spanish mussel culture 5,000 times the area involved in growing the shellfish.

There are two important consequences which are implicit from this argument. First, both intensive and extensive culture cannot be carried out in the same estuary. Closely adjacent cultures will inevitably compete for food. In any large estuarine system there is a finite potential yield which may be spread over a large region or, if tides and currents permit, concentrated into a very small area. While there are economic and logistic incentives for developing highly efficient and concentrated culture methods, particularly for the individual who controls a small portion of an estuary, these practices do not necessarily increase the yields of the entire system.

Second, it follows that high shellfish yields from one area of intensive cultivation cannot be extrapolated blindly to estuaries in general. It is tempting to show how shellfish culture can satisfy the world's food problem by multiplying the Japanese or Spanish raft culture results by the total area of the world's estuaries. This is clearly a fallacy, and should be avoided. There is no doubt but that improved technology can greatly increase shellfish production on both a local and world-wide basis, but the prospect must be approached with due caution and with an appreciation of the ecological principles involved.

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# DECREASE IN INCIDENCE OF *MINCHINIA NELSONI* IN OYSTERS ACCOMPANYING REDUCTION OF SALINITY IN THE LABORATORY[1]

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## ABSTRACT

*Sick oysters, selected from a population with high incidence of* Minchinia nelsoni *infection, were held in aquaria at different salinities for about 6 months. Salinities were about 7-8 ppt (running water), about 14-16 ppt (running water) and about 19-22 ppt (recirculating water). Section preparations for microscopic examination were made from preserved gapers and survivors. The incidence of infection in the 3 salinities were found to be respectively 5.5 per cent, 63.1 per cent and 88.8 per cent, indicating recovery of some oysters at the low salinity. These results agree with field observations which suggest that the parasite does not thrive at lower salinities, although some of the other factors which could have influenced the results were not controlled.*

## INTRODUCTION

Soon after the discovery in 1958 of *Minchinia nelsoni* (= "MSX") by Haskin, Stauber, and Mackin (1966), extensive surveys were undertaken by Rutgers University, Virginia Institute of Marine Science, the Bureau of Commercial Fisheries and Chesapeake Biological Laboratory to determine the distribution of this parasite. These surveys resulted in finding the organism only in oyster populations of the more saline waters of the estuaries. Andrews (1964) made a particularly thorough study of the relation of salinity to the distribution of *M. nelsoni* in the James River and recently considered the subject further in two additional studies, one on epizootiology (Andrews, 1966) and the other on history and distribution (Andrews and Wood, 1967). Results of the early field surveys led to the general feeling (among workers intimately concerned with the problem, who freely exchanged information) that low salinity is a barrier which limits the range of the parasite. Since a salinity barrier could have important economic implications, we started in the fall of 1960 an experiment designed to produce laboratory data on the role of the salinity factor in distribution of *M. nelsoni*.

Because of flaws in the experimental design (due largely to technical difficulties in controlling the environmental factors) and the desirability of obtaining more data, we have procrastinated for several years in publishing the work. The experiment was presented by the senior author, however, at a meeting of the Atlantic Estuarine Research Society in 1961. In spite of its limitations, it was apparently well received. So far as we are aware, no one else has carried on laboratory experiments to test the effect on *M. nelsoni* of varying the salinity. We suspect this has been due largely to a general acceptance of the results we reported in 1961. Nevertheless, we are advised that all available information on the subject needs to be in the published record for whatever value it may have as a factor in determining oyster management policies.

## MATERIALS AND METHODS

Seventy-two infected oysters from Mobjack Bay, Virginia, were obtained through the courtesy of Jay D. Andrews of Virginia Institute of Marine Science. They were small oysters (about 2 inches) which had been individually numbered and

[1] Contribution No. 378, Natural Resources Institute of the University of Maryland.

TABLE 1. *The experimental design.*

| Aquarium No.: | 1 | 2 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| Salinity ppt, approx. | 7-8 | 7-8 | 14-16 | 19-22 | 19-22 |
| Water running (R) or recirculating (C) | R | R | R | C | C |
| "Sick" oysters, 15 Oct. '60 | 0 | 20 | 20 | 12 | 20 |
| "Healthy" oysters (local), 15 Oct. '60 | 20 | 0 | 0 | 0 | 0 |
| "Healthy" oysters (local), 29 Nov. '60 | 0 | 0 | 0 | 6 | 6 |
| "Healthy" oysters (Potomac), 4 Nov. '60 | 10 | 10 | 10 | 0 | 0 |

weighed weekly under water for 3 successive weeks. They were selected by Andrews using his under-water weighing method (1961) as "sick" because they showed little or no increase in weight. These oysters were from "Plot 9" where he found *M. nelsoni* in 20-48% of 6 samples examined microscopically between 15 July 1960 and 20 December 1960. Because of this history these oyster were presumed to be essentially 100% infected. Since they were so few in number we decided against sacrificing a sample to obtain more direct evidence of the initial incidence of disease and intensity of infection. Our use of "incidence of disease" follows the definition given by Steinhaus and Martignoni, 1967. On 15 October 1960 the sick oysters were distributed, as indicated in Table 1, in aquaria (plexiglas, 1 ft X 1 ft X 2 ft long) supplied with water of 3 different salinities. At the same time, an aquarium (no. 1) was set up with 20 small (1-2 inches), healthy oysters collected locally, where *Minchinia* was never found. They were controls to test whether the parasite was being introduced into the aquaria with the running water. Other healthy oysters were distributed to various aquaria, as shown in Table 1, a few days later. This served the additional objective of exposing susceptible oysters to infection by placing them in proximity with the sick ones.

Aquarium no. 4 was supplied with running water pumped into the laboratory from the Patuxent River where the salinity varied from about 10-16 ppt. Except for the last 6 weeks (March and early April), when salinity dropped to the lower part of the range, it remained at about 14-16 ppt. The temperature, at first about 20°C, dropped gradually to about 0°C in late January and then gradually rose to about 10°C at the end of the experiment in mid-April.

The lower salinity in aquaria nos. 1 and 2 (about 7-8 ppt) was obtained by running equal

TABLE 2. Minchinia nelsoni *in oysters held at salinity of 7-8 ppt (Aquarium No. 2.)*

| No. of Oysters | Date of Death | No. of Days held | Incidence and Intensity of Infection* |
|---|---|---|---|
| | Oysters originally selected as "sick" | | |
| 1 | 17 Oct. | 2 | 3** |
| 1 | 22 Nov. | 38 | 0 |
| 2 | 12 Apr. | 178 | ? (boxes) |
| 16 | 12 " (sacrificed) | 178 | 0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0 |
| | (*M. nelsoni* incidence: 1/18 or 5.5%.) | | |
| | Oysters originally introduced as "healthy" | | |
| 10 | 12 Apr. (sacrificed) | 158 | 0,0,0,0,0,0,0,0,0,0 |

*The intensity of infection with *Minchinia* was subjectively estimated according to the following scale:
0 — none found.
1 — rare, one or very few found after thorough searching with oil immersion objective.
2 — light, several found after little searching.
3 — moderate, parasites easily found under oil.
4 — heavy, a few present in almost every oil immersion field.
5 — very heavy, many in almost every oil immersion field.

**Since the infected gaper which died in low salinity water did so in two days, it probably was beyond saving under any circumstances.

TABLE 3. Minchinia nelsoni *in oysters held at salinity of 14-16 ppt (Aquarium No. 4.)*

| No. of Oysters | Date of Death | No. of Days held | Incidence and Intensity of Infection |
|---|---|---|---|
| | Oysters originally selected as "sick" | | |
| 1 | 17 Oct. | 2 | 5 |
| 1 | 18 " | 3 | 3 |
| 1 | 21 " | 6 | 5 |
| 1 | 9 Nov. | 25 | 4 |
| 1 | 4 Mar. | 140 | ? (box) |
| 1 | 7 Apr. | 173 | 3 |
| 14 | 11 " (sacrificed) | 177 | 4,4,3,3,3,3,2,0,0,0,0,0,0,0 |
| | (*M. nelsoni* incidence: 12/19 or 63.1%.) | | |
| | Oysters originally introduced as "healthy" | | |
| 10 | 12 Apr. (sacrificed) | 158 | 0,0,0,0,0,0,0,0,0,0 |

parts of salt water from the river and fresh water from a well through a mixing tank and then into the aquaria. All running water was drained into a dry well, rather than into the river, to avoid the possibility of exposing the local oyster populations to disease.

Since high salinity running water was not available, recirculating water (from Gloucester Point, Va., and Snow Hill Public Landing, Md.) was used in aquaria nos. 5 and 6 and maintained at a salinity of about 19-22 ppt by adding distilled water periodically. These aquaria were maintained at room temperature.

Gapers were removed from the aquaria when found and fixed in FAA or Bouin. On 11 April 1961 the experiment was terminated. Since all oysters in aquarium no. 1 had remained healthy, and thereby served their purpose, they were discarded. All other survivors were fixed in FAA and, along with the gapers already preserved, were sectioned, stained with Heidenhain's hematoxylin and examined microscopically.

## RESULTS

The incidence of *M. nelsoni* in oysters which were originally selected as "sick" and which died during the course of the experiment or were finally sacrificed was very low in those held at low salinity (Table 2), considerably higher in those at medium salinity (Table 3) and very high at high salinity (Table 4). In other words, there was a positive relation between salinity and incidence of the parasite. There was, likewise, a positive relation between salinity and mortality. It must be emphasized, however, that there were other variables besides salinity, notably those which were unavoidably introduced by using both running and recirculating water.

The intensity of infection in oysters found to harbor *M. nelsoni* did not appear to correlate with any variable in the experiment. On the average, the intensity in infected individuals was about the same at one salinity as at another and about the same for gapers and survivors.

Many oysters in the running water (medium and low salinities) soon started growing and survivors generally showed good growth and good condition. Those in the recirculating water (high salinity) in which little or no food was available, did not show growth while condition became gradually poorer.

## DISCUSSION

Preferably, a sample of the "sick" oysters (had more been available) should have been sacrificed at first to establish the initial level of *M. nelsoni* infection. Since the oysters were selected as "sick" from a bed which showed 20-48% incidence in random samples, the incidence of *M. nelsoni* in these should have been very high. It is not surprising, therefore, that about 90% were found positive in aquaria nos. 5 and 6. It is extremely doubtful that this represents an increase over the initial incidence in view of the negative findings in oysters introduced as "healthy." The intensity of infection might be expected to increase in some oysters, especially some of those living under unfavorable conditions in aquaria nos. 5 and 6, but no evidence for such increase was produced.

It seems quite clear that there was recovery of some oysters correlated with, if not attributable to, lowered salinity. There were several other variables, mostly introduced by the use of recirculating water at the highest salinity and by using water from different sources, which could not be controlled. Any or all of these may have had an effect. Some of these were differences in available food, accumulated metabolic wastes,

TABLE 4. Minchinia nelsoni *in oysters held at salinity of 19-22 ppt (Aquaria Nos. 5 and 6 combined.)*

| No. of Oysters | Date of Death | No. of Days held | Incidence and Intensity of Infection |
|---|---|---|---|
| Oysters originally selected as "sick" | | | |
| 4 | 18-21 Oct. | 3-6 | ? (Boxes or decomposed) |
| 1 | 18 " | 3 | 1 |
| 2 | 25 " | 10 | 4,3 |
| 1 | 28 " | 13 | 4 |
| 2 | 29 " | 14 | 5,2 |
| 1 | 18 Nov. | 34 | 3 |
| 1 | 22 " | 38 | 0 |
| 1 | 8 Dec. | 54 | 2 |
| 3 | 26 " | 72 | 3,2,? (1 box) |
| 1 | 30 " | 76 | 2 |
| 1 | 12 Jan. | 88 | 3 |
| 1 | 15 " | 92 | 5 |
| 1 | 5 Feb. | 112 | 5 |
| 1 | 7 " | 114 | 3 |
| 2 | 26 " | 133 | 4,2 |
| 1 | 8 Mar. | 144 | 3 |
| 1 | 16 " | 152 | 3 |
| 1 | 20 " | 156 | 4 |
| 1 | 23 " | 158 | 4 |
| 1 | 27 " | 162 | 3 |
| 1 | 6 Apr. | 172 | 5 |
| 3 | 11 " (sacrificed) | 177 | 4,0,0 |
| (*M. nelsoni* incidence in oysters: 24/27 or 88.8%.) | | | |
| Oysters originally introduced as "healthy" | | | |
| 2 | 5 Jan. (sacrificed) | 67 | 0,0 |
| 1 | 15 " " | 77 | 0 |
| 1 | 7 Apr. " | 159 | 0 |
| 8 | 11 " " | 163 | 0,0,0,0,0,0,0,0 |

temperature, content and proportions of various metallic ions, etc. It is, therefore, not completely clear to which factor or combination of factors the recovery should be attributed. Whether the recovery was complete is also not clear. When *M. nelsoni* was apparently absent it may have been actually present in very reduced numbers or in an unrecognized latent stage.

The results of this experiment seem to justify an increased confidence in the theory arising from results of bar sampling, that low salinity somehow acts as a barrier tending to limit the range of *M. nelsoni*.

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# A HISTOCHEMICAL DEMONSTRATION OF GLYCOGEN, GLYCOGEN PHOSPHORYLASE AND BRANCHING ENZYME IN THE AMERICAN OYSTER [1]

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## ABSTRACT

*Oysters were sampled for glycogen using the modified PAS histochemical procedure of McManus. Two enzymes concerned with glycogen metabolism, glycogen phosphorylase and branching enzyme, were also demonstrated histochemically. Glycogen deposition coincided with centers of activity of the two enzymes suggesting that, as in mammals, these enzymes are bound to glycogen. Local concentrations of glycogen and enzymes were found in Leydig tissue in juxtaposition to metabolically active organs such as kidneys, gills, maturing gonad, mantle and alimentary canal. Much enzyme activity was concentrated in the systemic heart and adductor muscle. The advantages of such a wide-spread storage system capable of generating rapidly glycose-1-phosphate were discussed.*

## INTRODUCTION

Although several studies (Bargeton, 1941, 1942, 1943, 1955; Couteaux-Bargeton, 1947; Semichon, 1932) have been made on the histochemistry of glycogen in the oyster, no one has reported on the localization of the enzymes responsible for the metabolism of this storage product in shellfish.

Histochemical methods for the detection and localization of glycogen phosphorylase were first developed by Yim and Sun (1947) and later by Takeuchi and Kuriaki (1955). Takeuchi (1962) demonstrated that amylo-1, 4 ⟶ 1, 6-transglucosidase (branching enzyme) and uridine diphosphate glucose glycogen transferase (UDPG-glycogen synthetase) could be localized histochemically in a wide variety of tissues from rodents.

The investigations reported here will help shed additional light on the localization of glycogen in the oyster; further, the presence of two enzymes involved in glycogen metabolism, glycogen phosphorylase and amylo-1, 4 ⟶ 1, 6 transglucosidase, hereafter called branching enzyme, will be demonstrated and correlated with glycogen distribution.

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## MATERIALS AND METHODS

Oysters were collected monthly from Delaware Bay and from Chesapeake Bay; they were maintained in the refrigerator at 4°C. Tissues were sampled at various intervals after collection to study the effects of prolonged shell closure on glycogen content and distribution as well as on enzyme activity. Five to six oysters were sampled monthly throughout the year to detect seasonal changes in tissue concentrations of glycogen and the enzymes studied.

### *Glycogen*

Small pieces were taken from the visceral mass and processed in one of two ways: (1) fixed in FAA (formalin - 10 parts, absolute alcohol - 85 parts, glacial acetic - 5 parts) at 4°C for 18 hr; the tissue was washed, dehydrated, cleared, embedded in paraffin and cut at 10 $\mu$; or, (2) wrapped in aluminum foil and quenched in a slush of dry ice and acetone, cut at 15 $\mu$ in the cryostat at −25°C and fixed on glass slides in cold FAA for one hr. All sections were stained by the periodic acid-Schiff method modified after McManus (Pearse, 1961) which stains glycogen a deep red. Control sections were incubated in a 0.5% solution of alpha amylase for 30 min at 35°C before staining. Tissues that showed bright red granules which were absent in corresponding con-

trols, indicated sites of glycogen storage. Frozen sections fixed in FAA usually demonstrated intracellular glycogen distribution better than paraffin sections (in the latter, the glycogen granules typically tended to clump together in large masses in one corner of the cell, i.e., they showed polarity).

*Glycogen Phosphorylase*

The technique of Takeuchi and Kuriaki (1955) modified by Eränkö and Palkama (1961) was used with consistently good results. Fresh pieces of tissue were excised from the oyster, wrapped in aluminum then quenched in a slush of dry ice and acetone. The frozen tissues were then transferred to the cryostat, allowed to warm up to −25° C and serially sectioned at 20$\mu$. Sections were thawed on glass slides and alternate sections were fixed in ice-cold acetone for 30 sec then allowed to dry. All slides were incubated for 3 hr in a solution consisting of:

| | |
|---|---|
| glucose-1-phosphate | 100 mg |
| adenosine-5-phosphate | 10 mg |
| glycogen | 2 mg |
| sodium fluoride | 180 mg |
| polyvinyl pyrrolidone | 900 mg |
| insulin 40 i$\mu$ ml | 1 drop |
| absolute alcohol | 2 ml |
| 0.1 M acetate buffer pH 5.9 | 10 ml |

After incubation, sections were washed in distilled water, immersed in 40% alcohol for 2 min then air dried. Next they were immersed in 0.32 M sucrose for 5 min and finally "stained" for 5 min in Gram's iodine solution made in 0.32 M sucrose solution. The sections were mounted in iodine glycerol and kept in the refrigerator until examined. Control sections were incubated as above but with the glucose-1-phosphate omitted from the incubation solution. Newly-synthesized glycogen stained purple to red-brown in Gram's iodine solution and did not fade for several days if stored in the refrigerator. The chain length of the glycogen molecule was the main factor influencing the hue of the iodine complex: red color occurred with chains of 8 to 12 glucose units; lavender or purple with chains of 14 to 21 units, and a blue color with chains over 30 units (Swanson, 1947).

*Branching Enzyme*

Tissues were prepared and incubated for glycogen phosphorylase with separate controls run in solutions of alpha and beta amylase after incubation. Beta amylase (alpha - 1,4- glucan maltohydroase) will cleave alpha - 1,4 glycosidic linkages liberating successive maltose units; enzyme activity ceases when an alpha -1,6 bond is encountered. Alpha amylase (alpha-1, 4-glucan 4-glucanohydrolase) hydrolyses alpha - 1,4 glycosidic linkages at randon in the glycogen molecule yielding a mixture of branched and unbranched oligosaccharides (White, Handler and Smith, 1968). The product remaining after digestion with beta amylase demonstrates sites of branching enzyme activity.

## RESULTS

*Glycogen*

Tissues fixed in FAA, embedded in paraffin, and stained with the PAS reaction revealed glycogen as bright red granules which frequently coalesced to form large irregular masses in one side of the cell. This is known in classical cytology as the phenomenon of polarity. For more precise cytological studies it was found best to use the fresh-frozen technique which preserved well the particulate nature of the intracellular glycogen distribution (Fig. 1).

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FIG. 1. *Localization of glycogen in a Leydig cell Note concentrations in central cytoplasmic mass and in granules distributed along radiating webs of cytoplasm. PAS reaction. X1000.*

FIG. 2. *Section of stomach (S) and adjacent Leydig tissue (Ly). Note heavy concentration of glycogen under stomach (S) epithelium; Leydig tissue (Ly) also shows heavy deposits of glycogen. PAS reaction. X100.*

FIG. 3. *Section through immature gonad (G). Concentrations of glycogen can be seen around the gonoduct (Gd) as well as enveloping strands of gonads (G). PAS reaction. X100.*

FIG. 4. *Section of loop of intestine (I) showing high activity of glycogen phosphorylase in typhlosole (T). Weak activity may be seen in intestinal epithelium. X100.*

FIG. 5. *Section of visceral mass between stomach (S) and digestive gland (D) showing intense reactions for glycogen phosphorylase in the connecting Leydig tissue (Ly). X100.*

FIG. 6. *Heavy concentrations of glycogen phosphorylase surrounding ducts and tubules of digestive gland (D), which are negative for this enzyme. X100.*

FIG. 7. *Strong reaction of glycogen phosphorylase around developing gonad (G); enzyme is also concentrated in vicinity of gonoduct (Gd). X100.*

FIG. 8. *Section through large gonoduct (Gd) located under kidney (K). Marked concentrations of glycogen phosphorylase may be seen in ciliated epithelium of gonoduct (Gd). X100.*

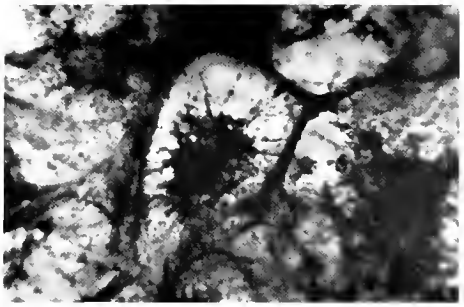

FIG. 1


FIG. 2

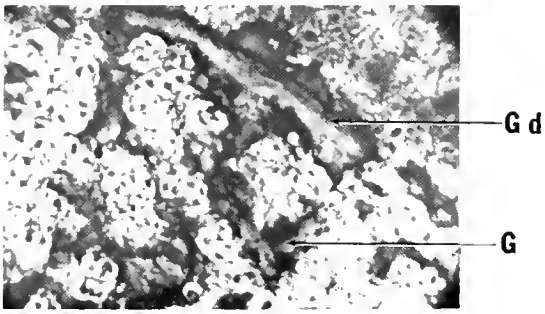


FIG. 3

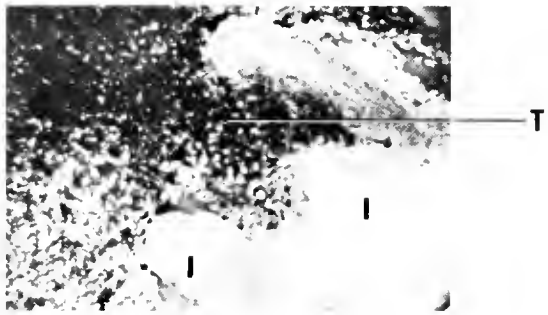


FIG. 4

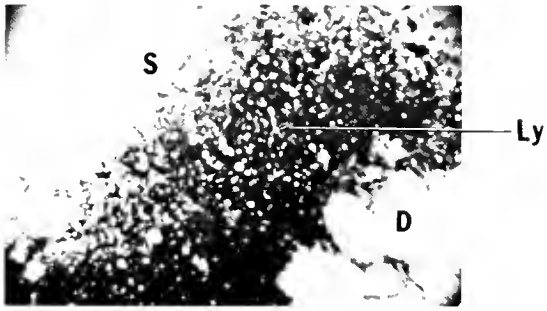


FIG. 5

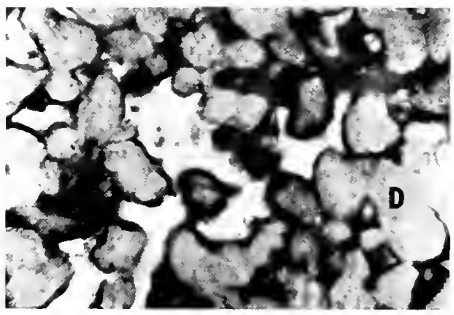


FIG. 6


FIG. 7

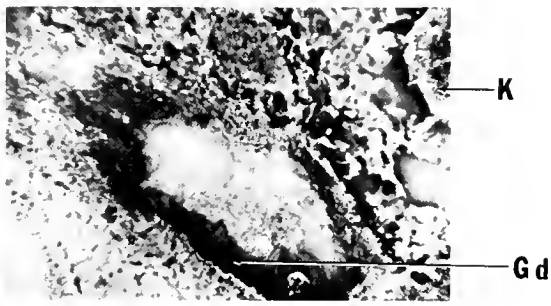


FIG. 8

Glycogen was concentrated in Leydig tissue in juxtaposition to stomach, intestine, style sac and other branches of the alimentary canal (Fig. 2); Leydig tissue in proximity to the mantle (Fig. 3), maturing gonads (Fig. 3) and gills also exhibited high glycogen levels. A Leydig cell under high magnification revealed glycogen distributed in the cytoplasm as small granules best seen in the radiating webs of cytoplasm and in the periphery of the cell (Fig. 1). Most glycogen was concentrated in the cytoplasm located in the middle of the cell known as the central cytoplasmic mass (Fig. 1). No glycogen has been detected in the vacuoles of Leydig cells.

*Glycogen Phosphorylase*

The distribution of glycogen phosphorylase followed quite closely glycogen localization. In active, feeding oysters, high activities of enzyme were found in the central cytoplasmic mass of Leydig cells located immediately adjacent to the alimentary canal. Figure 4 shows glycogen phosphorylase distribution in the vicinity of a loop of the intestine; similar patterns were found near the stomach, style sac, mid-gut and ascending intestine (Fig. 5). The gut epithelium was weakly reactive for this enzyme, which appeared in the basal portions of the epithelial cells (Fig. 5).

Leydig tissue that surrounded the duets and tubules of the digestive gland was active for this enzyme (Figs. 5, 6), although the glandular epithelium was negative. Leydig tissue lying adjacent to developing and mature gonad exhibited marked concentrations of glycogen phosphorylase (Fig. 7). Ciliated gonoduct epithelia also exhibited high enzyme activities (Fig. 8).

Leydig tissue in proximity to the mantle epithelium manifested a high activity for this enzyme. In addition, the mantle epithelium, especially in the vicinity of the ventral triplicated margin, was also quite reactive for glycogen phosphorylase (Fig. 9). The epithelium on the shell-side of the ventral mantle was more reactive than the corresponding epithelium on the gill-side. Further, the shell or outer lobe of the triplicated margin of the mantle exhibited higher activities than either the middle or inner lobes. The enzyme appeared to be concentrated in discreet areas located just under the outer limiting membrane of mantle epithelial cells (Fig. 10).

Leydig tissue at the base of the gills usually contained abundant amounts of this enzyme especially in the vicinity of the medial gill axis vein. Epithelia of medial food collecting furrows exhibited strong enzyme activities; gill epithelia in general lacked glycogen phosphorylase.

High activities of enzyme were present in the adductor muscle (Fig. 11). This organ appeared to contain the greatest concentrations of glycogen phosphorylase in the oyster. Kidney tubules also had high activities of enzyme (Fig. 12) particularly in apical portions of tubule cells, the lumen of the tubule and the connective tissue that closely invests outer portions of tubules (Fig. 12); renal sinuses also exhibited strong reactions for glycogen phosphorylase.

The systemic heart displayed high enzyme activity. Ventricular muscle fibers (Fig. 13) always manifested higher concentrations of enzyme than auricular fibers. The marked enzyme activity in blood spaces contiguous with heart muscle trabeculae probably represented a diffusion artifact (Fig. 13). Leucocytes, on the contrary, were never observed to contain enzyme (Fig. 13). As far as could be discerned, distributions of enzyme activity in the ventricle appeared to be even throughout; local concentrations apparently reflected the state of contraction at the time of

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FIG. 9. *Distribution of glycogen phosphorylase in mantle epithelium. High activities of enzyme are located in distal portions of epithelial cells just under cell membrane. X100.*

FIG. 10. *Detail of Fig. 9 showing glycogen phosphorylase in juxtaposition to cell membrane. X430.*

FIG. 11. *Section through adductor muscle showing distribution of glycogen phosphorylase in muscle fibers. Note absence of enzyme in blood (B) spaces. X100.*

FIG. 12. *Distribution of glycogen phosphorylase in kidney. Strong reactions may be seen in lumen of tubules; moderate reactions occur in the cytoplasm of the tubules. Blood (B) spaces surrounding kidney tubules are negative for this enzyme. Note marked concentration of enzyme in adjacent adductor muscle (A). X100.*

FIG. 13. *Glycogen phosphorylase in ventricular heart tissue. Note absence of enzyme in mass of leucocytes (L) and blood spaces (B). X100.*

FIG. 14. *Section through mantle (M) showing high activity of branching enzyme in Leydig tissue (Ly) supporting mantle (M). Strong reactions for this enzyme may also be observed in mantle epithelium. X100.*

FIG. 15. *Branching enzyme activity in adductor muscle. X100.*

FIG. 16. *Branching enzyme activity in ventricular myocardium. Compare with Fig. 13 to correlate distributions of branching enzyme and glycogen phosphorylase. X100.*

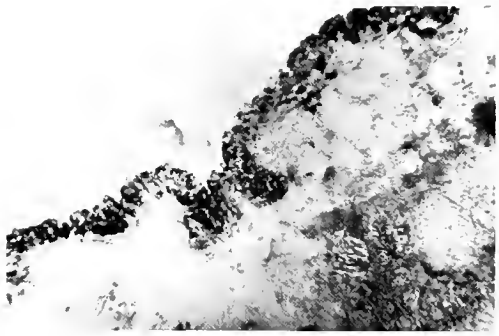

FIG. 9

FIG. 10


FIG. 11

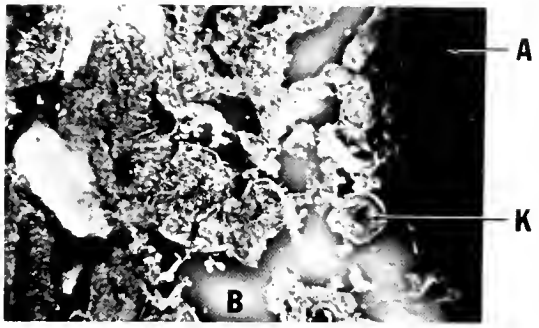


FIG. 12

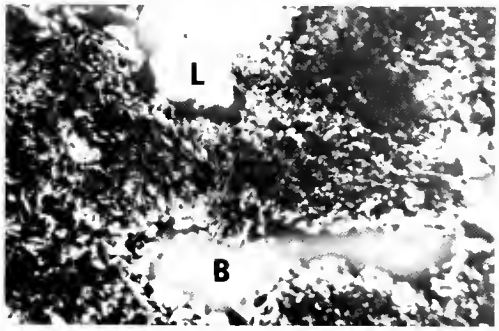


FIG. 13

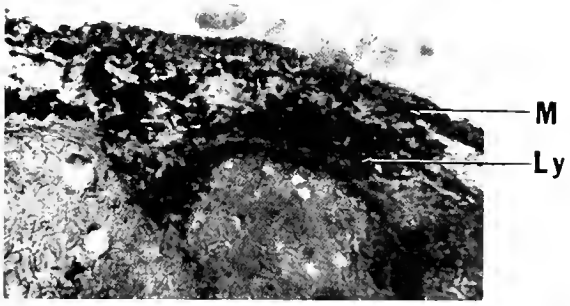


FIG. 14


FIG. 15

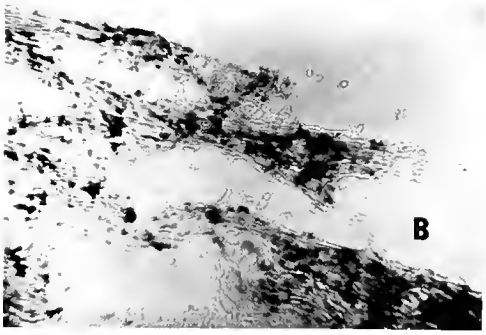


FIG. 16

freezing. The brown cells of the auricle did not exhibit enzyme activity.

*Branching Enzyme*

The distribution of branching enzyme was similar to that described for glycogen phosphorylase. High activities of enzyme were located in Leydig tissues proximal to mantle epithelia (Fig. 14), in ciliated epithelia of gonoducts and in Leydig tissue surrounding large arteries. Marked activities were also present in triplicated margins of the mantle, especially the shell lobe. Leydig tissues at the base of the gills were quite reactive. Branching enzyme activity appeared to be concentrated near the periphery of adductor muscle fibers as well as in the thin connective tissue that envelopes and separates them from adjacent blood sinuses (Fig. 15). The ventricle of the systemic heart exhibited a patchy distribution of branching enzyme (Fig. 16).

## DISCUSSION

*Glycogen*

Glycogen storage in the oyster, *Crassostrea virginica*, as in other oyster species (Bargeton, 1941) occurred as granules distributed throughout the cytoplasm of the Leydig cell. Due to the vesiculated nature of this cell, most of the glycogen was stored in the central cytoplasmic mass with isolated granules located along the webs of radiating cytoplasm (Fig. 1) and along the thin peripheral cytoplasmic border of the cell. Galtsoff (1964) stated, "In cells with a moderate amount of glycogen the latter can be seen in close contact with the protoplasmic network typical for vesicular cells."

Although all Leydig tissue stored glycogen, the richest deposits were found in the vicinity of the developing gonad (Fig. 3), branches of the alimentary canal (Fig. 2), digestive gland and mantle (Fig. 3). As the gonad matured the amount of stored glycogen in the area decreased, presumably supplying the energy and materials for gamete formation (Bargeton, 1941, 1942).

Glycogen is a highly polymerized, non-osmotic sugar which can be broken down into glucose-1-phosphate which is capable of entering immediately various metabolic pathways. Complete oxidation to carbon dioxide and water releases large amounts of energy, some of which can be captured in the form of high energy phosphate molecules such as adenosine triphosphate (ATP).

Glycogen is concentrated in oysters during the fall and early winter. It gradually declines during winter hibernation; this can be simulated by maintaining oysters for prolonged periods in cold storage. After 4 weeks' storage in the refrigerator most glycogen in the Leydig tissue of the visceral mass was depleted save that adjacent to the large arteries, the medial gill axis vein and mantle. Abnormally long hibernation periods will weaken and kill oysters as glycogen reserves drop to critical levels. Certainly, more work is needed on the intermediary metabolism of the oyster and other shellfish during these periods of prolonged shell closure.

*Glycogen Phosphorylase*

It is difficult to separate glycogen phosphorylase from branching enzyme activity since they both cleave glycogen but at different points: the former enzyme is specific for alpha 1, 4 linkages while the latter is specific for alpha 1, 6 bonds. Most newly-formed glycogen in the oyster stains red-brown to purple with iodine suggesting the presence of many branched chains in the molecule.

Glycogen phosphorylase degrades glycogen into units of glucose-1-phosphate by activating terminal glucose residues on the glycogen molecule. The equilibrium constant for this reaction is close to unity, hence the process can be reversed by adding glucose-1-phosphate to the incubation medium allowing the enzyme to synthesize glycogen.

It is well known that glycogen phosphorylase (Madsen and Cori, 1958) and branching enzyme (Leloir and Goldemberg, 1960) are bound to the "particulate" glycogen fraction in mammalian cell systems. The situation for molluscan cells has not been investigated but assuming it to be similar to the mammalian system, it was not surprising to note that the distribution of the two enzymes paralleled the pattern of glycogen storage. High activities were found in active metabolic areas such as the developing gonad, the stomach, intestine, style sac, kidney, the adductor and systemic heart muscles, gills and mantle.

The mantle epithelium (Fig. 9) does not have these enzymes uniformly distributed throughout its length; the shell-side of the ventral mantle epithelium exhibited a much higher enzyme activity than the dorsal portion (the gill-side of the ventral mantle had no activity). The enzyme concentrations appeared to increase ventrad and were quite marked in the vicinity of the triplicated margin, especially the epithelium of the shell lobe. This agreed well with the known shell-forming role of this area of the mantle. The energy released by the oxidation of newly-formed glucose-1-phosphate could contribute to the synthesis of ATP; the latter would be important in supplying energy requirements for shell formation.

The high activities of glycogen phosphorylase and branching enzyme immediately adjacent to the developing gonad (Fig. 7) would supply much glucose-1-phosphate to this active tissue. Upon its

oxidation, the captured energy in the form of ATP could support the extensive synthesis that occurs during gamete formation. It would be interesting to test for several enzymes of the phosphogluconate oxidative pathway in the gonadal area to see if glucose-1-phosphate were contributing to nucleotide synthesis via glucose-6-phosphate and, eventually, dexoxyribose. The high activities of glycogen phosphorylase and branching enzyme in proximity to the digestive gland and the various branches of the alimentary canal (Figs. 4, 5, 6) demonstrate the availability of glucose-1-phosphate for these active tissues.

The presence of glycogen phosphorylase and branching enzyme in the kidney tubules and its blood sinuses (Fig. 12) correlated well with the situation in the mammalian nephron: high activities of glycogen phosphorylase were present in the collecting tubules (Takeuchi, 1962). Since oyster kidney tubules have high levels of mitochondrial enzymes (Eble, 1965) and are apparently active metabolically, the presence of enzymes capable for forming glucose-1-phosphate is understandable.

The systemic heart and adductor muscle displayed high activities of glycogen phosphorylase and branching enzyme. The oxidation of the glucose-1-phosphate formed with subsequent ATP synthesis would provide the necessary energy-producing mechanisms for these active organs. Further, the high enzyme activities agreed well with corresponding tissues in mammals (Takeuchi, 1962; Bo and Smith, 1965; Shanthaveerappa, Waitzman and Bourne, 1966).

High activities of glycogen phosphorylase and branching enzyme were found in the Leydig tissue associated with the gills, especially in their base. The epithelium of the medial food collecting furrow of the gill apex also contained high activities of these enzymes. Since the gills of a eulamellibranch like *C. virginica* have a dual function, respiration and food-sorting, and since these functions require much ciliary work to perform effectively, a readily accessible storehouse of energy would be required. Further, vast amounts of mucus are secreted by the gills which requires both energy and precursor materials, both of which could be supplied by glucose-1-phosphate either as a result of its oxidation in the former instance or its enzymatic conversion to the polysaccharide moiety of mucus in the latter.

It becomes apparent, then, that glycogen, glycogen phosphorylase and branching enzyme have similar distributions, suggesting that these enzymes are bound somehow to glycogen as they are in mammals (Luck, 1961). The fact that glycogen and the enzymes studied are concentrated in or in juxtaposition to such active organs as the systemic heart, adductor muscle, kidney, maturing gonad, gills and the alimentary canal suggest a close support of tissues that are active metabolically with a storage product that is capable of rapidly generating glucose-1-phosphate. In higher animals such as mammals, glycogen is stored primarily in the liver where it is converted into glucose-1-phosphate which is then distributed throughout the body. The general reduction of the coelom in bivalves has resulted in the proliferation of Leydig tissue in the oyster which serves in many capacities: parenchymal, connective and storage. The fact that the vascular system of the bivalve mollusc (Eble, 1958; 1963[2]) is an open one with many large sinuses connecting the arteries and veins coupled with the extensive nature of the Leydig tissue, has resulted in local depots of glycogen storage obviating complicated transport problems. Thus conditions, both anatomical as well as physiological, are present in the oyster which favor the local storage of glycogen, together with those enzymes associated with its metabolism, in close proximity to active tissues rather than centrally located in a discreet organ. It is also interesting to note that both oyster and mammals have high levels of glycogen and glycogen phosphorylase in both heart and body muscles where energy demands are high and local supplies become a practical necessity.

[2] Eble, A. 1963. The circulatory system of the American oyster, *Crassostrea virginica* Gmelin. Ph.D. dissertation. Rutgers — The State University.

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# A SECOND MESENCHYMAL TUMOR FROM A PACIFIC OYSTER (*CRASSOSTREA GIGAS*) [1, 2]

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ABSTRACT

*A stalked benign tumor, consisting of an elongate, ovid mass with an irregular nodular surface, was observed in a Pacific oyster,* Crassostrea gigas. *It arose ventrally from the main body viscera, to which it was connected by a stalk, but lay dorsal to the pericardial sac anterior and to the left of the adductor muscle.*

*Histologically, the tumor was covered by an epithelial covering, consisting of tall columnar in some areas varying to cuboidal in others, and the body of the growth consisted of Leydig cells, muscle fibers and heavy deposits of collagenous fibers. The latter were often concentrically arranged. Structures superficially resembling digestive tubules, but consisting of collagen fibers and leucocytes, were observed and described as "pseudo-tubules". The area just beneath the epithelium was commonly characterized by a heavy leucocytic infiltrate resulting from trauma resulting from shell movement.*

## INTRODUCTION

Although records indicate that true neoplasms are rare in invertebrates, several have been observed in mollusks. Ryder (1887) reported a benign mesenchymal tumor originating in the pericardium of *Crassostrea virginica* but presented no illustration. Smith (1934) described and illustrated a similar growth in the same species. Sparks, Pauley, Bates and Sayce (1964a) reported a mesenchymal tumor in *C. gigas* and also published (1964b) an account of a fecal impaction of the rectum in the same species that superficially resembled a tumor. A unique internal fibrous tumor of possible gonadal origin was found in an oyster *(C. gigas)* by Pauley and Sayce (1968). Pauley, Sparks and Sayce (1968) described a nerve tumor associated with multiple watery cysts in a Pacific oyster. Rectal papillomas were reported in several soft-shell clams (*Mya arenaria*) by Hueper (1963). Taylor and Smith (1966) studied polypoid lesions on the foot of the gaper clam (*Tresus nuttalli*) which grossly were suggestive of neoplasia, but upon histological examination were revealed to be inflammatory hyperplasia. Two different types of tumorous lesions have been described on the foot of several freshwater mussels (*Anodonta californiensis*) by Pauley (1967a, 1967b).

A Pacific oyster from Humboldt Bay, California, was found to possess a pedunculated tumor. Since the literature indicates that tumors are of uncommon occurrence in mollusks and descriptions of a large number of these lesions are essential before a classification of their types can be made, a description of the gross and microscopic appearance of this neoplasm is presented.

## MATERIALS AND METHODS

The abnormal oyster with its lesion was fixed in Zenker's solution. The tumor was then photographed after it was pulled down to expose the

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[1] Contribution No. 305, College of Fisheries, University of Washington.

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[3] Present address: Biology Department, Battelle Memorial Institute, Pacific Northwest Laboratory, Richland, Washington.

adductor muscle (Fig. 1). The lesion was then examined histologically in five different areas (Fig. 2). The tissues were processed by standard methods and stained with either Mayer's hematoxylin and eosin or Mallory's trichrome.

## RESULTS

*Gross Description*

Grossly, the tumor consisted of an elongate, ovoid mass with an irregular, nodular surface (Figs. 1 and 2). It was soft and pliable in many areas and semi-solid, but still flexible, in other locations. The tumor was stalked and somewhat crescent shaped and measured 30 mm long, 13 mm deep, and varied from 15 to 20 mm in width. In its normal position, it was located to the left of and anterior to the adductor muscle, lying over the pericardial sac, and had protruded via a stalk through the left side of the mantle.

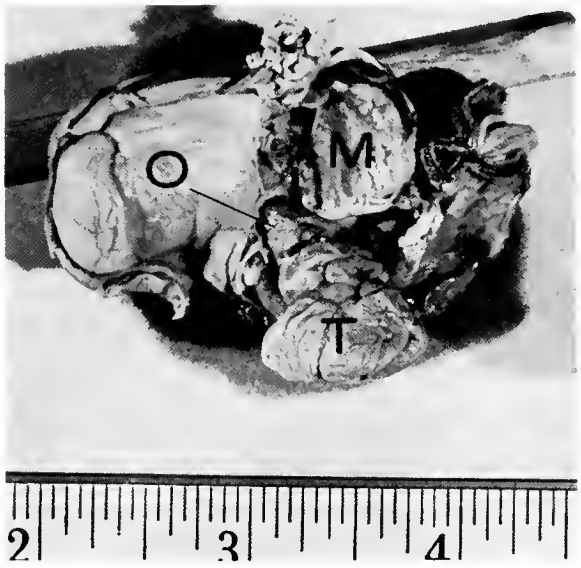


FIG. 1. Crassostrea gigas *from Humboldt Bay, California with pedunculated tumor (T) pulled down to show the ventral origin (O) of the lesion from the body beneath the adductor muscle (M).*

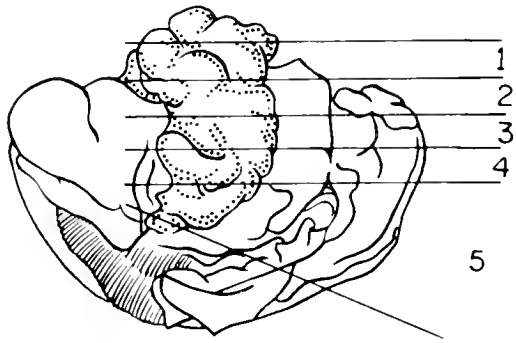


FIG. 2. *Normal position of the lesion showing the five areas of the tumor sectioned for microscopic examination.*

At first glance the tumor seemed to have its origin in the area of the pericardial sac. Closer observation, with the ventral part of the mantle cut away, revealed it had originated ventrally from the main body viscera via the suprabranchial chamber (Fig. 1). It appeared to be of glandular consistency, possibly originating from the digestive tubule area. After traversing the suprabranchial chamber, it made an upward arch to the left of the body and anterior to the adductor muscle over the side of the pericardial sac.

*Microscopic Description*

Five different areas of the tumor were examined microscopically as shown in Figure 2. A description of these areas is presented since their microscopic structure varies somewhat.

Section 1 possessed crypt-like depressions on that portion of the surface covered by tall columnar epithelial cells (Fig. 3), among which were many clear mucous cells. One area of the surface was traumatized with the surface epithelium missing. However, the major portion of the surface was smooth and covered by a single layer of cuboidal epithelium (Fig. 4). Beneath the surface epithelium there was an area of inflammation that was highly edematous, infiltrated by leucocytes, and contained both muscle and collagenous fibers. Internal to this area of inflammation were heavy deposits of collagen which formed the central core

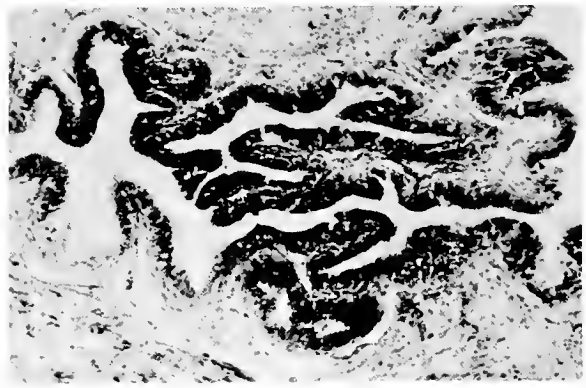

FIG. 3. *Section 1 of tumor with crypt-like depressions on that portion of the surface covered by tall columnar epithelial cells. Mallory's trichrome.*

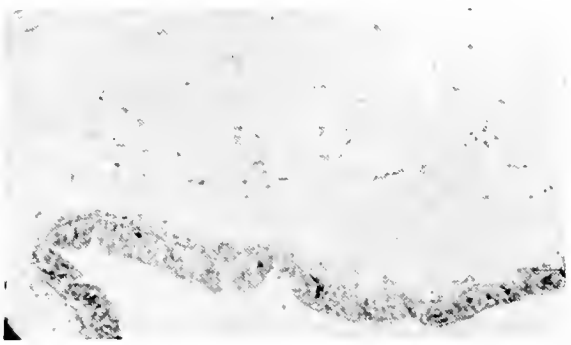

FIG. 4. *Section 1 of the surface tumor with a single layer epithelium, and underlying area of edema. Hematoxylin and eosin.*

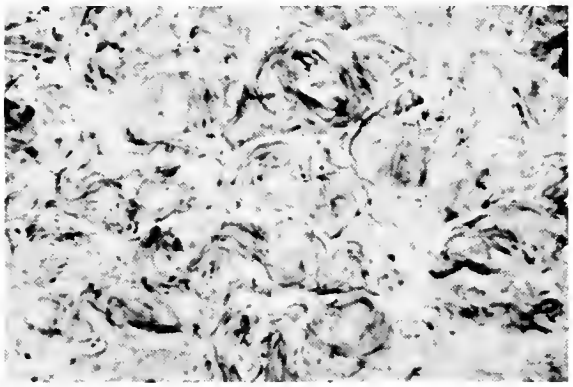

FIG. 5. *Heavy deposits of collagen in section 1 of tumor. Mallory's trichrome.*

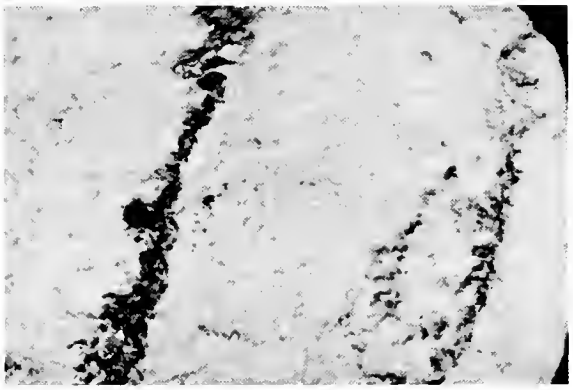

FIG. 6. *Concentric layers of muscle and collagen fibers with finer fibers running perpendicular to the layers within the body of the tumor from section 2.*

of the tumor (Fig. 5) and contained many pigment cells. Thus, in section 1, there were two major areas: (1) a central core composed mainly of collagen (Fig. 5) and (2) a peripheral region that was highly edematous (Fig. 4) and contained a few muscle and collagen fibers and some leucocytes. In between these two major regions was an area that contained a few disconnected Leydig cells among the edema.

Section 2 contained some epithelial crypts. Within the body of the tumor there were several concentric layers of muscle and collagen fibers (Fig. 6) with finer fibers running perpendicular to the concentric layers. In this region of the tumor there were structures that appeared under low magnification examination to be digestive tubules (Fig. 7). However, they were not epithelial structures, but were composed of collagen fibers and what

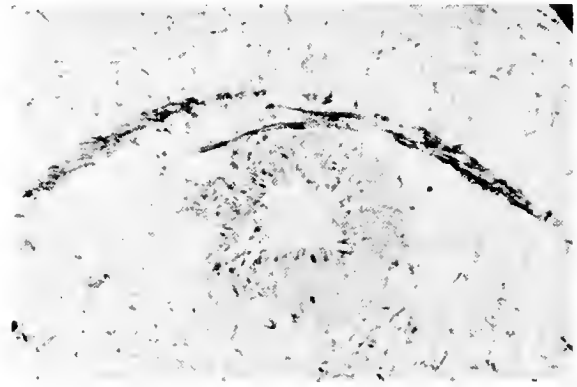

FIG. 7. *Section 2 of tumor with structures of resembling digestive tubules under low magnification. These were found not to be epithelial structures but "pseudo-tubules" composed of collagen fibers and apparent leucocytes (see same structures under high magnification in Fig. 8). Mallory's trichrome.*

appeared to be leucocytes (Fig. 8). We therefore call these "pseudo-tubules". This section was in general more cellular than section 1 and had many Leydig cells dispersed throughout it. The edematous areas and the heavily collagenous deposits beneath the epithelium indicate some trauma had occurred.

Section 3 is similar to section 2, but possessed a more convoluted epithelial surface (Fig. 9) with a more pronounced inflammatory reaction beneath the surface epithelium. The epithelium of the convoluted areas appeared to have an increased number of clear mucous cells. Pigment cells were present as in section 2, but differed from those observed in section 1.

Section 4 was similar to sections 2 and 3, with the concentric layers of muscle and collagen still present. However, there was much more edema in both the central and peripheral areas of section 4 than in sections 2 and 3.

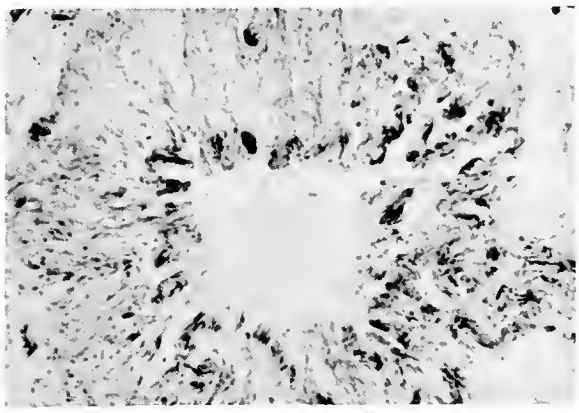

FIG. 8. *Section 2 of tumor showing "pseudo-tubules" with collagen fibers and apparent leucocytes. Mallory's trichrome.*

Section 5 had an almost normal Leydig cell area around the periphery. This peripheral area still possessed "pseudo-tubules" and the concentric layers (bands) of muscle and collagen. However, the bands were fewer and thinner in this section

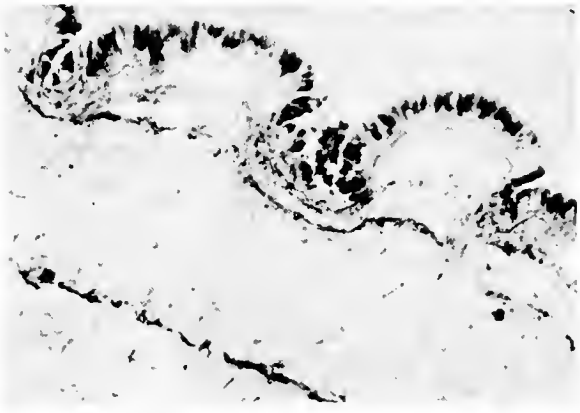

FIG. 9. *Section 3 of tumor showing convoluted epithelial surface with a pronounced inflammatory reaction beneath the surface epithelium.*

than in the other sections. Collagen deposits were interspersed among the Leydig cells near the surface epithelium. The central area of this section was highly edematous with a few widely dispersed Leydig cells.

## DISCUSSION

Grossly this tumor was similar to the large benign tumors observed on *Anodonta* (Butros, 1948; Collinge, 1891; Williams, 1890) and *Crassostrea* (Smith, 1934; Sparks *et al.*, 1964a). However, it differed microscopically from the other lesions by the presence of extensive collagenous areas and core, extensive edematous areas, concentric bands of muscle and collagen, and "pseudo-tubules". The inflammatory reaction associated with this tumor appears to be a common feature among benign lesions observed in bivalves and has been reported by several investigators (Butros, 1948; Pauley and Sayce, 1968; Smith, 1934; Sparks *et al.*, 1964a).

This tumor was apparently a benign growth, since all cell types present were normal and there was no sign of invasion into the body where the tumor had its origin. No mitotic figures were observed. Since the cause of this lesion is unknown it may be considered a spontaneous growth.

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# THE GENERAL DISTRIBUTION OF THE SURF CLAM AND OCEAN QUAHOG

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ABSTRACT

*The general distribution of surf clams,* Spisula solidissima *(Dillwyn), and ocean quahogs,* Arctica islandica *(Linnaeus), has been determined for the continental shelf of the Gulf of Maine and the Middle Atlantic Bight. Both species are concentrated in the latter area. Most surf clams inhabit the near-shore water to depths of 43 m, although greater depths have been recorded; most ocean quahogs are farther offshore, at depths of 25 to 61 m. In the middle Atlantic region, the average depth of surf clams and ocean quahogs were 29 and 42 m respectively. Both species occurred in deeper water at the southern end of their range than at the northern end.*

Two oceanic bivalves—the surf clam, *Spisula solidissima*, and the ocean quahog, *Arctica islandica*—exist in commercial quantities along the east coast of the United States, but their geographic distribution and abundance are ill-defined. Surf clam populations off New Jersey support the most important fishery of the two species at the present time. Among the bivalves, landings of surf clams were second only to oysters during the 5 years 1961-65 (Table 1), and reached a peak of 45 million pounds annually in 1966 and 1967 (Groutage and Barker, 1967; Yancey, 1968). Few people used surf clams for food before the 1950's and most were collected at low tide with hand rakes from the surf zone of beaches. Occasionally storms wash many surf clams ashore (Ropes, Chamberlin and Merrill, in press); such events probably suggested that many more inhabited the bottom farther offshore and that they could be taken on a commercial basis. Ocean quahogs have supported a modest fishery off Rhode Island over the past two decades—landings reached a high of 1.5 million pounds of meats in 1946, but since then the annual catch has usually been between 100 and 200 thousand pounds (Merrill, Chamberlin and Ropes, in press). Recently, industry has developed new consumer products which should stimulate the fishery for ocean quahogs.

Three species of *Spisula* occur in the continental shelf of eastern North America. *Spisula solidissima* has a range from the southern Gulf of St. Lawrence to Cape Hatteras, N. C. (Merrill and Webster, 1964). It is the largest species of *Spisula*; the shells of some are 200 mm long. *Spisula raveneli* (Conrad) is a smaller but distinct species (Jacobson and Old, 1966). It occurs south of Cape Hatteras and rarely attains a shell length much greater than 75 mm. A third species, *Spisula polynyma* (Stimpson), lives on the continental shelf of the north Pacific and north Atlantic oceans and attains a shell length of about 150 mm. Its center of abundance is more boreal than that of *S. solidissima*; it ranges from the Strait of Bell Isle to Long Island, but areas suitable for it in the middle Atlantic shelf were considered marginal by Chamberlin and Sterns (1963). Mixing of *S. solidissima* with the other two species in the area of the present fishery off Long Island, N. Y., New Jersey, and the Delmarva Peninsula is very unlikely.

*Arctica islandica* is a boreal species of North Atlantic origin. Reports of its occurrence in the Pacific Ocean have not been confirmed (Zatsepin and Filatova, 1961). It is the only living species in the family Arcticidae. On the American coast it occurs on the western Atlantic continental shelf from Newfoundland to Cape Hatteras (Nicol, 1951). MacPhail and Medcoff (1959) found commercial quantities in the southern Northumber-

TABLE 1. *The average annual landings of six east coast molluscan shellfish, 1961-65.*[1]

| Species | Thousands of pounds | Thousands of dollars |
|---|---|---|
| Oyster | 48,424 | 22,206 |
| Surf Clam | 35,835 | 2,454 |
| Sea Scallop | 21,804 | 10,422 |
| Hard Clam | 14,095 | 8,422 |
| Soft Clam | 9,770 | 3,296 |
| Ocean Quahog | 100 | 10 |

[1] Power, 1963; Power and Lyles, 1964; Lyles, 1965, 1966, and 1967.

land Strait. On the European coast the species is distributed from the White Sea and Barents Sea to the Bay of Cadiz on the southwest coast of Spain. It is abundant around the Faroe and Shetland Islands, the British Isles, and Iceland (Nicol, 1951; Zatsepin and Filatova, 1961) but is not commonly used for food outside of the United States.

Ocean quahogs are underutilized because the surf clam fishery has grown rapidly to meet the market demand for clam meats since World War II. Also, the surf clam is large and yields more meat per bushel than the ocean quahog. Extension of the clam fishery to include the ocean quahog, however, would provide the advantage to industry of not being dependent entirely upon the surf clam to fill the market demand for clam meats. A knowledge of ocean quahog distribution may help industry make objective decisions about developing this fishery.

Charts of the locations of surf clams (Fig. 1) and ocean quahogs (Fig. 2) from Cape Hatteras to Nova Scotia, Canada, were constructed from the latest information on their distribution without regard to numerical densities. The data were obtained from several sources: the records of the U. S. National Museum; the Museum of Comparative Zoology at Harvard University; sea scallop dredge samples (Merrill, 1962) from a middle Atlantic cruise of the R/V *Delaware*[1]; Campbell grab samples from cruises of the R/V *Gosnold* (Emery, Merrill and Trumbull, 1965; Wigley and Emery, 1968); hydraulic surf clam dredge samples from the spring and fall cruises of the R/V *Undaunted* in 1965 (Ropes, Chamberlin and Merrill, in press); and miscellaneous records of bottom samples by the R/V *Albatross*.

In the Gulf of Maine and vicinity of Cape Cod, surf clams and ocean quahogs are usually nearshore inhabitants, but Georges Bank is an exception. Ocean quahogs are found on southern Georges Bank and nearly to its eastern tip; surf clams are encountered less often than ocean quahogs and are through the central portion of Georges Bank to its northeastern tip.

Commercial concentrations of both species are indicated by their greater frequency of occurrence in the offshore bottom contiguous with the middle Atlantic States from New York through Virginia.

Bathymetric observations have been recorded for both species in the past. In the 1880's, the U. S. Fish Commission conducted extensive explorations along the continental slope, and Nicol (1951) reported 482 m as the greatest depth from which *Arctica* was dredged, but he did not state whether the catch contained live clams or only shells. Bush (1885) reported 15 to 234 m as the bathymetric range of live ocean quahogs; shell remains were found to 254 m. More recently, a live ocean quahog was taken at 256 m (43°50'N. Lat.; 67°45'W. Long.) on the 27 June 1961 R/V *Delaware* cruise. Bush (1885) reported depths to 33 m as the bathymetric limit of live surf clams and depths to 351 m for empty shells. From museum records, Merrill and Webster (1964) concluded that surf clams "live in depths as great as 70 fathoms" (128 m). A more recent deep record was of three live surf clams taken at 66 m (40°37'N. Lat.; 69°01'W. Long.) by th R/V *Albatross* II cruise on 8 December 1963. *Arctica*, then, inhabits a greater bathymetric range than *Spisula*.

An analysis of the data from the hydraulic dredge samples of the R/V *Undaunted* locates both species by depth in four areas (Fig. 3). The average depth at which surf clams were taken was 28.5 m. Depths of 12.3 to 42.7 m included 94% of the stations with surf clams. The average depth for surf clams increased from 21.2 m off Long Island, N. Y. to 32.1 m off Virginia and North Carolina. Ocean quahogs occurred most often at an average depth of 41.7 m. There was no single depth range where ocean quahogs were usually taken in all areas. Depths of 24.5 to 61.0 m included 97% of the stations with ocean quahogs. The average depth for ocean quahogs increased from 39.4 m off Long Island, N. Y. to 51.9 m off Virginia and North Carolina. Almost three-fourths of the stations contained one or both species of clams (Table 2). Mixing of the species was greatest at 30.6 to 42.7 m. In our studies, the deepest station for surf clams was at 61.6 m off the Virginia-North Carolina area; that for ocean quahogs was

[1] Merrill, A. S. and R. L. Edwards. Spring water temperatures and fishing log, Block Island to Cape Hatteras, *Delaware* Cruise No. 60-7, 11-21 May 1960. Manuscript Report Number 61-3 on file at the Bureau of Commercial Fisheries Biological Laboratory, Woods Hole, Mass.

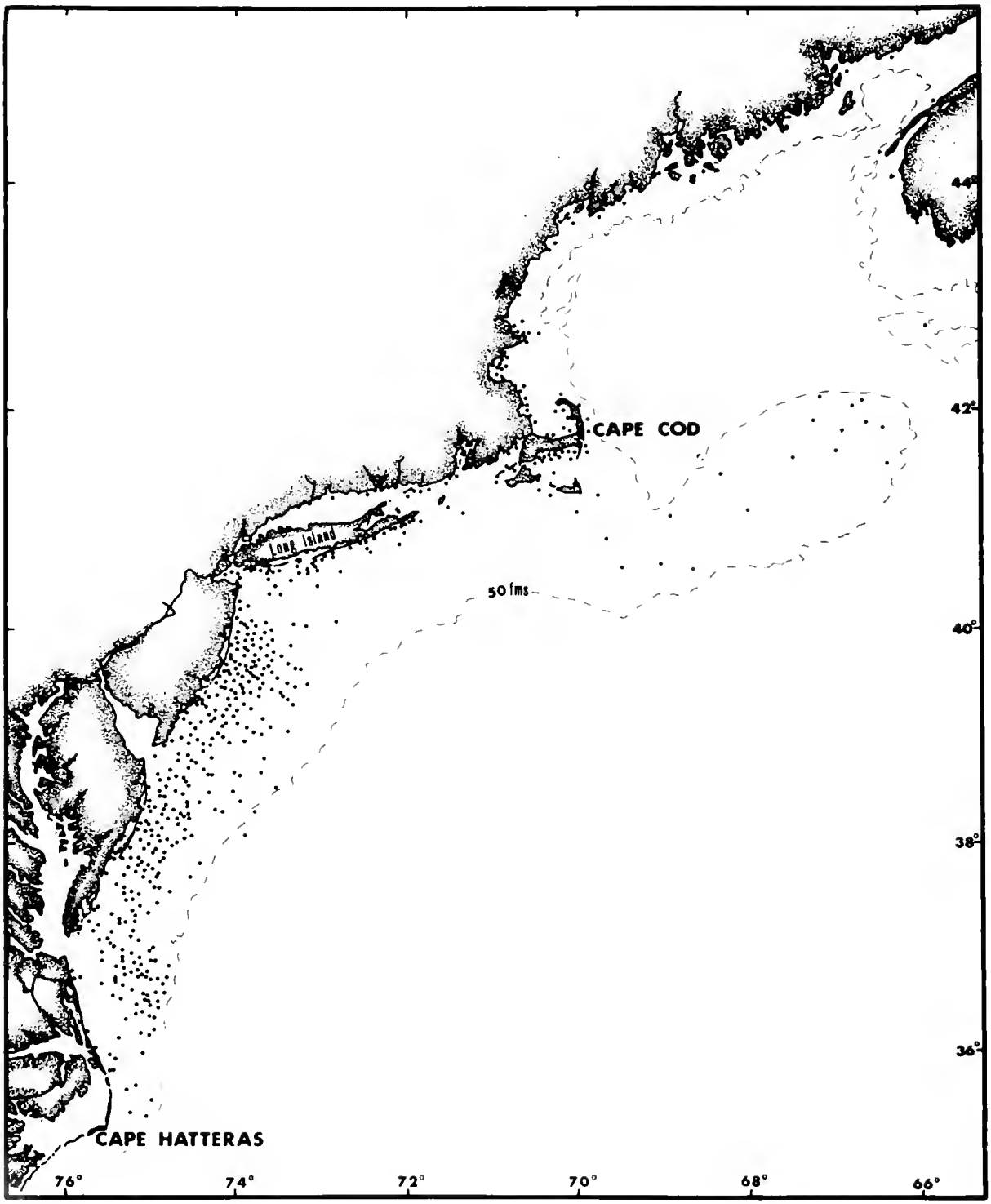


FIG. 1. *Distribution of surf clams in the Middle Atlantic Bight and Gulf of Maine.*

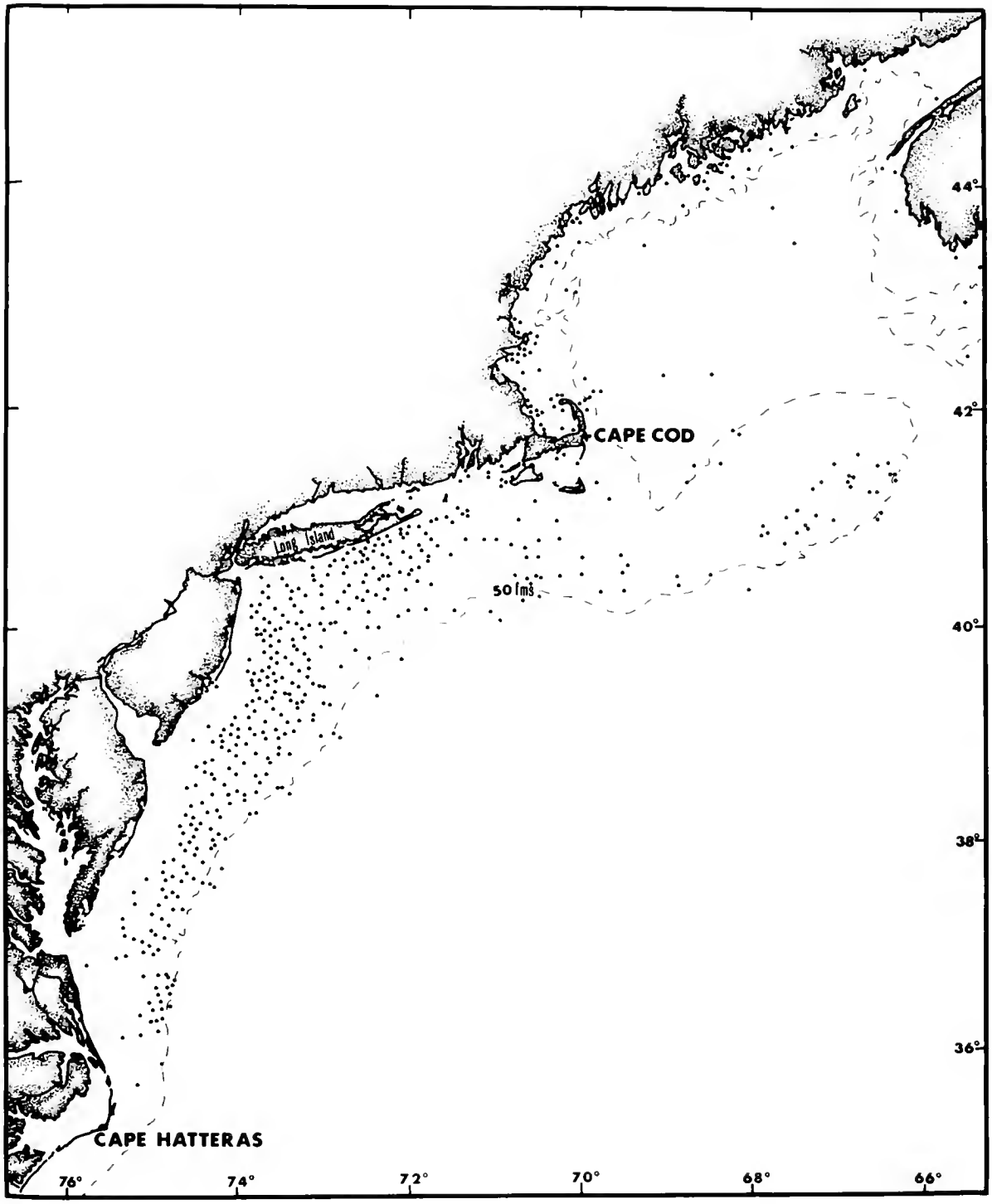


FIG. 2. *Distribution of ocean quahogs in the Middle Atlantic Bight and Gulf of Maine.*

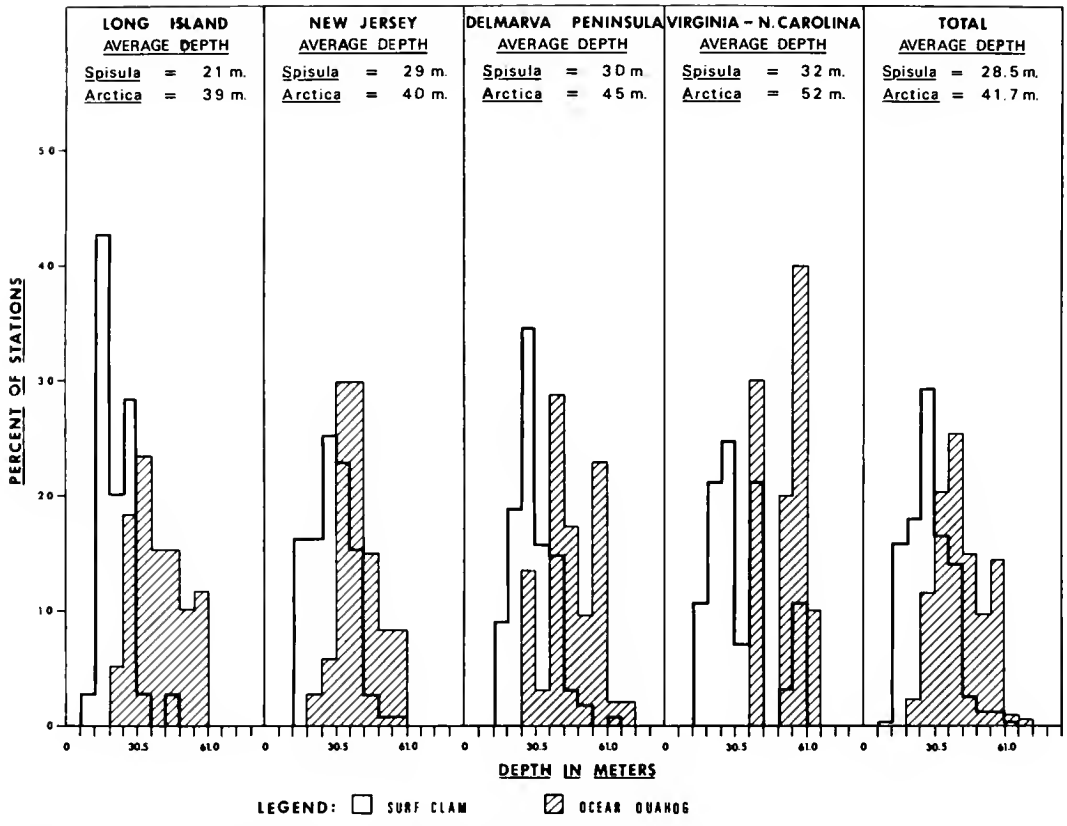


FIG. 3. *Comparative distribution of surf clams and ocean quahogs, by area and depth, in the Middle Atlantic Bight. (30.5 m = 100 ft).*

TABLE 2. *The distribution of surf clams and ocean quahogs by depth, R/V* Undaunted *— 13 May to 25 June and 23 October to 16 November 1965.*

| Station depth Meters[1] | Percentage of stations with: | | | | Total | |
|---|---|---|---|---|---|---|
| | *Spisula* alone | *Arctica* alone | *Spisula* & *Arctica* | Neither species | Number of stations | Percentage of stations |
| 6.2-12.2 | 0.5 | 0 | 0 | 3.1 | 6 | 1.0 |
| 12.3-18.3 | 22.6 | 0 | 0 | 18.5 | 80 | 13.5 |
| 18.4-24.4 | 23.5 | 0 | 5.4 | 24.7 | 97 | 16.4 |
| 24.5-30.5 | 35.7 | 9.6 | 14.0 | 18.5 | 133 | 22.5 |
| 30.6-36.6 | 9.7 | 9.6 | 33.3 | 16.1 | 89 | 15.1 |
| 36.7-42.7 | 6.8 | 20.9 | 31.2 | 5.6 | 77 | 13.0 |
| 42.8-48.8 | 1.4 | 21.7 | 6.5 | 3.7 | 40 | 6.8 |
| 48.9-54.9 | 0 | 13.9 | 4.3 | 1.9 | 23 | 3.9 |
| 55.0-61.0 | 0 | 22.6 | 4.3 | 5.6 | 39 | 6.6 |
| 61.1-67.1 | 0 | 0.9 | 1.1 | 1.2 | 4 | 0.7 |
| 67.2-73.3 | 0 | 0.9 | 0 | 0 | 1 | 0.2 |
| 73.4-79.5 | 0 | 0 | 0 | 1.2 | 2 | 0.3 |
| Number of stations | 221 | 115 | 93 | 162 | 591 | — |
| Average depth | 24.9 | 45.6 | 37.0 | 28.7 | 31.9 | — |

[1] Conversion factors: 6.1 m = 20 ft = 3.3 fm.

at 72.2 m off the Delmarva Peninsula area—neither observation approaches the record depth.

The shallow water and near shore habitat of surf clams has been an obvious factor in the success of the fishery. Surf clam vessels return to port each day with their catch, a practice which may not be practical in an ocean quahog fishery. Ocean quahogs have a widespread distribution along the continental shelf of the Middle Atlantic Bight; this distribution may offset the problem of fishing at greater depths and distance from ports. It is conceivable that the catch can be processed at sea, as are sea scallops, *Placopecten magellanicus*. Certainly some, if not most, of the technology developed by the surf clam industry could be used in an ocean quahog fishery.

## ACKNOWLEDGMENT

We thank Dr. Roland L. Wigley of the Bureau of Commercial Fisheries Biological Laboratory, Woods Hole, Mass.; Dr. Kenneth J. Boss, Curator of Mollusks at the Museum of Comparative Zoology of Harvard University; and Dr. Joseph Rosewater of the Division of Mollusks, U. S. National Museum, for records of the occurrence of surf clams and ocean quahogs along the northwestern Atlantic coast. Dr. J. Lockwood Chamberlin, Environmental Oceanographic Research Program, Bureau of Commercial Fisheries, Washington, D. C., identified and separated the two closely related species, *Spisula solidissima* and *S. polynyma*, in mixed samples from the Gulf of Maine.

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# AGE AND GROWTH OF LARGE SOUTHERN QUAHOGS FROM A FLORIDA ESTUARY [1]

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## ABSTRACT

*Data are presented on shell dimensions, weights, and age of 93 large southern quahogs,* Mercenaria campechiensis, *collected in Boca Ciega Bay, Florida. Shell length of four clams exceeded the previous size record for this species. The largest measured 179 mm in length, and the estimated age of the oldest was 22 years. Age of individuals was determined by counting growth lines on lateral and transverse shell surfaces. The transverse surface was exposed by sectioning. Average growth in the first 3 years produced clams 38 to 67 mm long early in the second year, 68 to 77 mm by the end of the second, and more than 77 mm in the third year. This rate of growth is at least three times that of northern quahogs.*

## INTRODUCTION

This report presents new information on the age and growth of southern quahogs (*Mercenaria campechiensis*) larger than 77 mm in total length (common name — chowders). A technique for determining the age of quahogs is described and a new size record for the southern quahog is recorded.

Growth of southern quahogs in Boca Ciega Bay was studied earlier by Taylor and Saloman (1968) by modal size frequency distribution, but only for clams not exceeding 6 years of age. To our knowledge there are no recorded studies of age and growth of southern quahogs beyond that age.

At one time a substantial commercial fishery for the southern quahog existed on the southwest coast of Florida near the Ten Thousand Islands, beginning in the late 1800's and continuing to about 1945 (Schroeder, 1924; Tiller, Glude and Stringer, 1952; Carpenter, 1967). The peak production from this industry was probably in 1922 when 205,625 bushels were harvested by hand and dredging. The last significant productive year was in 1945 when 78,000 pounds of meats were harvested. The extent and capabilities of this past fishery have stimulated interest in exploration of Florida waters for natural clam beds and in quahog culture. The morphometric information obtained in this study is useful in estimating age classes and potential meat production of clams in natural and planted beds. These data also show annual growth increments that may be expected in the culture of quahogs.

## PROCEDURE

Ninety-three quahogs were collected on 18 April and 10 May 1968, by treading and raking in shallow water (less than 1 meter) on two shoals within a small embayment in northern Boca Ciega Bay — 27°49'50"N. Lat.; 82°49'40"W. Long. (Fig. 1). Patches of sea grass, *Thalassia testudinum* and *Diplanthera wrightii*, grew on the bottom and sediments ranged from firm sand to soft, silty sand.

Shells of live and recently dead clams were cleaned before measurement of length, height and width to the nearest millimeter. Length is considered the greatest distance from the anterior to posterior margin of the valves; height is the greatest distance from the umbo to the ventral margin; and width is the greatest distance through both valves perpendicular to the other two axes. To record total weight and shell weight, live individuals were weighed on a solution balance to the nearest gram before and after the meat was shucked.

Age and growth of 93 quahogs between 6 and

[1] Contribution No. 53, Bureau of Commercial Fisheries Biological Laboratory, St. Petersburg Beach, Florida.

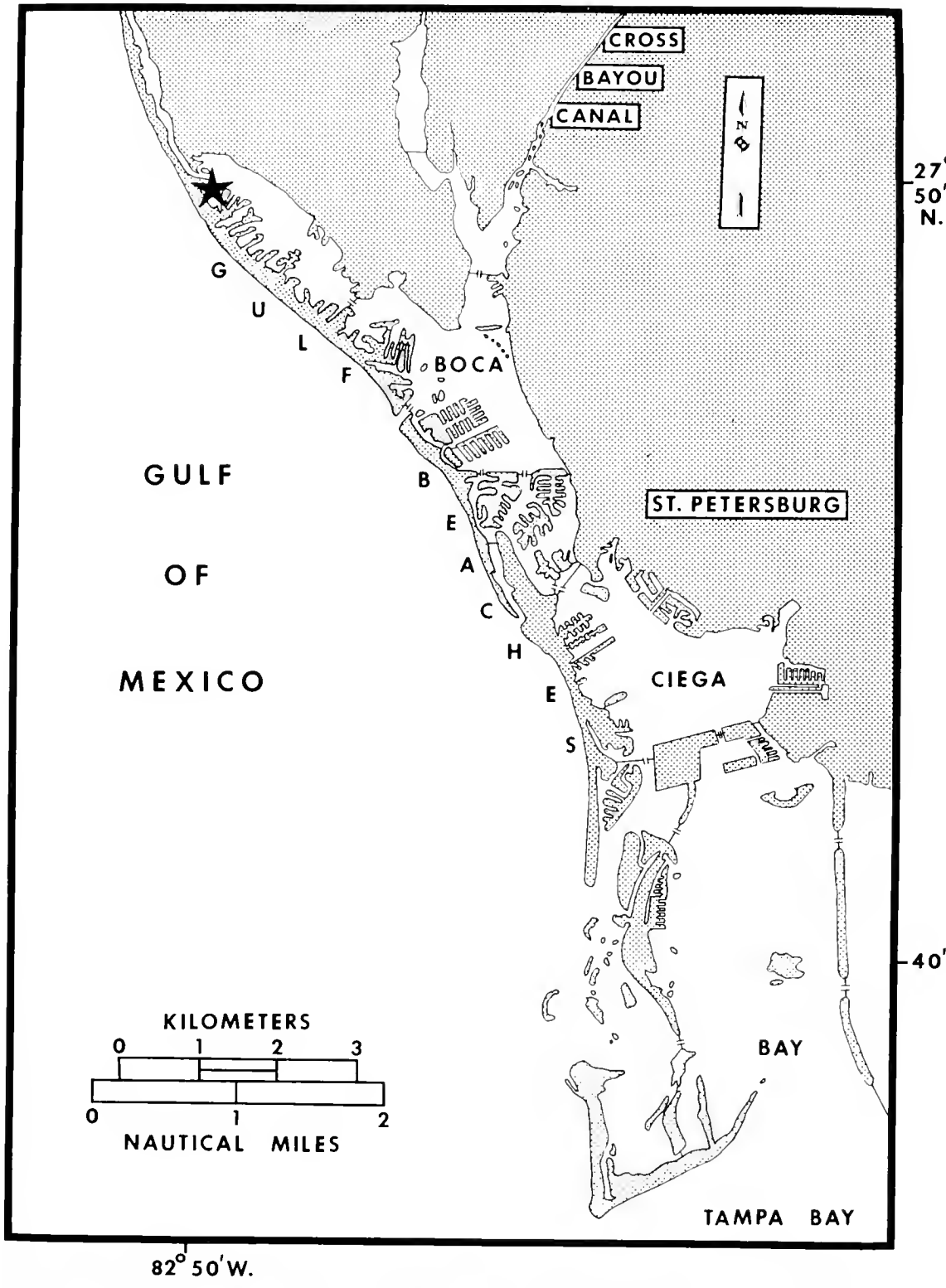


FIG. 1. *Location of sampling site (star) of large southern quahog clams in Boca Ciega Bay, Florida.*

22 years old were determined by counting annuli and measuring the distance between successive annuli from the umbo to the ventral edge. The position of closely set concentric grooves and ridges that mark the termination of annual growth on lateral shell surfaces was corroborated by examining the surface of transverse shell sections, as suggested by Shuster (1957) and illustrated in Figures 2 and 3. Pannella and Mac-

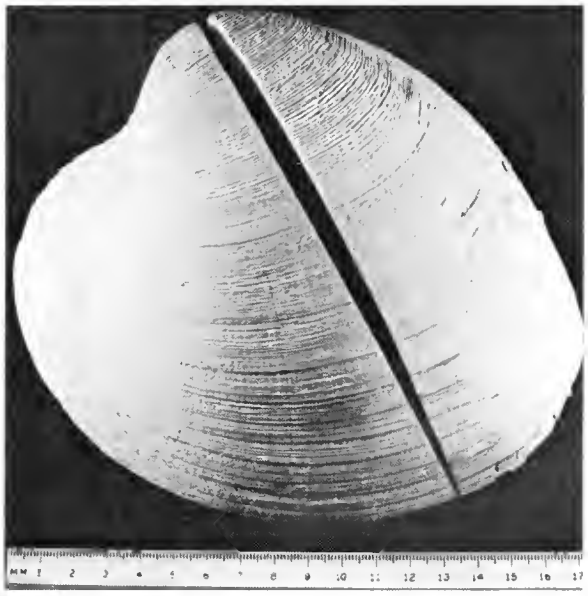

FIG. 2. *Largest live southern quahog collected (shell length, 172 mm). Transverse cut was made with a diamond-toothed saw between umbo and ventral margin.*

Clintock (1968) have further demonstrated (by cutting the clam from the umbo to ventral edge and examining the transverse surface) that *M. mercenaria* produces annual, breeding, lunar month, tidal, bidaily and daily growth patterns. The use of both criteria has increased the accuracy substantially over the use of one method.

Transverse sections were prepared with a diamond-toothed saw. This method of cutting did not cause cracking and was generally better than the embedding procedures suggested by Shuster (1957). Because of shell curvature, cumulative measurements of yearly growth gave a total shell height about 15% greater than the height determined from a single measurement between the umbo and ventral edge (Table 1).

Age determination based solely on lateral shell rings has been criticized by Ansell (1968). In earlier studies, Belding (1912) and Kerswill[2] counted growth lines on the outer shell surface

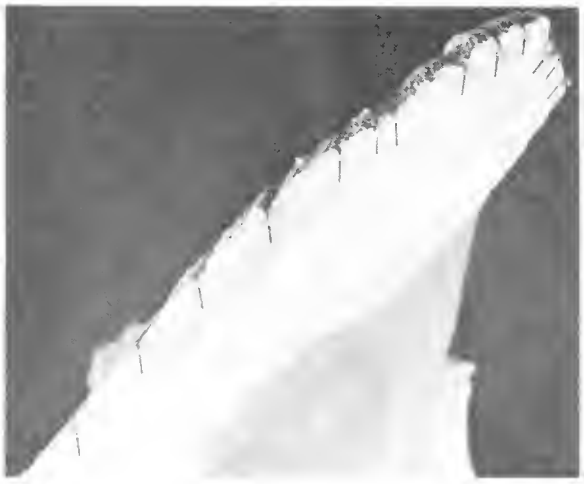

FIG. 3. *Transverse surface of southern quahog showing recurved ventral lip and relation of annuli on external and transverse surface (black lines).*

to determine age of the southern quahog, *M. mercenaria;* they found none over 25 years old. The same method has also been used to determine the age of pismo clams (Weymouth, 1923) and Pacific cockles (Weymouth and Thompson, 1931).

## RECORD-SIZE CLAMS

Four southern quahog clams exceeded the previous record of length. The largest live clam had a record shell length of 172 mm (6.8 in) and weighed 2,110 g (4.6 lbs); the second was 169 mm long (6.6 in) and weighed 2,485 g or about 5.5 lbs. Two large dead clams (valves and ligaments intact) were also collected. The largest measured 179 mm and the other 173 mm. The previous record of 168 mm (6.6 in) was reported for a specimen taken in the same collecting area (Sims, 1964), but the weight of that clam was 2,951 g (6.5 lbs).

The age of the oldest live clam collected was estimated to be 20 years. Valves of two recently dead clams indicated ages of 21 years and the age of a third was estimated at 22 years. Because of the fast growth rate and individual variation, the largest clams are not always the oldest. The largest collected (179 mm total length) was only 14 years old (Table 1).

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[2] Kerswill, Charles James. 1941. Some environmental factors limiting growth and distribution of the quahaug *Venus mercenaria* L. Doctoral Thesis, Univ. Toronto, 122 p.

TABLE 1. *Age, shell dimensions and weight of large southern quahog clams from Boca Ciega Bay, Florida.*

| Age group and item | Length mm | Height* mm | Accumulated height** mm | Width mm | Total weight g | Shell weight g | Weight of meat and fluids g |
|---|---|---|---|---|---|---|---|
| VI Group | | | | | | | |
| Average | 129 | 123 | 138 | 79 | — | — | — |
| Range | 126-134 | 120-126 | 133-143 | 79 | — | — | — |
| Number | 3 | 3 | 3 | 1 | — | — | — |
| VII Group | | | | | | | |
| Average | 120 | 117 | 136 | 76 | 866 | 608 | 258 |
| Range | 117-124 | 112-120 | 129-140 | 72-79 | 844-887 | 568-648 | 239-276 |
| Number | 3 | 3 | 3 | 3 | 2 | 2 | 2 |
| VIII Group | | | | | | | |
| Average | 124 | 123 | 141 | 78 | — | — | — |
| Range | 113-134 | 114-132 | 128-154 | 74-81 | — | — | — |
| Number | 2 | 2 | 2 | 2 | — | — | — |
| IX Group | | | | | | | |
| Average | 140 | 132 | 151 | 81 | 1,077 | 720 | 358 |
| Range | 131-147 | 128-136 | 137-158 | 79-82 | 995-1,159 | 688-751 | 307-408 |
| Nunber | 6 | 6 | 6 | 2 | 2 | 2 | 2 |
| X Group | | | | | | | |
| Average | 139 | 133 | 161 | — | — | — | — |
| Range | 138-139 | 129-136 | 157-165 | — | — | — | — |
| Number | 2 | 2 | 2 | — | — | — | — |
| XI Group | | | | | | | |
| Average | 140 | 136 | 160 | 87 | 1,290 | 820 | 470 |
| Range | 127-152 | 127-146 | 149-170 | 82-95 | 1,001-1,640 | 693-947 | 318-693 |
| Number | 8 | 8 | 8 | 5 | 5 | 5 | 5 |
| XII Group | | | | | | | |
| Average | 144 | 139 | 165 | 89 | 1,308 | 901 | 407 |
| Range | 126-158 | 124-153 | 140-183 | 85-93 | 1,195-1,464 | 776-1,025 | 383-439 |
| Number | 11 | 11 | 11 | 6 | 4 | 4 | 4 |
| XIII Group | | | | | | | |
| Average | 143 | 140 | 169 | 93 | 1,511 | 1,059 | 453 |
| Range | 124-161 | 123-158 | 145-189 | 84-104 | 1,039-2,001 | 763-1,387 | 276-614 |
| Number | 13 | 13 | 13 | 8 | 7 | 7 | 7 |
| XIV Group | | | | | | | |
| Average | 150 | 146 | 173 | 94 | 1,492 | 1,066 | 427 |
| Range | 133-179 | 130-168 | 156-194 | 86-102 | 1,167-1,912 | 826-1,378 | 341-534 |
| Number | 10 | 10 | 10 | 8 | 7 | 7 | 7 |
| XV Group | | | | | | | |
| Average | 147 | 143 | 173 | 96 | 1,536 | 1,150 | 386 |
| Range | 129-167 | 129-158 | 160-195 | 90-104 | 1,215-1,915 | 961-1,390 | 182-548 |
| Number | 9 | 9 | 9 | 5 | 5 | 5 | 5 |
| XVI Group | | | | | | | |
| Average | 147 | 145 | 172 | 97 | 1,709 | 1,212 | 497 |
| Range | 126-161 | 126-156 | 153-182 | 87-106 | 1,536-1,870 | 1,094-1,357 | 429-579 |
| Number | 10 | 10 | 10 | 8 | 7 | 7 | 7 |
| XVII Group | | | | | | | |
| Average | 159 | 154 | 182 | 101 | 1,938 | 1,333 | 605 |
| Range | 148-168 | 146-164 | 172-201 | 97-105 | 1,628-2,247 | 1,117-1,549 | 511-698 |
| Number | 6 | 6 | 6 | 2 | 2 | 2 | 2 |
| XVIII Group | | | | | | | |
| Average | 157 | 153 | 172 | 96 | 1,873 | 1 275 | 599 |
| Range | 156-158 | 152-153 | 171-173 | 95-97 | 1,821-1,925 | 1,248-1,301 | 520-677 |
| Number | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| XIX Group | | | | | | | |
| Average | 171 | 171 | 186 | 101 | 2,485 | 1,865 | 620 |
| Range | 169-173 | 170-172 | 181-190 | 99-102 | 2.485 | 1,865 | 620 |
| Number | 2 | 2 | 2 | 2 | 1 | 1 | 1 |
| XX Group | | | | | | | |
| Average | 166 | 158 | 188 | 102 | 2,037 | 1,421 | 615 |
| Range | 158-172 | 153-161 | 181-194 | 94-109 | 1,646-2,354 | 1,131-1,681 | 515-673 |
| Number | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| XXI Group | | | | | | | |
| Average | 161 | 158 | 189 | — | — | — | — |
| Range | 154-168 | 152-164 | 185-193 | — | — | — | — |
| Number | 2 | 2 | 2 | — | — | — | — |
| XXII Group | | | | | | — | — |
| Average | 154 | 153 | 181 | — | — | — | — |
| Range | 154 | 153 | 181 | — | — | — | — |
| Number | 1 | 1 | 1 | — | — | | |

*Height determined by a single measurement from umbo to ventral edge.

**Height based on accumulation of yearly increments of growth.

## AGE AND GROWTH

In Boca Ciega Bay, Florida, the southern quahog grows rapidly through its life. Very old clams, however, decrease in shell length and height with increasing age as shell margins recurve and became thick (blunt) — Tables 1 and 2. A more realistic measure of shell growth with age was obtained by recording shell height as the actual and summing the cumulative distance between successive annuli (Tables 1 and 2). A plot of these data produces a growth curve of the normal type that shows rapid growth among young animals followed by a progressively slower growth at least to age 22 (Fig. 4).

The relation of shell height to age shows that clam growth in the first 3 years is rapid and variable (Table 2). Assuming a life expectancy of at least 22 years, a clam exceeds 50% of its expected height in the third year and over 70% by the time it reaches age 5 (Fig. 4). Comparative height and length data for these clams and over 5,000 others from Boca Ciega Bay[3] show that clams attain an average length of 38 to 67 mm (little neck) early in the second year, 68 to 77 mm (cherrystone) by the end of the second year and

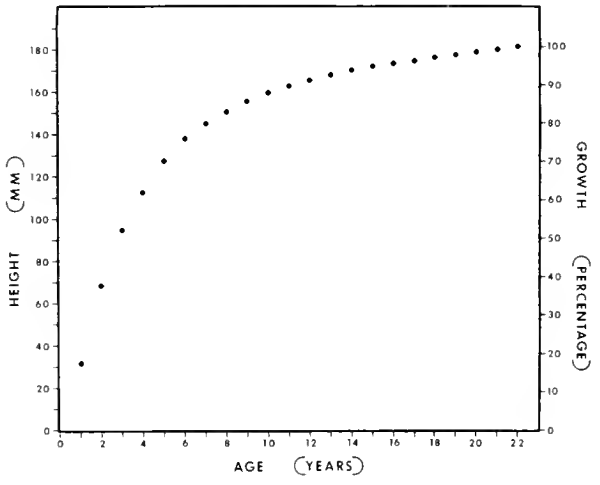


FIG. 4. *Mean growth and percentage of maximum height by age of southern quahog clams from Boca Ciega Bay, Florida.*

[3] Unpublished data on file at the Bureau of Commercial Fisheries Biological Laboratory, St. Petersburg Beach, Florida.

TABLE 2. *Mean growth in height*, range and standard deviation by age of large southern quahog clams from Boca Ciega Bay, Florida.*

| Age | Number of Clams | Increment of height | | |
|---|---|---|---|---|
| | | Mean (mm) | Range (mm) | Standard deviation |
| I | 93 | 31.5 | 10.2 - 63.7 | 13.8 |
| II | 93 | 37.4 | 12.8 - 61.0 | 12.6 |
| III | 93 | 26.0 | 11.1 - 53.6 | 9.1 |
| IV | 93 | 17.8 | 5.5 - 31.0 | 5.1 |
| V | 93 | 15.0 | 4.0 - 25.3 | 5.4 |
| VI | 93 | 10.4 | 1.4 - 21.7 | 4.6 |
| VII | 90 | 6.9 | 1.5 - 17.2 | 3.4 |
| VIII | 87 | 5.9 | 1.3 - 17.1 | 3.3 |
| IX | 85 | 5.1 | 1.4 - 19.9 | 3.3 |
| X | 79 | 3.8 | 1.0 - 9.7 | 2.3 |
| XI | 77 | 3.2 | 0.8 - 12.8 | 2.0 |
| XII | 69 | 2.8 | 0.6 - 8.6 | 1.6 |
| XIII | 58 | 2.5 | 0.9 - 9.0 | 1.6 |
| XIV | 45 | 1.9 | 0.7 - 7.6 | 1.2 |
| XV | 35 | 1.8 | 0.6 - 7.3 | 1.5 |
| XVI | 26 | 1.5 | 0.5 - 4.6 | 0.3 |
| XVII | 16 | 1.4 | 0.7 - 3.0 | 0.2 |
| XVIII | 10 | 1.7 | 0.5 - 4.4 | 1.5 |
| XIX | 8 | 1.2 | 0.4 - 3.2 | 0.3 |
| XX | 6 | 1.2 | 0.4 - 2.4 | 0.2 |
| XXI | 3 | 1.0 | 0.5 - 1.4 | 0.1 |
| XXII | 1 | 1.7 | 1.7 | 0.0 |

*Height based on yearly increments of growth.

above 77 mm long (chowder) in the third year (grade classifications by Godwin, 1967). This growth rate is about $3\frac{1}{2}$ times greater than that recorded for northern quahogs (Kerswill[2]) and more than twice that of northern quahogs transplanted to southern waters (Menzel, 1961).

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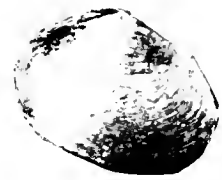

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# NUCLEIC ACID AND NUCLEIC PROTEIN PATTERNS IN VEGETATIVE STAGES OF THE HAPLOSPORIDAN OYSTER PARASITE, *MINCHINIA NELSONI* HASKIN, STAUBER AND MACKIN [1]

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ABSTRACT

*Nucleic acid and nucleic protein patterns in plasmodial stages of* Minchinia nelsoni *were studied in infected oyster hemolymph and sectioned tissue. DNA was found adjacent to the nuclear membrane in small (1 to 2μ) nuclei. The DNA appeared as a thin spherical structure with evenly spaced nodes. A similar, more ovoid DNA structure was generally found centrally located in large nuclei. From 1 to 4 DNA masses of variable size and shape were often found in the cytoplasm of individual parasites. The nuclear membrane, nucleolus, and cytoplasm were rich in RNA and in addition, a spherical RNA mass of variable size was often found in the cytoplasm. Histone was found in all DNA locations. The entire nucleus was often positive for protamine. The implications of these observations are discussed.*

## INTRODUCTION

*Minchinia nelsoni*, a haplosporidan parasite of the American oyster, *Crassostrea virginica*, was first reported in the spring of 1957 when approximately half the oysters planted on the New Jersey oyster grounds in Delaware Bay died within six weeks (Haskin, Stauber and Mackin, 1966).

Few cytochemical observations have been reported on members of the genus *Minchinia* or the closely related genus *Haplosporidium* as revised by Sprague (1963a). Sprague (1963b) reported the Feulgen nucleal test for DNA was not helpful in studying *Minchinia louisiana*, a parasite of the mud crab, *Panopeus herbstii*. The chromosomes of the parasite were so small and poor in chromatin that they could not be sharply resolved. Farley (1965) found acid-fast properties in the spores of *Minchinia costalis* (Wood and Andrews) another haplosporidan parasite of *C. virginica*, but in 235 oysters infected with *M. nelsoni* he found no acid-fast spores. The definitive spore stages of *M. nelsoni* were described by Couch, Farley and Rosenfield (1966), and Farley (1967) reported that the acid-fast reaction was probably an indication of spore maturity since the sporoplasm of immature spores first stained pink and at maturity a bright red.

No cytochemistry is described for the eight species of *Haplosporida* reported prior to 1935. Recently, Taylor (1966) reported a new species, *Haplosporidium tumefacientis*, a disease organism of the California sea mussel, *Mytilus californianus*. He found no Feulgen-positive structures in plasmodial stages of the parasite, but several small spots (endosomes) were weakly positive in the nucleus of the mature spore. No periodic acid-Schiff (PAS) positive material was found in plasmodial stages, but a thickened portion of the nearly mature spore wall and the cytoplasm of mature spores were strongly positive. The cytoplasm of mature spores had acid-fast properties.

Woolever (1966) reported cytochemical observations on the Haplosporidan, *Nephridiophaga blattellae*, which parasitizes the malpighian tubules of the German cockroach, *Blattella germanica*. Plasmodia were PAS-negative; developing spores were faintly positive, but mature spores were deeply PAS-positive and acid-fast. She reported observations on the Feulgen nucleal test

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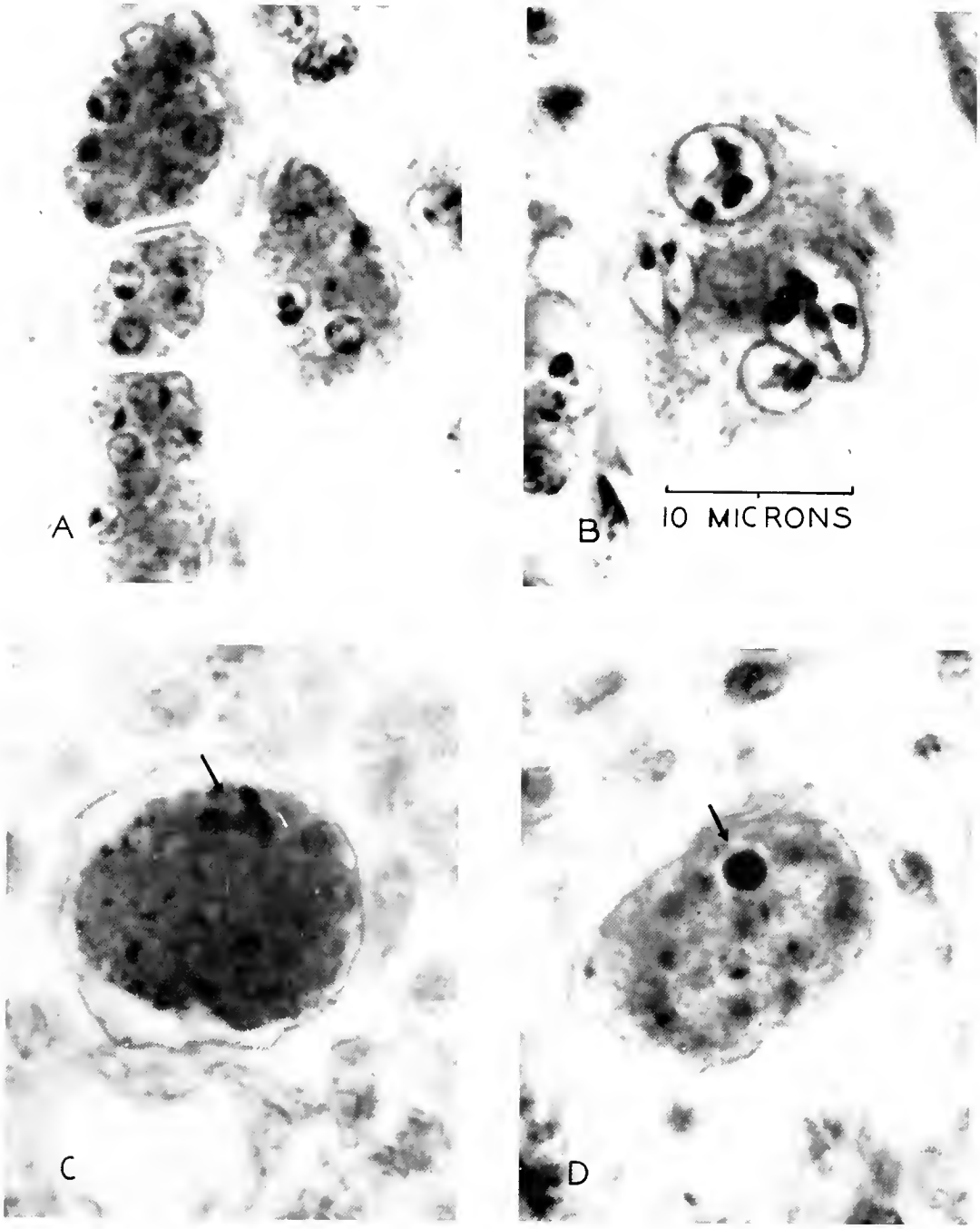


FIG. 1. M. nelsoni *plasmodia in oyster tissue all photographed at same magnification. Davidson's fixation and iron hematoxylin stain. A. Note spherical nuclei, each with a peripheral nucleolus and a variable number of smaller, peripheral endosomes; B. Note large, vacuous nuclei with a variable number of randomly spaced nucleoli; C. Note irregularly-shaped cytoplasmic DNA mass (arrow); D. Note spherical cytoplasmic RNA mass (arrow).*

and stressed the importance of this test in any description of life cycle stages.

Since 1958, thousands of oysters collected from Delaware Bay were found to be infected with *M. nelsoni*, but only four contained spores similar to the spore described by Couch *et al.* (1966). In the absence of the definitive spore stage, the organism has been identified on the basis of plasmodial characteristics. The cytochemistry of these plasmodial characteristics is the subject of this paper.

## METHODS

Since 1958, samples of 20 oysters each have been collected at monthly intervals from various planting grounds in Delaware Bay. Most samples were routinely fixed in Davidson's fluid (Shaw and Battle, 1957), sectioned at 5 $\mu$, stained with iron hematoxylin, acid fuchsin, and aniline blue (Gray, 1954) and examined for the presence of *M. nelsoni*. To investigate the cytochemistry of the parasite, some of the samples during this period were treated in the following manner: the hemolymph was collected from severed muscles of well-drained oysters. Equal portions from individual oysters were fixed in 15 ml of 10% filtered formalin sea water (10% F. S. W.) and in Zenker's acetic fixative. A portion of each oyster was then fixed in each of three fixatives — 10% F. S. W., Zenker's acetic, and Davidson's. Tissue was embedded in Paraplast [2] and sectioned at 5 $\mu$. Hemolymph samples were processed by spinning the cells down at 2000 rev/min and decanting the various reagents. The following cytochemical tests were performed.

*The Feulgen Nucleal Test* (Lillie, 1965). Hemocytes and tissue sections fixed in 10% F. S. W. and Zenker's acetic were hydrolyzed 16 min in 1 N. HCl at 50° C. Optimal hydrolysis time for tissue fixed in Davidson's was 8 min. Following hydrolysis, hemocytes or sections were stained 10 min in Schiff's reagent, rinsed three times in 0.5% sodium bisulfite, rinsed in tap water, counter stained in fast green, dehydrated through a routine alcohol-xylene series and mounted in Permount.

*The Azure B Differential Stain for Nucleic Acids* (Flax and Himes, 1952). Hemocytes and sections were brought to water and stained in 0.25 mg/ml Azure B adjusted to pH 4.0 with Lillie's citrate buffer. DNA stains a blue-green and RNA a deep blue-purple. Slides were then washed in buffered water and dehydrated in the usual manner.

*DNase Digestion* (Ris and Plaut, 1962). Hemocytes and sections were digested in deoxyribonuclease (Worthington, 1 x crystallized 0.3 mg/ml in 1/4 strength McIlvaine buffer at pH 7.0 with 4 x $10^{-3}$ mole of $MgSO_4$ per liter of solution), 5 hr at 40° C. Control slides, minus DNase, were prepared.

*RNase Digestion* (Flax and Himes, 1952). Hemocytes and sections were digested in ribonuclease (Worthington, crystallized in EtOH 10 mg/100 ml of 1/4 strength Sörensen's phosphate buffer pH 6.4) 4 hr at 40° C. Control slides, minus RNase, were prepared.

*The Alkaline Fast Green Procedure* (Alfert and Geschwind, 1953). This test demonstrates the nucleoprotein, histone, while extracting the more labile nucleoprotein, protamine. Sections fixed in 10% F. S. W. were hydrolyzed 15 min in 5% trichloroacetic acid at 90° C, followed by three 10 min washes in 70% ethanol. Slides were stained 10 min in a 0.1% solution of fast green adjusted to pH 8.2 $\pm$ 1 with sodium hydroxide. Slides were then differentiated 5 min in distilled water and dehydrated in the usual manner.

*The Picric Acid-Eosin Y Procedure* (Block and Hew, 1960). Since this test stains both histone and protamine it must be used in conjunction with the alkaline fast green procedure which extracts protamine. Sections fixed in 10% F. S. W. were hydrolyzed 6 hr in a saturated picric acid solution at 60° C. Without washing in 70% ethanol slides were stained 3 hr in eosin Y, brought to pH 8.2 $\pm$ 1 with sodium hydroxide. Slides were dehydrated in the usual manner.

*The Periodic Acid-Schiff (PAS) Test* (Casselman, 1959). In this test for carbohydrates, slides or hemocytes fixed in 10% F. S. W. were brought to water and thoroughly washed; oxidized 19 min at room temperature in 0.5% aqueous periodic acid; rinsed; stained 10 min in Schiff's reagent; rinsed in 0.5% sodium bisulfite; rinsed in tap water, then dehydrated in usual manner. Unoxidized controls were prepared.

*Giemsa* (Lillie, 1965). Hemocytes and tissue sections were routinely stained in Giemsa.

## OBSERVATIONS

After being fixed in Davidson's fluid and stained with iron hematoxylin, plasmodial stages of *M. nelsoni* are roughly spherical, usually from 4 to 30 $\mu$ in diameter with one to over 300 nuclei from 1.0 to 7.5 $\mu$ in greatest width. Because of the large size of some plasmodia the number of nuclei was difficult to assess. With 5 $\mu$ sections some of the largest plasmodia were sectioned three and four times. Approximately 100 nuclei were observed in some of these 5 $\mu$ sections and it was estimated that some plasmodia had over 300 nuclei. Fig. 2 A shows one such plasmodium, over 25 $\mu$ in width,

[2] Tissue embedding medium manufactured by Biological Research, Inc.

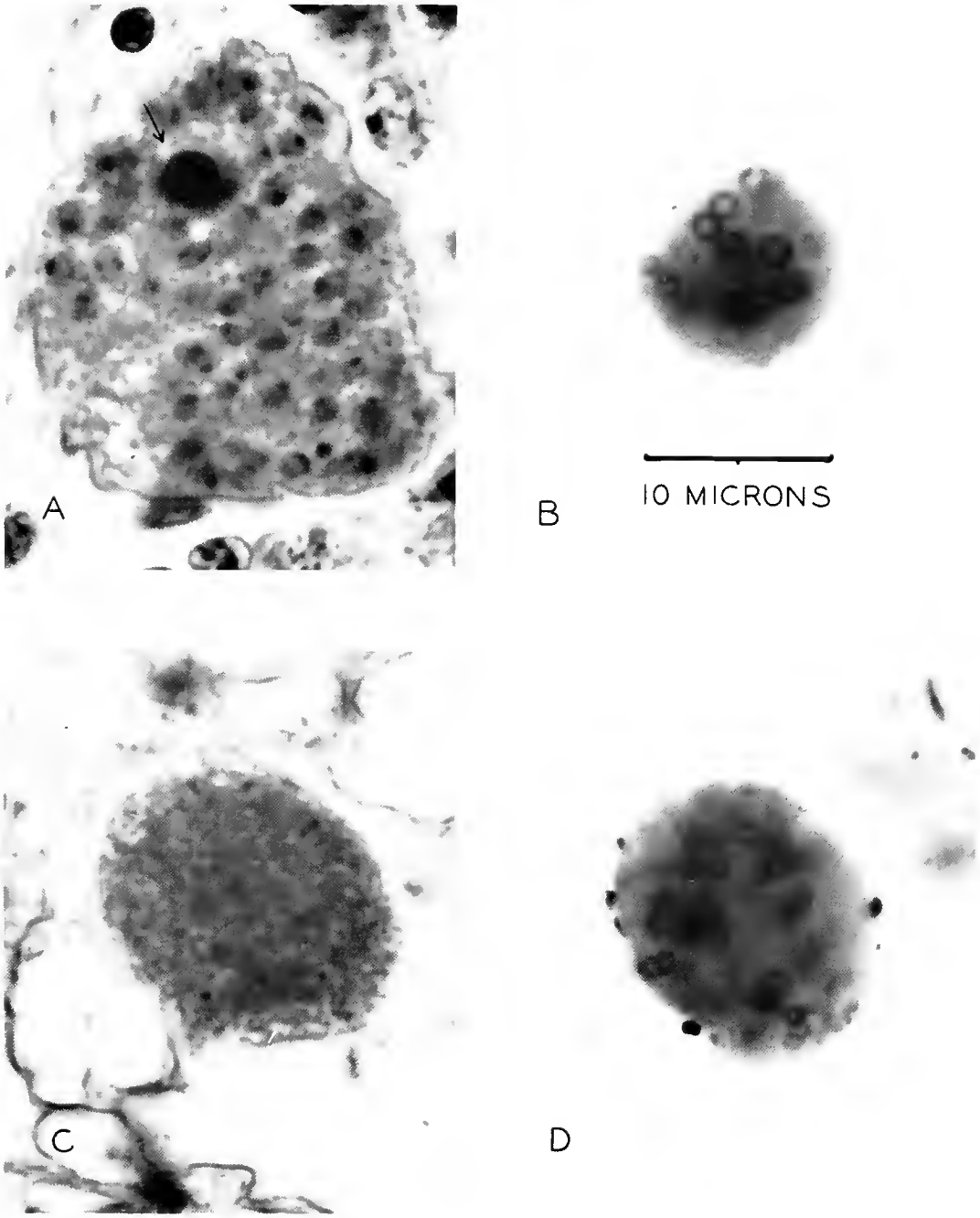


FIG. 2. M. nelsoni *plasmodia. A and C in oyster tissue. Davidson's fixation and iron hematoxylin stain. B and D in oyster hemolymph. 10% F. S. W. fixation and Feulgen nucleal stain. A. Note large number of nuclei and spherical cytoplasmic RNA mass (arrow); B. Plasmodium in hemolymph with Feulgen-positive nuclear DNA; C. Plasmodial type associated with presence of Feulgen-positive "rings"; Davidson's fixation and hematoxylin stain. Note small nucleoli and indistinct nuclear membranes; D. Plasmodium in hemolymph with clusters of small Feulgen-positive "rings".*

with approximately 50 nuclei visible in a field with a depth of focus of only 3 $\mu$.

Two nuclear types were most common: 1) small spherical nuclei, 1.0 to 2 $\mu$ in diameter with one prominent endosome adjacent to the nuclear membrane, an inconspicuous intradesmose, and a variable number of chromatin clumps regularly spaced on the nuclear membrane (Figs. 1A, 3A).

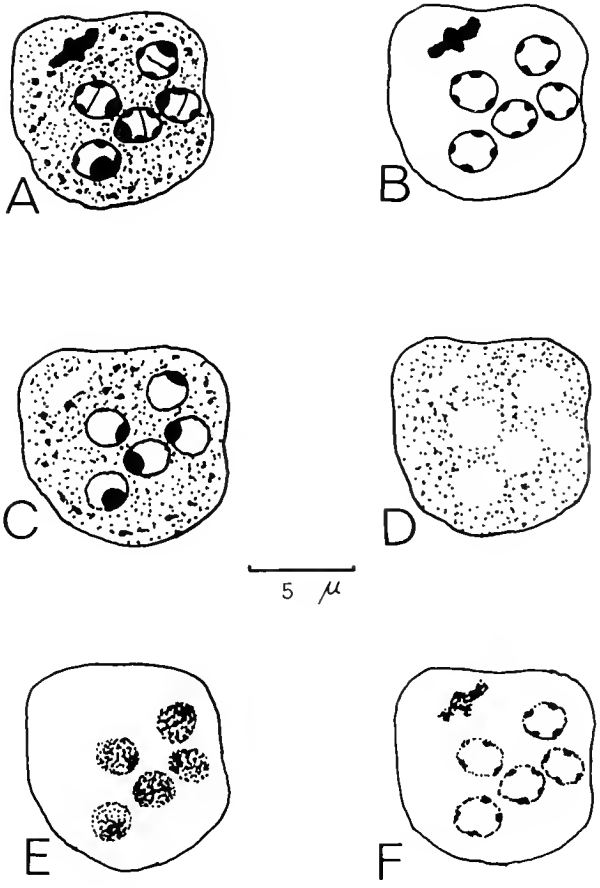


FIG. 3. *Idealized drawings of* M. nelsoni *plasmodium with small, spherical nuclei. A. Davidson's fixation and iron hematoxylin stain; B. DNA sites; C. RNA sites; D. PAS-positive sites; E. Protamine sites; F. Histone sites.*

2) Vesicular, spherical or ovoid nuclei, 2.0 to 7.5 $\mu$ in greatest width with a prominent intradesmose and a variable number of large endosomes generally near the center of the nucleus but also found adjacent to the nuclear membrane (Figs. 1B, 4A).

The Feulgen nucleal test for DNA was confirmed by extracting the Feulgen-positive sites with DNase. Tissue fixed in Davidson's or 10% F. S. W. showed incomplete digestion after 5 hr, but complete digestion after fixation in Zenker's acetic. The following structures in plasmodial stages of *M. nelsoni* were Feulgen-positive with the presence of DNA confirmed with DNase digestion. In the small spherical nuclei a thin film of DNA is always found adjacent to the nuclear membrane. Evenly spaced on this film are DNA nodes which correspond in size and position to the chromatin nodes of hematoxylin preparations (Figs. 1A, 3A, 3B). A similar DNA structure is often found in the larger vesicular nuclei, but it is more ovoid in shape, stains with less intensity and is more centrally located in the nucleus (Figs. 1B, 4A, 4B). No DNA is apparent in many of these larger nuclei. The alkaline fast green test indicated the presence of histone associated with the DNA in both nuclear types (Fig. 3F).

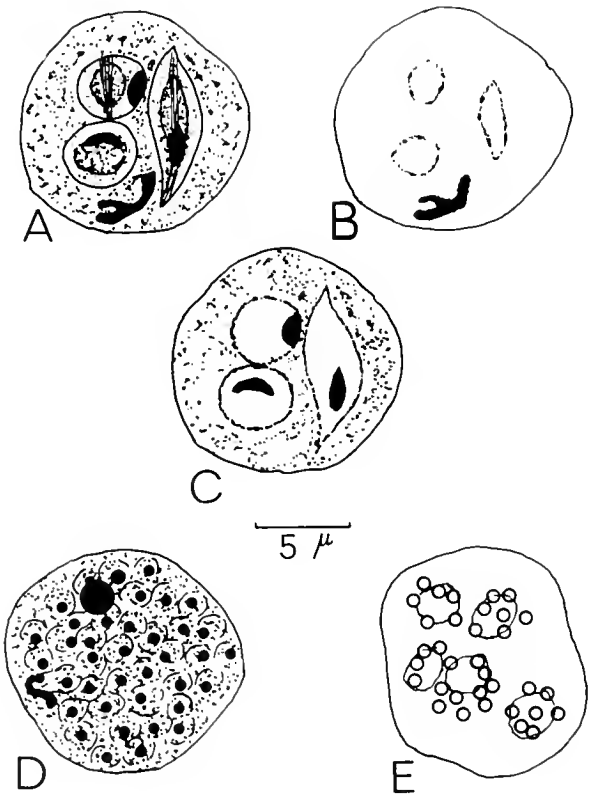


FIG. 4. *Idealized drawings of* M. nelsoni *plasmodia. A. With large vacuolated nuclei; Davidson's fixation and iron hematoxylin stain; B. DNA sites; C. RNA sites; D. Plasmodial type with Feulgen-positive "rings"; Davidson's fixation and iron hematoxylin; note small endosomes and indistinct nuclear membranes; E. Feulgen-nucleal stain; "rings" clustered around faintly positive nuclear DNA.*

A second type of DNA structure often found in plasmodial stages is an irregularly shaped mass, 0.5 to 4.0 $\mu$ in greatest width, located in the cytoplasm, generally in a peripheral position in the cell (Figs. 1C, 3A, 3B). From 1 to 3 such structures were found in some plasmodia, but were absent in many others. During a three-month period, September-November, 1964, the parasites in hemolymph samples were surveyed for the presence of this cytoplasmic DNA mass. The data indicate that 40 to 96% of the plasmodia from individual oysters had no apparent DNA mass; 4 to 54% had one; and 0 to 8% had two. Besides the variability in its size, shape and prevalence, this DNA structure varies in its cytochemistry. The structure is inconsistently positive for histone and may stain either red or blue with Giemsa. These observations suggest that the structure is unstable. The red-blue shift with Giemsa stain may indicate a change in the acid-base dominance of the DNA-histone complex.

A third type of Feulgen-positive structure was sometimes observed in *M. nelsoni* plasmodia from hemolymph samples. The structure was infrequently found, but when present was generally abundant in individual oysters. It appeared as intense Feulgen-positive "rings" 0.5 to 1.0 $\mu$ in diameter that apparently were spherical in shape. The smallest "rings" (Figs. 2D, 4E) were generally found in clusters, sometimes around Feulgen-positive structures identical in appearance to nuclear DNA (Fig. 3B). The largest "rings" (Fig. 2B) were intensely Feulgen-positive; however, if the hemolymph samples were stored several days in 70% ethanol much of the stain intensity was lost. This loss in stain intensity revealed node-like structures identical to nuclear DNA. The "rings" were found only in hemolymph samples. When tissue from these same oysters was embedded and sectioned, no "ring-like" structures were found, only the type of nuclear DNA illustrated in Fig. 3B. Apparently this intense-staining, Feulgen-positive material is quite labile, and confirmation of DNA with DNase digestion was not possible. Hematoxylin preparations of this tissue show unusual stages of the parasite. Many plasmodia have clusters of very small nuclei (less than 1 $\mu$ in diameter) with indistinct nuclear membranes (Figs. 2C, 4D). There is a central endosome in each nucleus which corresponds to the size of the Feulgen-positive "rings" found in the blood preparation. In tissue section, the Azure B stain for nucleic acid sometimes stains these small endosomes a light blue-green, the characteristic color of DNA, but most often they stain a deep blue-purple, the color of RNA. Treatment with RNase indicates that this material is resistant to digestion. A decrease in stain intensity was effected, but complete digestion was not always accomplished. The largest "rings" (Fig. 2B) were generally in great abundance in individual oysters, indicating synchronous development of the parasites.

RNA, as demonstrated with Azure B and confirmed with RNase digestion, was concentrated in four regions. As expected, the nucleoli in all stages of the parasite were rich in RNA, and with the exception of the stages associated with the Feulgen-positive "rings", were readily digested with RNase (Figs. 3C, 4C). RNA was heavily concentrated at the nuclear membrane and masked the DNA often found at this site. The cytoplasm varied from a fine granular material, rich in RNA, to a transparent material containing coarse RNA granules. Occasionally an infected oyster was found in which many of the plasmodia had one or more perfect spheres (1 to 5 $\mu$ in diameter) of RNA in the cytoplasm (Figs. 1D, 2A). Like the irregular cytoplasmic DNA mass described earlier, it stained either red or blue with Giemsa. This RNA sphere was readily digested with RNase.

The nuclei in plasmodial stages of *M. nelsoni* were conspicuously lacking in PAS-positive material. They were outlined by fine PAS-positive granules in the cytoplasm (Fig. 3D). The intensity of this test was considerably greater after 10% F. S. W. fixation than after Davidson's fixation.

Extensive tests for protamine were not conducted, but the hemolymph from five heavily infected oysters was examined. Protamine appeared to be evenly distributed in the nucleoplasm (Fig. 3E), and was particularly abundant in an infrequently found stage of the parasite where the nuclei appear to be "paired".

## DISCUSSION

The small amount of hemolymph available for cytochemical studies was a serious drawback because adequate control slides sometimes could not be made. The main advantage of this type of sample was that whole parasites could be studied rather than sections of parasites as in tissue preparations. The presence or absence of such structures as the cytoplasmic RNA and DNA masses could be determined only from whole parasites. The use of both tissue and hemolymph samples overcame the drawback of inadequate hemolymph controls.

Considerable variation was obtained with the various fixatives. The results of the Feulgen nucleal test were dependent upon the type of fixation and storage time of samples prior to test. The staining reaction was most intense a short time after hemolymph samples were fixed in 10% F. S. W. The intensity of the staining reaction decreased with time, and a considerable loss occurred when samples were stored for a number

of days in 70% ethanol. If tissue samples fixed in Davidson's were stored several weeks in Davidson's storage solution, all Feulgen-positive staining properties were lost. The DNA in *M. nelsoni* seems to be especially prone to loss in contrast to host DNA. Perhaps the poor results obtained with the Feulgen nucleal test by Sprague (1963b) and Taylor (1966) were due to this type of loss.

The plasmodial stages of *M. nelsoni* are similar to those of other species of *Minchinia*, such as *M. chitonis* (Debaisieux, 1920), *M. limnodrili* (Granata, 1914), *M. cernosvitovi* (Jirovec, 1936), *M. louisiana* (Sprague, 1963b); however, some significant differences were observed. The above authors illustrated nuclear division following development of a prominent achromatic structure. A similar achromatic structure occurred frequently in *M. nelsoni*, but division of the nucleolus followed by division of the nucleus, as described by the above authors, was rarely observed.

Farley (1967), in a proposed life cycle of *M. nelsoni*, related size of nuclei to stages in asexual nuclear division. He described a succession of events culminating in a nuclear division similar to that described by the above authors. He indicated, however, that nuclei in telophase were only occasionally observed.

Woolever (1966) found that without the Feulgen nucleal test it would have been possible to write a history of sexual processes in *N. blattellae* based upon an appropriate series of bodies revealed by other stains, particularly Giemsa. She stated that this was often done before the advent of the nucleal reaction. Hematoxylin is especially deceptive because it stains RNA and DNA indiscriminately, and the sequential changes in these nucleic acids must first be established before any description of nuclear events can be made.

In plasmodial stages of *M. nelsoni* the large, vacuous nuclei do not develop in a consistent manner. Endosomes number from one to more than four and are variable in size and position in the nuclei. The achromatic structure and even the nucleus are often "ragged" in appearance. This suggests an abortive nuclear division or a degenerating stage of the parasite. Even though the nuclei varied in appearance, the arrangement of the DNA, as revealed by the Feulgen test, remained as a spherical or ovoid structure with evenly spaced nodes (Figs. 3B, 4B). This suggests there is no fundamental difference in these nuclear variations.

The structure of the nuclear DNA was similar with all fixatives investigated. Perkins' (1968) study on the fine structure of plasmodial stages of *M. nelsoni* suggests that the DNA nodes correspond to the secondary endosomes he described. This nuclear DNA structure is so consistently found and so distinctive in appearance that its functional aspect must be considered.

The stages of *M. nelsoni* that are associated with the presence of the intense Feulgen-positive "rings" suggest that a form of nuclear fission may occur in addition to the type of nuclear division described by Farley (1967). The cluster of small "rings" may originate at the site of the DNA nodes on the nuclear membrane. The number of "rings" in each cluster corresponds to the number of DNA nodes and suggests that one parent nucleus may give rise to as many as 10 daughter nuclei in one division. The unstable properties of the "rings" suggests that they may be newly synthesized DNA. Perkins (1968) stated that evidence for nuclear division was rare, but indicated either elongation and pinching of nuclei or multiple fission. He showed an electron micrograph of four plasmodial daughter nuclei that were apparently products of multiple fission.

Other evidence indicates that multiple fission may occur. The small size and resistance to RNase digestion of the nucleoli found in plasmodial stages associated with the "rings" (Fig. 2B) suggest that the nucleoli are being resynthesized. Mauramatsu, Steele, Hodnett and Busch (1966) have shown the nucleolus to be the site of ribosomal RNA synthesis, and RNA still complexed with the DNA template is resistant to RNase. If multiple fission occurs in the manner described, the nucleolus and the nuclear membrane both disappear during division. This type of nuclear fission could explain the presence of the irregular-shaped cytoplasmic DNA mass and the spherical cytoplasmic RNA mass. These may be residual material from the parent nucleus following division, and are most often found in plasmodia with relatively small nuclei (Figs. 1C, 1D and 2A). The DNA in these small nuclei was much more intensely Feulgen-positive than in the large, vacuous nuclei and it is possible, as Woolever (1966) indicates for stages of *N. blattellae*, that this condition indicates recent division.

The DNA nodes found on the nuclear membrane in *M. nelsoni* may correspond to the nodes found in the macronucleus of the ciliate *Stentor coeruleus*. Schwartz (1935) has shown that a single node can support regeneration and survival. Recently, Zech (1966) has shown that each node is autonomous and contains at least one complete genome. In another diverse example of multiple genomes, Frankel (1966) has shown that bacteriophage $T_4$ nucleic acid exists in a replicating and a mature state. Replicating phage $T_4$ DNA is considerably longer than mature phage DNA and is thought to be a long molecule containing repeating units of information which give rise to terminally redundant individual chromosomes.

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# PRELIMINARY STUDY ON THE EPIZOOTIOLOGY AND HOST-PARASITE RELATIONSHIP OF *PARAMOEBA* SP. IN THE BLUE CRAB, *CALLINECTES SAPIDUS*

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ABSTRACT

*Infections with* Paramoeba *sp. (Sprague and Beckett, 1966, 1968) in blue crabs,* Callinectes sapidus, *were studied in the summer of 1968. Peeler (pre-molt) crabs were examined in commercial plants during a peak period of mortality, and hard (inter-molt) crabs taken by otter trawl were examined after the mortality subsided. Thirty-five percent (43 of 121) of the peeler and 8% of the hard crabs (12 of 156) were positive by hemolymph examination. The hemolymph was heavily parasitized in 31 peeler crabs and five of the hard crabs. The rate of infection decreased to a low level after the initial episode of serious mortality. Two groups of hard crabs dredged from Chincoteague Bay in November 1968 were negative for parasites in their hemolymph (0 of 30). Histopathologic studies will be required to determine whether there is a tissue phase of* Paramoeba *sp. that is not detectable by hemolymph examination.*

## INTRODUCTION

Unidentified amoeboid cells were discovered in dead or dying blue crabs, *Callinectes sapidus*, from Chincoteague Bay, Virginia, by Sprague and Beckett (1966). The abdomens of infected crabs were gray, and their appendages contained cloudy hemolymph and watery tissue. The disease became known as "gray crab disease" because of the condition of the exoskeleton. The amoeboid cell was later identified as a marine amoeba, *Paramoeba* sp. (Sprague and Beckett, 1968). The amoebae characteristically had typical nuclei and secondary nuclei or "Nebenkörper," and were morphologically similar to *Paramoeba eilhardi*, a free-living marine species described by Schaudinn (1896). After a comparative study of the two species, Sprague, Beckett, and Sawyer (in press) described the one in the crab as new.

The relationship of *Paramoeba* sp. to serious crab mortalities along the Atlantic coast is of special concern because of the economic value of these shellfish (Lunz, 1968). Although amoebae have been found routinely during peak periods of mortality, relatively little is known about their role in dead or dying crabs. The first attempt to estimate the number of infections during and after a mortality was made in Chincoteague Bay, Virginia, in the summer of 1968. The results of the study are summarized in this report.

## METHODS

Three groups of blue crabs collected in or near Chincoteague Bay, Virginia, in 1968 were examined for the presence of parasites in the hemolymph. Peeler crabs in late stages of ecdysis were supplied by commercial dealers during a peak of mortality in June; a collection of mostly hard crabs was taken by 10-minute tows with an otter trawl in July and August; and a collection of hibernating crabs was made in November by Mr. F. William Sieling III, Department of Chesapeake Bay Affairs, Annapolis, Maryland.

Smears from small amounts of hemolymph from a cut appendage were fixed, while moist, in Davidson's fixative. After 4 to 5 hr of fixation the slides were washed and stored in 95% alcohol for subsequent staining with a modified hematoxylin stain described by Mitchell (1966). Tissues from infected crabs were fixed in Davidson's, rinsed in 95% alcohol and stained with hematoxylin.

Size and sex were determined for crabs in each

TABLE 1. *Summary of Infections of Blue Crab,* Callinectes sapidus, *with* Paramoeba *sp.*

| Source | Number of Crabs | | Type of Infection[a] | | Type of Amoebae |
|---|---|---|---|---|---|
| | Total | Infected | Heavy | Light | (Large: small: mixed) |
| Snow Hill Public Landing, Md.[b] | 9 | 9 | 8 | 1 | 1:3:5 |
| Snow Hill Public Landing, Md.[c] | 12 | 12 | 11 | 1 | 10:2:0 |
| Snow Hill Public Landing, Md.[d] | 100 | 22 | 12 | 10 | 14:5:3 |
| Chincoteague Bay, Va.[e] | 156 | 12 | 5 | 7 | 6:3:3 |

[a]Heavy — one or more per field; light — less than one per field

[b]Peeler crabs, June 1968

[c]Peeler crabs, June 1968 (selected gray group)

[d]Peeler crabs, June 1968 (random group)

[e]Hard crabs, July, August 1968

of the three collections and the presence or absence of amoebae was noted. Photomicrographs of stained tissue sections were made with a Zeiss microscope, a dark green No. 58 filter and Kodak Plus X film.[1]

## OBSERVATIONS

### *Amoebic Infection in Peeler Crabs*

Two lots of peeler crabs were obtained during a period of mortality in June 1968. The first group consisted of nine symptomatic crabs selected from shedding floats by a commercial dealer at Snow Hill Public Landing, Maryland, on Chincoteague Bay (Table 1). The crabs were collected alive and stored in a refrigerator at 5°C, but were dead when examined 4 hr later. Hematoxylin-stained smears showed that all were infected with *Paramoeba* sp. Small amoebae measured 5 to 10 $\mu$, and large ones measured up to 23 $\mu$ (Table 2). Hemocytes were rare or absent in the hemolymph of heavily infected crabs.

The second group consisted of 112 peeler crabs supplied by a commercial dealer in Oxford, Maryland, that had been purchased from the dealer in Snow Hill Public Landing, Maryland. Most of the crabs were alive when hemolymph smears were made, but 86% died within the next 4 days. One hundred specimens in this group were selected randomly and 12 were selected on the basis of their gray color. Twenty-two of the random group and all 12 of the gray group were infected with *Paramoeba* sp. The infections ranged from light to heavy (Table 1) and the amoebae were of several size classes in different crabs (Table 2).

### *Amoebic Infection in Hard Crabs*

Amoebae were found in the hemolymph of only 12 of 156 hard crabs collected in Chincoteague Bay, Virginia, during the 2 months that followed the large-scale mortality in June 1968; only 5 of the crabs had heavy infections (Table 1). In two crabs the infections were so light that they were not discovered until stained smears were examined a second time. The low infection rate coincided with the absence of serious mortality in commercial crab operations. None of the crabs showed signs of gray discoloration, but several were inactive or lethargic when caught. The lethargic crabs were slow to recover when placed upside down. Five of the lethargic crabs, later found to be infected, died within 1 hr after capture. Signs of lethargy could not always be attributed to infection, however, because a few crabs were injured in the otter trawl.

Two groups of 15 crabs each were dredged from the bottom of Chincoteague Bay in November 1968; none had amoebae in the hemolymph. The hemolymph from these groups ranged from clear to pale orange or rust-colored. The orange color was not observed in crabs examined during the summer.

### *Observations on* Paramoeba *sp. in Crab Tissues*

Stained sections of gill, heart, intestine, hepatopancreas and muscle showed amoebae in the vascular spaces. Heavily infected crabs had amoebae in the vascular channels but organs or tissues were not infected (Figs. 1, 3, 5). Crabs with light infections usually had amoebae of the large type, some of which appeared to be surrounded by hemocytes or tissue cells (Figs. 2, 4, 6), and the intestinal wall appeared to be the

[1] Trade names referred to in this publication do not imply endorsement of commercial products.

TABLE 2. *Measurements (microns) of* Paramoeba *sp. from the Blue Crab,* Callinectes sapidus.[a]

| Source | Length | | Width | | Number |
|---|---|---|---|---|---|
| | Range | Mean | Range | Mean | |
| Hemolymph | 10.8 - 22.5[b] | 16.2 | 6.3 - 17.1 | 11.4 | 25 |
| Muscle | 9.0 - 21.6[b] | 12.6 | 5.4 - 12.6 | 8.9 | 20 |
| Muscle | 4.5 - 19.8[b] | 10.3 | 4.5 - 18.0 | 7.6 | 25 |
| Hemolymph | 4.5 - 16.2[b] | 9.4 | 2.7 - 11.1 | 6.6 | 25 |
| Heart | 4.5 - 13.5[b] | 9.2 | 3.6 - 10.8 | 7.2 | 25 |
| Hemolymph | 3.6 - 10.8[c] | 6.1 | 3.6 - 6.3 | 4.6 | 25 |
| Hemolymph | 2.7 - 7.2[c] | 4.9 | 2.7 - 4.5 | 3.8 | 25 |

[a]Different crabs used for each set of measurements

[b]Large type. [c]Small type

only solid tissue that was invaded by parasites (Fig. 4). Observations on crabs with light infections suggested a tissue response against the amoebae. Histopathologic studies of the infection are in progress to obtain further information on the pathogenesis of *Paramoeba* sp.

Stained slides and histologic sections of infected crab tissues showed that the nucleus and secondary body always were present in the amoebae. A few specimens showed two secondary bodies in addition to the nucleus. The large amoebae were lobose, ranged up to 23 $\mu$ in length, and often had clear vacuoles in the cytoplasm; the small ones were spherical and ranged up to 11 $\mu$ (Table 2).

Although most of the amoebae were in the vegetative stage of growth, dense spherical forms were observed on several occasions. The spheres were granular and showed a circumferential narrow band of ectoplasm that may have been a cyst wall. Nuclei and secondary bodies within the parasites were morphologically similar to those in the vegetative stages. The spherical forms may have been precyst stages as suggested by Sprague and Beckett (1966). The lack of differentiation in these stages indicated that maturation of the cyst probably occurred later in the year, or outside of the crab host. The probability of a free-living period of growth must be considered until the definitive life cycle for *Paramoeba* sp. is determined. Cytological studies to date do not permit the proposal of a tentative life cycle. The route of infection, the extent to which the amoebae reproduce in the crab, and the origin and function of the secondary nucleus are unknown. The secondary nucleus is a valuable and unmistakable diagnostic character for the amoebae, especially in very light infections.

*Influence of the Smear Techniques on Diagnosis*

Microscopic examinations of stained hemolymph smears at different focal planes showed that hemocytes of the crab attached to the surface of the glass slide, whereas amoebae often floated or remained suspended. Variations in the rate of settling accounted for several negative findings that later, upon closer examination, were determined to be positive. The positive cases were overlooked only in unusually light infections. The amount of hemolymph on the smears and the extent to which they were dried before fixation were important considerations. When smears were thoroughly dried before fixation the amoebae were more nearly in one focal plane, but their morphology was distorted and the nuclei were weakly stained. The best results were obtained with smears that were air-dried for several minutes, then fixed while moist.

## DISCUSSION

The discovery of *Paramoeba* sp. in dead or moribund crabs suggests a direct relationship between amoebic infection and mortality. At present, however, it is not possible to state whether amoebae are the direct cause of crab mortality, or whether amoebic infection is coincident to unknown stresses that lead to mortality.

Results of the present study showed that, after the large number of infections recorded for brief peaks of mortality, low levels of infection per-

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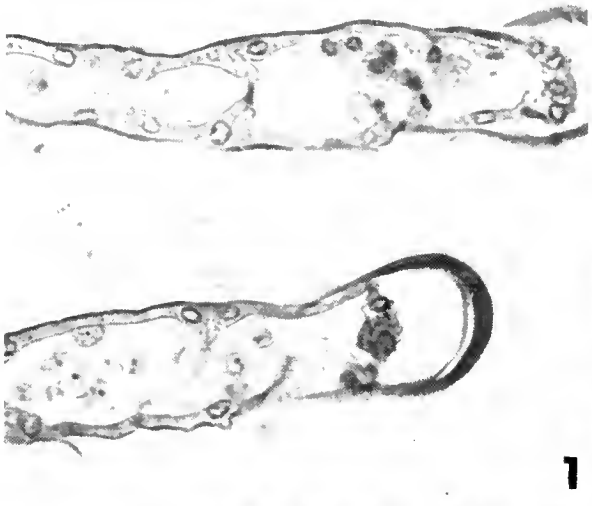
FIGS. 1 — 6 *Sections of* Callinectes sapidus, *infected with* Paramoeba *sp. Hematoxylin stain, 500X. Note large number of hemocytes in FIGS. 2, 4, 6.* FIG. 1 — *Gill filament of heavily infected crab with amoebae in vascular space;* FIG. 2 — *Amoebae in main branch of gill in crab with light infection;* FIG. 3 — *Large number of amoeba in intestinal wall;* FIG. 4 — *Amoebae in wall of intestine of crab with light infection;* FIG. 5 — *Large numbers of amoebae in vascular space of hepatopancreas;* FIG. 6 — *Small number of large amoebae in vascular space of hepatopancreas of crab with light infection.*



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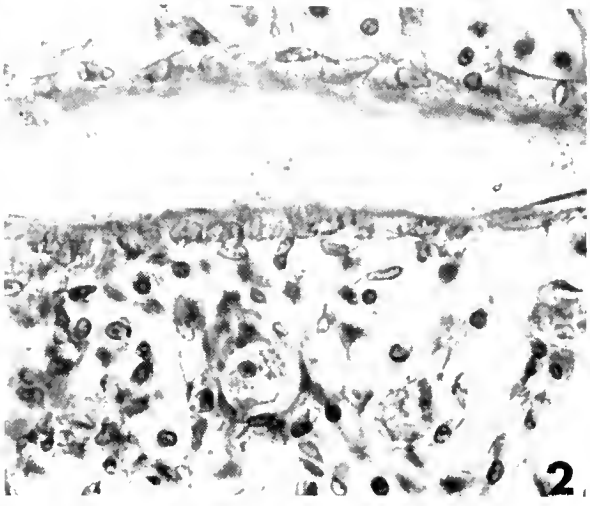

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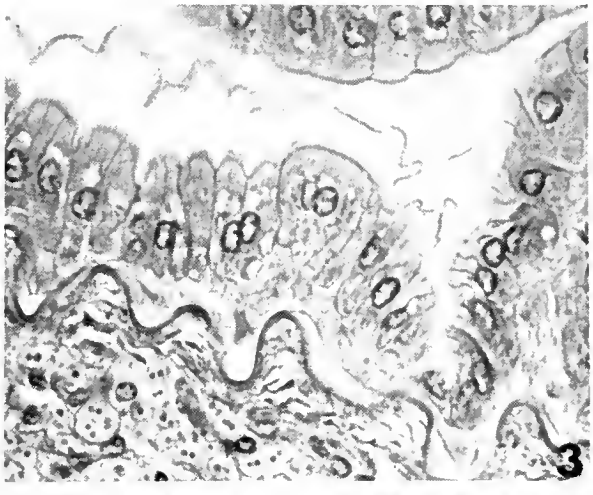

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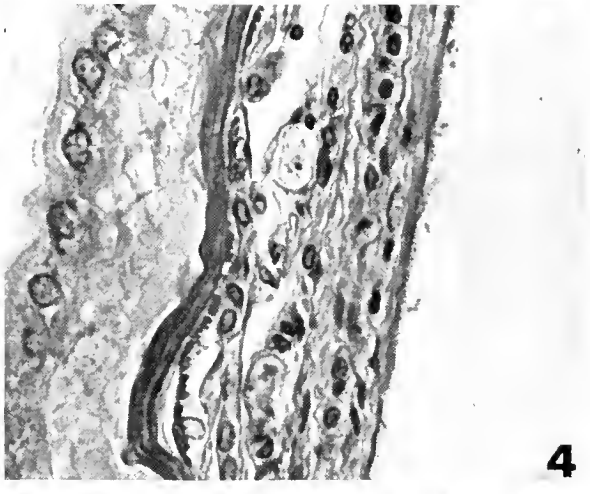

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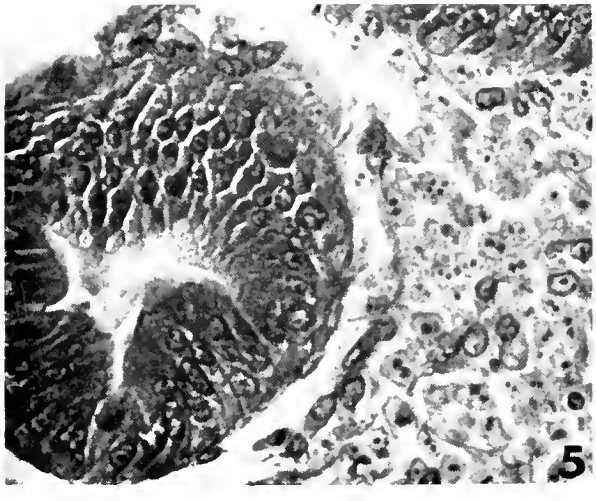

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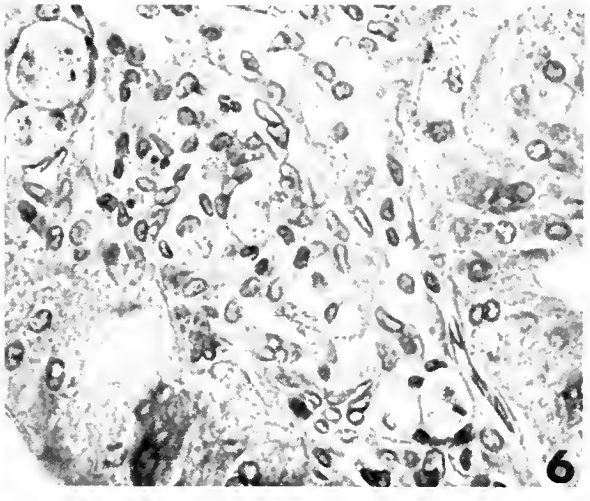

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sisted in crab populations throughout the summer and fall. It is likely that infected crabs died in Chincoteague Bay in the summer and fall, but not at a rate that was of commercial significance. The absence of amoebae in hemolymph of hibernating crabs suggests that amoebae are cleared from the circulating hemolymph during the winter. Histologic studies of tissues and organs are in progress to determine if hibernating crabs have tissue infections at times when amoebae are not in the hemolymph. The seasonal cycle of infection is being studied to determine whether new infections are initiated each year, or whether the blue crab is a reservoir for the amoebae during the winter.[2]

The presence of amoebae in the circulatory system of the crab host represents an unusual host-parasite relationship that may involve a cryptic tissue phase of growth. Until the complete life-cycle is discovered it may be speculated that *Paramoeba* sp. initially is a tissue parasite. Rapid proliferation in the tissues could lead to tissue destruction and subsequent entry of amoebae into the circulating hemolymph.

The presence of *Paramoeba* sp. in the intestinal wall of lightly infected crabs suggests that tissue infections may persist from one year to the next, and provide a pathway for re-infection. Future histopathologic studies may disclose whether crabs are exposed each year to new overwhelming infections, or whether small numbers of amoebae in the tissues initiate new infections. Cytologic studies have not disclosed the significance of the large and small form of the amoeba.

[2] *Paramoeba* sp. was found in tissues of 3 of 30 blue crabs dredged from Chincoteague Bay, Va., in January 1969 (personal communication, John A. Couch, Bureau of Commercial Fisheries, Biological Laboratory, Oxford, Maryland.)

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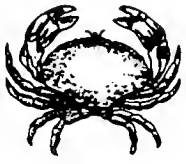

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# *MULINIA LATERALIS:* MOLLUSCAN FRUIT FLY?

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ABSTRACT

*Several characteristics of the clam,* Mulinia lateralis *(Say) (Family: Mactridae), make it adaptable for use in studies of shellfish genetics. It has a short generation period (approximately 60 days), a relatively high reproductive rate (3 to 4 million eggs at a single spawning), reasonable longevity (2 years), sex differentiation is readily discernible through the shell in live specimens in ripe gonad condition (eggs are pink to red to orange and sperm are white), is relatively easy to culture, is small (adults 2.7 to 20.0 mm long), and requires little space for rearing.*

Most commercial mollusks, such as the American oyster, *Crassostrea virginica*, the hard shell clam, *Mercenaria mercenaria*, the surf clam, *Spisula solidissima*, and the soft-shell clam, *Mya arenaria*, require a year or more to attain sexual maturity, have no externally distinguishable sex differentiation, and at least some are presumably protandric. To keep significant numbers of these mollusks under controlled conditions, as required for genetic studies, considerable food, space and flowing sea water are needed.

*Mulinia lateralis* (Say) (Family: Mactridae), commonly known as the coot or little surf clam, has a number of characteristics that could make it useful for determining genetic principles of shellfish. Factors which make it an efficient organism for such study include: (1) short generation time, (2) sex differentiation readily discernible through the shell in live specimens in ripe gonad condition, (3) relatively high reproductive rate, (4) ease of culturing, (5) small space requirements and (6) reasonable longevity. (See Calabrese, 1969, for supporting data.)[1]

I determined from laboratory experiments that *M. lateralis* has a very short generation time. In one experiment fertilized eggs were obtained on 31 October and some clams of this new generation released viable gametes on 8 December, just 39 days later. Usually, however, *M. lateralis* were reared from fertilized egg to sexual maturity from 51 to 135 days, and 60 days was the average generation time. It is conceivable that under ideal conditions a new generation can be established approximately every 39 days.

The eggs of this clam are pink to red to orange and sperm are white; thus, when these clams are ripe the coloration of the gonad, due either to eggs or sperm, is discernible through the thin shell in the umbone region. The gonad color seen through the shells of ripe individuals is the only evidence of sexual dimorphism. Although the maximum length of these animals is 15 to 20 mm, both male and female specimens as small as 2.7 mm were induced to spawn and released viable gametes. This production of eggs at such a small size also suggests that *M. lateralis* is not protandric as are certain other bivalve species.

The fecundity of *M. lateralis* is high, as judged by the number of eggs discharged by individual females at a single spawning, although variable. The number of eggs released appears to depend upon the size of the animal and the degree of development of the gonad at the time of spawning. The greatest number of eggs released by a single female was about 7 million, but from the color of the gonad it was evident that not all

[1] Calabrese, A. 1969. The early life history and larval ecology of the coot clam, *Mulinia lateralis* (Say) (Mactridae: Pelecypoda). A dissertation submitted to the graduate faculty of the University of Connecticut in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

the eggs had been released. Usually, a single female released about 3 to 4 million eggs per spawning.

*M. lateralis* larvae were reared to metamorphosis in containers as small as 1 liter, but 15-liter containers are preferred. Approximately 250,000 larvae can be reared to metamorphosis within 6 to 8 days in 15-liter containers. Post-metamorphosed clams can either be reared in the same containers as were the larvae or transferred to small trays with running water facilities. Maximum development of fertilized eggs to straight-hinge larvae and maximum growth of larvae of *M. lateralis* from Long Island Sound occur at 20.0 and 27.5°C, respectively.

This little clam can be reared easily and with little effort and space under laboratory conditions by methods similar to those described by Loosanoff and Davis (1963). Thus, it can be readily observed at any stage of development. Even though these clams grow rapidly in the laboratory, they achieve faster growth if placed outdoors in boxes of sand kept in tanks of running water during the warmer period of the year. Several different populations can be established within the time period of 3 to 4 months.

The maximum longevity of this clam appears to be 2 years. Several areas of known populations of live clams were sampled yearly over a period of 5 years. It was noted that, after flourishing for at least 1 year, the numbers of live specimens decreased precipitously until practically none could be found. Several populations were also maintained in laboratory holding facilities for at least 1 year and survived a maximum of 2 years.

Because of these factors relating to efficiency of experimentation, *M. lateralis* would be an excellent choice for genetic studies of shellfish.

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# BACTERIOLOGICAL CHANGES IN SHELLFISH MAINTAINED IN AN ESTUARINE ENVIRONMENT[1]

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ABSTRACT

*The accumulation and elimination of certain indicator bacteria by Pacific oysters,* Crassostrea gigas, *and Manila clams,* Tapes japonica, *stored in an estuarine environment under varying conditions were studied. Initially, pilot-scale experiments were conducted to investigate the effects of water temperature, tide and salinity on the bacteriological response of the shellfish. Shellfish and overlying water samples were collected simultaneously at various tide stages and examined for certain microorganisms of public health significance. Bacteriological tests included coliform and fecal coliform MPN's and 20°C plate counts using MacLeod's sea water agar. The results of these experiments indicated that both species of shellfish responded rapidly to bacteriological changes in water quality under all hydrographic conditions. Clams consistently accumulated coliforms and fecal coliforms to higher levels than did oysters. Concentration of the plate count group by both species was similar. Commercial-scale experiments also were conducted to determine the effect of loading on the response of shellfish. The results indicated that shellfish in all parts of the floats responded rapidly to changes in bacteriological quality of the water. Concentrations to unusually high levels were not found in any portion of the floats.*

## I. BACTERIAL ACCUMULATION-ELIMINATION RESPONSE OF PACIFIC OYSTERS *(CRASSOSTREA GIGAS)* AND MANILA CLAMS *(TAPES JAPONICA)* UNDER EXPERIMENTAL WET STORAGE CONDITIONS.

### INTRODUCTION

The response of shellfish under controlled laboratory conditions has been studied by several investigators (Kelly, Arcisz, Presnell and Harris, 1960; Kelly, 1961; and Beck, Kelly, Hoff and Presnell, in press). Their results have shown that oysters and clams exposed to polluted water rapidly ingest and concentrate bacteria to levels higher than that of the surrounding water, thus reflecting the bacteriological quality of the water. However, little work has been done in determining whether similar results would be obtained in shellfish maintained suspended in their natural environment under uncontrolled conditions. Moreover, the effects of changes in water bacterial density, water temperature, salinity and seasonal factors on the accumulation of bacteria by shellfish held under these natural conditions are not known. To this end, a two-part project was initiated to (1) study the effect of wet storage on shellfish with the use of a small-scale float maintained in a commercial shellfish growing area near the laboratory and (2) study the response of shellfish subjected to actual commercial wet storage procedures. The shellfish selected for ex-

[1] Prepared for presentation at 1968 National Shellfisheries Association Convention, 15-18 July 1968, Arlington, Virginia.

[2] A part of the Water Supply and Sea Resources Program of the National Center for Urban and Industrial Health, Cincinnati, Ohio.

perimentation were two commercially important species marketed extensively on the west coast — the Pacific oyster, *Crassostrea gigas*, and the Manila clam, *Tapes japonica*. A total of eight small-scale experiments were completed over a period of approximately 1 year.

## MATERIALS AND METHODS

*Float Constructon*

The shellfish float (Fig. 1) and accompanying baskets used in this study were similar to those described by Sparks and Chew (1961). Styrofoam-filled boxes constructed of marine plywood were used for flotation. Galvanized wire rope clips were bolted to the frame for support of the baskets. Each of the four baskets, measuring 36 in long, 17 in wide and 7 in high, was constructed of marine plywood covered with 0.5 in mesh expanded aluminum. The basket used for clam storage was divided into three equal compartments by two sheets of expanded wire mesh aluminum. Galvanized wire clips were attached to the four corners of each basket for suspension from the float. Each basket was suspended approximately 18 in from the top of the float and 12 in from the bottom by 0.25 in polyethylene rope. To protect against salt water corrosion, the entire float and the baskets were coated with an epoxy resin. As a safety precaution, a stabilizer

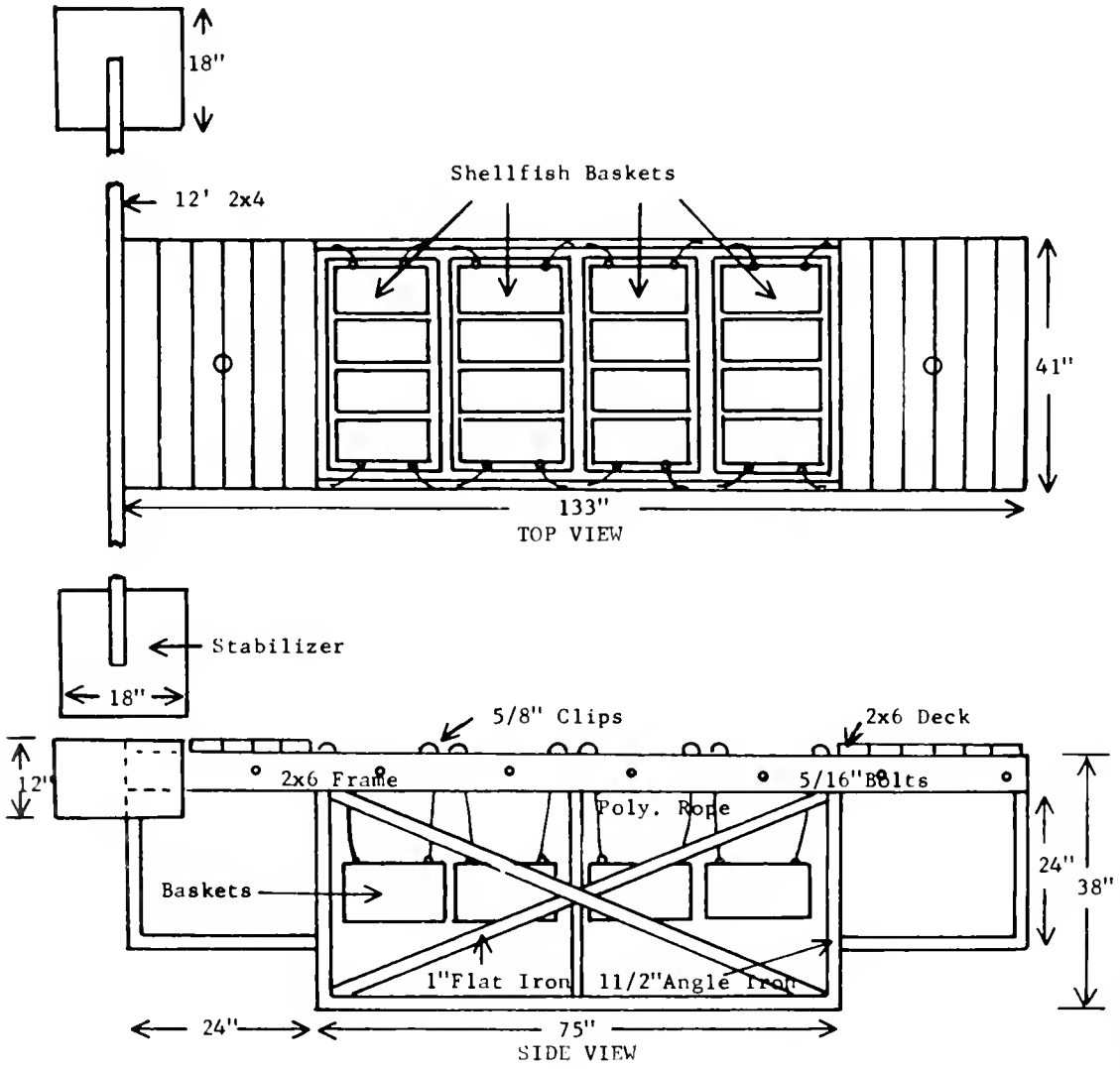


FIG. 1. *Shellfish float.*

consisting of a 12 ft long board with styrofoam-filled boxes at both ends was attached to the float.

*Location of Float*

The float used in these experiments was anchored at the southern portion of Burley Lagoon, immediately west of the laboratory (Fig. 2). Burley Lagoon is a long, narrow tidal estuary, encompassing approximately 370 acres, situated at the head of Henderson Bay and Carr Inlet in the southern part of Puget Sound, Washington. The lagoon is used extensively as a commercial oyster growing area. Two small fresh water streams enter the lagoon, one near the mouth (Purdy Creek) and the other at the head portion (Burley Creek).

The long axis of the float was situated in an east-west direction southeast of the power line towers and about 150 ft from the sandspit. A hydrographic study of the location by Kelley (1964) showed that a large strong eddy existed in the float area. Because of this eddy, the current always flows south along the west shore of the southern quarter of the lagoon and east along the west shore of the sandspit. Therefore, the water is always flowing in the same direction in the float vicinity on both ebb and flood tides. Freshwater runoff is contributed mostly by the two streams mentioned above. However, a few small seasonal streams are located on the west ridge of the lagoon. There are no private dwellings on the sandspit south of the float site.

To approximate commercial conditions, the float was anchored in an area where the baskets containing the shellfish would be exposed at lower low water. The float and baskets at this location were out of the water at about a +5.5 tide level.

*Shellfish*

Pacific oysters were harvested from an area 10 to 20 ft north of the float shown in Fig. 2. Since the oysters collected were relatively free of fouling organisms, they were placed directly in the float. If the degree of encrustation warranted the removal of barnacles, this was done at the laboratory prior to placing them in the baskets. A total of 450 oysters was distributed among three of the baskets. Manila clams were harvested by laboratory personnel near the mouth of Purdy Creek, east of the float. After rinsing in fresh water, 600 clams were placed in one basket.

*Shellfish Sampling*

Shellfish in the float were allowed to acclimate for a period of 24 hr before sampling was initiated. Following this, random oyster, clam, and accompanying sea water samples were collected in duplicate at various tide stages during two complete tide cycles. In experiments one through five, shellfish and water were collected at the low, mid, and high points of the first tide cycle and at the low and high points of the second cycle. In succeeding experiments, mid-tide samples were taken throughout the sampling period.

Duplicate sea water samples were collected just below the surface at opposite ends of the float immediately over the baskets and examined concurrently with shellfish. The collection of samples was always initiated on the lower of the daily low tides.

*Test Procedure*

Coliform and fecal coliform MPN determinations on shellfish and sea water were made according to American Public Health Association Recommended Procedures for the Bacteriological Examination of Sea Water and Shellfish, 3rd ed. (1962). Bacteriological analysis also included plate counts using MacLeod's sea water agar incubated at 20° C for 5 days. The medium was prepared using sea water which had been filtered once through filter paper. Salinity determinations were made with the use of either a portable salinometer, which also recorded temperature, or by the hydrographic method of Zerbe and Taylor (1953). In the eighth experiment, continuous temperature and salinity readings were obtained with the use of constantly recording instruments.

*Data Anyalysis*

The data from this report were analyzed by comparing the geometric mean and range of coliform and fecal coliform MPN's and the arithemetic mean and range of plate counts. Geometric means were computed by dividing the sum of the logarithms of each MPN value by the total number of samples taken in each experiment. The number of sampling intervals during each experiment was as follows: experiment one, 14; experiment two, 12; experiment three, 13; experiment four, 13; and experiments five through eight, 17 each. Since samples were taken in duplicate at each interval, the number of samples represented is therefore twice the number shown above for each experiment. The range of results, on the other hand, represents the extremes in each individual experiment. Another relationship between the shellfish and their surrounding water was established by computing mean accumulation ratios for each index. Each ratio was determined by dividing the mean levels of each index in the shellfish by the mean water concentration.

## RESULTS

A comparison by range and geometric mean of multiple sea water and shellfish samples collected during each experiment is shown in Figs. 3 and 4. Changes in "total bacteria" as determined by the plate count method using MacLeod's sea water

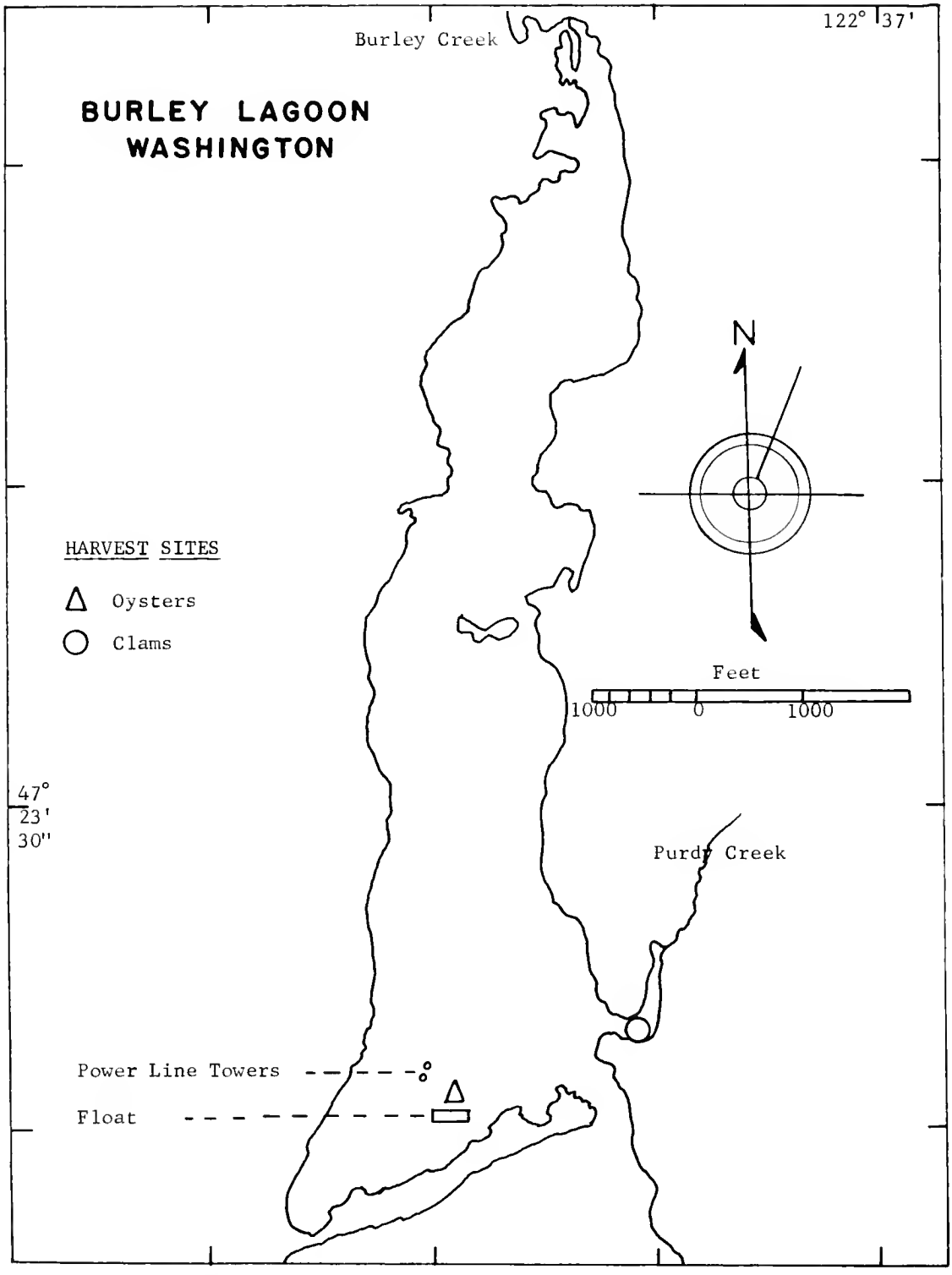


FIG. 2. *Location of float.*

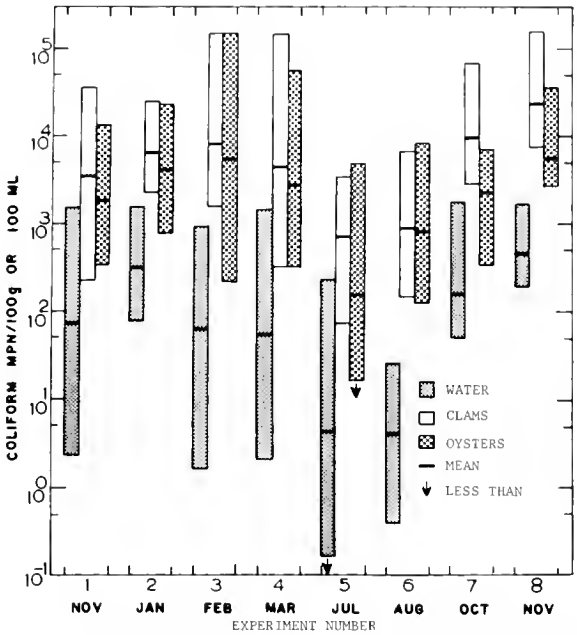


FIG. 3. *Comparison of mean and range of coliforms in shellfish and sea water (8 experiments).*

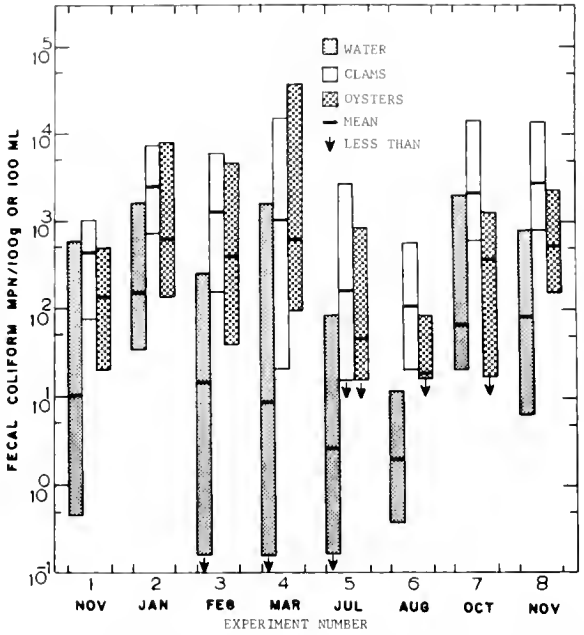


FIG. 4. *Comparison of mean and range of fecal coliforms in shellfish and sea water (8 experiments).*

agar are expressed as arithmetic means in Fig. 5. Experiments were conducted throughout more than one calendar year to encompass any climatological or seasonal influences on the bacterial response of the stored shellfish. Although the size and capacity of the storage float were small in comparison with commercial systems, many features were incorporated that correlated directly with commercial operation: duration of storage, storage depth, exposure time and distance from shore.

The mean levels and range of coliform bacteria in oysters and clams as compared to the levels in their overlying water environment are shown in Fig. 3. The coliforms in the water varied extensively from one seasonal experiment to the next, with the highest levels occurring in January (experiment 2) and November (experiment 8) of 1967 and the lowest during the summer months of July and August. Mean coliforms in both shellfish species generally followed the same pattern of change as that of the overlying water. However, in all eight experiments, mean levels in clams always exceeded those in accompanying oysters. The greatest mean accumulation of coliform in clams, and to a lesser extent in oysters, occurred during the months of July and August when corresponding levels in the sea water were at their lowest. In contrast, the lowest mean accumulation levels attained by both shellfish occur-

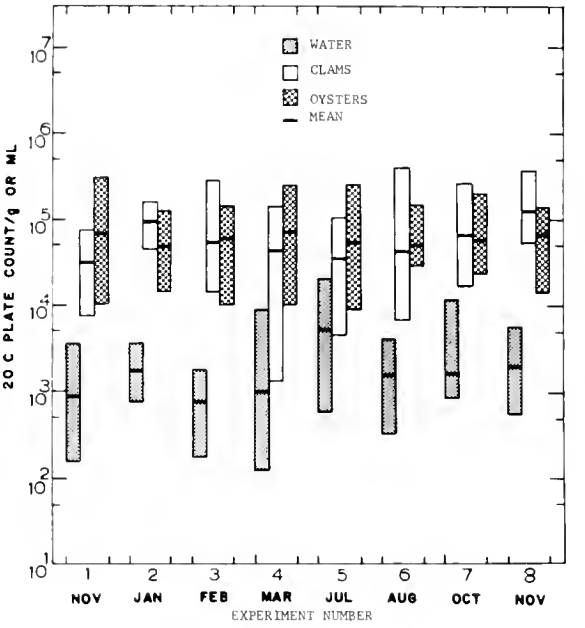


FIG. 5. *Comparison of mean and range of 20°C plate counts in shellfish and sea water (8 experiments).*

red during the month of January (experiment 2) when levels in the water were at their highest.

The mean fecal coliform levels and range of values encountered during each of the eight experiments are shown in Fig. 4. Fecal coliforms increased during the fall and winter months and decreased during the spring and summer. The seasonal patterns of both indices were about the same. Fecal coliform levels, however, were consistently lower than those of the preceding coliform group. As with the coliform group in the preceding Fig. 3, the greatest accumulation relative to the water occurred during the summer months when levels in the water were at their lowest. In contrast, the least accumulation by shellfish was observed during the winter (second experiment) when corresponding levels in the water were high. The higher accumulation with respect to the water observed during the summer months was probably associated with increases in water temperature and therefore activity of the animals. Fecal coliform levels, like coliforms, were persistently higher in clams than in oysters.

The 20° C plate count data are shown in Fig. 5. Levels of this index were much more uniform in shellfish and water than either of the multitube (MPN) determinations. In all eight experiments, variation in mean levels among shellfish was never more than 1 log, irrespective of the changes in water levels. Although relatively constant, the composition of the species making up the flora undoubtedly varied with changes in temperature, salinity, and other environmental factors. It is also interesting to note than in experiment 5, in which levels of coliform and fecal coliform in the water were at their lowest, corresponding mean plate counts were at their highest level. This increase in plate counts, however, did not appear to effect a similar increase in shellfish levels. Counts in oysters were higher than in clams except in two experiments.

A comparison of sea water and shellfish results

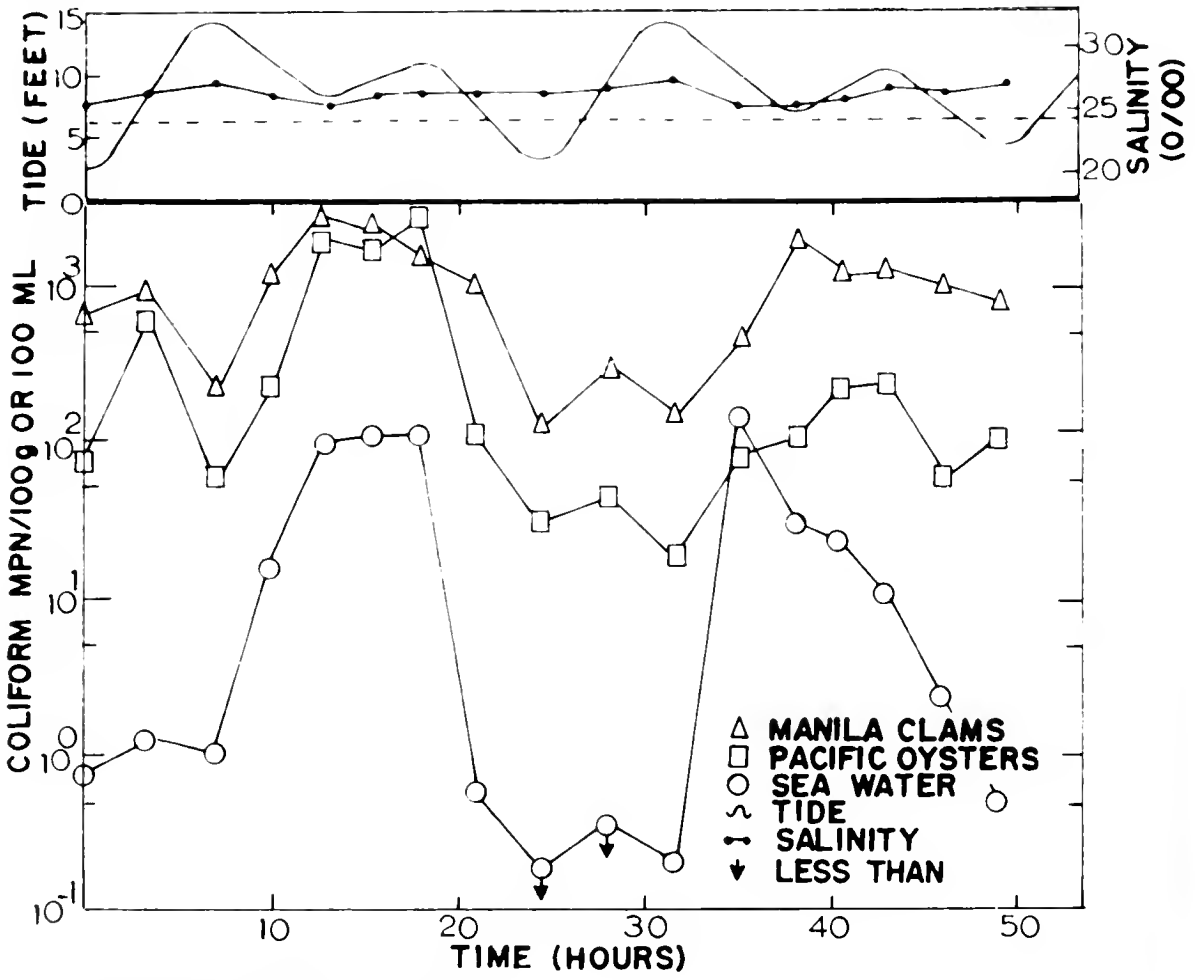


FIG. 6. *Changes in coliform MPN (Exp. 5).*

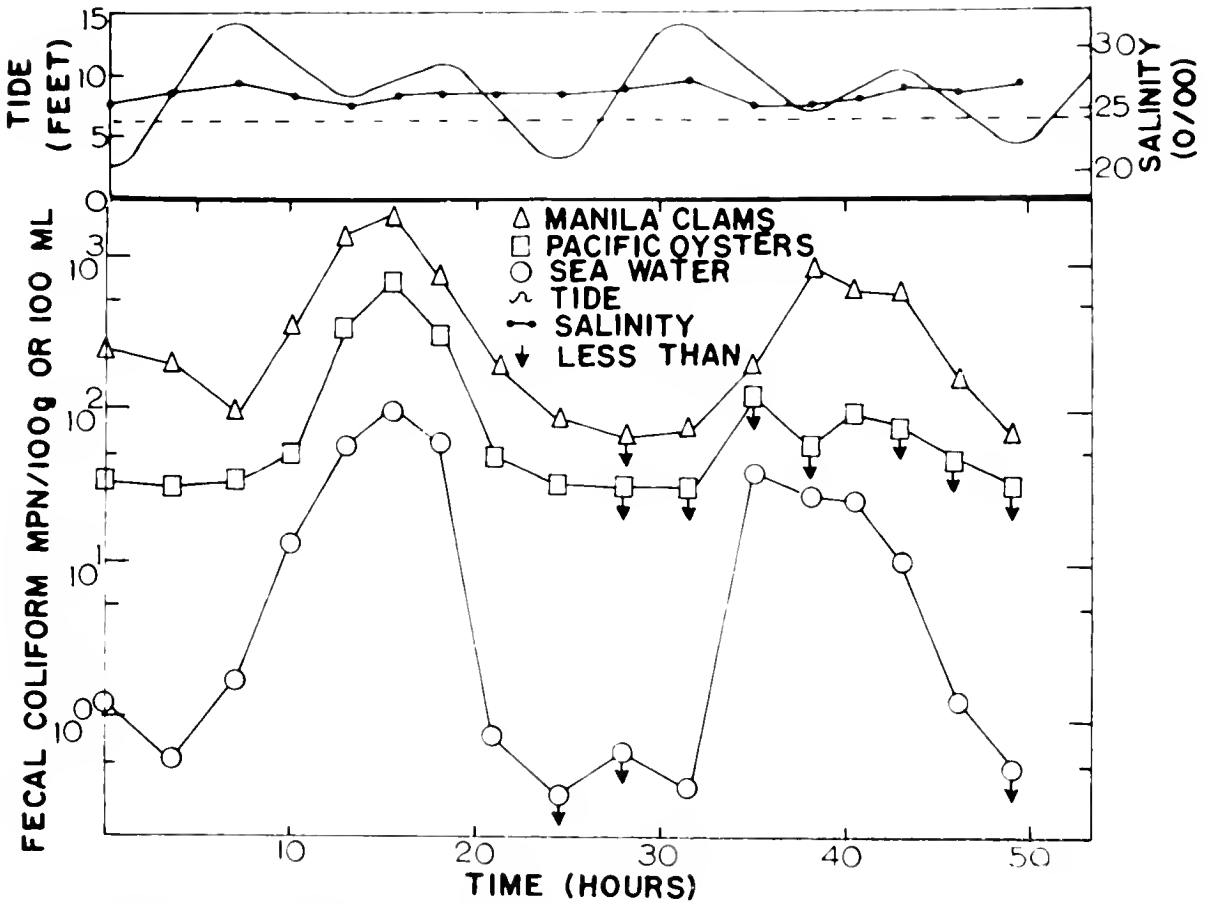


FIG. 7. *Changes in fecal coliform MPN (Exp. 5).*

from a typical experiment is shown by individual bacterial index in Figs. 6, 7, and 8. Changes in coliform and fecal coliform levels in the shellfish were extremely rapid and closely reflected changes in the surrounding water. It is apparent, however, that changes in the shellfish MPN's were not as great as those in the water. Considerable lag in shellfish response during elimination of these groups is indicated.

The accumulation ratios of oysters and clams were determined by dividing the mean concentration of each bacterial index in shellfish by the mean concentration in sea water. The results of these computations are shown in Table 1. The lowest mean coliform and fecal coliform ratios for both species were recorded during the second experiment conducted in the winter of 1966. During this experiment, water temperatures remained consistently low, ranging from 7.3-7.9° C. Nearly 11 in of rain fell during this month, with a correspondingly wide range in salinities. In experiment 4, under similar conditions of salinity and rainfall with only a slight increase in water temperatures, the coliform and fecal coliform ratios were much higher. The highest coliform ratios, however, in both clams and oysters occurred during the fifth and sixth experiments (summer series) when water temperatures were at their highest and the total rainfall was less than 1.0 in.

As shown in Table 1, clams consistently accumulated coliform and fecal coliform bacteria to higher levels than did oysters stored under identical conditions. This situation, however, did not occur with plate counts. The accumulation of microorganisms enumerated by the plate count method, unlike the coliform and fecal coliform groups, was more uniform throughout the series of experiments and less influenced by changes in season. Moreover, accumulation of these microorganisms by clams was no higher than that observed in oysters.

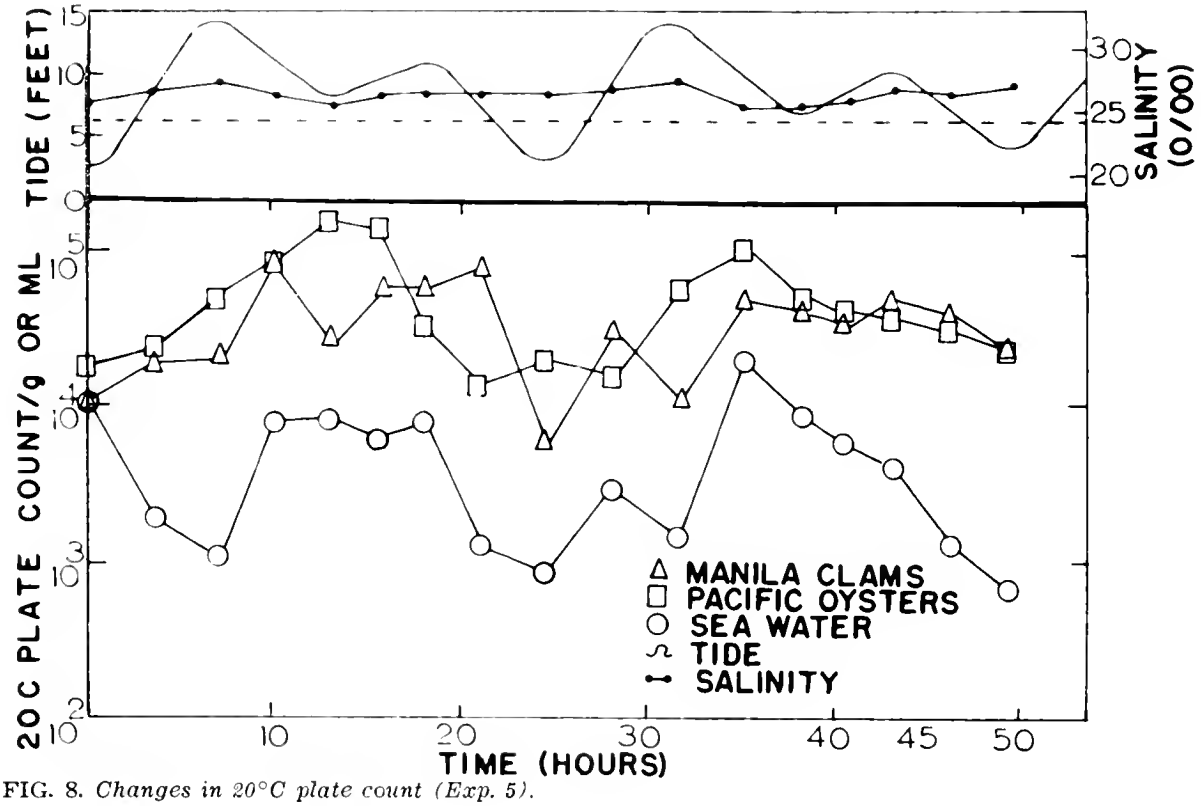


FIG. 8. *Changes in 20°C plate count (Exp. 5).*

## DISCUSSION

Bacterial levels in the sea water at the float site followed a normal seasonal cyclic pattern generally expected in southern Puget Sound: coliform and fecal coliform levels were generally highest during the fall and winter when considerable terrestrial runoff occurs and lowest in the spring and summer when light precipitation and slight runoff occur. These seasonal factors, nevertheless, had little effect on the number of plate count organisms. This index, unlike the coliform and fecal coliform groups, remained quite stable in both species of shellfish and their overlying water irrespective of fluctuation in water temperature, salinity or rainfall. The medium developed by MacLeod, Onofrey and Norris (1954) was designed primarily to grow microorganisms of marine origin found in coastal waters diluted by freshwater runoff. Since the levels of these microorganisms were little influenced by runoff and

TABLE 1. *Accumulation ratios of Pacific oysters and Manila clams.*

| Exp. No. | Date (Month) | Temp. Range[a] (° C) | Salinity Range[a] (ppt) | Total Precipitation[b] (inches) | Ratio: Mean Concentration in Shellfish / Mean Concentration in Water | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Coliform MPN | | Fecal Coliform MPN | | 20° C Plate Count | |
| | | | | | Clams | Oysters | Clams | Oysters | Clams | Oysters |
| 1 | Nov. '65 | 8.7-10.6 | 23.5-30.8 | 7.57 | 46 | 22 | 44 | 13 | 29 | 80 |
| 2 | Jan. '66 | 7.3- 7.9 | 18.2-27.6 | 10.61 | 23 | 13 | 18 | 4 | 44 | 27 |
| 3 | Feb. '66 | 7.0- 8.0 | 26.5-29.3 | 2.97 | 146 | 72 | 63 | 24 | 52 | 67 |
| 4 | Mar. '66 | 4.0- 9.0 | 18.7-28.5 | 8.38 | 111 | 70 | 132 | 88 | 39 | 52 |
| 5 | July '66 | 15.0-23.1 | 25.7-27.8 | 0.88 | 152 | 38 | 49 | 13 | 7 | 11 |
| 6 | Aug. '66 | 16.7-22.8 | 25.5-29.3 | 0.35 | 180 | 143 | 52 | 12 | 37 | 29 |
| 7 | Oct. '66 | 8.6-11.5 | 17.3-31.4 | 3.65 | 52 | 15 | 27 | 6 | 36 | 31 |
| 8 | Nov. '66 | 9.8-10.8 | 22.4-31.1 | 7.35 | 50 | 11 | 35 | 6 | 62 | 35 |

[a] Sea water in float.
[b] Obtained from climatological data — U. S. Dept. of Comm. U. S. A.

other seasonal factors, their persistence suggests the presence of a natural flora associated with the shellfish and their surrounding sea water. Similar observations with regard to a natural flora were made by Colwell and Liston (1960). Their conclusion was that the observed flora in Pacific oysters was not controlled solely by external environment, but was in fact typical of the oyster itself.

Accumulation ratios of shellfish maintained in their natural marine environment were higher than those observed in previous studies under laboratory conditions. In 11 such experiments with Pacific oysters, Kelly (1961)[3] obtained an average accumulation ratio of less than tenfold. Beck *et al.* (in press), in a series of 12 similar experiments, using Manila clams obtained accumulation ratios of 38-fold or less.

A number of factors may have contributed to the higher accumulation ratios observed in field experiments. For one, it was observed that a lag in shellfish response occurred during decreases in bacterial levels in the water (Jakubowski and Vasconcelos, 1966; Jakubowski, 1966). This lag, however, was more evident in shellfish coliform and fecal coliform MPN's than in plate counts. Although the bacterial levels in the water varied rapidly with changes in the tide, the levels in the shellfish did not decline as rapidly as did the levels in the water. This would tend to make the levels in shellfish higher than those in the water, thus tending to raise the value of each ratio.

The difference in concentration of microorganisms in both field and laboratory oriented experiments may have been due to another factor. In the laboratory experiments mentioned above, clams and oysters were exposed to sea water polluted with cultures of *Escherichia coli* maintained at a concentration of 1,000/100 ml of sea water (MPN of 1,000). In field experiments, the number of detectable coliform and fecal coliform bacteria in the water fluctuated with changes in the tide and rarely exceeded MPN levels of 800. Moreover, suspending shellfish under natural conditions for extended periods of time exposed them to ecological and environmental factors, such as tidal oscillations, climate, and wave action not normally duplicated in the laboratory. These factors may have had a direct or indirect influence on higher accumulation rates observed in these stored shellfish.

The results presented in this study could be of considerable interest to commercial operations. It is apparent that living oysters and clams placed in natural sea water rapidly reflect the bacteriological quality of that water. The degree of accumulation, however, is not solely dependent upon the concentration of bacteria found in the water but on the physiological activity of the shellfish themselves. This activity is usually intensified by increases in water temperature brought on by normal changes in season. Although the coliform and fecal coliform levels of the overlying water were higher in the winter, the degree of accumulation as reflected by the ratios was not as high proportionately as that observed during the summer.

## II. BACTERIAL ACCUMULATION-ELIMINATION RESPONSE OF PACIFIC OYSTERS (*CRASSOSTREA GIGAS*) *AND* MANILA CLAMS (*TAPES JAPONICA*) UNDER COMMERCIAL WET STORAGE CONDITIONS.

### INTRODUCTION

In some commercial operations in the Pacific Northwest, it is common practice to store market-size shellfish in shallow wooden floats near the processing plant prior to shucking or packing for market. The capacity of these floats can vary from 50 to 600 bushels, depending upon the particular needs of each grower. Wet storage enables the grower to harvest large quantities of shellfish during favorable tidal conditions, for processing or marketing at a later time, thus insuring a constant and ready supply. The placement of these storage floats, however, must be an important consideration since shallow waters near the shore are sometimes subject to terrigenous contamination from nearby streams.

Previous studies by Jakubowski and Vasconcelos (1966) using a small float with shallow baskets indicated that oysters and clams respond quickly to fluctuations in water bacterial density. This present investigation was undertaken primarily to study the effects of loading on the accumulation and elimination of bacteria by shellfish held under commercial wet storage conditions. Because of the diversity of storage facilities and procedures, experiments were conducted under actual field conditions at various commer-

[3] Kelly, C. B. 1961. Accumulation of bacteria by the Pacific and Olympia oyster. Paper presented at Shellfish Sanit. Res. Conf., Purdy, Wash.

cial plants in the Puget Sound area. Two preliminary reports covering portions of this study have been prepared (Vasconcelos, Hoff and Ericksen, 1968 and Vasconcelos and Ericksen, in press). Results presented in this report are based on data compiled from six experiments completed during the months of April, May, June, August and November of 1967 and March 1968.

## MATERIALS AND METHODS

*Float Construction*

The commercial floats utilized in this study were rectangular in design and constructed from wooden materials. Each float varied in size, shape and capacity. The bottom partitions were usually constructed of spaced, plank decking supported lengthwise by logs or wooden pontoons. The narrow ends were either partially or completely open to permit movement of sea water through the float. In some instances, metal screens were attached at one or both ends to prevent floating matter and scum from entering the float.

*Location of Floats*

Experiments 1 through 6 were conducted at three different geographical locations in the southern Puget Sound area. Experiments 1 through 3 involving Pacific oysters were performed in a tidal estuary (Minter Creek) located 5 miles southwest of the Pacific Northwest Marine Health Sciences Laboratory where extremes in salinity were known to occur. Since the float at this site was anchored in a natural stream bed, the oysters contained within were rarely out of water.

Experiment 2, in which Pacific oysters were used, was conducted at a plant located in a narrow tidal estuary known as Skookum Inlet, southeast of Shelton, Washington. The float utilized was anchored to a floating dock approximately 75 feet from the shucking house. Since the float was exposed at about a +7.0 tide, samples of sea water and shellfish were collected just before the float went dry.

Experiments 4, 5, and 6, in which Manila clams were used, were conducted in floats permanently anchored in an area 200 to 300 feet offshore from each respective plant. Since clams are usually stored longer than oysters, floats used exclusively for clams are usually located at lower tidal elevations in which the clams remain submerged during periods of extreme low water.

*Shellfish*

Commercially harvested oysters and clams were obtained during the harvesting and loading operations at each plant. In addition to commercial lots of shellfish, depurated oysters and clams were positioned in the floats at locations corresponding to those designated as commercially harvested. One week prior to each experiment, oysters and clams to be depurated were collected at locations adjacent to the laboratory. Following collection, the shellfish were washed in freshwater and, if needed, freed of fouling organisms. Depuration was accomplished by immersing the shellfish in tanks supplied with ultraviolet treated sea water for 5 to 7 days.

*Sampling*

Samples of commercially harvested and depurated shellfish were collected from two locations in the top and bottom layers of the floats during the low and high stages of one complete tide cycle of approximately 24 hours duration. In addition, water samples were collected from two locations inside the float prior to each sampling of shellfish. Samples of shellfish were also taken just prior to loading the floats. Therefore, a total of five samplings was made during each experiment. All samples were run in duplicate at the laboratory. The relative position of each sample is shown in Fig. 9.

To collect samples from the bottom layer without disrupting the storage system, 20 oysters or 30 clams were placed in small netted bags (2 ft by 2 ft) loosely tied at the corners with string. Each bag contained enough shellfish for a duplicate sample. Ropes were tied to the bags to retrieve them as well as to mark their location in the float. During each of the sampling tours sea

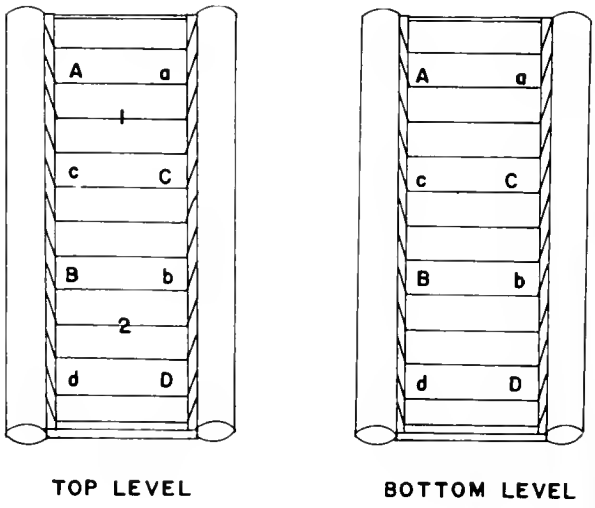


FIG. 9. *Sampling sequence and locations.*

water and bags containing either depurated or harvested shellfish were transported to the laboratory for bacteriological examination.

*Test Procedures*

Bacteriological examinations performed on shellfish and water samples consisted of quantitative coliform, fecal coliform, and 20° C plate count determinations. The coliform and fecal coliform tests were performed according to recommended procedures (American Public Health Association, 1962). MacLeod's sea water agar was used for plate counts with incubation at 20° C for 5 days (MacLeod, Onofrey and Norris, 1954). Salinity determinations were made using the hydrographic method described by Zerbe and Taylor (1953). Turbidity of the sea water was measured in Jackson Candle Turbidity Units (JTU) with the use of a Hach Laboratory Turbidimeter. Sea water temperature in the float was determined at each sampling interval.

*Data Analysis*

The data from the commercially harvested shellfish were compared by combining results from individual experiments. In each of the six experiments a mean value for each index was established for sea water and for shellfish taken from top and bottom layers of the floats. Geometric means were mathematically computed for all multitube MPN determinations, while arithmetic means were determined for plate counts. A total of 34 harvested and 34 depurated shellfish samples was collected during each experiment. Sea water samples were collected in duplicate at each of the five sampling periods for a total of 10 samples per experiment.

Mean accumulation ratios for each bacterial

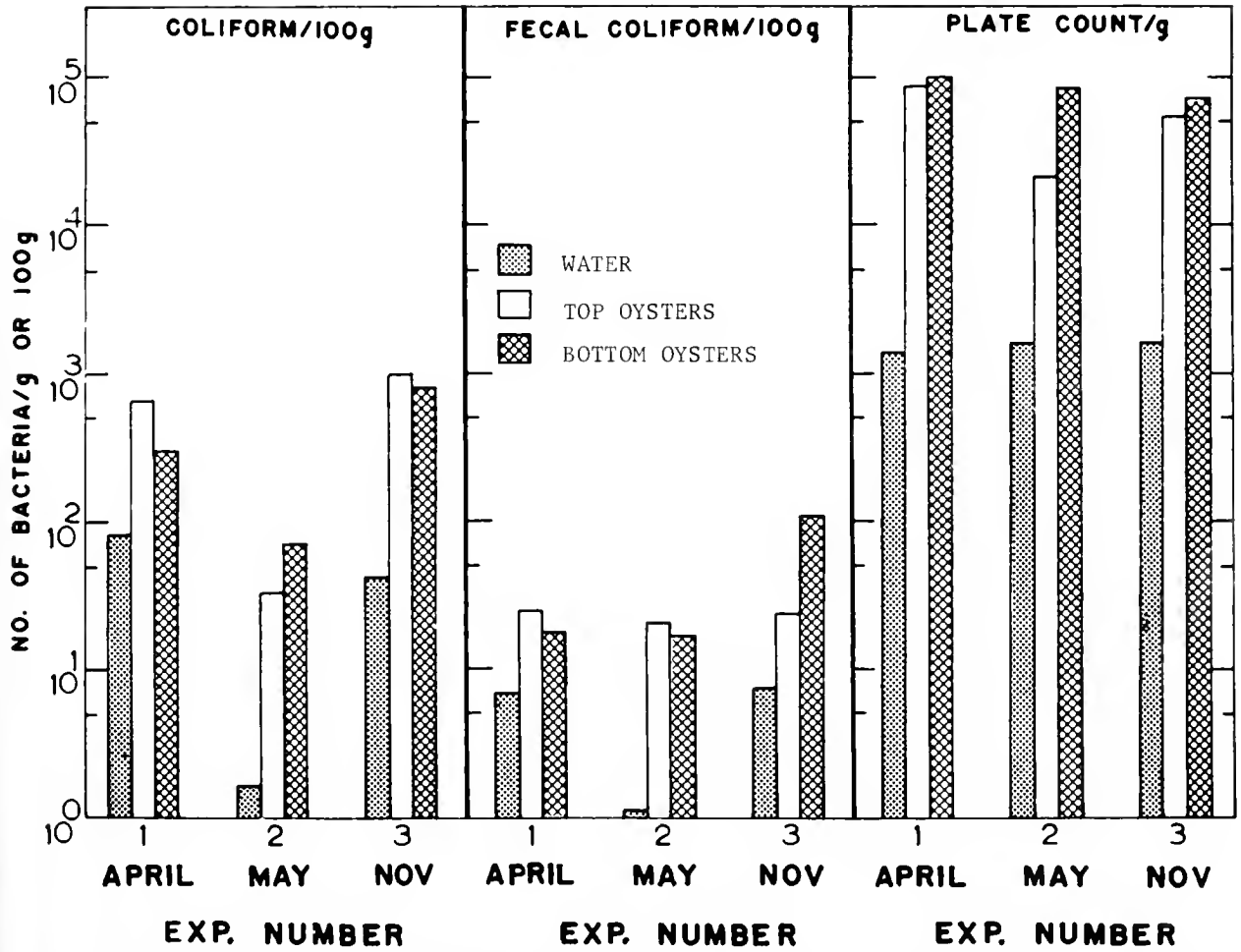


FIG. 10. *Comparison of mean coliform MPN's, fecal coliform MPN's and 20°C plate counts of harvested Pacific oysters and sea water (3 experiments).*

index were determined for those shellfish taken while the float was in the water. Each ratio represents the relationship between the shellfish and their overlying water during the course of the experiment. All mean ratios were calculated by dividing the mean levels of each index in the shellfish by the mean levels in the water.

## RESULTS

The mean coliform, fecal coliform, and plate count densities in sea water and harvested Pacific oysters from the first series of experiments are shown in Fig. 10. Since experiments were conducted at different commercial locations intermittently throughout the year, mean levels of coliform and fecal coliform MPN's in sea water varied extensively. This factor did not appear to affect the mean levels of plate counts in the water since the number of microorganisms enumerated by this test remained quite stable, varying by less than 500 per ml. In all three experiments, mean plate counts in oysters and their overlying water exceeded those of the coliform group. Mean levels of coliforms, in turn, exceeded those of the fecal coliform group. Although oysters at the top layers attained slightly higher mean levels of coliform and fecal coliform bacteria in two of the three experiments, the difference was very slight. From the mean concentrations shown here, the accumulation of coliform and fecal coliform bacteria was generally highest when levels in the water were low and lowest when levels in the water were high. The high accumulations observed during periods of low bacterial densities in the water can probably be attributed to increased shellfish activity brought on by higher water temperature associated with the season. The pattern of bacterial accumulation by oysters stored commercially is very similar to that described in Part I of this study.

The mean coliform, fecal coliform, and plate

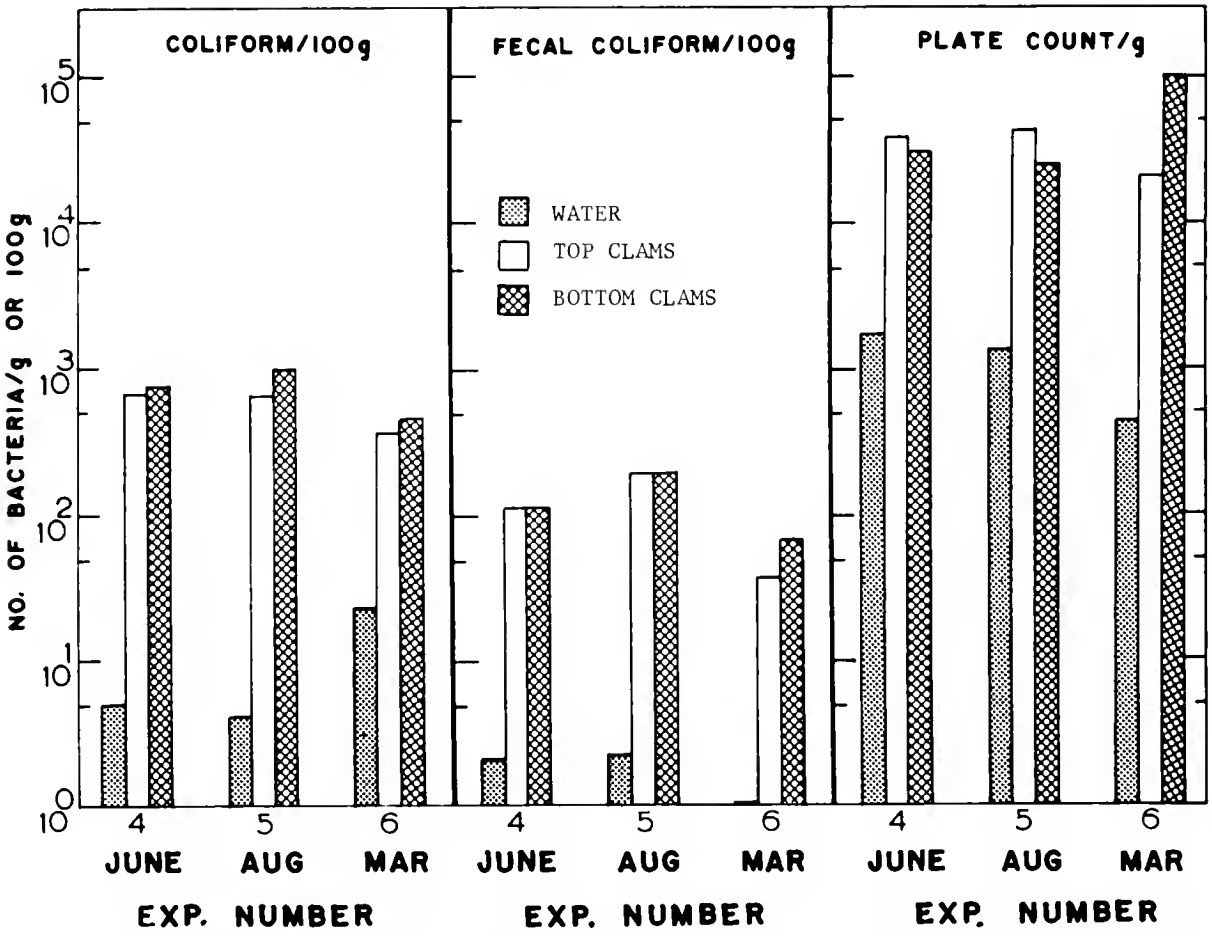


FIG. 11. *Comparison of mean coliform MPN's, fecal coliform MPN's and 20°C plate counts of harvested Manila clams and sea water (3 experiments).*

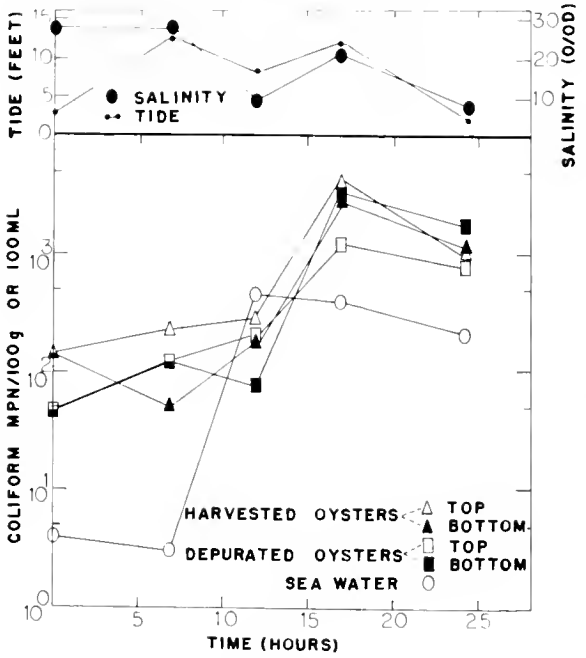


FIG. 12. *Changes in coliform MPN (Exp. 1).*

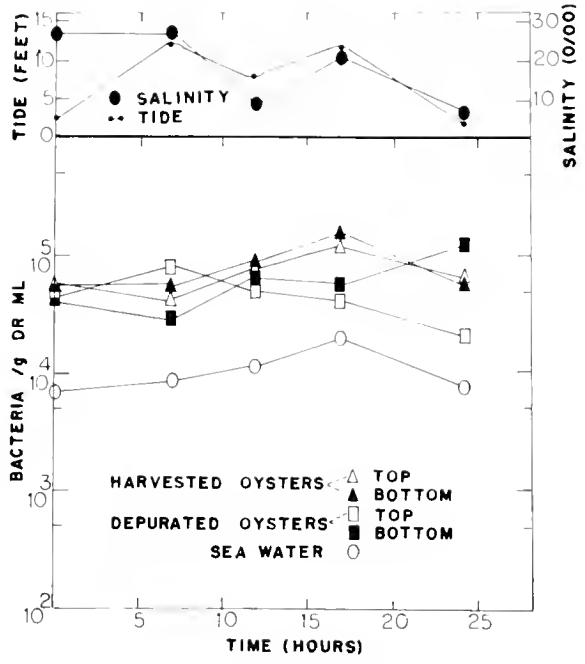


FIG. 14. *Changes in 20°C plate count (Exp. 1).*

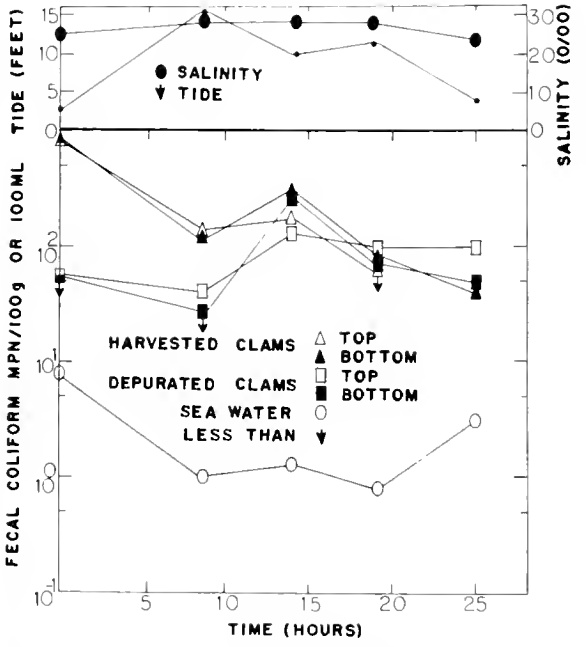


FIG. 13. *Changes in fecal coliform MPN (Exp. 3).*

count densities in harvested Manila clams from experiments 4 through 6 are shown in Fig. 11. Except for oyster experiment 2 conducted in May, coliform and fecal coliform levels in the water during clam storage were lower than those observed during oyster storage. Although the mean levels of these indices in water were lower, the levels accumulated by clams were tenfold to fifty-fold higher than those accumulated by oysters stored under similar conditions. Mean plate counts in the sea water during both oyster and clam storage experiments remained at about the same level, varying by less than 1 log between experiments. Accumulation of plate count organisms by clams was about the same during the three later experiments. The mean concentration of these microorganisms, however, was found to be slightly higher in oysters than in clams, even though concentrations in the sea water were similar. No definite differential pattern of bacterial uptake was demonstrated between clams on the top as opposed to those on the bottom.

Changes in coliforms, fecal coliforms, and plate counts from two typical experiments are shown in Figs. 12, 13 and 14, respectively. When individual bacterial indices were compared to changes in tide and salinity graphically rather than on a computed mean basis, the response of oysters and clams from both top and bottom appeared to be about the same. Counts were generally highest during low tides and lowest during high tides. The response time of both species of shellfish to changes in water was rapid. Plate counts were generally more stable and less influenced by fluctuations in tide and salinity.

As a comparative means of monitoring the rate and degree of bacterial accumulation, oysters and clams depurated to known low levels at the laboratory were placed in floats at positions corresponding to those of the commercially harvested shellfish (Fig. 9). At designated intervals, samples of depurated shellfish were taken concurrently with harvested shellfish. The results of these determinations are shown in Table 2. Since depurated samples were collected in duplicate, each recorded value represents the average of two determinations. The uptake of coliforms by both species of shellfish occurred rapidly within the first 7 to 8 hours of storage. Subsequent increases or decreases were generally attributed to factors such as salinity, temperature and rainfall. In experiment 5, depuration of the clams to indeterminate levels prior to their use in the experiment did not occur. Accumulation of coliforms was highest during experiments 3 (oysters) and 6 (clams) after 25 and 24 hours, respectively, and lowest during oyster experiments 1 and 2.

Ratios of bacterial densities of parameters in Pacific oysters to those in sea water from both depurated and harvested samples are shown in Table 3. The lowest coliform and fecal coliform accumulation ratios occurred in experiment 1. During this experiment, water temperatures were lower than at any other time. Due to a precipitation of 2.5 inches, salinities ranged from 5.6 to 27.5 ppt. In experiment 3, a similar wide range in salinity occurred, caused by a precipitation of 3.55

TABLE 2. *Bacterial change of depurated shellfish during wet storage.*

| Exp. No. | Shellfish | Hour | Tide | Coliform MPN | | Fecal Coliform MPN | | 20° C Plate Count | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Top | Bottom | Top | Bottom | Top | Bottom |
| 1 | Oysters | 0[a] | | 45 | 45 | <18 | <18 | 47,000 | 47,000 |
| | | 7 | High | 120 | 120 | 30 | <18 | 81,000 | 33,000 |
| | | 12 | Low | 190 | 77 | <18 | <18 | 57,000 | 72,000 |
| | | 17 | High | 1,200 | 3,200 | 20 | <18 | 45,000 | 61,000 |
| | | 24 | Low | 800 | 1,700 | 130 | 78 | 22,000 | 130,000 |
| 2 | Oysters | 0[a] | | <18 | <18 | <18 | <18 | 40,000 | 40,000 |
| | | 7 | High | 100 | 240 | <19 | <18 | 55,000 | 52,000 |
| | | 11 | Low | 180 | 78 | <18 | <18 | 100,000 | 91,000 |
| | | 18 | High | 510 | 70 | <18 | <18 | 77,000 | 19,000 |
| | | 25 | Low | 150 | 110 | <19 | <18 | 250,000 | 150,000 |
| 3 | Oysters | 0[a] | | <19 | <19 | <18 | <18 | 22,000 | 22,000 |
| | | 7 | High | 870 | 93 | 120 | <18 | 120,000 | 100,000 |
| | | 12 | Low | 1,600 | 630 | 170 | 51 | 79,000 | 60,000 |
| | | 18 | High | 620 | 1,200 | 160 | 150 | 41,000 | 120,000 |
| | | 25 | Low | 75,000 | 63,000 | 3,200 | 2,000 | 75,000 | 93,000 |
| 4 | Clams | 0[a] | | <55 | <55 | <18 | <18 | 11,000 | 11,000 |
| | | 8 | High | 430 | 120 | 40 | <29 | 23,000 | 19,000 |
| | | 14 | Low | 1,000 | 1,700 | 130 | 280 | 32,000 | 56,000 |
| | | 18 | High | 1,300 | 1,700 | 93 | 68 | 56,000 | 65,000 |
| | | 25 | Low | 600 | 790 | 93 | 45 | 33,000 | 30,000 |
| 5 | Clams | 0[a] | | 560 | 560 | 290 | 290 | 10,000 | 10,000 |
| | | 8 | High | 430 | 280 | 150 | 130 | 10,000 | 39,000 |
| | | 13 | Low | 910 | 620 | 200 | 150 | 30,000 | 57,000 |
| | | 18 | High | 1,800 | 1,700 | 210 | 250 | 33,000 | 35,000 |
| | | 25 | Low | 1,900 | 3,300 | 740 | 790 | 20,000 | 19,000 |
| 6 | Clams | 0[a] | | <18 | <18 | <18 | <18 | 30,000 | 30,000 |
| | | 7 | High | 730 | 750 | 68 | 120 | 35,000 | 38,000 |
| | | 12 | Low | 280 | 420 | <38 | 70 | 18,000 | 44,000 |
| | | 17 | High | 220 | 500 | <18 | 20 | 15,000 | 36,000 |
| | | 24 | Low | 1,300 | 180 | <29 | <18 | 38,000 | 48,000 |

[a] Same value for top and bottom samples.

TABLE 3. *Comparison of coliform, fecal coliform and 20°C plate count accumulation ratios in Pacific oysters.*

| Exp. No. | Date | Temp. Range[a] (° C) | Salinity Range[a] (ppt) | Turbidity Range[a] (JTU) | Total Precipitation[b] (inches) | Type of Shellfish | Position in Float | Ratio: Mean Conc. in Shellfish / Mean Conc. in Water: Coliform MPN | Fecal Coliform MPN | 20° C Plate Count |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Apr. '67 | 7.3-9.5 | 5.6-27.5 | 0.6-19 | 2.50[c] | Depurated Oysters | Top | 4 | < 3 | 44 |
| | | | | | | | Bottom | 4 | < 3 | 39 |
| | | | | | | Harvested Oysters | Top | 8 | 3 | 61 |
| | | | | | | | Bottom | 4 | < 3 | 71 |
| 2 | May '67 | 13.0-15.4 | 24.3-27.6 | 0.6-10 | 0.92[d] | Depurated Oysters | Top | 117 | <23 | 48 |
| | | | | | | | Bottom | 61 | <23 | 34 |
| | | | | | | Harvested Oysters | Top | <18 | <26 | 13 |
| | | | | | | | Bottom | 39 | <23 | 53 |
| 3 | Nov. '67 | 10.0-12.5 | 3.5-31.4 | 0.6-3.5 | 3.55[c] | Depurated Oysters | Top | 23 | 20 | 47 |
| | | | | | | | Bottom | 10 | < 7 | 55 |
| | | | | | | Harvested Oysters | Top | 23 | 3 | 25 |
| | | | | | | | Bottom | 19 | 14 | 41 |

[a] Sea water in float.
[b] Obtained from climatological data — U. S. Dept. of Comm.
[c] Wauna 3W Station.
[d] Shelton Station.

inches. The highest coliform and fecal coliform accumulation ratios occurred in experiment 2 during a period of high water temperature and little variation in salinity. Total precipitation during this experiment was less than 1 inch, the lowest of the three oyster experiments. Accumulation of all three indices by depurated oysters essentially followed the same pattern as that of harvested oysters.

Ratios of bacterial counts in Manila clams to counts in sea water are shown in Table 4. The highest coliform and fecal coliform ratios in clams occurred in experiments 4 and 5 during periods of relatively high water temperatures and low precipitation. During these experiments, water temperatures ranged between 16 to 20° C while total precipitation was less than 1 inch. As with oysters, the lowest coliform and fecal coliform ratios in clams occurred when water temperatures were relatively low. Except for plate counts, shellfish: water ratios were consistently higher in clams than in oysters. This situation persisted in both harvested and depurated shellfish regardless of their location in the float. Unlike coliforms and fecal coliforms, plate count ratios in both species remained more uniformly stable throughout this series of experiments.

## DISCUSSION

The response of shellfish to change in water bacterial density was about the same at all sampling positions in the float. Although subjected to physical pressures from above, oysters and clams in the bottom layers generally followed the same bacterial accumulation-elimination patterns as those at the top. The weight of the overlying shellfish had little effect on the pumping activity of the clams and oysters on the bottom. Besides the apparent accumulation response, the heavy deposition of feces and pseudofeces observed in and around the stored shellfish indicated that they were physiologically active, e.g. pumping, feeding and excreting. Since bacterial levels attained by bottom shellfish were not exceedingly higher than those on top, it is assumed that reingestion of the microorganisms excreted from above did not occur.

The accumulation ratios attained by oysters and clams held in commercial wet storage are comparable to those obtained in the small-scale experiments discussed previously in Part I. In both instances, a direct correlation between the accumulation of certain indices and seasonal changes in water temperature was observed.

Under commercial conditions, the highest coliform and fecal coliform accumulations in both species occurred when water temperatures were at their highest. Furthermore, the lowest accumulations occurred when water temperatures were at their lowest. A similar coliform accumulation pattern related to temperature was reported by Arcisz, Wattie and Dallas (1953) with quahogs harvested from a closed shellfish area. In this study, high numbers of coliform bacteria found in quahogs were attributed in part to increases in water temperature.

TABLE 4. *Comparison of coliform, fecal coliform and 20°C plate count accumulation ratios in Manila clams.*

| Exp. No. | Date | Temp. Range[a] (° C) | Salinity Range[a] (ppt) | Turbidity Range[a] (JTU) | Total Precipitation[b] (inches) | Type of Shellfish | Position in Float | Ratio: Mean Conc. in Shellfish / Mean Conc. in Water: Coliform MPN | Fecal Coliform MPN | 20° C Plate Count |
|---|---|---|---|---|---|---|---|---|---|---|
| 4 | June '67 | 16.4-20.4 | 22.9-28.2 | 2.1-5.2 | 0.84[d] | Depurated Clams | Top | 152 | 39 | 20 |
| | | | | | | | Bottom | 146 | 33 | 24 |
| | | | | | | Harvested Clams | Top | 134 | 52 | 22 |
| | | | | | | | Bottom | 142 | 52 | 17 |
| 5 | Aug. '67 | 17.3-19.0 | 26.7-30.6 | 1.4-4.7 | 0.02[d] | Depurated Clams | Top | 268 | 118 | 16 |
| | | | | | | | Bottom | 163 | 77 | 27 |
| | | | | | | Harvested Clams | Top | 161 | 86 | 30 |
| | | | | | | | Bottom | 239 | 86 | 19 |
| 6 | Mar. '68 | 9.0-11.5 | 16.6-22.6 | 1.3-5.9 | 8.43[d] | Depurated Clams | Top | 21 | <43 | 63 |
| | | | | | | | Bottom | 18 | <52 | 98 |
| | | | | | | Harvested Clams | Top | 16 | <38 | 49 |
| | | | | | | | Bottom | 19 | <67 | 233 |

[a] Sea water in float.
[b] Obtained from climatological data — U. S. Dept. of Comm.
[c] Wauna 3W Station.
[d] Shelton Station.

The bacterial index in shellfish and sea water least influenced by changes in water temperature and other seasonal factors was the 20° C plate count. Unlike its companion MPN indices, changes in plate counts were relatively unaffected by variations in water temperature, salinity or rainfall. Evidently, the microorganisms enumerated by the plate count method are indigenous to estuarine waters and influenced little by changes in hydrographic and climatological conditions. A similar pattern was described earlier at which time it was suggested that the persistence of these microorganisms was due to the presence of a natural bacterial flora associated with the shellfish and their surrounding estuarine water.

The pattern of change followed by each bacterial index was dependent to a large degree upon the geographical and biological conditions indigenous to each area. Since each experiment was conducted at different times of the year and at different locations, the physical and bacteriological factors affecting wet storage undoubtedly varied considerably. The response, however, of stored shellstock to changes in water bacterial density was about the same regardless of their relative position in the float. Depurated shellfish placed in corresponding positions rapidly attained coliform and fecal coliform levels comparable to those of the naturally harvested shellfish. Moreover, the patterns of uptake and elimination in both shellfish species were similar. No accumulation or retention of indicator bacteria to unusually high levels was observed in either depurated or commercially harvested Pacific oysters and Manila clams irrespective of their position in the floats.

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# TREATMENT OF SHELL CULTCH WITH POLYSTREAM TO INCREASE THE YIELD OF SEED OYSTERS, *CRASSOSTREA VIRGINICA* [1]

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## ABSTRACT

*A commercial-scale study was conducted on the Eastern Shore of Virginia during 1964, 1965 and 1966 to evaluate treatment of shell cultch with Polystream. Three intertidal reefs of the same approximate size were shelled with 600 to 1,000 bushels of cultch treated with Polystream; adjacent areas were shelled with similar amounts of untreated shells.*

*Results were evaluated for periods ranging up to 18 months on the basis of number of oysters attached per unit volume of shell; length and number of drilled oysters were also recorded. Treated shells consistently had more attached oysters than the controls at two of the plots. On the third, differences did not become apparent until the second year. Analysis suggests that treatment of cultch with Polystream by commercial growers may be economically feasible and may increase net profit.*

## INTRODUCTION

Certain chlorinated benzenes marketed under the name of Polystream have been reported successful in increasing production of the oyster *Crassostrea virginica* (Loosanoff, MacKenzie and Davis, 1960; Loosanoff, 1961; Davis, Loosanoff and MacKenzie, 1961; Shaw and Griffith, 1967). These authors reported a greater number of oyster spat attached to shells dipped in Polystream than on untreated control shells. It was not determined whether greater production was due to decreased predation, decreased fouling or other factors.

The present study was carried out in 1964-1966 to determine if it were economically feasible to use Polystream to increase production of oyster spat on commercially-planted shell cultch.

## DESCRIPTION OF AREA

The experiments were conducted in the northeast end of Hog Island Bay near Little Machipongo Inlet. A preliminary study was started in August 1964 and extended through November 1965; three large-scale experiments were started during July 1965 and lasted through August 1966. The preliminary experiment and part of the main experiment were carried out in an area called Tug Ames Shoal near High Shoal and Argyle Shoal drains. Other tests were conducted about 300 yards west on High Shoal marsh and on Argyle Shoal near Hodges Narrows, about 3/4 mile east. All three areas are man-made intertidal oyster reefs, less than 2 1/2 miles apart (Fig. 1). The Argyle Shoal oyster shell reefs were built on 6 mil polyethylene sheets. In other beds the shell base was placed directly in the mud. Temperatures during the experiment ranged from 2 to 33.6°C and salinities from 30.2 to 35.4 ppt. The tidal amplitude was 4.5 feet and the reefs were exposed for about 90 minutes each low tide.

## METHODS

In all experiments the cultch was oyster shell from shucking house shell piles accumulated during the previous fall and winter. In all studies handling of shells and application of Polystream were carried out by the crews of the oyster companies who planted the treated and untreated

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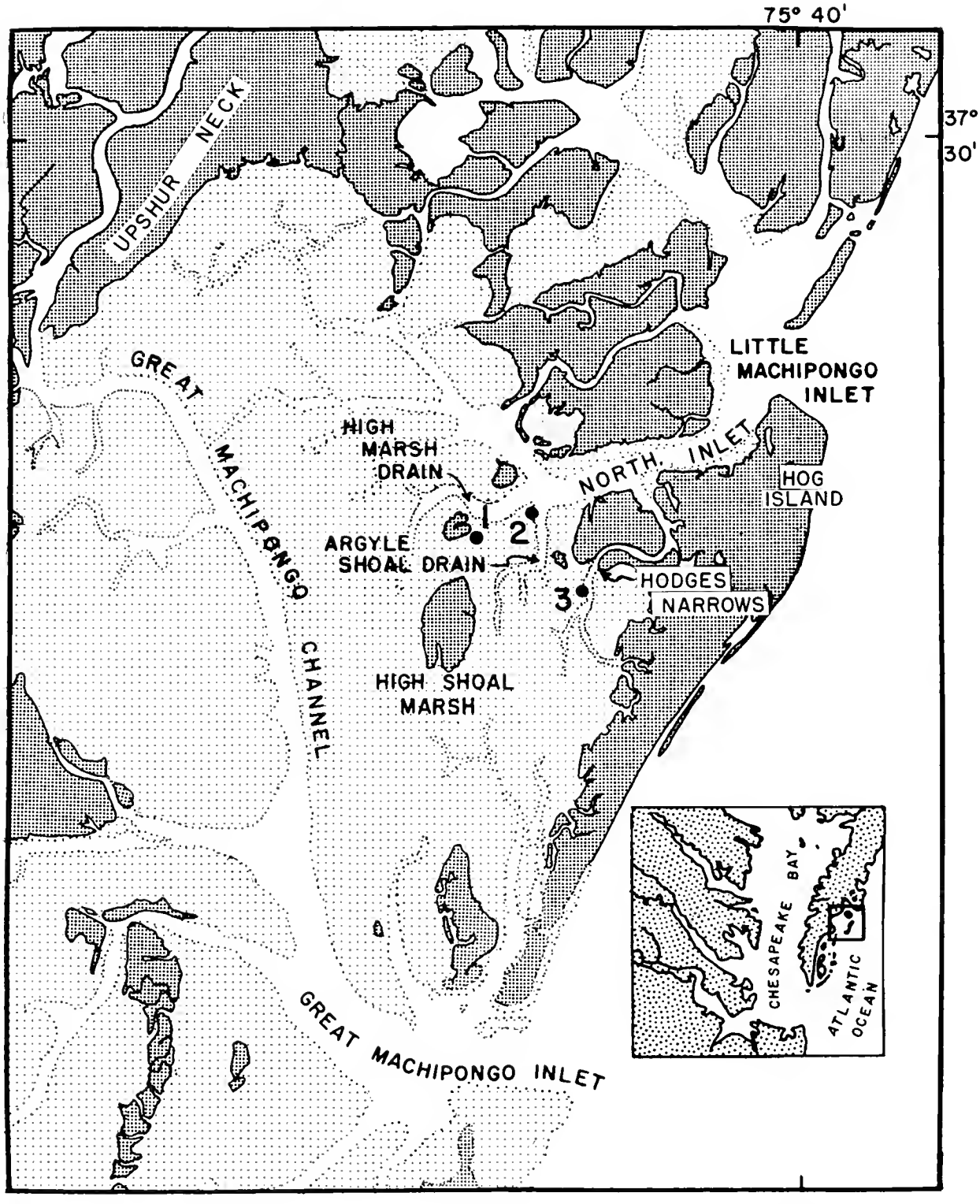


FIG. 1. *Location of test areas. (1) High Shoal Marsh, (2) Tug Ames Shoal, (3) Argyle Shoal.*

cultch on their oyster reefs. Variations in the methods employed from test to test were dictated by the equipment or procedures of each of the participating companies. Observations and evaluation of results were carried out by personnel of the Virginia Institute of Marine Science. The unit of measure used in this study was the Virginia oyster bushel equivalent to 1.397 standard bushel. Details of each study are shown in Table 1.

*Application of Polystream to Oyster Shells*

The shells were treated by wetting with undiluted Polystream. In the 1964 experiment, Polystream was sprayed from a commercial orchard sprayer as the shells were being transported on a conveyor. The Polystream caused rapid deterioration of the rubber belt and the rubber sprayer parts, and the method was discontinued. In subsequent experiments the shells were wetted with Polystream as they were being loaded onto barges by wheel barrows, or dumped from a truck in small (about 5 bu) batches. In this latter method each load or batch was treated by sprinkling it with Polystream using a water can fitted with a sprinkler head. Examination of treated shell piles indicated that both methods achieved complete coverage. Control shells were loaded onto separate barges and were not contaminated by the chemical.

Inhalation, long exposure, or long physical contact with the chemical are potential health hazards. Protective coats, boots, gloves and gas masks were offered to the labor force involved in handling the chemical.

*Methods of Planting Cultch*

Treated and untreated shells were planted in June and July to receive a possible early set. In every case, treated shells were planted within one day following application of Polystream. Two methods were used to distribute the oyster shell over the bottom. In the first, shells were shoveled from the barge into the water. During this process the laborers imparted a horizontal motion to the shovel. This technique, called "broadcasting," resulted in a rather uniform distribution of shell over the bottom as later inspection of the exposed ground revealed. In the second method, employed on High Shoal, shells were washed off the slowly moving barge with a stream of water from a fire hose. On the first low tide of the following day, the shell plantings were inspected and raked down to give level, uniform coverage. The control shells were distributed in the same manner on a nearby reef. In each case, the control and treated shells formed similarly shaped reefs and were spread at the same thickness and equally exposed at low tide.

*Assessment of Oyster Drill Predation*

In the preliminary experiment, a one-bushel random sample was taken from test and control plots in September 1964 and again in November 1965. Oyster spat were counted and a random subsample of 100 oyster spat was measured. In the 1965 experiment, 1/4 bushel samples were taken from five equidistant points on each test and control area at intervals of from one to four months. The shells, number of spat, and drilled spat in each sample were counted. Length of 25 oysters in each 1/4 bushel sample was taken to the nearest 0.1 mm.

*Residual Polystream Assessment*

A bio-assay was made of the tissues of oysters to determine the uptake of Polystream. In July 1966, after approximately one year, samples of oysters were collected from experimental and control plots. After carefully cleaning the shells to remove all visible traces of mud, the meats were removed, frozen and later shipped to Hooker Chemical Company for analysis. Samples of mud obtained from the control and experimental plots were also sent for analysis.

TABLE 1. *Details of treatment, exposure, location and sponsor of treated and untreated shell cultch on Eastern Shore, 1964, 1965 and 1966.*

| Station location | Period shells on the reef | Hours after treatment before planting | Control cultch (bu) | Test cultch (bu) | Polystream applied (gal) | Sponsor |
|---|---|---|---|---|---|---|
| Tug Ames Shoal | 6/17/64-9/17/64 | 24 | 600 | 600 | 50 | H. M. Terry Co. |
| Tug Ames Shoal | 6/29/65-8/31/66 | 24 | 600 | 600 | 45 | H. M. Terry Co. |
| Argyle Shoal | 7/7/65-8/31/66 | 6 | 700 | 1000 | 50 | Ballard Fish and Oyster Co. |
| High Shoal Lump | 7/12/65-7/31/66 | 24 | 800 | 800 | 50 | H. L. and R. L. Bowen Oyster Co. |

## RESULTS AND DISCUSSION

*Preliminary Tests*

During the preliminary experiments in 1964, an extremely light set of oysters occurred about the end of July and continued through October. It was apparent on casual observation that shells treated with Polystream had more and larger seed oysters than did shells on the control area. The algae *Aghardiella*, *Gracilaria*, and *Ulva* were abundant on shells of both plots, but the quantity appeared greater on the treated shells. Sponges (*Halichondria* sp.) appeared equally abundant on shells in both areas as did a bright orange flatworm. Xanthid crabs were not observed on the treated shells although they were abundant on the control. Hard clams were observed in both areas but were only partially buried in the bottom on the treated area.

A one-bushel sample taken from the treated area on 17 September 1964 had 332 oyster spat averaging 16.0 mm in greatest diameter; a comparable control sample had 89 spat with an average size of 11.6 mm. On 3 November 1965, 18 months after the shells were planted, a one-bushel random sample gave further evidence of increased production on treated shells. There were 576 spat on shells in the bushel of treated cultch; of this total, 225 were of the 1964 set and 351 had set in 1965. The control had 189 spat per bushel, of which 18 were of the 1964 set and 171 of the 1965 set. The preliminary experiment ended when the shells were moved to a growing area shortly after the sample was taken.

*Main Test*

During the main studies begun in July 1965, sets were heavier at all stations at comparable periods than in the preliminary study. Results partially agreed with the preliminary study since at Argyle and High Shoals treated shells consistently had greater sets than untreated shells. At Tug Ames Marsh differences were not apparent in the initial phase of the study, but during the second year treated shells had heavier sets (Table 2).

At the end of the study during September 1966 when oysters were large enough to be harvested for seed purposes, size on the three plots ranged in mean length from 19.5 to 40.8 mm. During the study, however, there appeared to be no consistent difference in mean length at any single area between those from test and control plots.

Number of drilled spat was greatest at Tug Ames Shoal. In this location the drilled spat varied from 0.10 to 4.90% of the total number of live oysters. At High Shoal where drilled spat were least abundant, mean counts varied from 0.00 to 2.80% of the total; differences between test and control plots were not evident.

A possible explanation for greater numbers of spat on treated shells over controls, with no obvious difference in drill damage, may be due to damage by young drills. Predation by newly emerged drills is often overlooked and is difficult to assess through field experiments. Newly emerged drills appear in the nearby Chincoteague Bay of Virginia during May, June and July, and even when less than 5 mm in length, will attack oyster spat smaller than 10 mm in diameter (Carriker, 1955). Unfortunately, the right valve of a small oyster spat will usually break away from the left valve shortly after the spat has been killed, leaving no evidence of predation. If we assume the chemical is detrimental to newly hatched drills, a greater survival of the early (July) oyster set will occur, resulting in greater numbers of spat.

The treated shells in each of the three areas had different concentrations of Polystream (Table 1). This evidently made no difference in the results since the Tug Ames area had the highest concentration (650 lb per 600 bushels of shell) and showed the least difference in survival. Apparently even the lowest concentration used (650 lb per 1,000 bushels) was sufficient to show a mean difference between treated and untreated shell.

*Bio-Assay Studies for Chemical Residue*

Samples of oyster tissue and mud collected one year after the experiment was established were analyzed by the Hooker Chemical Company. Analysis of the three experimental areas showed less than 0.1 ppm Polystream in oysters from treated and control areas. Mud samples collected from High Shoal and Tug Ames Shoal contained less than 0.1 ppm. Mud collected from the test area at Argyle Shoal showed 0.14 ppm. Shells in this latter area were planted on a sheet of 6 mil polyethylene; all other plantings were made directly on mud substrate.

Further tests were made on 2 September 1966 to ascertain if Polystream imparted an undesirable odor or flavor to the meat of oysters. A bushel sample of oysters and attached shells was obtained from treated and control plots. The oysters were steamed and a panel of six participants was unable to discriminate between oysters taken from test plots and those taken from the control plots.

*Estimate of Costs and Profit derived from Treating Cultch with Polystream*

By mid-summer in 1966 oysters at the three stations were of sufficient size for harvest. Consequently, estimates of yields in terms of bushels are based on data for July, August, and September of that year.

Analysis of data from Table 2 indicates that

TABLE 2. *Total number of spat in five quarter bushels for stations in Polystream study, Eastern Shore, 1965-1966.*

| | Aug. 1965 | Sept. | Oct. | Nov. | Dec.* | Jan.* 1966 | May* | July | Aug.* | Sept. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Tug Ames Shoal | | | | | |
| Treated | 225 | 353 | 1178 | 2267 | 1847 | 2110 | 1446 | 1513 | 1710 | 1157 |
| Control | 300 | 476 | 1199 | 2235 | 1944 | 2125 | 1528 | 1455 | 1304 | 1019 |
| | | | | | High Shoal | | | | | |
| Treated | 43 | 102 | 714 | 1569 | | | | 1371 | | 1165 |
| Control | 55 | 37 | 184 | 810 | | | | 325 | | 703 |
| | | | | | Argyle Shoal | | | | | |
| Treated | 103 | 196 | 1420 | 2736 | 2252 | 2302 | 1330 | 1612 | 1305 | 1598 |
| Control | 64 | 136 | 1250 | 1333 | 1538 | 1010 | 680 | 843 | 913 | 938 |

*Data for High Shoal not obtained.

number of spat per 1/4 bushel sample during the last three months were increased from 26 to 42%, with the last month for combined stations showing a 30% increase (Table 3). Consequently, for the purpose of calculating the possible economic value of Polystream treatment, the latter figure will be used.

To derive estimates of the cost of planting shell, harvesting oysters, and value of "seed," representatives from several oyster companies and personnel of the Virginia Marine Resources Commission were interviewed. It was found that costs vary with locality due to differences in value of the shell, transportation and labor costs. Variation in value of shell was the most significant factor contributing to differences in the cost of planting. Estimates of yields of seed oysters per bushel of planted shell were taken from the 1965-1966 experiments and from data on commercial production from industry. On the Eastern Shore of Virginia such estimates are difficult to establish since oysters are selected from the plantings by hand and only spat which has reached a preferred size is harvested. This size, locally called "brush," is about one or two inches long and from 2,000 to 2,500 filling a Virginia bushel, depending on whether they grew singly or in bunches. Oysters not attaining proper size at time of harvesting are left for another year even if new shell for the attachment of spat is to be scattered on the rock. This method of harvesting causes a wide range of estimates of production of from 200 to 600 bushels of seed per 1,000 bushels of planted shell. For the purpose of estimates, we used a return of 500 bushels at the end of 14 months in an oyster setting area. This value was chosen as typical of yields obtained by many growers in the immediate region. Cost of harvest is 30 cents per bushel and sale price is estimated as $1.50 per bushel. Calculations using these production and cost figures are shown in Table 4. They indicate that profits from planting 1,000 bushels of shell would be about $350. Treatment of shells with Polystream would add to production costs, not only for the cost of chemicals and labor, but also because of the 30% increase in yield. However, even when the additional costs are added to the base cost, profits are calculated at $384 per 1,000 bushels of planted shell. This latter figure would mean an increased profit of $34. Some of the experimental areas were selectively harvested as explained previously, and in each case the treated area had greater production.

Increasing the number of spat per shell to a given per cent does not necessarily mean an equal increase in oysters. Larger spat tend to push other spat off the shell; predation, smothering and mortality will continue to various degrees throughout the life of the oyster. If an oyster grower is interested in replanting the seed on a growing ground, he would prefer the greater number of oysters per shell, especially if they

TABLE 3. *Per cent increase in number of spat associated with treatment.*

| Station | July 1966 | August 1966 | September 1966 | Three months combined |
|---|---|---|---|---|
| Tug Ames Shoal | 2 | 24 | 12 | 13 |
| Argyle Shoal | 48 | 30 | 41 | 41 |
| High Shoal | 76 | | 32 | 59 |
| Combined stations | 42 | 26 | 30 | |

TABLE 4. *Comparative cost analysis and profit expectancy from planting 1,000 bushels of Polystream-treated and untreated oyster shell for seed production.*

| | Cost or profit in dollars Treated | Cost or profit in dollars Control |
|---|---|---|
| *Planting* | | |
| Planting 1,000 bushels shell at 25c per bushel | 250 | 250 |
| Polystream cost, 650 lbs. | 111 | |
| Shipping costs | 25 | |
| Extra labor for treatment | 10 | |
| *Harvesting* | | |
| Cost of harvesting at 30c per bushel | | |
| Yield—500 bushels untreated area | | 150 |
| Yield—650 bushels treated area | 195 | |
| Total Cost Planting and Harvesting | 591 | 400 |
| *Value of Harvest* | | |
| Value per bushel—$1.50 | | |
| Yield—500 bushels untreated area | | 750 |
| Yield—650 bushels treated area | 975 | |
| Net Profit | 384 | 350 |

were growing well. However, if the seed were sold, a greater number of oysters per bushel would not necessarily increase the price paid per bushel since this is primarily a function of supply and demand. Polystream treatment of shell would be of greatest value in marginal oyster setting areas or heavy predation areas where it might make a difference between survival of a year class of oysters or complete failure. This type of area is considered a greater risk and most oystermen would be reluctant to increase the cost of placing shells in these locations.

*Potential Use of Polystream*

Polystream showed promise in a setting area with intensive drill predation, but has several disadvantages. It degrades very slowly, having the potential of unknown cumulative effects on the entire eco-system; it is nonspecific, killing most of the benthic invertebrate community, upsetting the natural balance in treated areas; and it is a potential health hazard to the people using it (Haven, Castagna, Chanley and Whitcomb, 1966). Its use is presently restricted to experimental in most states. Although these experiments show good results, there is at present no information on long-term or repeated use in an area.

## SUMMARY AND CONCLUSIONS

1. Treated cultch showed a mean increase in numbers of living oysters over control groups. In two out of three studies the difference was apparent all through the experiment; in the third, this became apparent at the end of the study.

2. Size of spat on test and control groups was similar.

3. Increased production associated with treatment was estimated at 150 bushels per 1,000 bushels of shell.

4. The study has shown that treatment of cultch by commercial growers may be economically feasible on the Eastern Shore of Virginia and may increase net profits on seed production programs.

## ACKNOWLEDGMENTS

The authors wish to thank the Ballard Fish and Oyster Company, Harvey L. and Robert L. Bowen Oyster Company, H. M. Terry Oyster Company, and Walker Brothers, Inc. These four oyster firms of Willis Wharf, Virginia, generously provided boats, equipment, shells, labor and oyster reefs, and gave of their time unstintingly for these studies. Without their interest and help, this work would not have been accomplished. We are also indebted to Hooker Chemical Company for furnishing the Polystream and providing the analysis of residual chemicals. We are especially grateful to H. M. Terry, Jr., whose personal interest and suggestions were invaluable.

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# TRACE METAL ACCUMULATION BY THE AMERICAN EASTERN OYSTER, *CRASSOSTREA VIRGINICA* [1]

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## ABSTRACT

*A 20-week study was conducted early in 1968 to corroborate an earlier experiment on uptake by* Crassostrea virginica. *About 200 November-harvested oysters were placed in each of eighteen 120-liter tanks making use of a flow-through sea water system. During the acclimation and experimental periods the sea water was maintained at a flow rate of 2.5 l/min and a 20 ± 1°C temperature with salinity variable but usually 31 ± 2 ppt. Beginning in January, the oysters were subjected to continuous levels of the nitrate salts of zinc (0.1 and 0.2 ppm), copper (0.025 and 0.05 ppm), cadmium (0.1 and 0.2 ppm) and chromium (0.05 and 0.1 ppm). Each week oysters were removed for analyses.*

*Information was obtained on trace metal accumulation rates, on mortalities, shell growth and on the general appearance of the oysters. The most obvious effects were observed in the copper-and the cadmium-exposed oysters. The bodies of the oysters exposed to copper became bluish-green in color and their shells showed excellent growth, mantle edge pigmentation increased and mortalities were slightly higher than in the controls. Cadmium-exposed specimens were emaciated, showed very little shell growth, lost pigmentation of the mantle edge and coloration of the digestive diverticulae and suffered high mortalities.*

## INTRODUCTION

We have been involved in investigations concerned with the maintenance of healthy seafood resources at the Northeast Laboratory since 1963, when the first of several studies was initiated, including a series on trace metals in shellfish. That series of studies was the basis for this paper. The general rationale for initiating these efforts was recognition of the fact that the chemical content of the hydrosphere, particularly in the coastal areas subjected to pollution, was an important part of the environment of estuarine species, including shellfish, and should be investigated (Houser, 1965).

The possible public health significance of chemically polluted estuarine waters, especially in relation to shellfish, was recognized at least a decade ago (Jensen, 1959). Three years later, Jensen (1962) announced plans of the Public Health Service to study trace metals and other chemicals in shellfish at two newly authorized Shellfish Sanitation Research Centers (Dauphin Island, Alabama and Narragansett, Rhode Island).

Our studies started during the summer of 1963, on the quahaug in Raritan Bay, New York New Jersey and then laboratory-based studies followed with the dedication in 1964 of the new facility in Rhode Island. The primary objective was to obtain much more comprehensive data on the levels of certain chemicals in shellfish than hitherto available. Accordingly, studies were carried out on a coast-wide sampling of shellfish, North Carolina through Maine, and on trace metal accumulation under controlled laboratory conditions utilizing atomic absorption spectrophotometry. Although the prime emphasis was on the ultimate levels attained by shellfish, associated observa-

[1] Contribution No. 30 from the Northeast Marine Health Sciences Laboratory, Narragansett, Rhode Island.

tions yielded additional information which was first reported by Pringle, Hissong, Katz and Muluwka (1968) and Shuster and Pringle (1968). Further observations made in 1968 are reported herein and discussed in relation to the earlier studies.

The most extensive data on trace metals in a single species of American shellfish were found in studies on the American oyster, *Crassostrea virginica*. The principal contributions were by Coulsen, Levine and Remington (1932), Chipman, Rice and Price (1958), McFarren, Campbell and Engle (1962), Galtsoff (1964) and Pringle *et al.* (1968); all summarized in Table 1. These data, although obtained using different analytical procedures, provided an overview of trace metal levels in the American oyster and useful reference levels for the interpretation of our experimental results.

Meanwhile, the health significance of trace metals in the environment, principally water and foods, had been investigated by Schroeder, Nason, Tipton and Balassa (1967) during the past decade.

Cognizance of the information provided by the cited studies, as well as a general knowledge of the actual and potential industrial metal-contain-

TABLE 1. *A comparison of trace metal levels in American eastern oysters from Atlantic coast waters, Maine through North Carolina.*

| Element | Source of data* | Trace metal levels (in ppm of oyster wet tissue weight) Range | Average |
|---|---|---|---|
| Zinc | (1) | 204.4 - 4120 | 1404 |
| | (2) | 310.0 - 4000 | 1641 |
| | (3) | 710 - 2760 | 1468 |
| | (4) | 740 - 1332 | 1018 |
| Copper | (1) | 6.83 - 517.4 | 133.4 |
| | (2) | 8.80 - 520.0 | 137.5 |
| | (3) | 84 - 600 | 230 |
| | (5) | 34.4 - 137.2 | 78.5 |
| Iron | (1) | 30.42 - 238.1 | 66.92 |
| | (3) | 23 - 183 | 57 |
| | (5) | 24.9 - 47.1 | 34.8 |
| Manganese | (1) | 0.14 - 15.00 | 4.30 |
| | (3) | 1.5 - 10.2 | 3.7 |
| | (5) | 1.05 - 3.00 | 2.3 |
| Cadmium | (1) | 0.08 - 7.78 | 2.40 |
| Lead | (1) | <0.12 - 2.29 ** | |
| Chromium | (1) | <0.12 - 3.40 *** | |
| Nickel | (1) | <0.12 - 1.74 **** | |
| Cobalt | (1) | - <0.12 | <0.12 |

*Note:* Except for source (1), the series of values from the other sources were determined by methods other than atomic absorption spectrophototometry.

(1) Pringle *et al.* (1968): 30 samples, Maine through North Carolina (1965 through 1967).

(2) McFarren *et al.* (1962): New Hampshire through North Carolina (1960).

(3) Galtsoff (1964): Long Island Sound 1933 to 1935): from dry weight data divided by 5.

(4) Chipman *et al.* (1958): Connecticut through Georgia; from dry weight data divided by 5.

(5) Coulsen *et al.* (1932): Rhode Island through New Jersey (1933).

*Adapted from Shuster and Pringle (1968).

**Only four samples exceeded 0.12 ppm lead.

***Only five samples exceeded 0.12 ppm chromium.

****Only this value exceeded 0.12 ppm nickle.

ing wastes, directed our attention toward studies mainly on zinc, copper, cadmium, lead and chromium. Two or more experimental concentrations of these metals were selected from a series of doubling concentration within the range of 0.025 to 0.2 ppm in sea water. This was done in anticipation that some of the resultant levels of uptake by the shellfish, after prolonged exposure to constant dosage in a flow-through sea water system (Fig. 1), would be comparable to the higher levels recorded in the field survey. The experimental data thus obtained from two such exxperiments (Pringle *et al.*, 1968 and Shuster and Pringle, 1968) revealed that the accumulation by oysters of zinc, copper and chromium produced 20-week uptake levels (Fig. 2) comparable to the highest found during the field survey (Table 1). For cadmium (17-week duration due to high mortalities) and the earlier study of lead (10-week exposure: Pringle *et al.*, 1968), the resulting experimental levels greatly exceeded the survey values.

Our experiments were limited in duration mainly by the number of oysters that could be ac-

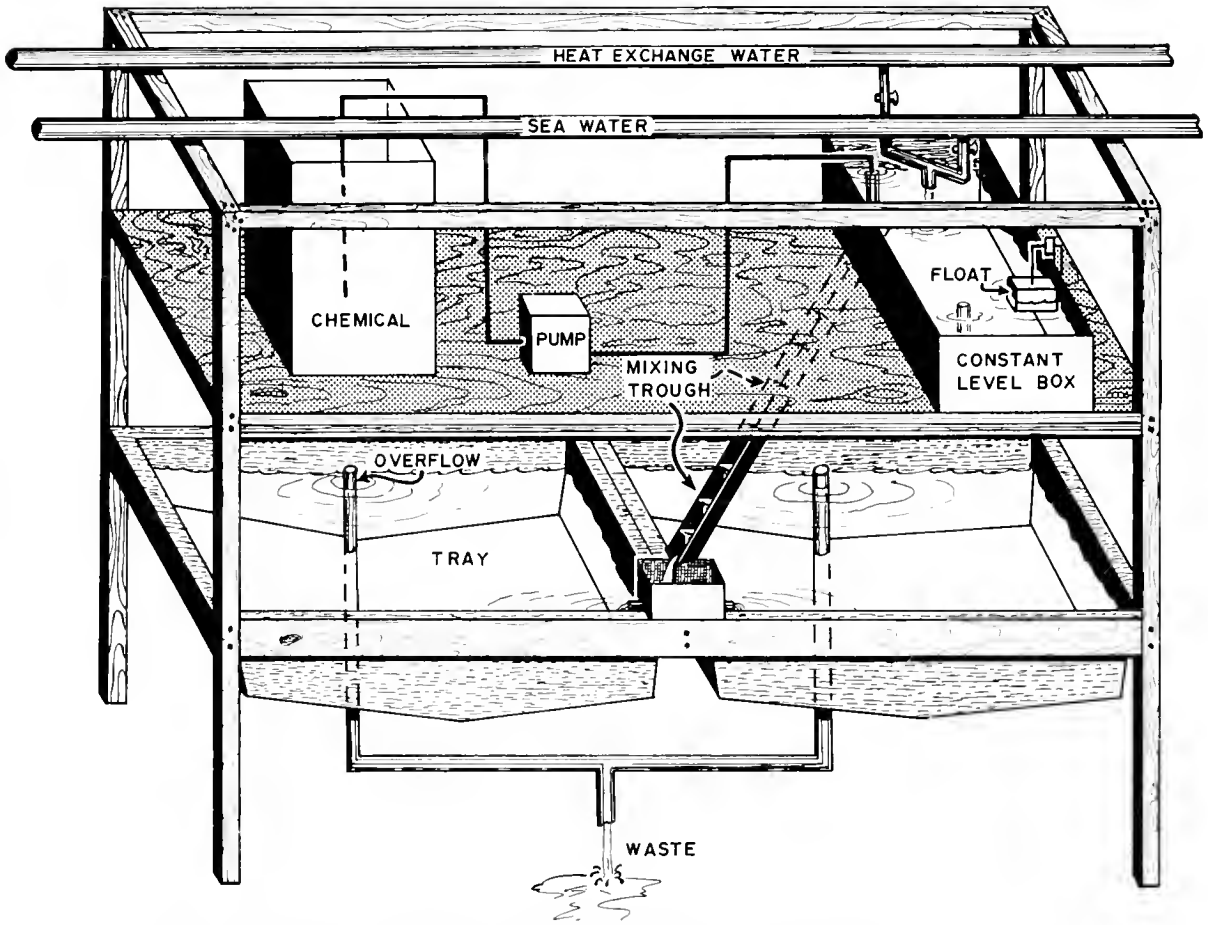


FIG. 1. *One unit of the two-tiered experimental system (from Pringle* et al., *1968): on the upper tier a metered quantity of a trace metal solution was added to the stream of water flowing from the constant level box into the mixing trough, which had a series of baffles. The chemical flow was controlled by the sea water level — constant water level was maintained by an overflow pipe but if the water level diminished a float-controlled mechanism shut off the chemical flow. The shellfish were held in a pair of 120-liter tanks on the lower tier. Prior to the A-68 experiment the two overhead pipes (heated sea water and ambient-temperature sea water) were replaced by open, rectangular troughs. One tray contained the 150-oyster population which was monitored for mortalities; the other tray was started with 252 specimens that were sampled weekly, throughout the experiment, for the analyses.*

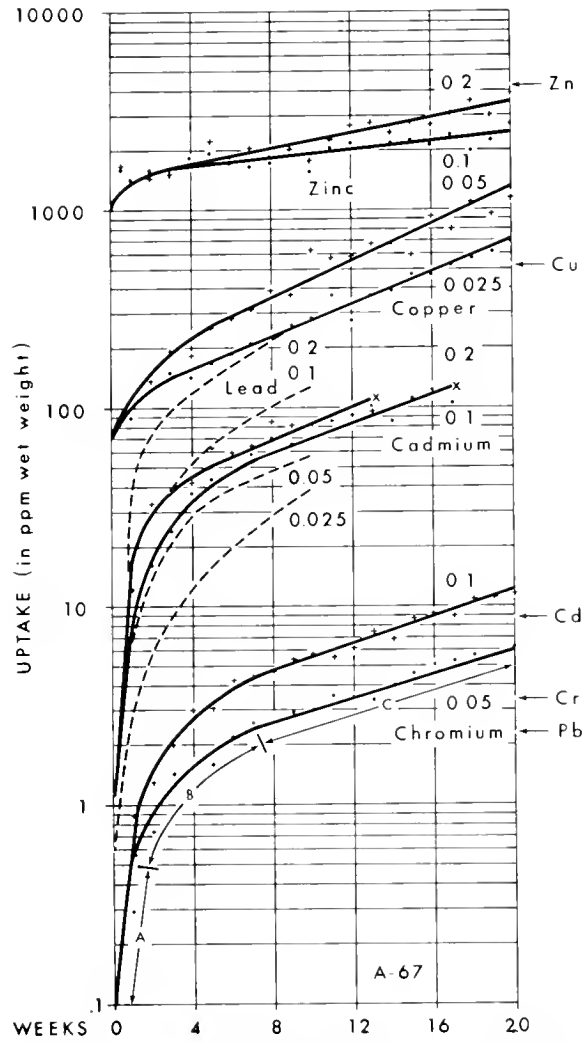


FIG. 2. *Semi-logarithmic plots of the A-67 and the 1966 lead data showed the relative changes in the rate of accumulation of several trace metals by the oyster. Three readily recognizable phases of uptake were indicated on the 0.05 ppm chromium curve by the capital letters A, B, and C. X's were used to mark the termination, due to heavy mortalities, of the cadmium-uptake studies. The arrows on the right margin of the graph marked the maximum levels we found in oysters from natural areas (see Table 1). The 10-week lead uptake curves were shown as dashed lines; the average initial level for each of these was 0.6 ppm lead.*

comodated by the experimental design. It was a matter of the space available versus the number of oysters required for analyses.

Among the questions that arose during consideration of the experimental design was what environmental conditions would be most appropriate to simulate. It was decided to provide an environment that would grossly approximate the effective growing season at northern latitudes and/or a suitable period of time for trace metal uptake. Butler (1953) had pointed out that the length of the oyster growing season varies with latitude: 7 months in the Canadian maritimes and 8 months in Long Island Sound. Hence, the water temperature was controlled by heat exchange equipment at 20 ± 1°C during the acclimation and the experimental periods which were of 20-week duration.

The earlier studies (Pringle *et al.*, 1968, and Shuster and Pringle, 1968) and our former experiences with oysters indicated that the hydroclimate, particularly temperature and salinity, the dosage and duration of exposure to metals and the physiological condition and activity of the shellfish, undoubtedly were among the parameters affecting the metal uptake and the levels ultimately attained in the tissues. Apparently the converse situation, depletion, was governed by the same parameters. Depletion data reported by Pringle *et al.* (1968) indicated that loss of trace metals from the tissues, when shellfish were no longer subjected to dosed-water levels of the metals, proceeded more slowly at lower (4 to 12°C) than at higher water temperatures (20°C). In addition, a direct relationship existed between the level of accumulated metal and the time for depletion -- the higher the tissue metal level, the longer the time required for depletion. Ikuta (1968b) found that depletion of zinc and copper by the Pacific oyster, *Crassostrea gigas*, was also related to the levels of accumulation and that the loss of zinc was more immediate but only one-half as rapid as the rate of copper decrease.

The design of the 1967 experiment (see Pringle *et al.*, 1968) was modified and then used as the basis for another experiment which was conducted early in 1968. The modifications permitted a more accurate determination of mortalities and of shell growth. In addition, a few specimens were examined each week for histopathological changes while fresh tissue samples obtained from other oysters were studied to learn if the trace metals exerted any effect on certain enzyme systems in the oyster. These latter two observations are being reported separately; this report deals only with the uptake rates and the associated mortalities, shell growth, and changes in the external appearance of shucked oysters.

## METHODS

Our third long-term experiment on the oyster, reported herein for the first time and designated as A-68 in subsequent discussion, was patterned

after the procedure adopted for the second study (henceforth cited as A-67). Namely, in A-68, November 1967-harvested oysters were acclimated to the laboratory flowing-water system for several weeks (see Fig. 1). In that system the sea water was maintained at 20 ± 1°C, with salinity variable but usually 31 ± 2 ppt, depending upon conditions in Narragansett Bay at the laboratory sea water intake.

On 9 January 1968, all of the oysters were carefully re-examined and an exact number of large-sized specimens, approximately 100 mm in length, were placed in each tank. At that time the shell margins of all the oysters were lightly ground to a blunt edge in the manner described by Butler (1965). During the course of the experiment the new shell was trimmed from the specimens sampled each week for the chemical analyses. These trimmings and the shells were stored for future analyses on trace metal content of the shells.

The oysters were exposed to metered quantities of the nitrate salts of two concentrations each of four trace metals (zinc: 0.1 and 0.2 ppm; copper: 0.025 and 0.050 ppm; cadmium: 0.1 and 0.2 ppm; chromium: 0.05 and 0.10 ppm) in eight pairs of 120-liter Fiberglas[2] tanks. One of the paired tanks contained 150 oysters at the beginning of the experiment, with 252 specimens in the other tank. The 150 shellfish population was used to monitor mortality; the oysters were observed twice daily and gapers removed immediately from both tanks.

Nine oysters were removed at weekly intervals throughout the A-68 experiment, except that additional specimens were removed during the first week. These extra samplings occurred on the initial day of the experiment and on the fourth day. Six of the oysters from each tank were utilized as a pooled sample for chemical analyses, shell growth measurements, and other observations; three specimens were taken for the histopathological examinations.

Each of the weekly samples of six oysters from each concentration of metal were shucked into glass pie plates nested in ice. After color photography, the "iced" specimens were drained of fluids for 5 min on a No. 10 U. S. Standard Sieve and the pooled sample homogenized. Sufficient amounts of the homogenized, cold tissues were removed for the enzyme analyses. The remaining material was weighed for a "wet weight" determination and then frozen and lyophilized. The oysters were photographed on 35 mm color film on the initial day of the experiment and at 5-week intervals thereafter.

Observations on the condition of the shucked meats were subjective and limited to fullness and translucency of the body. The meat was firm and all tissues were opaque in good oysters; fair individuals had a tendency, less than 25%, towards translucent tissues; and poor oysters were greater than 25% translucent.

Each sample of the dried, lyophilized material was weighed and the percentage of solids calculated. The per cent solids varied from one 6-animal lot to another. For example, when pre-experimental pools of six oysters each were reduced to a dry weight, 16 such lots of the A-67 experiment averaged 16.02 ± 0.57% solids, while 9 lots of A-68 oysters were 21.38 ± 0.98 per cent. Generally, for quick computation, one can divide dry weight data by 5 to obtain approximate wet weight values (see Table 1, footnotes 3 and 4).

The samples for atomic absorption analyses were weighed out as soon as possible after lyophilization in order to avoid any moisture uptake which would upset the actual wet to dry weight relationship. Subsequently, these samples were wet digested to remove the organic material. To do this, 10 ml of concentrated nitric acid was added to approximately 0.5 gram of dried tissue in a 30 ml micro-Kjeldahl digestion flask. The material was digested for 2 to 3 hr until practically colorless. The digested samples were then diluted to 50 ml and analyzed by atomic absorption spectrophotometry.

Since a major objective was to record trace metal levels in shellfish under natural and experimental conditions, our baseline values were always derived from analyses on shellfish. Thus, the lowest levels observed in the coastwide survey established baselines of minimum levels in oysters from their natural habitat. When oysters were being acclimated in the laboratory prior to uptake trials, their pre-experimental levels of the trace metals became the experimental baseline values.

The difficulty of correlating tissue levels in shellfish with the ambient environmental water levels was demonstrated by the study of Ikuta (1968a) on the Pacific oyster. He found that the tissue levels of zinc and copper steadily rose in oysters transplanted into an area receiving industrial and mining wastes, during a 125-day period, July to November, 1965. But the resulting five sets of data, based on monthly shellfish and water samples, did not demonstrate any definite correlation between the tissue levels and the water samples. That is, the five oyster samples were more revealing of the probable long-term situation of the two trace metals in the water than the actual water samples.

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[2] Mention of commercial products does not imply endorsement by the Public Health Service.

In our experience and for the above reason, it seemed that an extensive temporal sampling of environmental waters, with much less frequent shellfish samples, and a comparison of the results with the more precise information tabulated from experimental studies would be required to obtain a more realistic approximation of the environmental situation. This was reinforced by the understanding that tissue levels may be affected by seasonal or any other variations in contaminant levels to which the shellfish were exposed (Chipman *et al.*, 1965) as well as the seasonal or other physiological conditions of the shellfish. As a result, we concentrated on determining tissue levels in the oyster.

For the most part, whenever sufficient data were available, only their range and arithmetic average were determined. In the case of the tissue uptake of trace metals, however, the data that described Phase C of each accumulation curve (see Figs. 2 and 3) were analyzed by least squares regression on a computer (see Table 5) to determine the degree to which the data described a line, and if they did to a high degree, to find the line of best fit.

## RESULTS AND DISCUSSION

*Accumulation*

The levels of trace metals in oyster tissues prior to each experiment or in non-dosed controls during the course of the 1968 experiment were summarized in Table 2. A comparison of these data with those obtained from coastwide surveys (Table 1) showed that the oysters selected for experimental uptake were comparable to the "average" determined by the surveys.

Our data on the experimental increase of five trace metals in the tissues of the American oyster were obtained during three experiments (on lead in 1966, Table 3; and for zinc, copper, cadmium and chromium in 1967 (A-67) and 1968 (A-68), Table 4). Least squares regression analyses, summarized in Table 5, were performed on the bulk of the A-67 and A-68 data and were utilized to construct the curves in Figures 2 and 3.

The A-67 and A-68 uptake data were generally comparable; where differences were seen, these probably were due to the higher initial levels of the trace metals in the A-68 oysters. The most evident differences were in the uptake of zinc and copper; their high initial A-68 levels in the tissues apparently precluded the usual pattern of rapid uptake during the first week or so. A similar situation was reported by Ikuta (1968a). He introduced Pacific oysters from Urashiro Bay with an initial tissue level of 30.5 ppm copper into a metal-polluted area, Akamizu Inlet, where the tissue level rose to 190.8 ppm in 125 days. That

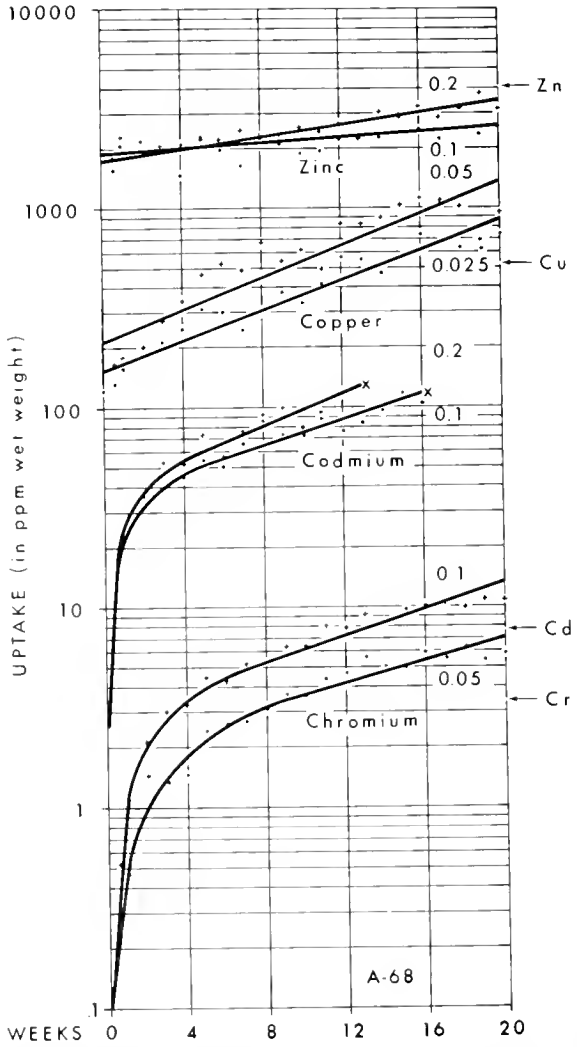


FIG. 3. *Semi-logarithmic plots of the A-68 experimental data showed the relative changes in the rate of accumulation of four trace metals by the oyster (see Fig. 2 for an explanation).*

increase followed an exponential function (log y = 0.0069x + 1.4399).

The full sets of data for both 20-week experiments (Table 4) have been utilized to prepare graphs showing the rate of change in the rate of uptake; hence, the curves (Figs. 2 and 3) showed the per cent change in the rate of uptake. In other words, the general curve showed an exponentially increasing rate and the data, as plotted, suggested an equation of the form $y = a(1 - e^{-bt})$.

Each curve usually showed three segments or phases in the rate of change in the rate of uptake. During the first week or so (Phase A) the rate of uptake increased at a rapid and contant rate,

TABLE 2. *Trace metal levels in oysters held in the flow-through sea water system (see Fig. 1), but not exposed to experimental dosages of the metals.*

| Experiment | Metal | Number of analyses | Levels (in ppm of wet tissue weight) Range | Mean |
|---|---|---|---|---|
| A-67 | Zinc | 7 * | 792 - 1895 | 1036 |
| | Copper | 7 * | 51.31 - 90.40 | 71.02 |
| | Cadmium | 7 * | 0.21 - 2.19 | 1.17 |
| | Chromium | 7 * | <0.12 | <0.12 |
| A-68 | Zinc | 6 * | 1426 - 2171 | 1708 |
| | | 22 ** | 1433 - 2317 | 1884 |
| | Copper | 6 * | 92.00 - 140.3 | 121.4 |
| | | 22 ** | 93.71 - 151.9 | 121.1 |
| | Cadmium | 6 * | 2.56 - 2.76 | 2.64 |
| | | 22 ** | 2.02 - 3.43 | 2.80 |
| | Chromium | 6 * | <0.12 | <0.12 |
| | | 22 ** | <0.12 - 1.08 | <0.19 *** |
| O-A | Zinc | 4 | 1563 - 2247 | 1786 |
| | Copper | 4 | 107.5 - 153.3 | 128.7 |
| | Cadmium | 4 | 2.54 - 3.37 | 2.98 |
| | Chromium | 4 | <0.12 | <0.12 |

*Analyses on oysters being acclimated prior to experiment.
**Analyses on experimental control oysters.
***Arithmetic mean based upon an assumed level of 0.10 ppm for all samples determined to be <0.12; only four samples had levels >0.12

while during Phase B the rate of uptake increased at a decreasing rate, and in Phase C the constant rate of uptake increase was less rapid than during Phase A. Phase A or both Phase A and B may be missing, however, depending upon the initial tissue level of the metal. This, as mentioned earlier, was demonstrated by the A-68 zinc- and copper-exposed oysters and by the Pacific oysters placed in Akamizu Inlet (Ikuta, 1968a).

Phase C of the data was analyzed by least squares regression and, as the data in Table 5 illustrated, all the correlations were high, except for the uptake in 0.1 ppm zinc (A-68) which was 0.06794. The R square values ($R^2$) showed that in all cases except for the A-68, 0.1 ppm zinc (46.2%), 72.8 % or more of the variation in log uptake was attributable to differences in time. The largest vertical deviation, as represented by the standard error of estimate, was only 1.226 ppm and that was for the 0.05 ppm copper data in A-68. Even

TABLE 3. *Accumulation of lead by the American eastern oyster experimentally exposed to four levels of lead in the flow-through sea water system (see Fig. 1), 19 April through 28 June 1966.*

| Week | Accumulation of lead in oysters (in ppm of wet tissues) 0.025 ppm | 0.05 ppm | 0.10 ppm | 0.20 ppm |
|---|---|---|---|---|
| 0 | 2.48 | 3.91 | 2.12 | <0.12 |
| 3/7 | 2.45 | 6.92 | 12.31 | 22.03 |
| 1 | 2.90 | 7.28 | 13.91 | 37.66 |
| 2 | 5.30 | 16.64 | 37.10 | 74.08 |
| 3 | 10.00 | 20.48 | 26.68 | 110.20 |
| 4 | 12.57 | 30.45 | 56.80 | 119.90 |
| 5 | 17.00 | 31.62 | 71.00 | 136.60 |
| 6 | 17.10 | 37.45 | 83.75 | 130.00 |
| 7 | 21.18 | 28.14 | 80.35 | 190.00 |
| 8 | 30.20 | 42.85 | 115.20 | 216.65 |
| 9 | 36.90 | 43.20 | 123.15 | 274.30 |
| 10 | 35.10 | 57.59 | 102.85 | 276.75 |

TABLE 4. *Summary of two 20-week periods of trace metal accumulation by the American eastern oyster. The levels of the metals in the oyster tissue (in ppm wet weight) were tabulated in reference to the weekly interval of sampling and the level of the metals in the experimental sea water system during the 1967 (A-67) and 1968 (A-68) studies.*

| Week | Zinc | | | | Copper | | | | Cadmium | | | | Chromium | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.1 ppm | | 0.2 ppm | | 0.025 ppm | | 0.05 ppm | | 0.1 ppm | | 0.2 ppm | | 0.05 ppm | | 0.1 ppm | |
| | A-67 | A-68 | A-67 | A-68 | A-67 | A-68 | A-67 | A-68 | A-67 | A-68 | A-67 | A-68 | A-67 | A-68 | A-67 | A-68 |
| 0 | 1036 | 1708 | 1036 | 1708 | 71.02 | 121.4 | 71.02 | 121.4 | 1.17 | 2.64 | 1.17 | 2.64 | <0.12 | <0.12 | <0.12 | <0.12 |
| 4-7 | 1640 | 1798 | 1679 | 1563 | 84.75 | 130.9 | 100.2 | 166.7 | 6.28 | 15.39 | 11.30 | 18.01 | 0.76 | <0.12 | 0.77 | 0.53 |
| 1 | 1456 | 2065 | 1381 | 2265 | 89.56 | 157.7 | 105.5 | 179.2 | 6.59 | 22.63 | 12.16 | 24.17 | 0.30 | 0.47 | 0.90 | 1.10 |
| 2 | 1561 | 2186 | 1470 | 1856 | 124.2 | 144.4 | 138.7 | 201.4 | 16.30 | 36.58 | 33.44 | 39.18 | 0.75 | 1.46 | 1.30 | 2.24 |
| 3 | 1538 | 1936 | 1519 | 2030 | 152.2 | 215.1 | 195.8 | 272.2 | 24.47 | 40.06 | 38.89 | 53.53 | 1.48 | 1.36 | 2.04 | 3.02 |
| 4 | 1831 | 2095 | 1761 | 1474 | 144.5 | 250.3 | 184.7 | 341.5 | 37.83 | 45.76 | 42.64 | 52.54 | 1.56 | 1.47 | 3.03 | 3.25 |
| 5 | 1956 | 2293 | 2234 | 2267 | 170.1 | 315.2 | 257.7 | 459.5 | 44.66 | 54.62 | 64.03 | 72.90 | 1.61 | 2.42 | 3.10 | 4.45 |
| 6 | 1736 | 1996 | 1811 | 2200 | 189.4 | 301.8 | 286.7 | 522.6 | 47.42 | 51.60 | 60.18 | 56.34 | 2.23 | 2.62 | 4.33 | 4.31 |
| 7 | 1666 | 1613 | 2055 | 2451 | 213.7 | 243.2 | 334.1 | 485.5 | 58.39 | 66.41 | 64.87 | 75.89 | 2.62 | 2.71 | 4.49 | 5.25 |
| 8 | 1732 | 2229 | 1869 | 2307 | 222.0 | 306.0 | 396.6 | 669.7 | 71.41 | 85.88 | 87.02 | 90.75 | 2.66 | 3.12 | 4.76 | 5.35 |
| 9 | 1911 | 2135 | 2037 | 2343 | 263.2 | 370.7 | 375.6 | 542.3 | 66.73 | 73.92 | 83.04 | 99.88 | 2.97 | 3.65 | 5.44 | 6.36 |
| 10 | 1570 | 1877 | 1767 | 2531 | 288.8 | 334.9 | 626.6 | 617.5 | 71.03 | 72.74 | 84.21 | 79.16 | 3.04 | 3.66 | 5.81 | 6.40 |
| 11 | 2035 | 1918 | 2269 | 2413 | 371.9 | 418.2 | 576.1 | 489.3 | 87.50 | 88.58 | 92.11 | 93.98 | 3.56 | 4.40 | 5.60 | 8.06 |
| 12 | 2189 | 2251 | 2660 | 2642 | 279.9 | 563.1 | 584.5 | 833.4 | 82.70 | 77.77 | 93.40 | 115.8 | 3.56 | 4.67 | 6.21 | 7.84 |
| 13 | 2059 | 2366 | 2733 | 2222 | 372.3 | 558.4 | 675.6 | 824.9 | 101.5 | 84.39 | 96.50 | 125.8 | 3.40 | 5.63 | 7.47 | 9.20 |
| 14 | 2139 | 2229 | 2445 | 3054 | 396.3 | 470.0 | 683.2 | 884.3 | 87.66 | 99.06 | * | * | 4.05 | 5.07 | 6.87 | 8.47 |
| 15 | 2212 | 2859 | 2740 | 2912 | 474.6 | 559.7 | 589.7 | 1020 | 111.6 | 118.2 | * | * | 4.67 | 4.94 | 8.81 | 9.75 |
| 16 | 2118 | 2475 | 2667 | 3233 | 478.9 | 737.0 | 931.4 | 1109 | 122.5 | 105.7 | * | * | 5.12 | 5.99 | 9.33 | 10.04 |
| 17 | 2382 | 2224 | 3033 | 2869 | 524.8 | 664.2 | 901.3 | 1079 | 106.7 | * | * | * | 5.35 | 5.51 | 8.98 | 10.46 |
| 18 | 1970 | 3314 | 3528 | 3159 | 568.7 | 630.0 | 1076 | 1001 | * | * | * | * | 5.69 | 6.21 | 10.94 | 9.92 |
| 19 | 2206 | 2340 | 2976 | 3743 | 611.8 | 622.8 | 942.9 | 682.9 | * | * | * | * | 5.64 | 5.43 | 11.12 | 10.94 |
| 20 | 2708 | 2560 | 3813 | 3185 | 691.8 | 715.6 | 1125 | 943.8 | * | * | * | * | 6.28 | 5.81 | 11.49 | 10.87 |

*No oysters available due to heavy mortalities.

TABLE 5. *Summary of least squares regression analysis of Phase C (see Figs. 2 and 3) of the uptake of metals by the oyster.*

| Number of points used in analysis | Trace metal (in ppm) | | Correlation between week and log uptake (Multiple R) | Standard error of estimate (ppm) | Multiple R squared ($R^2$) | Equation of line of best fit (for Phase C) | y-intercept (x = 0) * | y at x = 0 * |
|---|---|---|---|---|---|---|---|---|
| A-67: | | | | | | | | |
| 21 | 0.1 | Zn | 0.8531 | 1.091 | 0.7277 | $\log y = 0.00978x + 3.18295$ | 1524 | 1909 |
| 21 | 0.2 | Zn | 0.9341 | 1.112 | 0.8730 | $\log y = 0.01899x + 3.15539$ | 1430 | 2215 |
| 19 | 0.025 | Cu | 0.9900 | 1.080 | 0.9802 | $\log y = 0.04054x + 2.03554$ | 108.5 | 276.0 |
| 18 | 0.05 | Cu | 0.9668 | 1.163 | 0.9347 | $\log y = 0.04509x + 2.19618$ | 157.1 | 443.7 |
| 14 | 0.1 | Cd | 0.9598 | 1.112 | 0.9212 | $\log y = 0.03602x + 1.49125$ | 30.99 | 71.03 |
| 12 | 0.2 | Cd | 0.9388 | 1.143 | 0.8813 | $\log y = 0.04186x + 1.50652$ | 32.10 | 84.16 |
| 15 | 0.05 | Cr | 0.9873 | 1.056 | 0.9747 | $\log y = 0.03139x + 0.17858$ | 1.509 | 3.037 |
| 15 | 0.1 | Cr | 0.9861 | 1.060 | 0.9724 | $\log y = 0.03237x + 0.42800$ | 2.679 | 5.646 |
| A-68: | | | | | | | | |
| 22 | 0.1 | Zn | 0.6794 | 1.136 | 0.4616 | $\log y = 0.00755x + 3.26502$ | 1841 | 2190 |
| 22 | 0.2 | Zn | 0.8858 | 1.122 | 0.7846 | $\log y = 0.01462x + 3.24154$ | 1744 | 2442 |
| 22 | 0.025 | Cu | 0.9572 | 1.187 | 0.9162 | $\log y = 0.03757x + 2.18181$ | 152.0 | 361.0 |
| 22 | 0.05 | Cu | 0.9054 | 1.336 | 0.8198 | $\log y = 0.04100x + 2.31757$ | 207.8 | 534.0 |
| 15 | 0.1 | Cd | 0.9474 | 1.126 | 0.8975 | $\log y = 0.03282x + 1.54593$ | 35.15 | 74.84 |
| 12 | 0.2 | Cd | 0.9334 | 1.142 | 0.8711 | $\log y = 0.03972x + 1.57947$ | 37.97 | 94.77 |
| 16 | 0.05 | Cr | 0.9324 | 1.127 | 0.8694 | $\log y = 0.02710x + 0.29424$ | 1.969 | 3.675 |
| 18 | 0.1 | Cr | 0.9589 | 1.130 | 0.9196 | $\log y = 0.03258x + 0.46467$ | 2.915 | 6.173 |

*These two points, found by solving the equation of best fit by antilogs, were used to plot the lines of best fit on the semilog graphs (Figs. 2 and 3).

though a limited number of data points were available (which reduced the reliability of the analyses), the least squares regression analyses strongly suggested the linearity of Phase C. Thus, Phase C of the curves, as shown in Figures 2 and 3, was the line of best fit as determined by a statistical analysis of weeks versus the log of the metal uptake.

Shuster and Pringle (1968) found that, in general for each doubling of the environmental level of a trace metal, the tissue level of the metal approximately doubled. They pointed out that the doubling of the accumulation for each doubling of the water level was adversely affected, as in the case of the A-67 cadmium-exposed (0.2 ppm) oysters, when the tissue level of the metal reached an obvious disastrous amount. Further limitations were indicated by the results of the A-68 experiment. Those results, specifically from the zinc- and copper-exposed oysters, indicated that doubling occurred only when the initial tissues levels were low and only when the uptake curve had entered Phase C.

The cadmium uptake data also showed that the maximum level attained by oysters before their death was a few hundred ppm and that an uptake by a factor of 2 logs occurred in oysters with an initial tissue level of less than 1 in about 15 weeks in dosages of 0.1 and 0.2 ppm. If the initial tissue level had been 30 to 50 ppm cadmium, then uptake probably would have proceeded like that of zinc and copper during the A-68 experiment (see Fig. 3), until the lethal tissue levels were approached.

*Mortalities*

Although Shuster and Pringle (1968) were able to report only relative mortalities, their A-67 data were comparable to those ascertained with more certainty from the A-68, 150-oyster populations (Table 6). Since cadmium had the most demonstrable effect in both studies, only data for the cadmium-exposed oysters and the aberrant mortality in the A-68 control were considered further.

When graphed, A-67 and A-68 mortalities due to exposure to 0.2 ppm cadmium revealed similar curves (Fig. 4). The difference between the location of the two curves probably was due to the manner in which the data were collected. In the A-67 study it was necessary to calculate relative mortalities, whereas a direct per cent value was obtained from the A-68 data. During the A-67 experiment both dead and live oysters (for chemical analyses) were removed from each tank. This dual loss of oysters rapidly reduced the number of surviving oysters, hence hastened the conclusion of the studies. By contrast, one of the paired-tanks was observed for mortalities while specimens for analyses were taken only from the other during the A-68 experiment. This difference in collecting the data probably was the main reason for the position of the A-67 curve to the left of the A 68 mortality curve. Both sets of data, however, suggested that if a 100% mortality occurred in a tank during the 20-week experiment, then one-half of the oysters had died by about the mid-way point of the ultimate survival time.

The oysters in the control tank showed higher-than-expected mortalities but by the time this had become an obvious trend and the situation corrected, it was too late in the experiment to substitute suitable new oysters. A continuing search for the difficulty finally implicated the sea water pipes

TABLE 6. *A summary of mortalities during the 20-week, A-68 experiment; each tank was started with 150 oysters.*

| Metal | Concentration in sea water (in ppm) | Mortality during 4-week periods | | | | | Total dead | |
|---|---|---|---|---|---|---|---|---|
| | | 0-4th week | 5-8th week | 9-12th week | 13-16th week | 17-20th week | Number | % |
| Zinc | 0.1 | 0 | 2 | 4 | 2 | 3 | 11 | 7.3 |
| | 0.2 | 0 | 1 | 5 | 2 | 2 | 10 | 6.7 |
| Copper | 0.025 | 1 | 4 | 6 | 2 | 2 | 15 | 10 |
| | 0.05 | 5 | 1 | 10 | 3 | 3 | 22 | 14.7 |
| Cadmium | 0.1 | 2 | 15 | 64 | 36 | 9 | 126 | 84 |
| | 0.2 | 6 | 19 | 91 | 25 | 9 * | 150 | 100 |
| Chromium | 0.05 | 3 | 1 | 0 | 4 | 1 | 9 | 6 |
| | 0.1 | 2 | 8 | 5 | 3 | 3 | 21 | 14 |
| Control | | 3 | 12 | 29 | 27 | 14 | 85 | 56.7 |
| Control rerun ** | | 1 | 2 | 2 | 3 | 1 | 9 | 6 |

*Last survivors died during the 19th week.
**2 July — 19 November 1968; acclimated from day of arrival, 13 June 1968, to start of rerun.

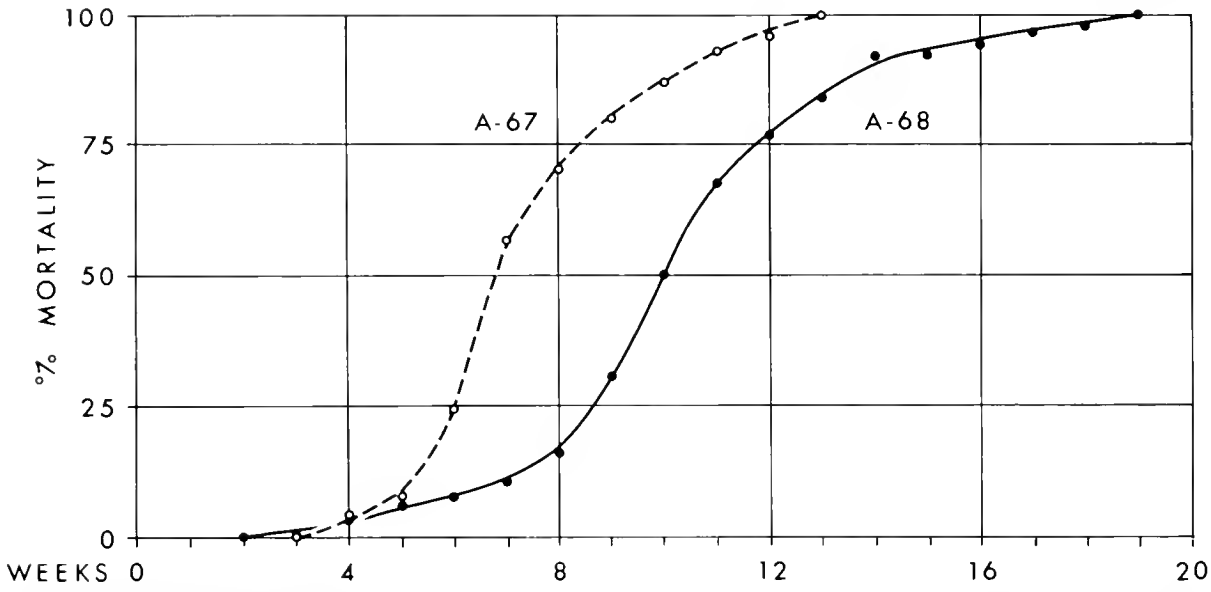


FIG. 4. *Cumulative mortality, in %, of the 176 (A-67) and the 150 (A-68) oysters that had been exposed to 0.2 ppm cadmium.*

which served only the control tanks. Tests on sulfide levels during the 13th week of the experiment showed that the sea water flowing into the control tanks, 0.06 mg/l, was twice that entering the experimental tanks. When the pipes leading to the control tanks were disassembled a mass of living and moribund mussels, *Mytilus edulis*, were found and then promptly removed. This cleansing of the pipes, so late in the experiment, did not noticeably alter the mortality trend in the control tanks. Later in the year a second collection of oysters from the original harvest site was placed in tanks that had been used for experiment populations in the A-68 uptake study. Although this second control group (designated as O-A) furnished additional data, the time of year of its harvest and the presumed associated biological conditions seemed to rule out complete comparability with the original A-68 oysters. The O-A data were summarized in Table 2 and 6. If the O-A, 20-week mortality of 6% was a valid base, then at least the 14 and 14.7% mortalities in the 0.1 ppm chromium and the 0.05 ppm copper, respectively, were excessive deaths probably related to the levels of the trace metals.

Overall, the mid-period (9 to 12th weeks) of the experiment usually was the one with the peak mortalities. The only exception occurred in the 0.1 ppm chromium, where the 5 to 8th week period had the highest number of deaths.

*Effects on the Shell Growth*

Shuster and Pringle (1968) reported three trends that occurred in shell growth during the A-67 experiment: 1) cadmium-exposed oysters had not developed as much new shell as the oysters exposed to the other metals, 2) considerable shell growth occurred in the zinc-, copper-, and chromium-exposed specimens and 3) the copper-exposed oysters had the most shell growth. Those trends, however, were based on limited observations — weekly measurements on two specimens from each water level of the trace metals, starting with the seventh week of exposure. Comparable, but more extensive data resulted from the A-68 experiment.

The A-68 data were obtained from weekly measurements of six specimens from each of the trace metal environments. A summary of that data (Table 7) provided information on 24 oysters for each metal in 4-week periods. Most notable were the time-shell-growth relationships for: 1) the number, hence per cent, of oysters which had shell growth, 2) the trace metal environment in which the least (and most) growth occurred, and 3) the environment in which the lowest (and highest) average shell growth occurred.

During the first 4 weeks of the A-68 experiment, only 50% or less of the oysters grew any shell. Except for the cadmium-exposed and the control oysters this lack of shell growth changed markedly during the next 4-week period. Even so, at the end of the 20-week experiment, there were still several oysters in each tank that evidently had not grown any new shell. In terms of the most

TABLE 7. *A summary of shell growth during the 20-week, A-68 experiment; measurements (in mm) from the 6 oysters sampled each week for the chemical analysis.*

| Metal | | Shell growth during 4-week periods | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration | | 0-4th week* | | 5-8th week | | 9-12th week | | 13-16th week | | 17-20th week | |
| in sea water (in ppm) | | Range ** | Avg. *** | Range | Avg. | Range | Avg. | Range | Avg. | Range | Avg. |
| Zinc | 0.1 | 0(12)-6 | 1.8 | 0( 2)-9 | 3.1 | 0( 3)-11 | 4.1 | 0( 3)-13 | 4.8 | 0( 3)-15 | 6.0 |
| | 0.2 | 0(17)-6 | 0.6 | 0( 2)-9 | 3.5 | 0( 2)-13 | 4.5 | 0( 3)-11 | 5.6 | 0( 0)-10 | 4.6 |
| Copper | 0.025 | 0(15)-7 | 0.9 | 0( 3)-9 | 2.9 | 0( 2)-14 | 4.8 | 0( 4)-13 | 5.2 | 0( 3)-12 | 5.0 |
| | 0.05 | 0(17)-5 | 0.9 | 0( 3)-9 | 4.5 | 0( 5)-12 | 4.4 | 0( 3)-12 | 5.2 | 0( 6)-13 | 3.6 |
| Cadmium | 0.1 | 0(23)-1 | 0.04 | 0(16)-8 | 0.9 | 0(22)-1 | 0.07 | 0(14)-4 | 0.7 | *** | |
| | 0.2 | 0(24) | | 0(22)-1 | 0.06 | 0(23)-2 | 0.09 | *** | | *** | |
| Chromium | 0.05 | 0(17)-9 | 1.2 | 0( 7)-9 | 2.7 | 0( 6)-11 | 2.7 | 0( 3)-14 | 5.9 | 0( 6)-12 | 4.2 |
| | 0.1 | 0(18)-5 | 0.7 | 0( 2)-7 | 2.7 | 0( 3)-7 | 3.1 | 0( 1)-8 | 3.4 | 0( 4)-9 | 3.1 |
| Control | | 0(20)-2 | 0.3 | 0(12)-3 | 0.5 | 0(20)-2 | 0.3 | 0(18)-2 | 0.3 | 0(10)-20 | 3.0 |

*No growth for any oyster was observed during the first week.
**The numbers within the parentheses are the numbers of oysters which had no shell growth during the period.
***Average, to nearest 0.1 mm, except for the cadmium-exposed oysters.
****No oysters available due to heavy mortalities.

oysters showing some shell growth, the zinc-exposed oysters had the best record.

As in the A-67 experiment, the least shell growth occurred in the oysters exposed to the 0.2 ppm cadmium. Overall, the best shell growth was that of the zinc-exposed oysters, but the copper-exposed specimens grew nearly as well. The poor growth of the control oysters was most unfortunate; the background for this was discussed under mortalities. As a result we lacked information on what the "normal" growth would have been. It was also apparent, by the time the A-68 oysters reached the last 4-week experimental period (17 to 20th week), that additional new growth was minimal. That lack of new growth might have been a normal slowdown, but it was probable it resulted from a combination of several factors, not the least of which may have been the cumulative effects of a low level of available food and the increasing levels of trace metals in the tissues. Some of the changes noted in the growth data may have resulted also from repeated damage to the fragile, thin edge of new shell during the weekly washing of the oysters and tanks to flush out accumulated debris including feces and pseudofeces. It is possible too that there was a bias in the sampling of the oysters since those with or without shell growth, particularly in the early stages of the experiment, may have been differentially selected unintentionally.

*Changes in General Appearance*

Our weekly examination of six shucked oysters from each of the trace metal environments revealed some changes of body color, in mantle edge pigmentation, and of general body appearance. Color changes were most obvious in the copper- and the cadmium-exposed oysters. The cadmium-exposed oysters deteriorated to the poorest condition of any of the metal-exposed specimens.

As noted by Galtsoff (1964), the intensity of color in "green" oysters is directly related to their copper content. The general sequence of uptake (see Table 4, which gives the average tissue levels for pooled lots of six oysters) and related coloration has been further delineated by our experiments. By the end of the first week all of our A-68 oysters exposed to 0.05 ppm copper had green-tinted bodies and a tissue level of 179.2 ppm. Only two of those in the 0.025 ppm tank had such coloration (tissue level of 157.7 ppm copper). Similar greenish tints occurred, however, among all of the other lots of oysters during the 20-week experiment but only the copper-exposed oysters developed the deeper green and blue-green coloration. A definite pale green coloration had developed in all of the oysters exposed to 0.05 ppm copper (tissue level of 272.2 ppm) by the end of the third week. A "moderate" green occurred in these oysters by the fourth week (tissue levels of 341.5 ppm copper). The first blue-green oysters in the 0.05 ppm sample (tissue level of 459.5 ppm) occurred in the fifth week; only one of the 0.025 ppm copper-exposed oysters was blue-green in the sixth-week sample (tissue average of 301.8 ppm). By the seventh week, five of the six 0.05 ppm specimens were blue green; with a tissue level of 485.5 ppm. A noticeable blue-green coloration was already present in the A-67 oysters exposed to 0.05 ppm copper when we first recorded our observation on color changes in that experiment (seventh week

of exposure). At that time their tissue levels averaged 334.1 ppm copper. A greenish tint was observed a week later in the A-67 oysters exposed to the 0.025 ppm solution; those oysters had an average tissue level of 222.0 ppm. The differences between the time of appearance of the green colors in the A-67 and A-68 oysters could have been due to the differences in the initial zero-day tissue levels (71.02 ppm copper in A-67; 121.4 ppm in A-68). Galtsoff (1932) found that oysters turned green in a 0.13 ppm copper solution within three weeks.

By the end of the A-67 experiment, most of the oysters "bled" globs of blue green material resembling semi-coagulated vertebrate blood. Galsoff (1964) had reported that a large proportion of the copper in oysters was in the blood cells; Shuster and Pringle (1968) postulated that it may be bound in a proteinate complex.

The cadmium-exposed oysters lost the coloration usually associated with oysters in good condition more rapidly than those in the other trace metal environments. The cadmium specimens looked "faded." This paleness extended even to their digestive diverticulae which retained only a hint of their original dark, greenish-brown color.

Galtsoff (1964) noted that pigmentation of the mantle edge differed from specimen to specimen, even among oysters from the same stock and environment. This was also seen in pre-experimental-shucked samples of our oysters. But, once the experiments were well-underway, our subjective observations revealed that there were less differences among the oysters within each lot. Also, there was a more noticeable difference among the several lots of oysters. For example, after several weeks, the mantle edges of the cadmium-exposed oysters were much paler than they had been initially, whereas those of the copper-exposed specimens had become the most deeply pigmented of all the oysters.

Emaciation was most apparent in the cadmium-exposed and the A-68 control oysters. The reason for the poor condition of the controls was discussed under the topic of mortalities. Those cadmium-exposed oysters which survived into the second half of the 20-week experiments (A 67 and A-68), besides being emaciated and in a poor condition, were also pale in color as previously noted. In the 1966 experiment, those oysters subjected to the high sea water levels of lead (0.1 and 0.2 ppm) had a general atrophy of tissues near the termination of the 10-week experimental period (Shuster and Pringle, 1968).

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# THE MALACOLOGY OF PHILIPPINE OYSTERS OF THE GENUS *CRASSOSTREA* AND A REVIEW OF THEIR SHELL CHARACTERS

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ABSTRACT

*Detailed studies on the soft anatomy of seven species of locally occurring oysters include two specific characters besides those used by others. One is the formation of a collar around the anal port, and the other is the predominating pigment of the circumpallial region. The form and expansion of the anal fold and the circumpallial pigmentation are very useful characters in identifying the species.*

*All the specimens studied possessed the generic features of* Crassostrea: *well developed promyal chamber; the heart is not wrapped around the rectum; and moderately to markedly recessed below the hinge.*

*The original descriptions of* Ostrea iredalei *Faustino and* Ostrea malabonensis *Faustino are purely conchological. The soft parts of these two species are for the first time described here in detail. These two species properly belong to the genus* Crassostrea.

## INTRODUCTION

The need to update the taxonomic status of Philippine oysters is long overdue. From the time Dr. Leopoldo A. Faustino first described the species *iredalei* which he assigned to the genus *Ostrea* in 1932, no work has yet been done to revise the classification of the locally occurring oysters up to the present time, and since then the species afore-mentioned together with all the others remained classified under the genus *Ostrea*. Recent workers on oysters still mention the supposedly twelve species of ostreids in the Philippines under that genus (Blanco, Villaluz and Montalban, 1951; Sarenas, 1952; Blanco and Montalban, 1956[1]; Blanco, 1956[2]; EFD:PFC, 1967[3]).

Authorities on the nomenclature of oysters at present recognize only three living genera of ostreids: *Ostrea* Linnaeus, *Crassostrea* Sacco, and *Pycnodonte* Fisher de Waldheim. For the generic descriptions and historical background of ostreid taxonomy, the reader is referred to the works of Galtsoff (1964) and Thomson (1954).

All the specimens of seven species examined in this study fit the generic details of *Crassostrea* and they shall therefore be referred to this genus throughout the text.

## DIAGNOSTIC MALACOLOGICAL CHARACTERS

*Generic Features*

The genera of living oysters today recognized in the zoological nomenclature are differentiated malacologically by at least four characters: (1) presence of promyal chamber, (2) diameter of the gill ostia, (3) auricular fusion of the heart and recto-ventricular relation and (4) per cent surface area of the adductor muscles (Thomson, 1954).

As observed among the Australian and

[1] Blanco, G. J. and H. R. Montalban. 1956. Scheme of oyster farming in the Philippines. Bur. Fish., DANR, Manila, 10th issue, 12 p. (processed).

[2] Blanco, G. J. 1956. Philippine marine shell resources. Bur. Fish., DANR, Manila, 22 p. (processed).

[3] Estuarine Fisheries Division. 1967. Oyster farming. Philippine Fish. Comm., DANR, Manila, 8 p. (processed).

American specimens, these genera may be briefly differentiated from one another as follows. Those of the genus *Crassostrea* possess distinct promyal chamber. This chamber is also present in the genus *Pycnodonte* but absent in *Ostrea*. Being larviparous, the latter genus has relatively large gill ostia in contrast to the narrower openings of *Crassostrea* and *Pycnodonte* (Galtsoff, 1964; Thomson, 1954). Pycnodontes are easily recognized by the penetration of their recta through their ventricles, a condition never found among the species of the other two genera.

Thomson (1954) noted that among the Australian species at least, the fusion of the auricular walls at varying degrees is of important generic feature. It is interesting to note that in the present work, with all the specimens studied so far belonging to the genus *Crassostrea* this anatomical feature had lost its generic significance since the different species showed very wide variation from almost complete fusion to complete separation of the auricular walls, e.g.*Crassostrea iredalei* (Faustino) has auricles very widely to almost completely separate; *C. malabonensis* (Faustino) has auricles very broadly joined; and *C. lugubrius* (Sowerby) has about 50% of its

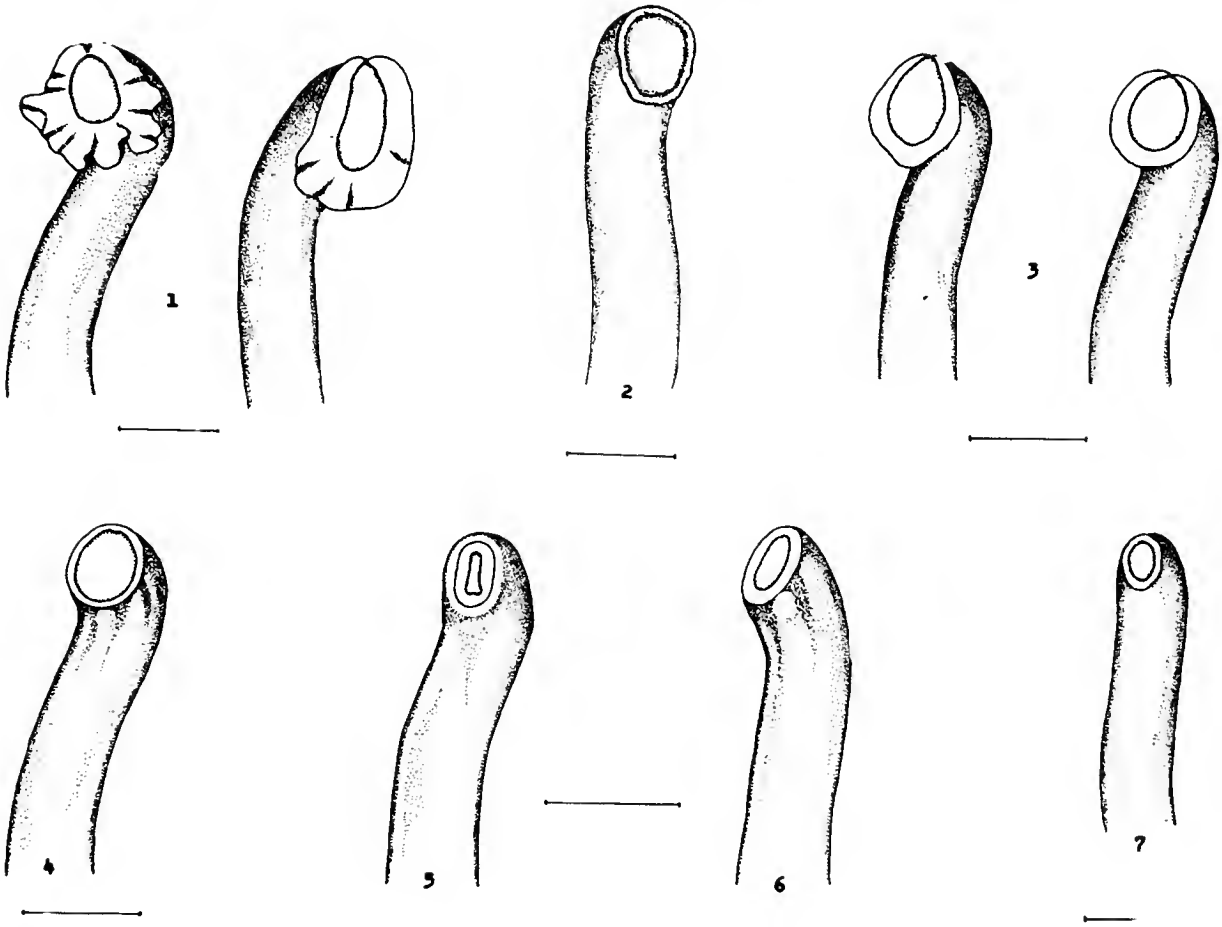


Plate I. ANAL FOLDS

Note: All magnification lines each = 2 mm

Fig. 1 — *Crassostrea iredalei*
Fig. 2 — *Crassostrea malabonensis*
Fig. 3 — *Crassostrea tuberculata*
Fig. 4 — *Crassostrea echinata*
Fig. 5 — *Crassostrea lugubrius*
Fig. 6 — *Crassostrea palmipes*
Fig. 7 — *Crassostrea amasa*

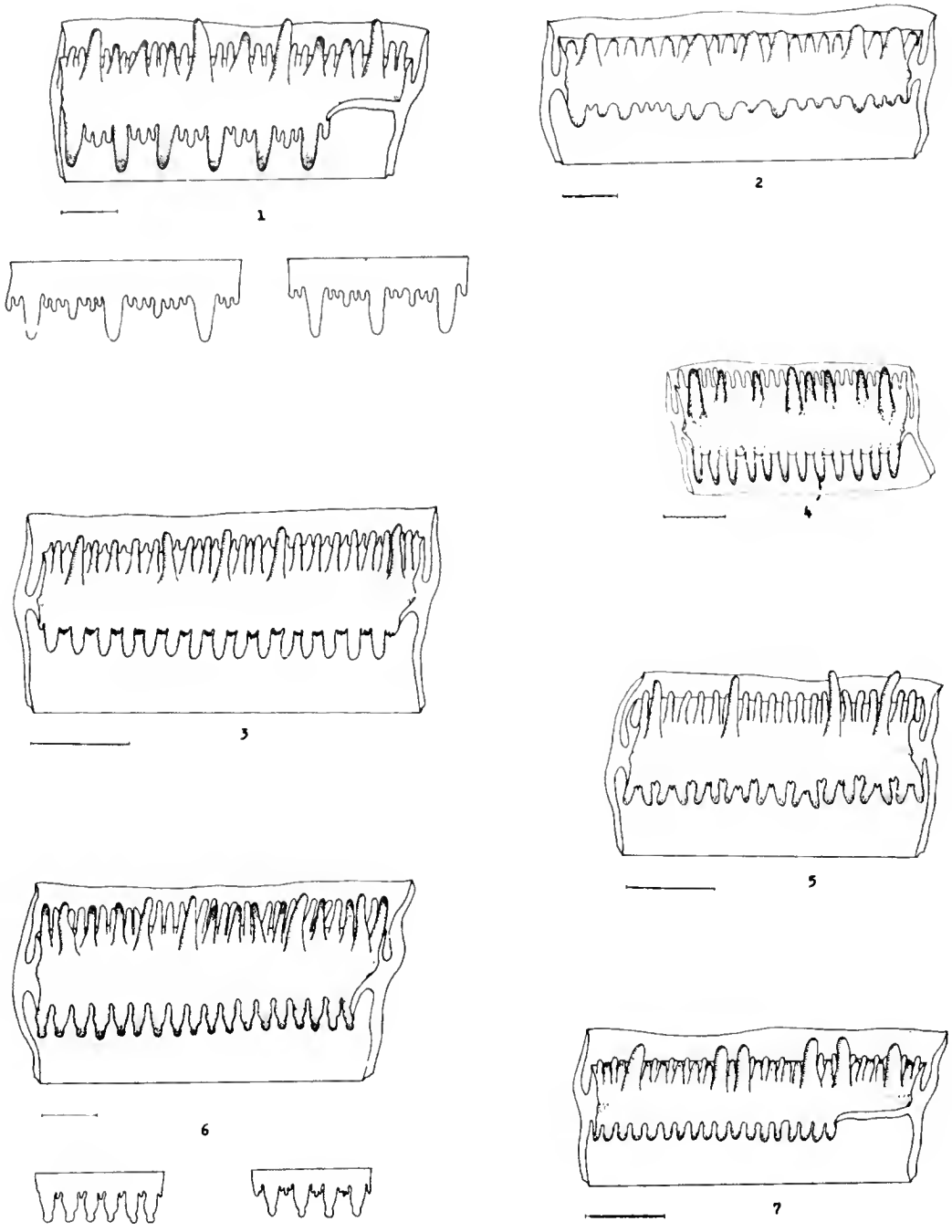


Plate II. ARRANGEMENT OF TENTACLES (Semi-diagrammatic)
Note: All magnification lines each = 2 mm

Fig. 1 — *Crassostrea iredalei*
Fig. 2 — *Crassostrea lugubrius*
Fig. 3 — *Crassostrea palmipes*
Fig. 4 — *Crassostrea malabonensis*
Fig. 5 — *Crassostrea amasa*
Fig. 6 — *Crassostrea echinata*
Fig. 7 — *Crassostrea tuberculata*

auricular length united posteriorly (Pl. III, figs. 1, 2, and 4). The condition of the auricles was studied through careful cardiectomy and slide preparation of the cardiac tissues.

Per cent surface area of the adductors was also observed to be a generic diagnosis among the Australian specimens (Thomson, 1954). The catch muscle area which is nearer the hinge is said to be more than 50% in *Ostrea*, about 30% in *Crassostrea*, and less than 30% in *Pycnodonte*. Among almost all the locally occurring specimens of the seven species studied, the catch muscle area ranged from 50% and up so that this character does not apply to the Philippine oysters of the genus *Crassostrea*.

*Specific Feature*

Identification of the species becomes more conclusive by considering the following features of the soft parts in corroboration to some important conchological characters: (1) gill count, which refers to the number of filaments per plica of the gills, (2) number of rows of tentacles on the middle fold and tentacular arrangement on the inner mantle fold; (3) predominating pigment or pigments of the mantle folds; (4) Length of rectum relative to the dorsal side of the adductor; (5) pigmentation of the circumpallial region and (6) form and expansion of the anal fold or collar.

Galtsoff's (1964) observation that "other characters such as ridges of the mantle, pigmentation of the tentacles and their size and spacing are variable, and in my opinion have no taxonomic values" is true and very clearly noticed in this work. However, it was observed here that predominating pigment or pigments in the general mantle fold area is quite persistent specifically and this actually varied from one species to another and may therefore he used taxonomically. The arrangement of tentacles on the inner fold and the number of rows of tentacles on the middle fold are useful characters (Plate II), but such features as relative size of tentacles and their distribution are insignificant for purposes of identification.

More attention is due the last two characters in the list. The deposition of predominant pigment along the circumpallial line of the mantle is observed to be diagnostic to the species. This pigmentation may be in thin to narrow lines, sometimes very bold or diffused all along.

The form and expansion of the anal fold is very useful in identifying the species (Plate I). Similar observation was made by Hynd (1955) in four species of *Pinctada*. Among the ostreids under study, this fold forms a collar around the lip of the anus. The degree of its expansion and shape vary according to species.

## DATA AND DEFINITE LOCI OF SOFT PARTS FOR STUDY

There is a need for consistency in choosing definite loci of the soft parts wherein the data for diagnostic features may be taken. Such consistency is particularly imperative in counting the rows of tentacles of the middle mantle fold and in preparing sections of the gills for filament count per plica. In this work, it was noticed that the occurrence of the tentacles in rows on the middle fold of either mantle lobe is more normally discernible at a locus nearer the junction of the mantle. Farther from this site the tentacles occur very irregularly. The portion of the gills taken from each oyster specimen was regularly cut from the mid-length of the right outer and inner demibranchs and then carefully treated and prepared for sectioning. There was better conformity of filament counts in sections taken within the first two or three millimeters of the marginal area of the gill plate portion. Filament counts were erratic from plica to plica in sections taken from near the base of the gills.

Conformity in the choice of definite loci of the soft parts from specimen to specimen was strictly adopted here, not only in the inspection of the tentacles and the gills but also in the observation of pigment deposition.

The data on visible pigmentation of the mantle folds and the measurements of the ova were taken from live specimens. A 6X-micrometer eyepiece was used to take the diameters of the ova in temporary mounts without cover slips under a 10X-low power objective. The same eyepiece and objective were used in taking the sizes of the ostia by measuring the perpendicular distances in-between the filaments. As the degree of shrinkage of the gills after fixation is not known, the measurements of the ostia presented here are not corrected to that effect. This accounts for the big discrepancy in the sizes of the ova and ostia of each species.

Tentacular arrangement of specimens fixed in 5% formalin was observed with the use of a 20X-hand lens.

In the study of the union of auricles, each series of longitudinal sections was carefully inspected. Those sections which showed maximum fusion of auricular length were selected to yield the information needed. Typical examples for the species are shown in Plate III.

The oyster heart is rather delicate. To separate this organ from the rest of the soft parts without any damage, more particularly to the auricles, the tissues to which the ventricle was anteriorly and the auricles posteriorly connected were cut and extricated intact with the organ. The whole thing was then transfered to a petri dish a id


Plate III. UNION OF AURICLES (Camera Lucida; Longitudinal Sections)
Note: All magnification lines each = mm. Inserted broken lines indicate length of auricular fusion; A — auricle; V — ventricle.

Fig. 1 — *Crassostrea iredalci*
Fig. 2 — *Crassostrea malabonensis*
Fig. 3 — *Crassostrea tuberculata*
Fig. 4 — *Crassostrea lugubrius*
Fig. 5 — *Crassostrea echinata*
Fig. 6 — *Crassostrea palmipes*
Fig. 7 — *Crassostrea amasa*

then the heart was freed of unnecessary tissues before it was finally treated for sectioning.

## MALACOLOGY AND CONCHOLOGICAL REVIEW OF PHILIPPINE OYSTERS

### *Crassostrea iredalei* (Faustino)

Plate IV, Figs. 1, 2, 4

*Ostrea iredalei* Faustino, 1932, pl.l, figs. 1-4.

Right circumpallial line of the mantle surface facing the shell is orange brown to reddish brown; predominating tint of mantle folds is brown to dark brown. Tentacular arrangement: IF[4] has only one row; longer tentacles alternating with much smaller finger-like ones in groups of 3 (4)[5]; MF[6] has three rows of tentacles. Gill plates are gray or grayish; gill count[7] is 12 to 16 (18). Ova are 70 to 90 $\mu$; diameter of ostia 20 to 38 $\mu$. Auricles of the heart are completely separate or almost so (Pl. III, Fig. 1). Rectum reaches one-half around the dorsal side of the adductor; anal fold well expanded beyond the walls of the rectum and sub-oval in shape (Pl. I, Fig. 1); tip of the rectum is well free from attachment.

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[4] Inner fold of the mantle.

[5] Seldom occurring in that order.

[6] Middle fold of the mantle.

[7] Number of gill filaments per plica.

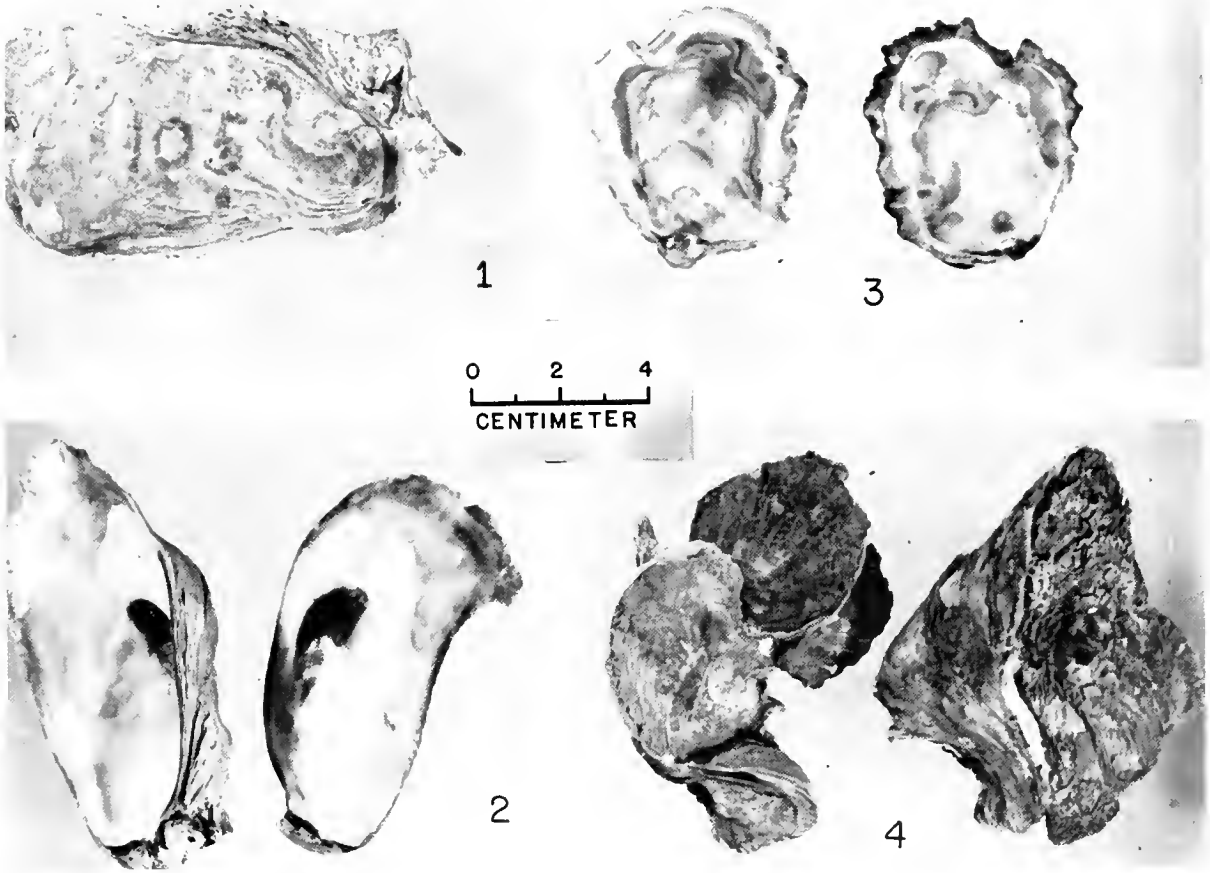


Plate IV. PHILIPPINE OYSTERS

Fig. 1 — *Crassostrea iredalei*, singly grown on the bottom mud.
Fig. 2 — *Crassostrea iredalei*, internal view of left and right valves.
Fig. 3 — *Crassostrea malabonensis*, internal view on left and right valves.
Fig. 4 — *Crassostrea iredalei*, (left) younger specimens growing in bunch and (right) older specimen extricated from a bunch.

Slight variation of IF tentacles was observed among specimens grown in bunches as follows: largest tentacles occurring at regular intervals with 3, 5 or 7 smaller tentacles in-between, the middle one of which is tallest and flanked by 1, 2 or 3 finger-like tiny tentacles on both sides as the case may be (Pl. II, Fig. 1).

Shell outline is very variable but typically elongate and slipper shaped when grown singly on or close to the bottom mud; margins not anywhere denticulate; inner surface generally soft and chalky, or with broad patches of chalky white; valves are equilateral, very foliaceous, and blunt or rounded at the lip. Muscle scar deep purple, curved on the side nearer the hinge, and displaced postero-dorsally. Periphery of inner surface of both valves yellowish to apparent yellow gold or yellowish white sometimes with streaks of purple. External surfaces yellowish white and smudged with purple or predominately tinted purple. Right valve almost flat but not operculate; left valve deep and slightly recessed below the hinge. Hinge plate squatting. Beak of left valve more produced than that of the right.

*Habitat:* All over Bacoor Bay of Cavite Province, Luzon Is. and possibly all oyster beds throughout the country.

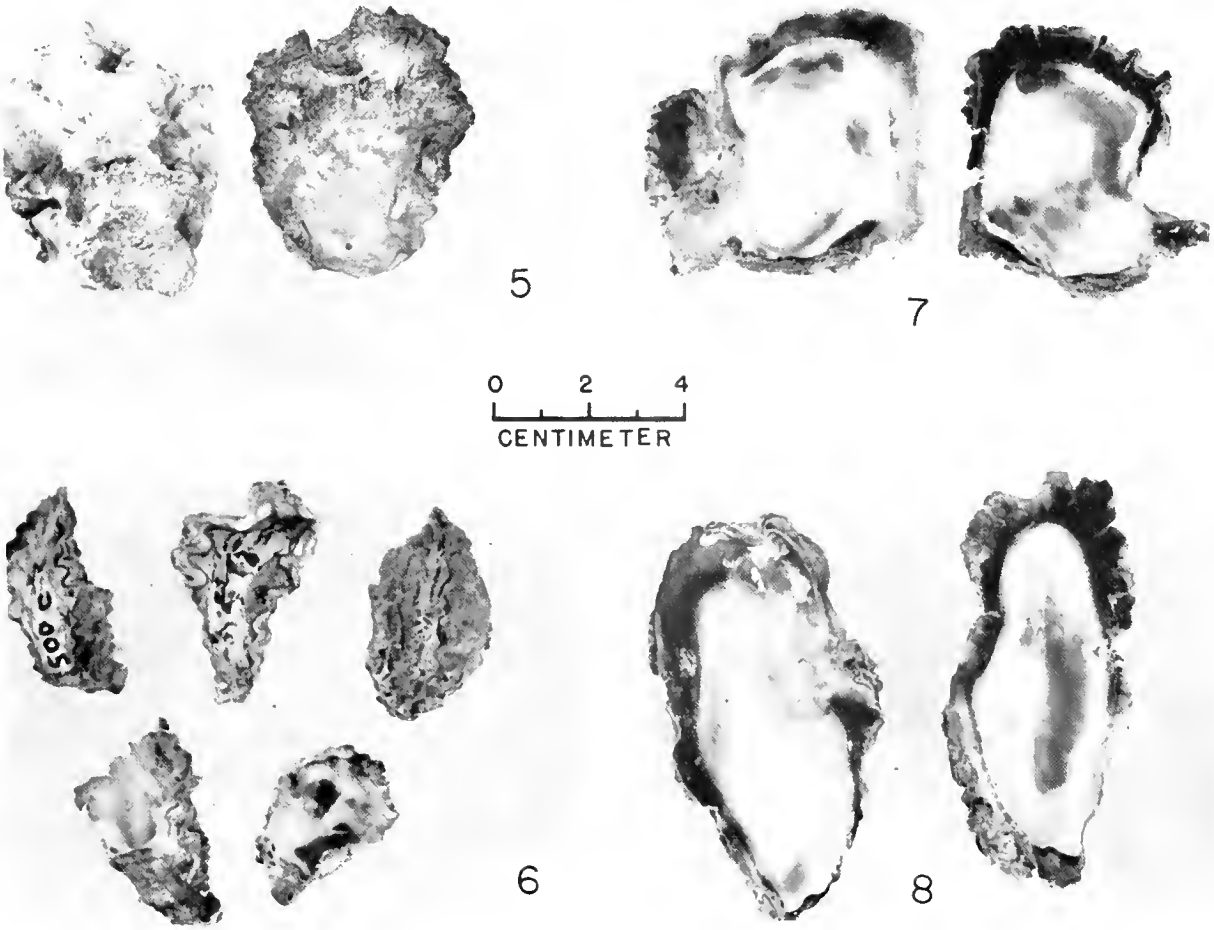


Plate V. PHILIPPINE OYSTERS

Fig. 5 — *Crassostrea malabonensis*, external view of left and right valves; specimen of Pl. IV, Fig. 3.

Fig. 6 — *Crassostrea amasa*, upper three are the more common forms extricated from a matted bunch; (lower) left and right valves of a specimen with extremely deep recess below the hinge.

Fig. 7 — *Crassostrea echinata*, internal view of left and right valves.

Fig. 8 — *Crassostrea echinata*, internal view of left and right valves.

*Dimensions:* (Adult) Length — 10 to 15 cm.
Width — 4 to 6 cm.

*Crassostrea malabonensis* (Faustino)
Plate IV, Fig. 3; Plate V, Fig. 5

*Ostrea malabonensis* Faustino, 1932, pl. 2, figs. 3-5.

Circumpallial region of the inner surface of mantle lobes light to deep yellow gold; predominating pigment of mantle folds is a suffusion of yellow and brown, sometimes dark brown. Tentacular arrangement: IF has only one row of tentacles uniform in size, well spaced with web-like membranes in-between; MF has three rows of tentacles. Gill plates and palps are white; gills with bluish tinge near the fusion of mantle lobes; gill count is 11 to 12; diameter of ostia 19 to 28 $\mu$; ova 56 to 75 $\mu$ in diameter. Auricles of the heart joined by more than one-half of the auricular length (Pl. III, Fig. 2). Rectum reaching about one-half along the dorsal side of the adductor; anal fold is absent (Pl. I, Fig. 2); anal port is not pinched; tip of the rectum is not bent away but attached to the adductor.

Shell outline is variable, oftentimes subtrigonal or oblong; valves inequilateral; rim of valves irregularly crenulate; right valve foliaceous but centrally smooth. Left valve distinctly plaited with large irregular folds; deep and cupshaped, typically hoof-like when grown uncrowded; markedly recessed below the hinge. Denticles present on both sides of the hinge region, seldom dentate at the lip margin. Muscle scar is curved on the side nearer the hinge and markedly displaced dorsally; scar is conspicuous on right valve but oftentimes not on the left valve. Inner surface of the rim of left valve with large patches of gray to dark gray; inner surface of both valves with blotches of dirty yellow or greenish brown; external predominating color usually purplish blue or gray. Hinge plate small; beaks unequal.

*Habitat*: Bacoor Bay, Cavite Province.

*Dimensions:* (Adult) Length — 5 cm.
Width — 4 cm.

*Crassostrea tuberculata* Lamarck
Plate VI, Fig. 11

*Crassostrea tuberculata* Lamarck (see Thomson, 1954,p. 157, pl. 8, fig. 3).

*Ostrea tuberculata* Lamarck, 1819.

*Ostrea cucullata* Lamarck, 1819. Born (in Rasalan, 1937,pl. 1, figs. 1-2, p. 269-278: Shell generally subtrigonal, solid, rather plaited, whitish toward apex, purple toward margin. Lower valve extends deeply beyond flat opercular upper valve; interior yellowish brown with slight purple tinge. Upper valve brownish near base, purple toward margin; denticulated to about two thirds from hinge line. . . .)

Inner surface of the circumpallial region with deposits of pigments similar to those found in the mantle folds; predominating tint of mantle folds yellow gold with smudges of gray or brown. Tentacular arrangement: IF has one row of almost uniform tentacles close to each other; MF has three rows of tentacles. Gill plates bluish gray which fades toward the palps; gill count is 10 to 14; ova 50 to 80 $\mu$ in diameter; ostia 19 to 28 $\mu$. Auricles of the heart joined by at least three-fourths of the auricular length (Pl. III, Fig. 3). Rectum reaching about one-third along the dorsal side of the adductor; anal fold sub-oval in form and only slightly expanded; sometimes absent (Pl. I, Fig. 3).

Shell outline sub-quadrate; valves inequilateral; inner surface margin of right valve conspicuously denticulated around. Rim of valves apparently but irregularly crenulated. Both valves sparely foliaceous at the periphery. Central part of right valve somewhat washed out or smooth. Muscle scar on right valve conspicuous, of alternate strips of white and brown; scar on left valve almost inconspicuous; curved on the side nearer the hinge and markedly displaced postero-dorsally. Left valve moderately deep and recessed below the hinge. External color purplish brown obscured by smudges of copper-green; inner surface of valves smooth, hard, white with smudges of yellowish brown; rim beyond the line of denticles black, sometimes lightly colored. Hinge plate broadly squatting; beaks sub-equally produced.

*Habitat*: Bacoor Bay, Cavite Province and Puerto Galera, Mindoro Is.

*Dimensions:* (Adult) Length — 4 to 5 cm.
Width — 2.5 to 4 cm.

*Crassostrea amasa* (Iredale)
Plate V, Fig. 6

*Ostrea glomerata* Gould (in Blanco *et al.*, 1951, p. 42: Shell thick, irregular, sharp ribbed, with margin dentated, wrinkled or lobed. Upper valve opercular, compressed with thick concentric laminae. Lower valve cucullated, purple. Hinge generally attenuated, produced, pointed. Differs from *O. cucullata* Born in having numerous strong ribs. Inside edge purple or black. Lateral margin denticulated.)

*Saxostrea amasa* Iredale, 1939.

*Crassostrea amasa* (Iredale) Thomson, 1954, p. 154, pl. 7, figs. 1-2.

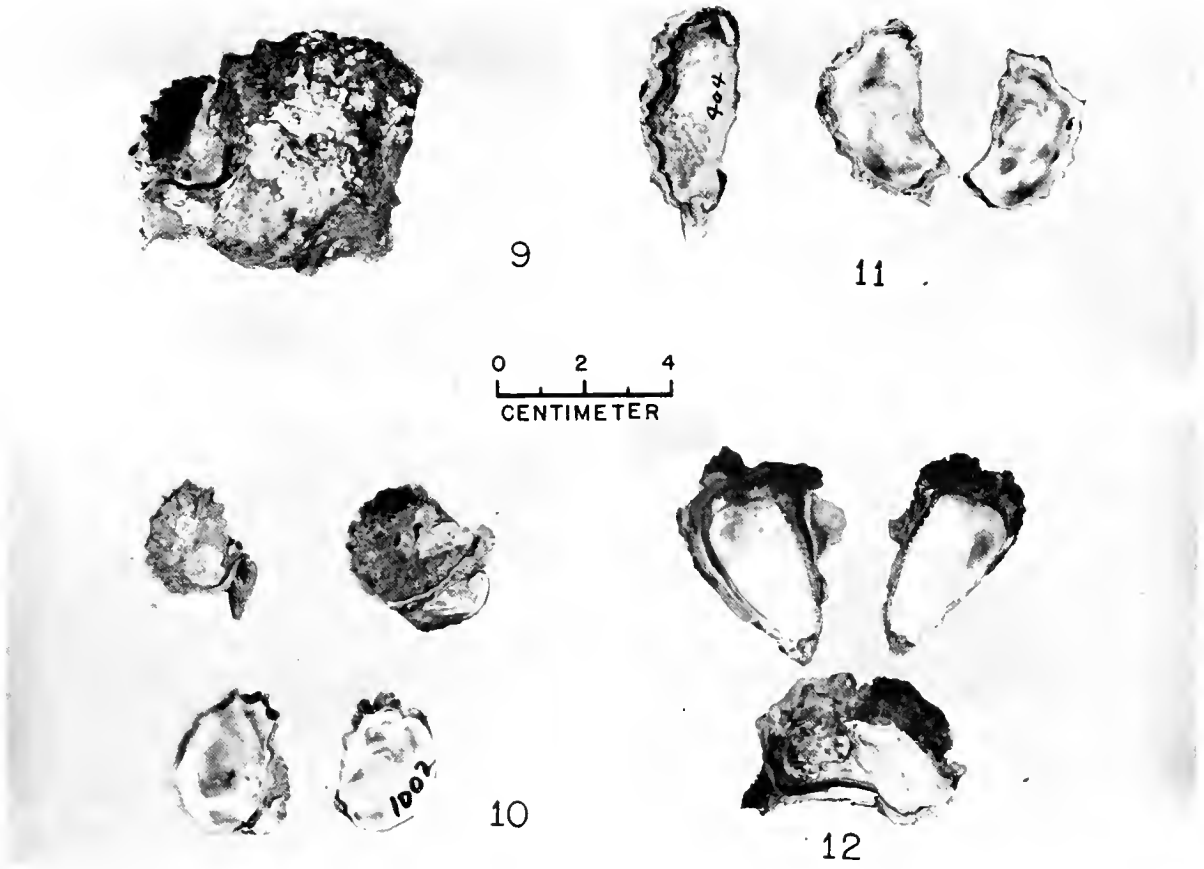


Plate VI. PHILIPPINE OYSTERS

Fig. 9 — *Crassostrea echinata*, left and right valves intact; specimen of Pl. V, Fig. 7.

Fig. 10 — *Crassostrea palmipes*, (upper) specimens with left and right valves intact; (lower) internal view of left and right valves.

Fig. 11 — *Crassostrea tuberculata*, (upper) left and right valves intact, and (lower) internal view of left and right valves.

Fig. 12 — *Crassostrea lugubrius*, (upper) left and right valves intact, and (lower) internal view of left and right valves.

Circumpallial region with heavy deposits of yellow gold on both surfaces; predominating pigments of mantle folds yellow gold and brown; other shades like gray may be present. Tentacular arrangement: IF has one row of tentacles in pairs at angular positions and each pair close to one another; MF has only two rows of tentacles. Gill plates and palps clear white to hyaline; gill count is 10 to 13(14); ova 40 to 55 $\mu$ in diameter; those of ostia 19 to 38 $\mu$. Auricles of the heart markedly pinched at the auriculo-ventricular junction; thin, membrane-like, and globose; united posteriorly by at least 60% of the auricular length (Pl. III, Figs. 7-9). Adductor distinctly large in proportion to body surface; circular in outline. Rectum reaching almost the entire dorsal side of the adductor; anal fold absent or degenerate (Pl. I, Fig. 7); anal port is pinched.

Shell of three very distinct characteristic outlines: (1) dorso-ventrally wedged with the dorsal contour convex and ventral contour straight or slightly concave; this is the case when attached singly; (2) antero-posteriorly wedged with a very deep recess below the hinge of the left beak giving it the appearance of a horn cup; (3) moderately deep cup-like and acuminated toward the beak, its dorsal and ventral walls sub-equal. Forms (2) and (3) are developed when growing in thick bunches. When dorso-ventrally wedged, the dorsal region of the left valve forms a deep wall with very prominent ribs of 8 to 10, sometime as few as 7 to as many as 12; Shells of other

ecomorphs have obscured ribs.

Valves are thick; rim of right valve finely dentated around; dentition usually in groups corresponding to the crenulations of the lower valve. Right valve operculate, its outer rim produced into flattened processes fitting the crenulations of the left valve; its lamellae arranged into radial rows from the beak, forming very distinctive furrows radiating from a common point.

Muscle scar dark or deep purple on the right valve but almost indistinct on the left valve; very much dorsally displaced. Inner surface is smooth, resistant to scratch; external predominating color light reddish brown to purple, sometimes obscured by smudges of copper green; rim of both valves deep purple. Hinge plate outstandingly tall and the beak of the left valve very much more produced beyond that of the right.

*Habitat:* Puerto Galera, Mindoro Is.

*Dimensions*: (Adult) Length — 3 to 5 cm.
Width — 2 to 3 cm.

*Crassostrea echinata* (Quoy and Gaimard)
Plate V, Figs. 7-8; Plate VI, Fig. 9

*Ostrea echinata* Quoy and Gaimard, 1835; (in Blanco *et al.*, 1951, p. 42: Shell compressed, thin, acuminated towards hinge, roundish beneath. Lower valve sometimes deeply concave; upper valve armed with tubeshaped purple outstanding spines. The species occurs attached to rocks just below high tide mark on both coasts of the Pacific Ocean and Japan Sea.)

*Crassostrea echinata* (Quoy and Gaimard) Thomson, 1954, pl. 5, fig. 4; pl. 6, figs. 1-4.

Circumpallial region of both surfaces and of both mantle lobes brown to dark brown near the inhalent chamber and fading toward the hinge; predominating pigment of the mantle folds is brown with shades of pink or red, even yellow or gray. Tentacular arrangement: IF has only one row of uniform and close together tentacles; MF has three rows of tentacles. Gill plates dark blue gray, sometimes purplish, fading toward the palps; gill count very variable. Ova 60 to 75 $\mu$ in diameter; those of ostia 19 to 38 $\mu$. Auricles of the heart broadly united by about two-thirds of the auricular length (Pl. III, Fig. 5). Rectum reaches about one-third of the dorsal side of the adductor; anal fold is slightly expanded (Pl. I, Fig. 4); anal port is not pinched.

Shell outline is very variable due to persistant ecomorphism; may be oblong to elongate or subcircular to sub-quadrate; margin of right valve with denticles on both sides near the hinge line; denticles may be present at the lip region. Rim of valves vaguely crenulate or irregularly undulate; right valve foliaceous or concentrically lamellate at the periphery, smooth or washed at the center. Muscle scar curved on the side nearer the hinge and displaced mid-dorsally; color of scar usually follows that of the inner surface of the valves. Inner surface of both valves smooth, white, with or without smudges of dirty yellow or dark brown. Rim beyond the line of denticles black or purple. Hinge plate nearly taller than broad; outgrown denticles may still be present on the plate.

*Habitat:* Bacoor Bay, Cavite Province and Puerto Galera, Mindoro Is.

*Dimensions*: (Adult) Length — 6 to 8.5 cm.
Width — 4 to 5.5 cm.

*Crassostrea lugubrius* (Sowerby)
Plate VI, Fig. 12

*Ostrea lugubrius* Sowerby (in Blanco *et al.*, 1951, p. 43: Shell thin, obliquely subtrigonal, slightly auriculated, greyish purple, obscurely rayed, rather smooth, shining whitish within. Anterior side produced at ventral margin. Upper valve flattened, subopercular. Lower valve convex, obscurely ribbed, expanded at margin beyond upper valve. Muscular impression reniform, nearly black.)

Circumpallial region of both lobes of the mantle heavily invested with yellow pigments, with or without shades of light brown; predominating tint of mantle folds are brown and yellow. Tentacular arrangement: IF has only one row of well spaced tentacles irregular in size. Gill plates purple, fading toward the palps; gill count 8 to 14; ova 30 to 40 $\mu$ in diameter; those of ostia 19 to 40 $\mu$ . Auricles of the heart broadly united by at least one-half of the auricular length (Pl. III, Fig. 4). Rectum reaches one-half along the dorsal side of the adductor; anal fold thick but narrow, sometimes absent, pinched (Pl. I, Fig. 5).

Shell outline varies according to mode of attachment which is usually buttress roots and trunks of *Rhizophora* submerged during high tide; valves very much inequilateral; margin of right valve with fine denticles around; denticles may not be conspicuous at the lip region. Rim of left valve distinctly lamellate, with the wing-like thin plates laterally overlapping by 2, 3, or even 5; right valve is washed on its external surface. Scar is sub-acuminate and curved on the side nearer the hinge; not distinctly colored on either valve; displaced mid-dorsally. Left valve moderately deep, slightly recessed below the hinge; right valve operculate, flat, its rim is upturned to fit the steep walls of the rim of left valve. External predominating color brownish purple to deep purple. Inner surface of valves smooth, strongly iridescent; sometimes white; hard. Hinge plate

moderately tall; beaks are sub-equal.

*Habitat*: Puerto Galera, Mindoro Is.; Estuaries of Malabon and Navotas in Rizal Province; Bulacan; and the Bicol Region of Southern Luzon.

*Dimensions*: (Adult) Length — 4 to 5 cm.
Width — 2 to 3.5 cm.

*Crassostrea palmipes* (Sowerby)
Plate VI, Fig. 10

*Ostrea palmipes* Sowerby (in Blanco *et al.*, 1951, p. 44: Shell much compressed sub-quadrate, thin, very inequivalve, inequilateral, fulvoid, rayed with purple or black. Anterior side very short, sloped. Dorsal margin straight while ventral margin rounded, lobed. Umbos small, acuminated. Upper valve small, smooth. Lower valve more expanded, radiately striated, ribbed, tuberculated, produced at margin, flattened interstices.) Also in Talavera and Faustino, 1933, p. 1-39, pl. 3, figs. 1-3.

Circumpallial region of either surface of both mantle lobes gray or blue-gray; predominating pigment of mantle folds brown with shade of gray. Tentacular arrangement: IF has one row of tentacles uniform in size, with or without three diminutive tentacles in-between; MF has three rows of tentacles. Gill plates gray to blue-gray; gill count is 8 to 10(12); diameter of ostia 28 to 38 $\mu$; those of ova about 80 $\mu$. Auricles of the heart broadly united by at least two thirds of the auricular length (Pl. III, Fig. 6). Rectum reaches one-third along the dorsal side of the adductor. Anal fold is absent or degenerate (Pl. I, Fig. 6); tip of the rectum bent away from the adductor.

Shell outline sub-oval to sub-quadrate; valves very unequal; rim of left valve crenulate; only right valve moderately foliaceous. Solitary specimens have dorsal margins straight and ventral margins rounded. Radial folds or ribs much more prominent on the left valve than on the right valve. Rim of valves with fine denticles on the sides nearer the hinge region. Muscle scar moderately curved on the side nearer the hinge; displaced mid-dorsally; not very prominent on both valves. Left valve deep and cup shaped; its umbonal feature somewhat similar to family *Cardiidae*. Right valve flat, its rim upturned fitting the crenulation of the left valve. External predominating color is gray to bluish or yellowish gray. Internal surfaces of both valves smooth; hard; white with patches of dirty yellow; rim gray to dark gray. Hinge plate broadly squatting and beaks sub-equally produced.

*Habitat*: Puerto Galera, Mindoro Is.; oyster beds in Rizal, Cavite, Bulacan, Pangasinan and the neighboring provinces in Luzon Is., Samar.

*Dimensions*: (Adult) Length — 2 to 3.5 cm.
Width — 2 to 3 cm.

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ADDENDUM

## KEY TO THE SPECIES OF PHILIPPINE OYSTERS OF THE GENUS *CRASSOSTREA* BASED ON MALACOLOGICAL CHARACTERS

THE GENUS: Promyal chamber is present and the rectum does not penetrate the heart.

1. Three rows of tentacles on middle mantle fold ..... 2
   Two rows of tentacles on middle mantle fold ..... 3
2. Rectum reaches one-half of the dorsal side of adductor ..... 5
   Rectum reaches one-third of the dorsal side of adductor ..... 4
3. Inner mantle fold with uniform tentacles in pairs at angular positions (Pl. II, Fig. 5); rectum reaches almost the entire dorsal side of adductor ..... *Crassostrea amasa*
   Inner mantle fold with tentacles irregular in size, well spaced in-between (Pl. II, Fig. 2) ..... *C. lugubrius*
4. Gill count 10-14; both circumpallial pigmentation and predominant pigment of mantle folds yellow gold ..... *C. tuberculata*
   Gill count 8-10(12); circumpallial pigentation gray to blue-gray; predominant pigments of mantle folds brown and gray ..... *C. palmipes*
5. Circumpallial pigmentation predominantly brown ..... 6
   Circumpallial pigmentation deep yellow gold; anal fold absent ..... *C. malabonensis*
6. Gill count 12-16(18); predominant pigment of mantle folds definitely brown; anal fold well expanded, sub-oval ..... *C. iredalei*
   Gill count very variable;predominant pigment of mantle folds reddish brown; anal fold slightly expanded or not at all ..... *C. echinata*

## KEY TO THE SPECIES OF PHILIPPINE OYSTERS OF THE GENUS *CRASSOSTREA* BASED ON CONCHOLOGICAL CHARACTERS

THE GENUS: Recessed below the hinge; muscle scar displaced dorsally; when dentate, only right valve possesses denticles and the left valve the corresponding pits.

1. Dentate ..... 2
   Not dentate; valves foliaceous, blunt or rounded at the lip region; large: length 10-15 cm, width 4-6 cm ..... *Crassostrea iredalei.*
2. Crenulate ..... 3
   Not crenulate; inner surface of valves iridescent; length 4-5 cm, width 2-3.5 cm ..... *C. lugubrius.*
3. Rim of valves gray to dark gray ..... 4
   Rim of valves black or purple ..... 5
4. Inner surface of valves white, patched with yellow; much smaller than *C. malabonensis*; length 2-3.5 cm, width 2-3 cm ..... *C. palmipes.*
   Inner surface of valves with abundant blotches of greenish yellow or greenish brown; length 4-5 cm, width 2-3.5 cm ..... *C. malabonensis.*
5. Right valve partly foliaceous without distinctive pattern ..... 6
   Right valve with small lamellae arranged in radial rows forming distinctive furrows radiating from the beak ..... *C. amasa.*
6. Scar displaced mid-dorsally, its color usually follows that of the valve; length 6-8.5 cm, width 4-5.5 cm ..... *C. echinata.*
   Scar displaced postero-dorsally; of alternate strips of white and brown; length 4-5 cm, width 2.5-4 cm ..... *C. tuberculata.*

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# INFLUENCE OF LIGHT ON LARVAL SETTLEMENT OF AMERICAN OYSTERS[1, 2]

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ABSTRACT

*It has been reported that oyster larvae show a marked preference for attaching to the undersurface of test plates and shells. Other investigators have reported that oyster larvae prefer the uppersurfaces of test plates and shells. Laboratory experiments were conducted to determine whether or not the reported setting preferences were associated with the avoidance of light. Eyed and ready-to-set oyster larvae* Crassostrea virgincia *(Gmelin) were placed in setting aquaria that contained experimental and control test shells.*

*Experimental shells were illuminated from above and below with 50 foot candles of light. Control shells in the same setting aquarium received 50 foot candles of illumination from above and no illumination from below. During twenty-four hour experiments, when undershell illumination was held at 50 foot candles, the ratio, oyster larvae settlement on the undersurface of lighted experimental shells: oyster larvae settlement on the undersurface of unlighted control shells was 4:96. When undershell illumination was reduced to 25 foot candles, the ratio of undersurface settlement on lighted experimental shells increased to 20:80.*

## INTRODUCTION

There is ever increasing evidence that the planktonic larvae of some polychaetes, echinoderms and mollusks are capable of testing various bottom substrates, and even delaying metamorphosis, until the appropriate setting substrate is encountered. Among others, Wilson (1952) and Scheltema (1961) have presented sound evidence that some larvae are capable of exercising a choice in the selection of setting sites. The ability of larvae to exercise some choice in the selection of setting sites might indicate that larvae respond to some kind of physical or chemical stimulus from the environment. *Crassostrea virginica* larvae develop pigmented eye spots and a ciliated foot only one or two days prior to setting. Galtsoff (1964) reports that both these sensory organs are resorbed one day after oyster larvae have set. The larval sensory organs may thus be used for detection and selection of setting sites. Definitive investigations of factors influencing setting are warranted because settlement, metamorphosis, and subsequent survival of oyster spat, are intimately associated with the selection of setting sites.

The state of knowledge concerning the setting behavior of oysters has been reviewed by Korringa (1952) and brought up to-date by Galtsoff (1964). From these reviews, it appears that there is a lack of consistency in the reported observations of various investigators concerning the influence of light.

Nelson (1926) reported that the eyed larvae of *C. virginica* are light sensitive and that stimulation by light causes heavy attachment in shaded areas. Prytherch (1934), after eight years of ex-

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[2] A portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the M. S. degree from Florida State University.

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perimentation could find no evidence that the larvae of *C. virginica* are sensitive to variations of light intensity or color. Hopkins (1937) concluded that light is not an orienting factor in the setting of *Ostrea lurida*, and Korringa (1952) reported that vertical distribution and setting of the larvae of *O. edulis* is not affected by light.

Buter (1955), Hidu (1967[4]) and Shaw (1967) have reported that upper substrate surfaces are preferred by *C. virginica* larvae in the field. Crisp (1967) reported that *C. virginica* larvae settle preferentially on the underside of shells and on the smooth inner surface of oyster shell. Yet, it has been reported by Cole and Knight Jones (1949) that the larvae of *O. edulis* show a marked preference for setting on the undersurfaec of test plates or shells. Hopkins (1935) reported that the larvae of *O. lurida* show the same preference, and Schaefer (1937) has reported that the larvae of *C. gigas* have the same tendency. Medcof (1955), Sieling (1950) and Nelson (1928) have reported that *C. virginica* larvae show a marked preference for setting on the undersurface of submerged objects. Pomerat and Reiner (1942) stated that oyster larvae do not differ from many other fouling organisms that attach in greater abundance to the undersurface of test plates held in horizontal position.

## METHODS

All of the oyster larvae used in these investigations were reared at the Florida State University, Alligator Harbor Marine Laboratory of the Department of Oceanography. The laboratory oyster culture methods were similar to those described by Loosanoff and Davis (1963) but modified in the following manner:

Straight hinge oyster larvae were reared to early umbo size on seawater that was passed through a 38 $\mu$ sieve. Early umbo size larvae were then reared to the eyed setting size on seawater that was passed through an 88 $\mu$ sieve. Culture waters were changed every other day with no addition of supplemental cultured algae foods. The numbers of ready-to-set oyster larvae that were used in each experiment were somewhat dependent on the availability of setting size larvae. Generally, approximately 50% of the larvae used in each experiment appeared to attach during the experimental period. All oyster setting experiments were conducted in seawater that had been passed through an 88 $\mu$ sieve.

A plexiglas setting aquarium was placed over a light box that was illuminated by a 15 watt cool/white fluorescent bulb. The bottom of the setting aquarium was completely blackened with heavy black tape. At four staggered locations, a 14 mm circular hole was cut in the black tape to allow light to shine up and under four experimental shells. The amount of light illuminating the underside of each of the experimental shells was measured at 50 foot candles. No measurable increase in water temperature occurred under the illuminated shells. Four staggered control shells in the same aquarium received no measurable amount of illumination from below. The setting aquarium with test shells is shown in Figure 1. Ready-to-set oyster larvae were placed in the aquarium having a setting substrate of clean, single valves of the sunray venus clam, *Macrocallista nimbosa* (Solander). Test shells were either well scrubbed green shells with the periostracum intact or bleached and dried old shells with the periostracum intact or removed. The shells were washed in freshwater, air dried, and rinsed in filtered seawater just prior to placing them in the setting aquarium. All shells were used in only one experiment. Each row of test shells was supported approximately 3.6 mm from the bottom of the setting aquarium by plexiglas rods.

Experiments were conducted over 24 or 48 hour time periods and undershell light intensity was varied. Ambient lighting in the laboratory was measured at 50 foot candles, and the regular laboratory lights usually illuminated the uppersurface of control and experimental shells. However, on 28 August and again on 5 September 1967 the regular laboratory lights were inadvertently turned off sometime during the night. No appreciable difference between undersurface settlement ratios was observed.

Fluorescent lighting proved to be adequate for the variations of light intensity that were used in these experiments. Future investigators might consider the use of incandescent lighting since incandescent bulbs would permit greater variation in light intensity.

## RESULTS AND DISCUSSION

In all experiments, *C. virginica* larvae showed a marked preference for setting on the smooth undersurface of unilluminated control shells. Less than 5% of the total number of larvae setting in any experiment were found attached to the uppersurface of control and experimental shells. The following discussion pertains only to the actual numbers of oyster larvae that were found firmly attached to the undersurface of control and ex-

[4] Hidu, H. 1967. Inshore settlement of several marine invertebrates at the Cape May shore of Delaware Bay, New Jersey, with special reference to the American oyster *Crassostrea virginica* (Gmelin). Ph.D. Dissertation. Rutgers — The State University, New Brunswick, New Jersey.

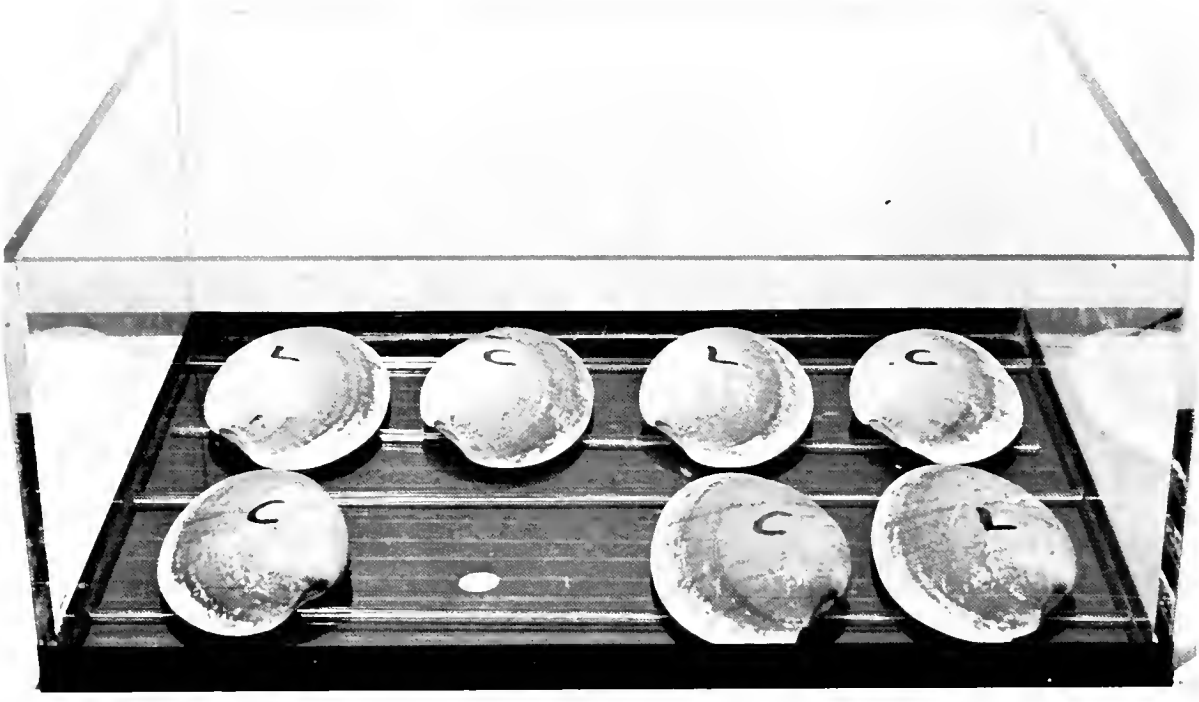

FIG. 1. *Photograph of setting aquarium with one experimental shell removed to show undershell light source.*

perimental shells in the same setting aquarium.

The setting intensity of oyster larvae on the undersurface of test shells illuminated with 50 foot candles of light is presented in Table 1. These data indicate that ready-to-set *C. virginica* larvae tend to avoid the undersurface of test shells that are illuminated from below with 50 foot candles of light. In the series of experiments, the ratio of

TABLE 1. *Twenty-four hour undershell setting intensity of* Crassostrea virginica *larvae in relation to undershell illumination of 50 foot candles.*

| Date | Temp. °C | Sal. ppt | Spat Set on Undersurface of Experimental Shells (50 Foot Candles Illumination) | | | Spat Set on Undersurface of Control Shells (No Illumination) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Total | Range | Av./Shell | Total | Range | Av./Shell |
| 18 Aug | 27 | 31 | 7 | 0-5 | 2 | 40 | 1-23 | 10 |
| 19 Aug. | 27 | 30 | 19 | 3-7 | 5 | 651 | 97-219 | 163 |
| *28 Aug. | 27 | 30 | 25 | 5-7 | 6 | 464 | 97-127 | 116 |
| 30 Aug. | 27 | 29 | 27 | 5-9 | 7 | 924 | 139-2468 | 231 |
| * 5 Sept. | 27 | 30 | 31 | 3-19 | 8 | 381 | 45-94 | 65 |
| 6 Sept. | 27 | 30 | 29 | 5-11 | 7 | 82 | 16-25 | 20 |
| 28 Sept. | 26 | 30 | 26 | 4-9 | 6 | 1081 | 187-397 | 270 |
| Total | | | 164 | | | 3623 | | |
| Percentage | | | (4.4%) | | | (95.6%) | | |
| Ratio, Experimental: Control 4:96 | | | | | | | | |

*Regular laboratory lights turned off sometime during the night.

oyster larvae settlement on illuminated undersurface: larvae settlement on unilluminated undersurfaces was 4:96.

Experiments were also conducted in which the intensity of undershell illumination was halved to 25 foot candles. The results of these experiments are presented in Table 2. These data indicate that ready-to-set oyster larvae attached more readily to the undersurface of experimental shells when the intensity of undersurface illumination was reduced. In this series of experiments, the ratio of larvae settlement on illuminated undersurfaces: larvae settlement on unilluminated undersurfaces was 20:80. The undershell setting ratio increased from 4:96 to 20:80 when undershell illumination was reduced from 50 foot candles to 25 foot candles.

Most of the undershell illumination experiments were terminated at the end of a 24 hour time period. Experiments were also allowed to run for 48 hours (Table 3) to determine if setting oyster larvae would eventually become accustomed to illuminated undershell surfaces.

These data indicate a significant increase in the total number of larvae that attached to the undersurface of experimental shells and a corresponding increase in the number of larvae that attached to the undersurface of control shells during the 48 hour period. The 48 hour ratio of larvae settlement on undersurfaces of illuminated test shells: settlement on unilluminated test shells was 6:94 and almost identical to the ratio obtained during 24 hour, 50 foot candles experiments. It would appear that oyster larvae do not become accustomed to undershell illumination.

## CONCLUSIONS

1. The eyed larvae of *C. virginica* are light sensitive.
2. Ready-to-set oyster larvae showed a definite preference for setting on the unilluminated undersurfaces of control shells.
3. The undershell setting intensity of *C. virginica* larvae increased from a ratio of 4:96 to a ratio of 20:80 when undershell illumination was reduced from 50 to 25 foot candles.
4. Oyster larvae do not become accustomed to undershell illumination of 50 foot candles.

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TABLE 2. *Twenty-four hour undershell setting intensity of* Crassostrea virginica *larvae in relation to undershell illumination of 25 foot candles.*

| Date | Temp. °C | Sal. ppt | Spat Set on Undersurface of Experimental Shells (25 Foot Candles Illumination) | | | Spat Set on Undersurface of Control Shells (No Illumination) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Total | Range | Av./Shell | Total | Range | Av./Shell |
| 16 Sept. | 27 | 29 | 119 | 18-46 | 30 | 352 | 52-185 | 88 |
| 18 Sept. | 27 | 30 | 220 | 3-187 | 55 | 486 | 6-225 | 121 |
| 28 Sept. | 27 | 30 | 111 | 4-85 | 28 | 910 | 83-347 | 227 |
| Total | | | 450 | | | 1748 | | |
| Percentage | | | (20.5%) | | | (79.5%) | | |
| Ratio, Experimental: Control 20:80 | | | | | | | | |

TABLE 3. *Forty-eight hour undershell setting intensity of* Crassostrea virginica *larvae in relation to undershell illumination of 50 foot candles.*

| Date | Temp. °C | Sal. ppt | Spat Set on Undersurface of Experimental Shells (50 Foot Candles Illumination) | | | Spat Set on Undersurface of Control Shells (No Illumination) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Total | Range | Av./Shell | Total | Range | Av./Shell |
| 4 Sept. | 27 | 29 | 100 | 15-42 | 25 | 1530 | 93-731 | 382 |
| Percentage | | | (6%) | | | (94%) | | |
| Ratio, Experimental: Control 6:94 | | | | | | | | |

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# PHOSPHATE ADSORPTION BY KAOLIN IN SALINE ENVIRONMENTS[1]

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ABSTRACT

*The adsorption of dissolved inorganic phosphate on pure kaolin and on the silt-clay sediment fraction from Point Judith Pond, Rhode Island, was studied in laboratory experiments. Temperature, salinity, pH, and the phosphate to kaolin ratio were varied to determine the effects of these environmental factors on adsorption.*

*The adsorption of phosphate onto kaolin was depressed by increased salt concentration, high pH and low temperature. In all experiments the adsorption reaction was essentially complete after 24 hr. The calculated heat of reaction from temperature dependence data indicates that fixation is associated with exchangeable hydroxyl groups on the edges of the clay crystal. Point Judith Pond sediment displayed a markedly different phosphate adsorptive capacity. This is possibly due to the clay mineral content of the sediment and to a depressant effect on the adsorption reaction by associated organic matter.*

## INTRODUCTION

Phosphate adsorption by clay minerals has been studied by soil scientists for many years. This adsorption phenomenon in kaolin clays is associated with hydroxyl groups in the clay crystal (Dean and Rubins, 1947; Sieling, 1946; Stout, 1939).Quantitative studies have demonstrated that there are sufficient exchangeable hydroxyls in the kaolin crystal to account for the phosphate adsorption reported by most of these workers (Halevy, 1964). It has also been shown that at very low concentrations, <3ppm P, the exchange with hydroxyls predominates the adsorption reaction (Muljadi, Posner and Quirk, 1966).

Limnologists have demonstrated that phosphate adsorption and exchange occur on bottom sediments (Holden, 1961; Hutchinson and Bowen, 1950; Livingstone and Boykin, 1962; Rigler, 1956). Hepher (1958) found that phosphate fixation (i.e., loss from solution) was directly related to concentration of colloidal material found in bottom muds.

Work by oceanographers in the estuarine and inner continental shelf regions indicates that clays play a role in phosphate adsorption and exchange (Pratt, 1949; Rittenberg, Emery and Orr, 1955; Rochford, 1951). Seshappa (1953) has shown that muds from the Malabar Coast of Western India contain several hundredfold as much phosphate per unit volume as the water layer directly above them and attributed increases in the phosphate content of the water during periods of great ocean disturbances to agitation and suspension of phosphate rich muds. Laboratory experiments with Chesapeake Bay sediments (Carritt and Goodgal, 1954) and with sediments from Doboy Sound, Georgia, (Pomeroy, Smith and Grant, 1965) suggest that an adsorption reaction can occur between suspended solids and phosphate. Pomeroy's study indicates that the rate and magnitude of the adsorption reaction are large enough to be ecologically significant.

Despite the numerous papers cited, there have been no studies which clearly demonstrated the presence of adsorption reactions between pure clay minerals and ionic phosphate species under saline conditions in the absence of microorganisms. The primary purpose of this work was to

[1] Contribution No. 1310 of the Rhode Island Agricultural Experiment Station.

examine the reactions of phosphate with kaolin under varying conditions of temperature, pH, salinity, and different phosphate to kaolin ratios and to propose the possible mechanism for fixation of phosphate to kaolin. A secondary purpose was determining absolute amounts of phosphate adsorbed with time.

## METHODS AND MATERIALS

Investigations were made using kaolin (K-3) obtained from the Fisher Scientific Company. Kaolin was selected because of its comparatively stable crystalline characteristics under high salt concentrations and the very short time required to reach equilibrium with the surrounding saline environment. To establish a homogeneous ionic surface (NaCl), the clay was washed with 0.1 N HCL followed by suspension in a 3.5% NaCl solution. The clay was adjusted to pH 7.0 by additions of dilute NaOH. The neutral suspension was then centrifuged and excess salts removed from the kaolin by washing with 80% $CH_3OH$. The clay was air dried and used in all subsequent tests.

The general procedure consisted of weighing 50 mg of the treated clay into 50 ml polypropylene centrifuge tubes previously treated with N-butyl-methacrylate polymer (K & K Laboratories, Inc.). Centrifuge tubes were coated to prevent adsorption of phosphate by the plastic surface. To each tube was added 15 ml of 1 ppm P solution at pH 7.0 containing various concentrations of NaCl or, for one comparative case, using artificial sea water (Lyman and Fleming, 1940). The clay suspensions were shaken on a Burrell Wrist-Action Shaker at shaker setting five. The tubes were then centrifuged and phosphate remaining in the supernatant was measured by the colorimetric method of Dickman and Bray (1940) using a Beckman DU Spectrophotometer at 735 m $\mu$ and 0.03 mm slit width. Using this method with 1 cm cells it is possible to measure phosphorus concentrations as low as 0.05 ppm in the presence of high salts.

The amount of adsorption was calculated as the difference between initial and final phosphate levels in the water. Adsorption curves were constructed by plotting the per cent adsorbed as a function of time.

## RESULTS AND DISCUSSION

Increased chlorinity decreased the amount of phosphate removed from solution (Fig. 1) and also increased the flocculation of the clay. The decrease in adsorption may be due to: 1) increased chloride competition for phosphate fixation sites; 2) less clay remaining in suspension because of increased flocculation including probable reduction of exposed surfaces due to flocculation; or 3) decrease in the activity of the phosphate ion with increasing salt concentration. Since it has been demonstrated that chloride is not significantly adsorbed on a kaolinitic type soil colloid at neutral to alkaline pH's (Mattson, 1931), it is thus unlikely that chloride competition with phosphate for exchange sites on the clay would have a significant effect on the phosphate adsorption at the neutral pH used in our experiments. To determine the effect of high salt concentration on flocculation the reaction tubes were agitated strongly enough to break up the flocs. The increased agitation rate resulted in increased adsorption of phosphate. This suggested that the decrease in adsorption was due mainly to increased flocculation of the clay.

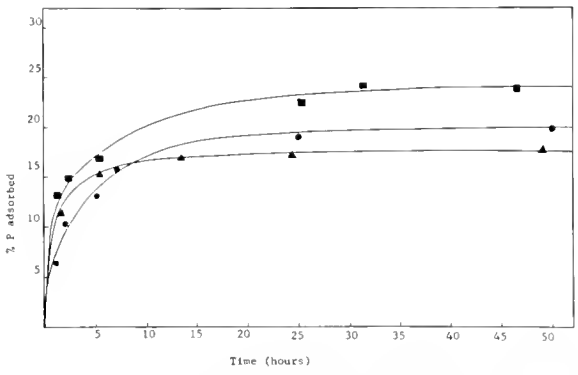


FIG. 1. *Effect of chlorinity on adsorption. Initial conditions; 50 mgm kaolin, 15 ml 1 ppm P solution, temperature 23°C, pH 7.0.* ■, *5%;* ●, *19%;* ▲, *30%.*

Figure 2 compares phosphate adsorption in the presence of NaCl (Cl 19%) and artificial sea water solution of the same chlorinity. The same

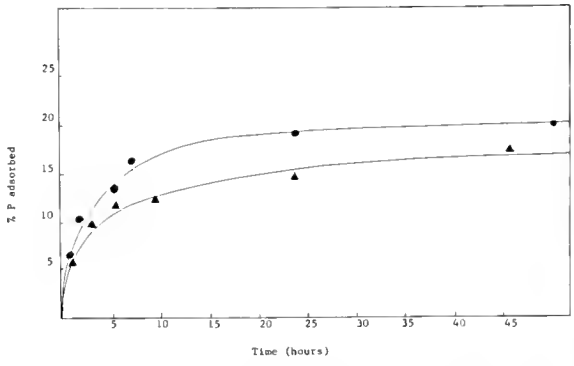


FIG. 2. *Effect of an artificial sea water solution on adsorption. Initial conditions; 50 mgm kaolin, 15 ml 1 ppm P solution, temperature 23°C, pH 7.0.* ●, NaCl 19% Cl; ▲, artificial sea water 19% Cl.

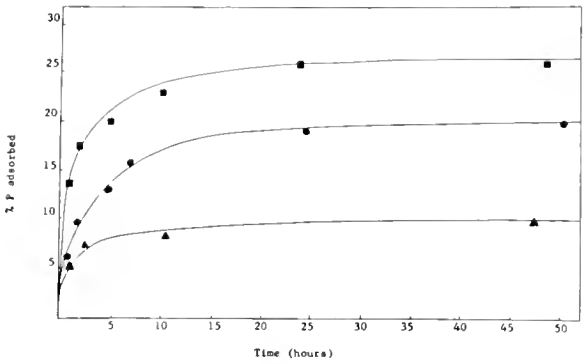


FIG. 3. *Effect of temperature on adsorption. Initial conditions; 50 mgm kaolin, 15 ml 1 ppm P solution, temperature 23°C, pH 7.0.* ▲*2°C;* ● *23°C;* ■ *65°C.*

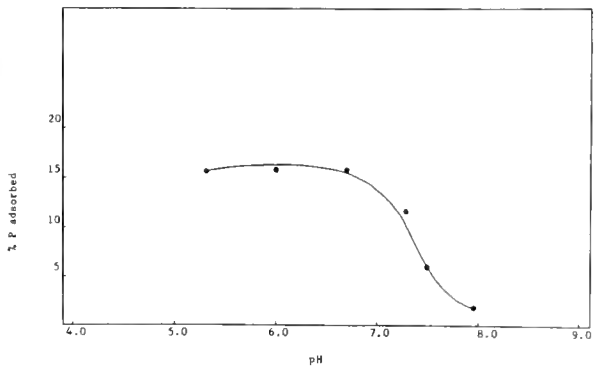


FIG. 4. *Effect of pH on adsorption. Initial conditions; 50 mgm kaolin, 15 ml 1 ppm P solution, 0.002 M Tris malcate buffer, 19% Cl, temperature 23°C, time 24 hr.*

shaped curves were obtained but the artificial sea water solution resulted in a rather uniform decrease in the amount of phosphate removed by the kaolin over a period of 70 hr. At least two explanations for this phenomenon may be advanced, increased flocculation by divalent cations or competition by polyvalent anions for the phosphate adsorption sites on the clay crystal.

It might be expected that despite the sulfate concentration almost 1,000 times greater than that of the phosphate ion, adsorption of phosphate would not be reduced due to sulfate competition because sulfate adsorption at pH 7.2 is 0.002 meq (Mattson, 1931). Carritt and Goodgal (1954) also show that the presence of phosphate reduced sulfate adsorption, indicating that a competition does exist favorable to phosphate and thus it is doubtful that at pH 7.0 sulfate would compete to any great extent with the phosphate for exchange sites. From this evidence it is believed that the small decrease in adsorption is due to increased flocculation of the clay by divalent cations (Krauskopf, 1967) resulting in a diminished surface area for phosphate reaction.

More pronounced effects on adsorption are related to temperature changes (Fig. 3). The increase in phosphate adsorption at higher temperatures agrees with previous work (Carritt and Goodgal, 1954).

Using a 0.002 M Tris-maleate buffer system it was possible to create salt solutions varying in pH from 5.30 to 8.15. Adsorption decreased as the pH changed from acid to alkaline conditions (Fig. 4). Over the pH range covered in this study maximum phosphate adsorption occurred in the range in which the $H_2PO_7^-$ ion is the predominant species. It is questionable whether it is valid to extrapolate the phosphoric acid dissociation curves of Kester and Pytkowicz (1967). By examination of their curve for $H_2PO_7^-$ ionization at 0.68 M NaCl it is evident that at 0.54 M NaCl (the molarity used in this study) the dissociation constant for the second phosphoric acid hydrogen will be shifted further toward the alkaline pH range. Kester and Pytkowicz (1967) state that their measured dissociation constants may not be valid when some other variable is introduced into the system, in this case the introduction of kaolin. A decline in phosphate adsorption may result for any decrease in the monovalent ion or may occur only after the monovalent ion drops below some limiting level.

The effect of increasing the phosphate to clay ratio on the amount of phosphate adsorbed from solution is shown in Figure 5. Within the limits of the ratios used, doubling the amount of clay

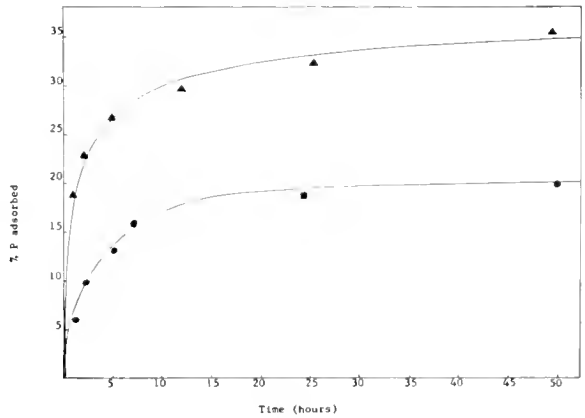


FIG. 5. *Effect of phosphate to kaolin ratio on adsorption. Initial conditions; 15 ml 1 ppm P solution, temperature 23°C, pH 7.0, 19% Cl.* ●*50 mgm kaolin;* ■ *100 mgm kaolin.*

almost doubled the amount of phosphate adsorbed. The increased tendency of the suspensions to flocculate at higher clay concentrations may reduce phosphate adsorption per gram of clay.

To compare the adsorption of P by a natural material to the adsorption of the element by kaolin, a sample of sediment was obtained from Point Judith Pond, a shallow salt pond in southern Rhode Island. The sediment was air dried and passed through a 325 mesh sieve (0.043 mm openings) to obtain the fine silt and clay separates. The material also contained some organic matter. The sediment material adsorbed considerably more than did the kaolin (Fig. 6). This greater adsorption was probably due to the clay fraction being composed chiefly of illite which has four to five times greater anion exchange capacity than kaolin. Studies by Eroshchev-Shak (1962) demonstrate that illite is the predominant clay in the North Atlantic. Also, soils found in the northeastern United States are generally high in illite; thus it is likely to be present in adjacent waters.

To remove possible effects of organic matter such as the blocking of anion exchange positions or the reduction of surface area by cementing action, the sediment was treated with 30% $H_2O_2$ on a steam bath at 90° C for 48hr. This sediment freed of organic matter adsorbed far more phosphate than untreated sediment (Fig. 6). This indicates strongly that adsorptive properties of sediments are due to exchange sites on the clay rather than on organic materials and supports previous observations by de Haan (1965) who found that the adsorptive capacity of organic coated illite increased after it was treated with 30% $H_2O_2$.

By plotting the log of amount of phosphate adsorbed up to 50% of maximum fixation at 23 C and 65°C against time, two straight lines are obtained (Fig. 7). The linearity indicates a first order reaction (Daniels and Alberty, 1966) and the slope of such a plot equals K/2.303 in which K is the rate constant of the reaction. The slope was calculated by the method of least squares and the values of K thus obtained were substituted into the Van't Hoff equation to obtain the $\Delta$ H or heat of reaction:

$$\log \frac{K_2}{K_1} = \frac{\Delta H}{2.303R} \frac{(T_2 - T_1)}{T_2 T_1}$$

The calculated heat of reaction is less than 1.5 Kcal/mole. This is very low and characteristic of an ion exchange reaction. This suggests that the heat of reaction is due to the displacement of water molecules by the larger phosphate ion. Muljadi *et al.* (1966) reported a low heat of reaction and attributed the small gain in entropy to the rearrangement of two water molecules from around a central ion; in this case the central ion is the hydroxyl group.

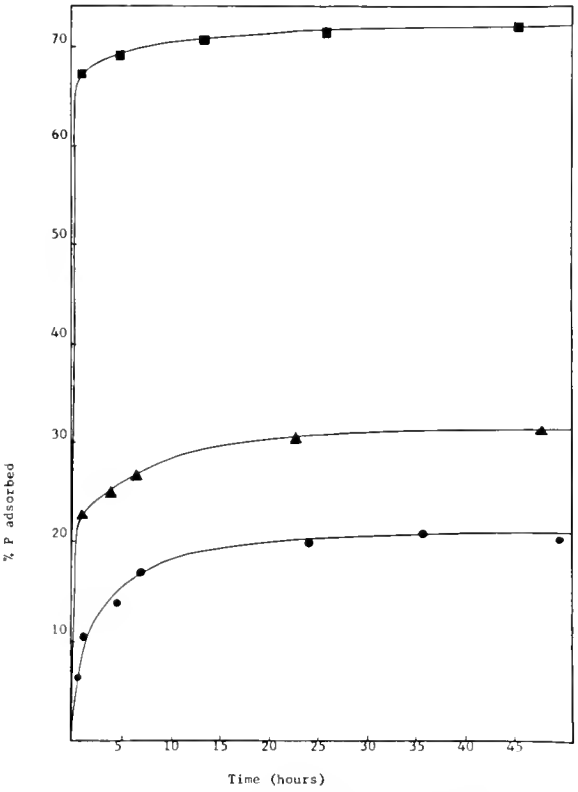


FIG. 6. *Comparison between the capacity of Pt. Judith Pond sediment and pure kaolin to adsorb phosphorus. Initial conditions; 50 mgm of kaolin or sediment, 15 ml 1 ppm P solution, 19‰ Cl, temperature 23°C, pH 7.0.* ● *kaolin;* ▲ *untreated sediment;* ■ *30% $H_2O_2$ treated sediment.*

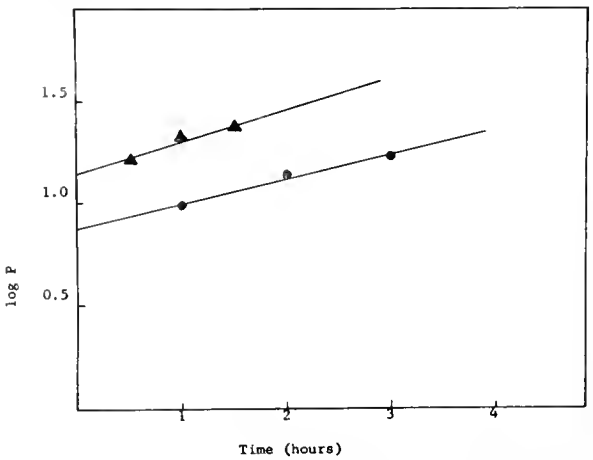


FIG. 7. *A plot of log P versus time to determine order of reaction and to calculate heat of adsorption. Points obtained from temperature dependence curves.* ● *23°C* ▲*65°C.*

## CONCLUSIONS

Kaolin adsorbed appreciable amounts of phosphate from our test solutions. From heat of adsorption data it is evident that phosphate is held in an exchangeable form. The largest amounts of phosphate are adsorbed under conditions of high temperature, low salinity and pH 5.0 to 6.5—conditions that are rarely found in oceanographic or estuarine situations. Destruction of the organic matter and the exposure of exchange sites on clay from Point Judith Pond caused a great increase in the adsorptive capacity of these sediments.

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# GROWTH, SURVIVAL AND SOME EFFECTS OF A DENSE RAZOR CLAM SET IN WASHINGTON

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## ABSTRACT

*Setting of the Pacific razor clam,* Siliqua patula *Dixon, reached record levels north of Grays Harbor, Washington, in 1966. Growth rate was greatly reduced when the set persisted strongly for 7 months. Plankton appeared to be diminished by the set, and availability ("show") of larger clams was reduced. Survival of the 1966 set was poor when measured at age 1.5 years. Transplants made to a beach where natural setting was poor resulted in modest contribution.*

## INTRODUCTION

Setting intensity of the Pacific razor clam, *Siliqua patula* Dixon, has varied considerably in past years. McMillin (1924) described the heaviest previous set of 1,450 clams per ft² at Copalis Beach, Washington in August 1923. The set survived for 4 months at a density above 450 per ft², then suffered 95% mortality during a severe storm from 4 to 6 December. Monthly screening samples between 1952 and 1959 revealed annual setting highs ranging from 0 to 155 per ft², but showed that high abundance levels did not persist for more than 2 consecutive months (Tegelberg, 1964). There was considerable variation in the time of setting, but abundance of over 10 per ft² was found only after July.

The presence of an unusual razor clam set was investigated on 14 June 1966 at Mocrocks Beach, Washington (Fig. 1). One's first impression was that a shipload of grain had washed ashore as the clams averaged 6 mm in length. Patches of clams up to several inches deep and several acres in extent were found for a period of weeks along the beach during low tides. Examination of the patches revealed that 20 to 80% of the clams were alive. At Copalis Beach, just south of Mocrocks Beach, smaller patches of clams with similar survival rates were found during the same period. Similar surface patches have been noted by McMillin (1924) in 1923. Quayle (personal communication)[1] found concentrations of dead and dying clam sets in July and August 1961 following heavy setting at Long Beach, Vancouver Island, B.C.

The record set, which was confined to the beaches north of Grays Harbor, was unusual in that it was early and persisted at a high level for many months. Although dense populations are known to be adversely affected from lack of food, razor clams have not been considered previously in this regard because of the rich supply of plankton found along the Washington beaches. Rapson (1954), however, had noted a "reduction in growth rate in dense beds" of the toheroa (*Amphidesma ventricosum*), a New Zealand clam found in sand beaches similar to those occupied by the razor clam. Concern over possible effects of the record razor clam set prompted a study of the growth and survival of this year class, and its effect on other year classes present on the same beaches. The unusual availability of set clams in surface patches facilitated a transplant to Twin Harbors Beach (south of Grays Harbor) where natural setting was poor. The result of the transplant was also investigated.

## METHODS

The principal razor clam area in Washington is geographically separated into four ocean beaches known as Long Beach, Twin Harbors Beach, Copalis Beach and Mocrocks Beach (Fig. 1).

[1] Dr. D. B. Quayle, Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.

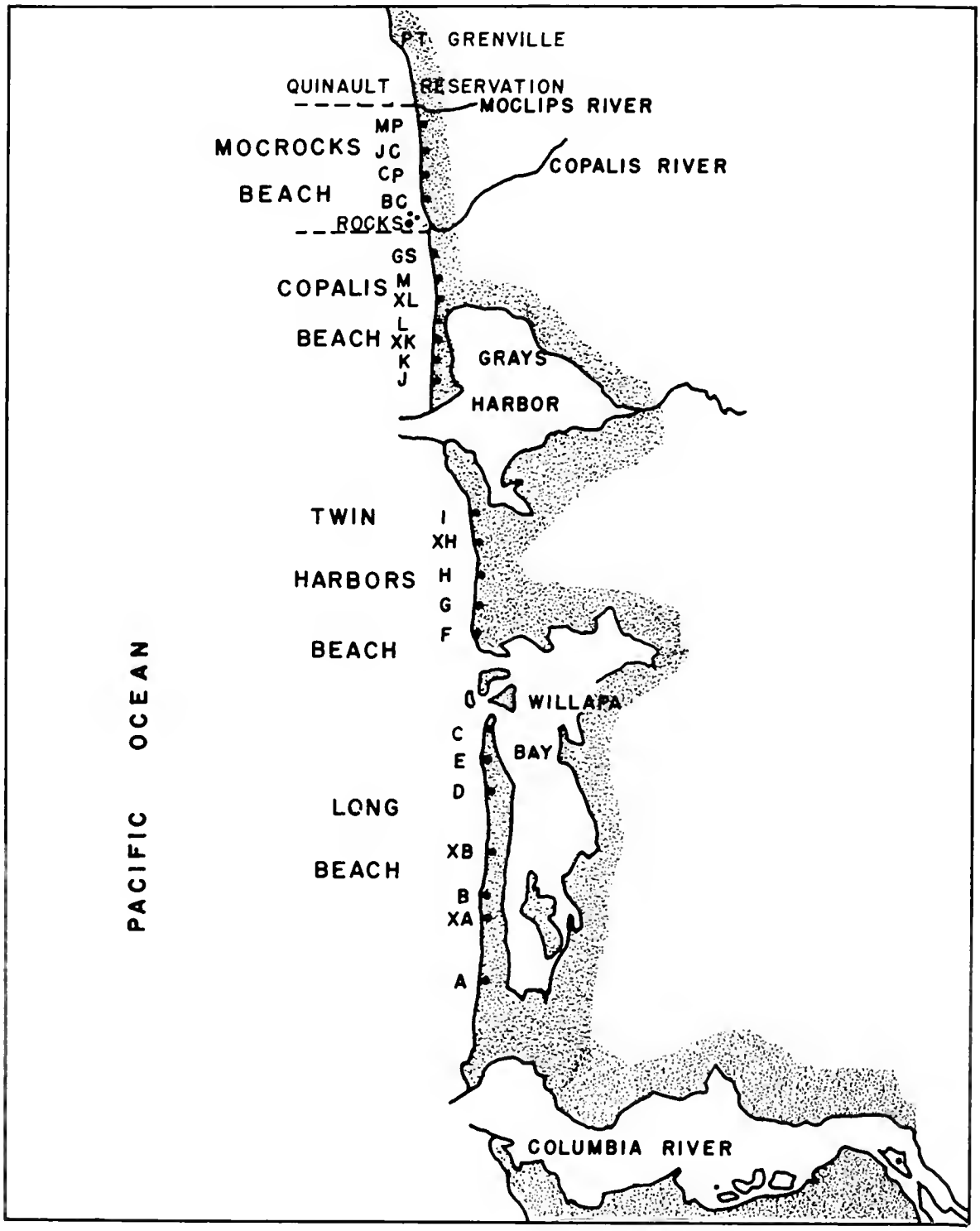


FIG. 1. *Sampling stations at the principal Washington razor clam beaches.*

Mocrocks Beach, extending from Copalis River to Moclips River, has become an important producer only within the past decade. Much of the data presented became available from regular sampling programs at these beaches. Monthly screening samples, as described by Tegelberg (1964), were taken in 3 to 5 lettered areas (Fig. 1) at each beach to determine the abundance and size of set clams. Surface patches of set clams were avoided, and individual screenings were 2 ft² rather than 4 ft² in areas of dense set. Numbers were estimated from total volume and counts of live clams in sub-samples of 5 to 40 ml. Beginning in September, weight was used instead of volume. Set clams were measured on millimeter graph paper by use of a needle punch.

An annual population census is carried out each fall in the lettered sampling areas. This involves marking and planting razor clams (Tegelberg, 1964) in 9-foot-wide strips divided into 100-ft sections that extend the width of the available clam bed. Recovery is accomplished by locating and digging individual clams whose presence is revealed by a depression or hole called a "show". Population estimates for each 9- by 100-ft section are calculated from mark and nonmark recoveries after achieving minimum mark recovery of 20%. Growth and rate of show of marked clams were used to determine effects of the dense set, wherein Long Beach and Twin Harbors Beach, which had moderate or light sets, were considered controls. Strength of the 1966 year class in 1967 was determined from the sport catch and abundance

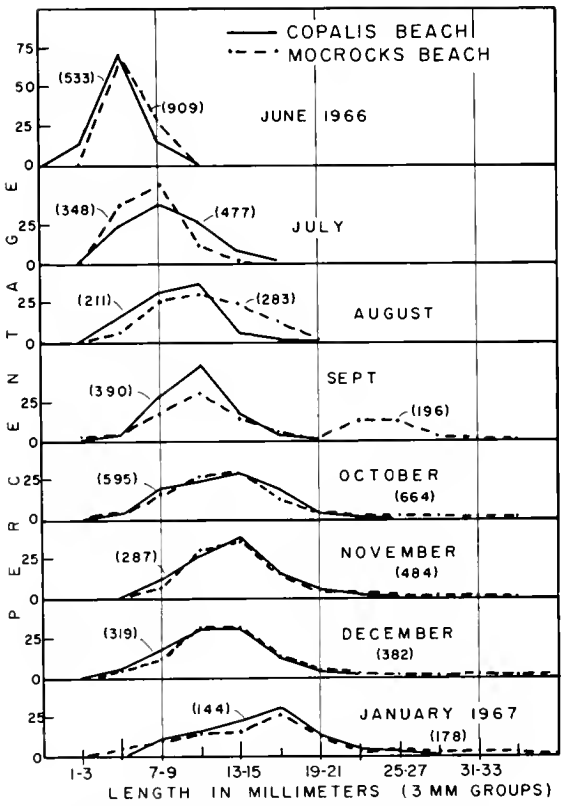


FIG. 3. *Monthly length composition of razor clams screened at Copalis and Mocrocks Beaches, June 1966-January 1967. Sample size is given.*

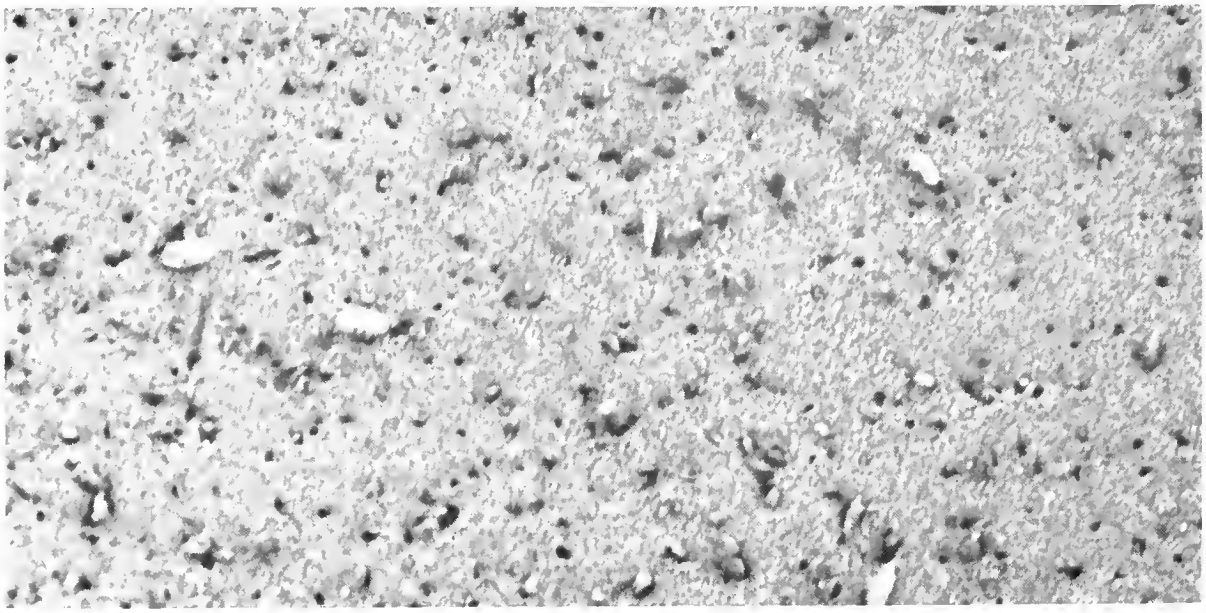

FIG. 2. *Typical sand appearance during the abundant 1966 set.*

index (clams per yd²) obtained in the fall census.

Length measurements were taken of sport-caught razor clams, and of clams taken by random digging methods — including the nonmarked clams in the fall census. Length frequencies by beach were used to compare growth of the 1966 year class.

Water samples were taken in the surf in December 1966 for determination of plankton composition and chlorophyll *a*. The method of Strickland and Parsons (1960) was used for chlorophyll *a* determinations, except an HA (0.45 $\mu$) millipore filter was used rather than AA (0.8 $\mu$). General notations were made of unusual occurrences of other species.

Set clams for transplant were collected from surface patches having a high percentage of live clams. These were shovelled into tubs and boxes, trucked to Twin Harbors Beach, and broadcast on that beach during the same flood tide. During initial transplants, clams were spread in wet areas or in small areas roughened by a rake in an effort to facilitate digging-in. Gross numbers were estimated from total volume and subsample counts of live clams.

## RESULTS

### *Timing and Density of 1966 Setting*

Countless holes and partly-exposed clams were evidence of the dense set established in the sand at Moerocks Beach (Fig. 2). Individual screening samples in June yielded 115 to 13,600 live clams per ft², or an average of 3,685 (Table 1). The percentage of dead clams was low in June except in area MP (Fig. 1) where high mortality was evident. Abundance of set clams dropped somewhat in July and sharply in August, then increased to another peak in September (average 2,262 per ft²). Despite normal stormy periods in October through December, set density remained above 400 per ft² for an unprecedented period of 7 months. Subsequently, the density at Moerocks Beach dropped to less than 3 per ft² in February 1967 after severe storms that caused some beach erosion.

Copalis Beach setting began in June, but was at a lower level than at Moerocks Beach (Table 2). Abundance of set clams peaked at an average 863 per ft² in July, dropped sharply to 123 per ft² in August, peaked again at 1,385 per ft² in September, and remained above 100 per ft² through December. Severe mortality followed and density at Copalis Beach dropped to 0.2 clams per ft² in February 1967. Considerable variation was evident between samples at both beaches, but average values are believed to satisfactorily show the abundance changes by month.

The biomass was high for 7 months at Moerocks Beach because of the dense set. Excluding the surface patches, estimates were about 7,000 lb per acre in June, July and August, followed by a sharp rise to 20,000 lb per acre in September, or

TABLE 1. *Numbers of razor clams per ft² taken in monthly screening samples at Moerocks Beach, June 1966 - February 1967.*

| Sampling location | | 1966 | | | | | | 1967 | |
|---|---|---|---|---|---|---|---|---|---|
| | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. |
| BC 600* | 3,740 | | | | | | | | |
| 700 | | 5,032 | 141 | 1,919 | 567 | 577 | 456 | | 3 |
| 800 | | | | | | 750 | | | 7 |
| 900 | 2,082 | 1,480 | 201 | 4,727 | 1,350 | | 516 | | |
| CP 600 | 13,629 | | | | | | | 21 | |
| 700 | | 6,316 | 313 | 3,234 | 893 | 534 | 820 | 25 | 2 |
| 800 | | | | | | 183 | | | |
| 900 | | | 453 | 3,485 | 1,694 | | 566 | | 2 |
| 1000 | | 581 | | | | | | | |
| JC 600 | | | | | | | | 12 | |
| 700 | 5,456 | | | | 656 | | | | |
| 800 | | | | | | | | | |
| 900 | 619 | | | | 1,342 | | | | |
| MP 600 | 115 | | | | | | | | |
| 700 | | 1,240 | 1,287 | 30 | 343 | 124 | 55 | 15 | 1 |
| 800 | 155 | | | | | | | 17 | |
| 900 | | 703 | 796 | 178 | 695 | 344 | 115 | | 1 |
| Average | 3,685 | 2,559 | 532 | 2,262 | 943 | 419 | 421 | 18 | 2.7 |

*Distance (feet) seaward of baseline.

TABLE 2. *Numbers of razor clams per ft² taken in monthly screening samples at Copalis Beach, May 1966 - February 1967.*

| Sampling location | | 1966 | | | | | | | | 1967 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. |
| J | 1700* | | | 540 | 42 | | | | | | |
| | 1900 | | | 3,102 | 402 | | | | | | |
| K | 1600 | | | | | 66 | 211 | 87 | 5 | 2 | 0.5 |
| | 1700 | 0.7 | 726 | | 24 | | | 135 | | | |
| | 1800 | | | | | 2,389 | 387 | | 5 | | 0.0 |
| | 1900 | 0.5 | 10 | | 132 | | | | | | |
| L | 1300 | | 170 | | 39 | 101 | 414 | 431 | 152 | | 0.3 |
| | 1400 | 0.3 | 425 | | 82 | | 421 | | | 22 | |
| | 1500 | 0.0 | | | | | | 266 | 156 | | 0.3 |
| M | 1200 | | | 140 | 61 | 1,890 | 324 | 420 | 272 | 34 | 0.0 |
| | 1300 | 0.0 | 157 | | | | 702 | | | | |
| | 1400 | 0.0 | 106 | 210 | 36 | | | 172 | 88 | | 0.0 |
| GS | 1200 | | 575 | | | | 523 | 543 | 214 | 13 | 0.3 |
| | 1300 | | | 1,155 | 127 | 2,478 | 442 | | | | |
| | 1400 | | 350 | | | | | 221 | 89 | | 0.3 |
| | 1500 | | | 618 | 309 | | | | | | |
| | 1700 | | | 274 | 98 | | | | | | |
| Average | | 0.3 | 315 | 863 | 123 | 1,385 | 428 | 284 | 123 | 18 | 0.2 |

*Distance (feet) seaward of baseline.

nearly 10 times the average biomass of a typical razor clam population. Biomass declined thereafter, reaching a level of 5,000 lb per acre in December. The same trends were noted at Copalis Beach, but biomass was about half the level of Mocrocks Beach. The monthly size compositions of set clams at Copalis and Mocrocks Beaches are plotted in Figure 3. Although the principal mode remained similar for the two beaches from June 1966 through January 1967, some larger set clams were evident at Mocrocks Beach after July. Inspection of size by area revealed that the larger clams were from area MP, the northernmost sampling area (Fig. 4), and this area was the only atypical one of the nine sampled. The second mode in September in Figure 3 was caused by measuring a disproportionately high number of clams from area MP. At Mocrocks Beach, the lowest abundance of set clams was usually found in MP, a factor that may have promoted faster growth of clams in this area.

Setting at Long Beach was 1 to 2 months earlier than at other beaches. Sampling in May 1966 revealed 2.5 clams per ft² averaging 9 mm in length. Abundance increased to about 5 per ft² in June and 9 per ft² in July, then declined through November when sampling was terminated. Early sets of this magnitude had not been measured at Long Beach since 1955-1957 (Tegelberg, 1964). Length frequencies indicated that set clams taken through November were predominantly from the early set (Fig. 5).

*The Transplant to Twin Harbors Beach*

Sampling at Twin Harbors Beach revealed poor setting in 1966, with monthly averages of 0.0 clams per ft² in May and under 0.2 clams per ft² in early June. The poor natural set at this beach, coupled with the availability of clams in surface patches at Mocrocks Beach, resulted in experimental transplants of several million clams to sampling areas I and XH (Fig. 1) on 16 and 17 June 1966. Approximately 50% were alive when transferred, and many were observed digging into the sand after dispersal on soft, wet beach. An inspection two hours later revealed that encouraging numbers had dug in and were established in area I. Since no set clams had been found in 8 ft² just prior to the transplant, the catch of 22 live clams in 28 ft² on 21 June revealed some success, but also indicated either high mortality or considerable dispersal, or both. Additional transplants were made on 21, 22, and 23 June. On 23 June, an estimated 335 million clams were transplanted by beach residents and fisheries personnel to the middle part of Twin Harbors Beach. Sampling of 30 ft² in the middle part of the beach on 30 June revealed only 8 clams (0.3 per ft²). On 15 July, 15 clams averaging 12 mm in length were taken from 18 ft² in the same area (0.8 per ft²). Regular sampling areas (G, H, I) averaged 1.1 clams per ft² on 15 July, or the highest average since 1957 (Tegelberg, 1964) and five times higher than in June prior to the transplant. The increased number of clams in July was apparently the result of the

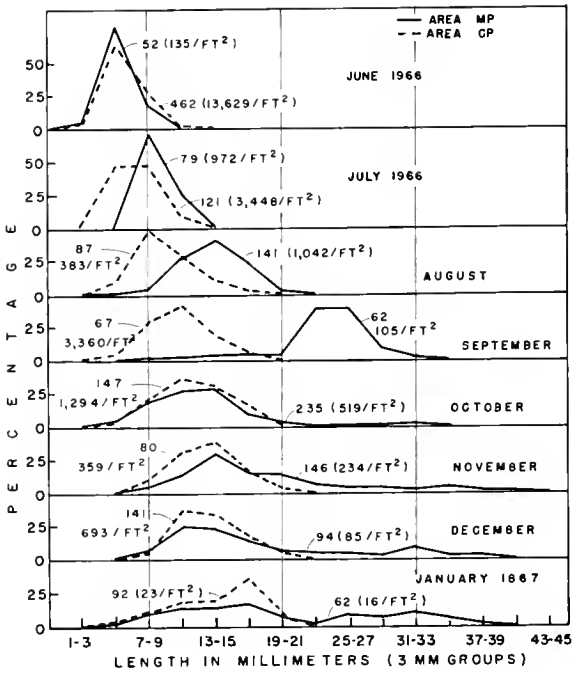


FIG. 4. *Monthly length composition of razor clams screened in areas MP and CP, Mocrocks Beach. Sample size and set density are given.*

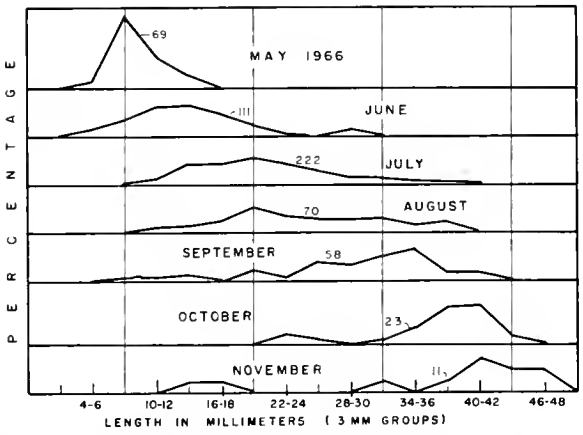


FIG. 5. *Monthly length composition of razor clams screened at Long Beach in 1966. Sample size is given.*

transplants. The average then dropped to between 0.1 and 0.2 clams per ft² from August to October.

*Growth of 1966 Year Class*

The use of average length of set clams as a measure of growth assumes that the early sets in 1966 were not supplemented appreciably by later setting; this was supported by the predominance of one mode in the length frequencies (Figs. 3 and 5). Growth of the set clams appeared to be extremely slow at Copalis and Mocrocks Beaches. The only exception was in MP area of Mocrocks Beach (Fig. 1) where one group reached approximately 25 mm length by September. Although this group was evident through January 1967 (Fig. 4), it was masked by an infusion of smaller clams in October 1966 - presumably from offshore.

The average lengths of clams taken in screening are plotted for Copalis, Mocrocks and Long Beaches in Figure 6; too few clams were taken to plot the size at Twin Harbors Beach for the same period. Average length at Long Beach progressed from 9 mm in May to 21 mm in July, 32 mm in September and 42 mm in November. Since screening is selective for clams less than 20 to 25 mm in length, this is considered to be a minimal representation of growth. Hirschhorn (1962) reported similar growth for Oregon set clams when he followed a length mode from 10 mm to 25 mm in 2 months. He then noted development of a bimodal size distribution due to a new set. During a 6-month period (June - December) the set clams at Copalis and Mocrocks Beaches grew only from 6 mm to 15 mm, indicating that growth rate was far below normal.

Length frequencies of random and sport samples were useful in comparing size of the 1966 year class in 1967 at Copalis and Twin Harbors Beaches. The 1966 year class, including the transplants, was first apparent in 1967 in a random dig made in April at Twin Harbors Beach, but this group (75 to 90 mm) was being avoided by the sport fishermen, who select larger clams (Fig. 7). Recruitment to the fishery occurred mainly in July and August, and the mode for the 1966 year class advanced to approximately 98 mm by late July and 104 mm in October 1967.

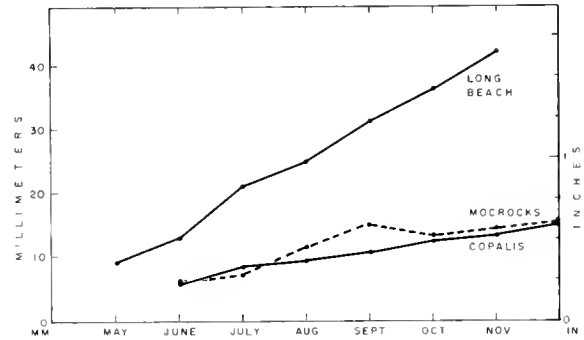


FIG. 6. *Monthly average length of clams screened at Long Beach, Copalis Beach, and Mocrocks Beach in 1966.*

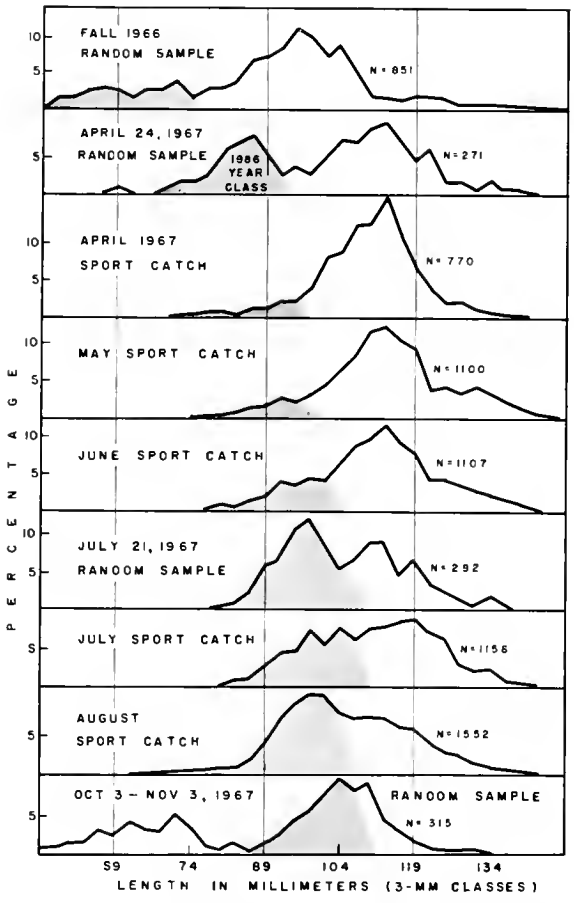


FIG. 7. *Length frequencies of razor clams sampled at Twin Harbors Beach, fall 1966 - fall 1967. The shaded area approximately delineates the 1966 year class.*

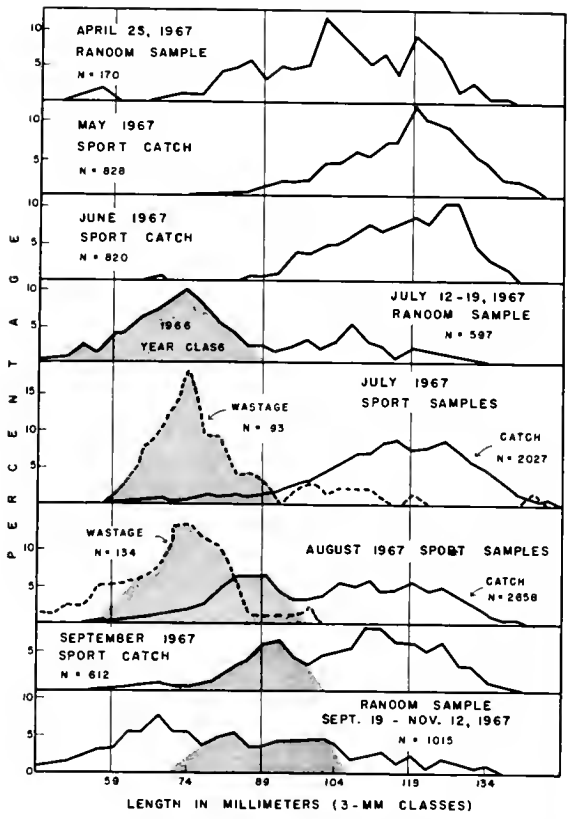


FIG. 8. *Length frequencies of razor clams sampled at Copalis Beach in 1967. The shaded area approximately delineates the 1966 year class.*

At Copalis Beach the 1966 year class was not evident in a small random sample in April, but showed strongly in July 1967 (Fig. 8). Average size was about 72 mm in July, or 26 mm (1 inch) smaller than at Twin Harbors Beach. Larger clams of the 1966 year class entered the sport catch at Copalis Beach in August and September, and smaller clams of this year class were discarded by diggers (wastage). The length frequency for fall 1967 indicated a size range of 73 to 104 mm for the 1966 year class, but a distinct mode was lacking. At age 1.5 years, clams of the 1966 year class at Copalis Beach averaged 10 to 15 mm smaller than the 1956 year class (June set) at this beach (Tegelberg, 1964) and the 1966 year class at Twin Harbors Beach. The 50 to 73 mm clams at both Copalis and Twin Harbors Beaches in fall 1967 apparently were fast-growing clams from an early 1967 set.

*Growth and Show of Older Clams*

Marked clams planted in September 1966 at Mocrocks and Copalis Beaches were recovered during the censusing period ending in December and subsequently from the sport fishermen. The growth of mark recoveries from Copalis Beach, excluding those at liberty 45 days or less, is listed in Table 3. Growth was very poor through January as recoveries grew an average of only 0.0 to 2.5 mm. Improved growth coincided with heavy mortality of the set, since February recoveries had grown an average of 1.5 to 6.0 mm. The possibility that this was a natural slowdown of growth because of winter was ruled out by the fact that marked clams at Long Beach and Twin Harbors Beach grew about 10 times as much as marked clams at Copalis or Mocrocks Beaches (Table 4). Although recovery numbers were relatively small, the results are considered to be meaningful because growth at Washington beaches has been described by Tegelberg (1964) as consistently fastest at Copalis Beach.

A high surf in mid-September 1966 altered the

TABLE 3. *Growth of marked clams planted at Copalis Beach in September 1966.*

| Planted length (mm) | Average growth (mm) by recovery month and days out | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nov. 46-75 | | Dec. 76-105 | | Jan. 106-135 | | Feb. 136-165 | | March 166-195 | | April 196-225 | | May 226-255 | | June 256-285 | |
| | $\bar{x}$ | n | $\bar{x}$ | n | $\bar{x}$ | n | $\bar{x}$ | n | $\bar{x}$ | n | $\bar{x}$ | n | $\bar{x}$ | n | $\bar{x}$ | n |
| 70- 79 | | | | | | | 4.5 | 2 | | | | | | | | |
| 80- 89 | 0.8 | 5 | | | 0.0 | 2 | | | 7.0 | 1 | 15.0 | 1 | | | | |
| 90- 99 | 1.0 | 3 | 0.0 | 1 | 2.5 | 4 | 6.0 | 2 | | | | | | | 20.0 | 2 |
| 100 109 | 0.0 | 2 | 0.0 | 1 | | | 5.0 | 1 | | | | | 10.5 | 2 | | |
| 110-119 | 0.0 | 2 | 0.0 | 1 | 0.0 | 1 | 1.5 | 2 | | | 5.0 | 1 | | | | |
| 120 129 | 0.3 | 3 | 0.0 | 1 | 1.0 | 2 | 1.5 | 4 | 2.0 | 1 | | | | | 4.0 | 1 |
| 130-139 | | | 0.0 | 1 | | | | | | | | | | | | |

$\bar{x}$ = Average growth in mm. n = Number of clams.

beaches from the normal undulating summer topography of bars and lagoons to the typical flat topography of winter. The beaches south of Grays Harbor maintained a normal firm condition, but the beaches north of Grays Harbor became very soft. This soggy condition was attributed to the activity of the set clams in the upper 1 or 2 inches of sand. Older clams seemed reluctant to show, and shows did not maintain their identity in the soft sand. As a result, catch per sport digger dropped sharply after 15 September.

The poor show of older clams at Copalis and Mocrocks Beaches was further substantiated in the recovery digging portion of the fall census between 15 September and 9 December 1966 (Table 5). Total mark recoveries of 17.2% and 22.8% were made at Copalis and Mocrocks Beaches, respectively. Despite greater effort than usual, these recoveries were the lowest in 15 years of similar sampling, and the census was not satisfactorily concluded at these beaches. In contrast, total mark recoveries of 37.8% and 47.7% were made at Long Beach and Twin Harbors Beach, respectively. Recovery per dig was approximately 20% at these beaches compared to 3.8% at Copalis and 6.3% at Mocrocks. Thus, the show of clams at the beaches with the dense sets was approximately one-fourth that of the "control" beaches, while in normal years recovery percentages are similar at all four beaches.

Sport checks in February 1967 indicated that digging had improved considerably, coinciding with the sharp drop in set abundance. On 23 February and 7 March in sections of two areas used in the fall census at Copalis, marked clams (12-16 September plants) were recovered at a rate of 16% per dig, indicating that the show had improved substantially.

*Survival and Contribution of the 1966 Year Class*

At Copalis Beach the 1966 year class contributed somewhat to the late-summer 1967 fishery. However, the catch per digger trip was low (9 clams) in August 1967 and wastage was not severe. These facts indicated that the 1966 year class was not particularly strong, and this was borne out by the lack of a significant mode in the fall 1967 length frequency (Fig. 8). The over-all abundance in fall

TABLE 4. *Growth by beach for marked razor clams planted in fall 1966 and recovered after 46 to 75 days at liberty.*

| Planted length (mm) | Growth in millimeters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mocrocks Beach | | Copalis Beach | | Twin Harbors Beach | | Long Beach | |
| | $\bar{x}$ | n | $\bar{x}$ | n | $\bar{x}$ | n | $\bar{x}$ | n |
| 60- 69 | 1.0 | 2 | | | | | | |
| 70- 79 | 1.0 | 1 | | | 9.3 | 4 | 14.0 | 1 |
| 80- 89 | 0.0 | 1 | 0.8 | 5 | 7.5 | 19 | 7.9 | 7 |
| 90- 99 | 0.0 | 2 | 1.0 | 3 | 5.9 | 26 | 4.7 | 3 |
| 100-109 | 0.2 | 6 | 0.0 | 2 | 5.7 | 7 | 5.0 | 2 |
| 110-119 | 0.5 | 4 | 0.0 | 2 | 2.8 | 4 | | |
| 120-129 | 0.0 | 5 | 0.3 | 3 | 0.7 | 3 | 0.0 | 1 |
| 130-139 | 0.0 | 1 | | | | | | |
| 140-149 | 0.0 | 1 | | | | | | |

$\bar{x}$ = Average growth. n = Number of clams.

TABLE 5. *Recovery of razor clams marked and planted 29 August to 16 September 1966 and recovered in the fall census.*

| Beach | Number of marks planted | Recovery | | | | | Per cent recovery per dig |
|---|---|---|---|---|---|---|---|
| | | Dates | Number of sections | Avg. no. digs per section | Total No. | % | |
| Mocrocks | 535 | 9/29-12/8 | 18 | 3.6 | 122 | 22.8 | 6.3 |
| Copalis | 639 | 9/28-12/9 | 22 | 4.5 | 110 | 17.2 | 3.8 |
| Twin Harbors | 415 | 9/17-11/12 | 16 | 2.1 | 198 | 47.7 | 22.7 |
| Long Beach | 645 | 9/15-11/15 | 21 | 1.9 | 244 | 37.8 | 19.9 |

1967 was below the 15-year average for Copalis Beach. The abundance of the 1966 year class alone was approximately 0.7 clams per yd² at age 1.5 years, assuming a size range of 73 to 104 mm for this group. An inspection of comparable fall census data revealed that the 1966 year class ranked 11th in strength in the past 16 years. Wide year class strength has been evident, ranging from about 0.2 to 3.8 clams per square yard at age 1.5 years. Survival of the 1966 set was thus poor, and the 1966 year class was expected to make only a mediocre total contribution to the Copalis fishery. Similiarly, Quayle (personal communication[1]) noted that a disappointing year class resulted from the excellent 1961 set at Long Beach, Vancouver Island. At Copalis Beach, however, McMillin (unpublished report, 1926[2]) stated "the 1923 class was one of the largest resulting from any spawning that has been observed".

The 1966 year class did not enter the 1967 catch to any extent at Mocrocks Beach. Low abundance of this year class became evident when the overall abundance in fall 1967 reached the lowest level for 9 years of sampling. Survival was at about the same level as at Copalis Beach, and the below-average 1966 year class was expected to make a modest contribution to the Mocrocks fishery.

The 1966 year class at Twin Harbors Beach (Fig. 7) contributed approximately 33% of the July 1967 sport catch and 50% of the August catch. The 1967 contribution from this year class was estimated at 700,000 clams, including wastage, or approximately 30% of the total 1967 sport catch. Although this year class comprised between 40 and 50% of the population in fall 1967, its abundance level was only 0.3 clams per yd² and overall abundance at this beach was the second lowest in 15 years. Estimated contribution to the 1968 catch at Twin Harbors Beach was 400,000 clams during a poor season. Although judged to be about 50% of average, the 1966 year class which included the transplants made a worthwhile contribution to the fishery for two seasons.

*Unusual Occurrences*

The water at Copalis and Mocrocks Beaches was unusually clear from late summer through December 1966. Notably absent were the dense blooms of *Chaetoceros armatus* which color the water and create a brown scum on the beach, particularly during periods of high surf. This inshore diatom appears to be the principal food organism available to the razor clam during the period October to April (Tegelberg and Magoon, unpublished data). Water samples collected in December north of Grays Harbor contained chlorophyll *a* in amounts averaging 2.9 to 8.6 mg/m³ (Table 6). These were much lower values than found at Twin Harbors Beach and Long Beach where *C. armatus* occurred in high concentrations. The chlorophyll *a* values at Copalis Beach were considered to be abnormally low, and were lower than any values obtained from biweekly water samples in 1965 when chlorophyll a ranged from 54 to 1,060 mg/m³ at Copalis Beach and 18 to 2,143 mg/m³ at Mocrocks (Magoon, unpublished data). Further, the Copalis Beach chlorophyll *a* concentrations in 1965 always exceeded those at Twin Harbors Beach for comparable dates. *C. armatus* did not appear in quantity north of Grays Harbor until January when the set had decreased in numbers.

Throughout the period of dense set, shorebirds of the sandpiper group (Scolopacidae) were observed in great numbers feeding on razor clams. In addition, large numbers of gulls, principally the glaucous-winged gull, *Larus glaucescens*, actively fed on the set clams. Thousands of sea ducks, especially the surf scoter, *Melanitta perspicillata*, and white-wing scoter, *M. deglandi*, congregated just off the beach, presumably attracted by the set. The heaviest concentrations of gulls and scoters were noted where the largest set clams were found — i.e. the northern part of the beach represented by sampling area MP (Fig. 1).

Small Dungeness crabs, *Cancer magister*, were

[2] McMillin, H. C. Unpublished report, 1926. Observations on the Pacific razor clams (*Siliqua patula*) of the State of Washington from April 1, 1925 to March 31, 1926.

TABLE 6. *Average chlorophyll a concentration (mg/m³) at Washington ocean beaches in December 1966.*

| Date | Mocrocks Beach | | Copalis Beach | | Twin Harbors | | Long Beach | |
|---|---|---|---|---|---|---|---|---|
| | No. of samples | mg m³ | No. of samples | mg/m³ | No. of samples | mg/m³ | No. of samples | mg/m³ |
| 9, 10 Dec. | 1 | 2.9 | 2 | 4.3 | 10 | 1,082.1 | - - - | - - - |
| 22-24 Dec. | 4 | 8.6 | 12 | 6.7 | 10 | 409.5 | 22 | 15.3 |

observed in unusually large numbers in shallow, inshore lagoons where they fed on set clams. Screening for set clams occasionally captured Dungeness crabs, an extremely unusual occurrence. On one occasion, 149 Dungeness crabs 36 to 75 mm in carapace width were taken incidental to screening for set clams.

Crab fishermen reported many small clams in sand brought up with crab pots from as deep as 18 fathoms north of Grays Harbor. One fisherman reported that his pots were sitting in dead juvenile shells and that the shells were clinging to his buoy lines.

## DISCUSSION AND CONCLUSIONS

McMillin (1924) noted that the heavy 1923 set resulted from nearly complete, mass spawning the end of May. Timing and density of the 1966 set suggest that mass spawning resulted from the unusually warm weather the first week of April, and that high survival and northerly setting were related to ocean conditions.

Variations in abundance of set clams appeared to be correlated to beach topography, the high mortality, and shifting of set clams within the beach area. The surface patches of clams and the sharp increase in set abundance in September indicated that set clams were being supplied to Mocrocks and Copalis Beaches from a vast reservoir of clams that had originally set outside the available beach. It is concluded that the shifting or dispersal of set clams and the contribution of set originating from outside the available beach also pertain to normal years.

Growth of the 1966 set clams was very slow for the 7-month period of high abundance, and growth and show of marked clams were shown to be adversely affected from September through December. It is concluded that these effects were directly attributable to the record set which cause overpopulation and drastic reduction of the plankton supply. Failure of *C. armatus* to appear in quantity during this period has never before been observed at these beaches. Rapson (1952, 1954), who described dense blooms of *C. armatus*, attributed certain instances of low plankton quantities to large populations of toheroa and tuatua on New Zealand beaches.

Survival of sets is considered to be a function of size reached before winter storms strike. Because of subnormal growth, the 1966 set clams were small and suffered high mortality in January and February 1967. Subsequently, the 1966 year class at Copalis and Mocrocks Beaches ranked low in abundance and made a disappointing contribution to the razor clam fishery at these beaches.

The transfer of set clams made a worthwhile addition to the poor natural set at Twin Harbors Beach. The 1966 year class, including the unknown transplant survivors, contributed over 1 million clams to the sport fishery at Twin Harbors Beach in 1967 and 1968. Since this year class was about half of average strength, it is concluded that any seeding program would have to be on a very large scale. Since razor clams rarely occur in surface patches, chances are remote for another transplant.

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# ASSOCIATION AFFAIRS

## ANNUAL CONVENTION

The National Shellfisheries Association 1968 Convention was held jointly with the Oyster Institute of North America and the Oyster Growers and Dealers Association of North America, Inc. from July 14 to 17 at Arlington, Virginia.

Officers and Executive Committee members elected for the term 1968-1969 were:

| | |
|---|---|
| President | Harold H. Haskin |
| Vice-President | Albert K. Sparks |
| Secretary-Treasurer | Fred W. Sieling |
| Members-at-Large | Roy Drinnan |
| | James E. Hanks |
| | R. Winston Menzel |
| Co-Editors, NSA Proceedings | |
| | Arthur S. Merrill |
| | William N. Shaw |

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

Dr. R. Winston Menzel has been appointed Membership Chairman for the Association. We have lost 3 members by death, Dr. Robert Coker, Dr. George Moore and Mr. R. H. Gregory. The membership is now 232, with 63 active library subscribers and 4 abstract companies.

Dr. Walter Chipman and Dr. Sewell H. Hopkins were elected to Honorary Membership in the National Shellfisheries Association. Mr. Victor Kennedy received the "Thurlow C. Nelson" award for his work with soft shell clams (*Mya arenaria*).

The Oyster Institute of North America presented plaques to Congressman John Blatnik, Congressman John Dingell, Senator Edmund Muskie and Mr. Murray Stein for their contributions to the right to market shellfish.

The Pacific Coast Section of the NSA met jointly with the Pacific Coast Oyster Growers Association on 22-24 August 1968 at Harrison Hot Springs, British Columbia. Officers of the Section elected for the term 1968-1969 were:

| | |
|---|---|
| Chairman | Kenneth K. Chew |
| Vice-Chairman | John C. Hoff |
| Secretary-Treasurer | Robert B. Herrmann |

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

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