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ABSTRACTS OF TECHNICAL PAPERS PRESENTED AT THE 1970 NSA CONVENTION

PROGRESS IN STUDIES OF THE CALICO SCALLOP [1]

Donald M. Allen

U. S. Department of Commerce
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Tropical Atlantic Biological Laboratory
Miami, Florida

Commercial concentrations of the calico scallop, *Argopecten gibbus*, are located primarily off the east coast of Florida, and to a lesser extent, off North Carolina and in the northeastern Gulf of Mexico. The development of mechanical shuckers has increased interest in this resource, and annual yields of 15 million pounds of edible meats have been predicted for the Florida east coast beds near Cape Kennedy.

The calico scallop program of the National Marine Fisheries Service is composed of two parts: resource assessment and biological research. Distribution and abundance of scallops are assessed by exploratory dredging, observations from submarines and by use of a bottom scanning device called RUFAS (Remote Underwater Fishery Assessment System). Biological studies include those on reproduction, larval development, spat setting, growth and age, mortality and environmental relationships. Most of these observations are made at buoy-marked underwater sites on the Cape Kennedy grounds.

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[1] Contribution No. 183, National Marine Fisheries Service, Tropical Atlantic Biological Laboratory, Miami, Florida.

THE EFFECTS OF TEMPERATURE, SALINITY AND FOOD ON THE DEVELOPMENT OF THE LARVAE OF BUTTER CLAMS, (*SAXIDOMUS GIGANTEUS*, DESHAYES)

Neil Bourne

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Biological Station
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The butter clam, *Saxidomus giganteus*, is the most important commercial species of clam in British Columbia. In the last 30 years recruitment has been very minor and sporadic in the southern part of the province although moderate recruitment has occured in recent years in the northern part of the province. To explain some of these recruitment patterns, a project was undertaken to study the larval development of butter clams and the effects of temperature, salinity and food on growth and survival.

To date little success has been achieved spawning conditioned adult butter clams in the laboratory. Larvae for most experiments have been obtained by stripping eggs and sperm from conditioned adults and treating the eggs with 0.1 N ammonium hydroxide. The larvae have been reared under combinations of four temperatures, 10, 15, 20 and 25°C; three salinities, 20, 25 and 29 ppt; and fed different concentrations of three species of cultured algae. Optimum growth was attained when the larvae were reared at 15 and 20°C, although survival was better at the lower temperature. No differences in growth rates were observed when the larvae were raised at salinities of 20, 25 and 29 ppt but faster growth was found when the larvae were fed a mixture of two or three species of algae rather than a single species. Under optimum conditions, settlement occured in 20-25 days when the larvae were 230-250 μ shell length.

MEASUREMENT OF THE OXYGEN REQUIREMENTS OF A LARGE POPULATION OF *MERCENARIA MERCENARIA* [1]

Walter J. Canzonier

Oyster Research Laboratory
N. J. Agricultural Experiment Station
Rutgers University
New Brunswick, New Jersey

Oxygen consumption of a population of 100 clams was measured continuously in a closed perfusion system. In a series of seven experiments consumption ranged from 4.1-9.5 mg/clam/hr with a mean of 5.7. These values agree closely with those obtained by others using individual clams (3.6-10mg/clam/hr).

Based on observations of continuously monitored clam activity and pumping observed in individuals, a value of 65% saturation was determined to be the threshold below which clams would be excessively inhibited.

Using these values it is possible to calculate minimum volumes of seawater required for the operation of large scale aquarium systems (e.g. depuration plants). Assuming a relatively high activity level of 70%, values determined ranged from 220-280 litres (58-73 gal) per bushel (200 clams) per hour at 17°C.

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[1] Research supported by U. S. Public Health Service contract FD00086.

EFFECT OF ARTIFICAL SHELL DAMAGE ON SEX DETERMINATION IN OYSTERS

Nancy W. Davis and Robert E. Hillman

William F. Clapp Laboratories
Battelle Memorial Institute
Duxbury, Massachusetts

One-half bushel of oysters was received from Marion, Massachusetts in August, 1969. Upon arrival, 30 oysters were randomly chosen for sex determination. Two hundred oysters were then divided equally into two groups and suspended in trays in the Clapp Laboratories channel in Duxbury Bay. The shells of the experimental group were filed weekly (except during the months of November, 1969 through April, 1970) in order to force the continuous deposition of new shell, while the controls were left unfiled.

On 1 June 1970, smears were made from 50 oysters of each group and the sex ratios were compared. The filed oysters contained 36 males and 14 females compared with 20 males, 24 females and 6 undifferentiated in the control group.

It is felt that forced shell deposition in the experimental group limited the available energy required for gonad development, and since less energy is required for sperm formation than for egg formation, a greater number of males resulted in that group. The significance of this phenomenon to oyster genetic studies is discussed.

HISTOCHEMISTRY OF GONADAL REGRESSION AND SUBSEQUENT MATURATION IN THE ATLANTIC SURF CLAM, *SPISULA SOLIDISSIMA* [1]

Albert F. Eble

Department of Biology
Trenton State College
Trenton, New Jersey

The partially-spawned and spent phases of the testes revealed much glycogen and PAS-positive material in the thickened walls of the alveoli as well as in spermatogonia present in the periphery of the alveoli. Glycogen phosphorylase, acid phosphatase and NADH dehydrogenase were concentrated in spermatogonia.

The ovary during these stages exhibited enzyme and glycogen patterns similar to the testis: oogonia displayed much enzyme activity as well as moderately-high levels of glycogen.

Residual gametes in the gonads appeared to be resorbed by leucocytes which became highly PAS-positive during the process.

The early active phase of the testes showed much glycogen in the cytoplasm of spermatogonia and primary spermatocytes; similar distributions occurred with glycogen phosphorylase. Acid phosphatase and NADH dehydrogenase displayed high activity in primary spermatocytes which lined up in rows toward the center of the alveolus.

The ovary in the early active phase contained much glycogen in the alveolar walls which contained the oogonia as well as in the growing oocytes attached to the walls. High activities of glycogen phosphorylase, acid phosphatase and NADH dehydrogenase were present in oogonia and oocytes.

The late active phase of the testes marked the beginning of sperm production. Glycogen and glycogen phosphorylase became concentrated in the area of mature sperm which occupied the middle of the alveolus. Acid phosphatase remained active in the spermatocytes located in the periphery of the alveolus but was quite weak in the mature sperm zone. NADH dehydrogenase

displayed high activities in the mature sperm with moderate activity in the zone of spermatocytes.

The ovary in the late active phase showed oocytes growing into the lumen of the alveolus. Glycogen and glycogen phosphorylase increased in activity in growing oocytes; much PAS-positive, alpha-amylase resistant material also began to accumulate in oocyte cytoplasm. Acid phosphatase activity was low in growing oocytes in contrast to high activity in the alveolar lining. NADH dehydrogenase manifested high activity in growing oocytes.

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[1] The work presented here was supported by contract #14-17-0003-185 with the National Marine Fisheries Service.

OPTIMAL PUMPING RATE CONDITIONS FOR OYSTERS AS DETERMINED BY A FORTRAN IV PROGRAM

D. W. Ehresmann, C. E. Whalen and G. Claus

Offshore/Sea Development Corporation
New York, New York

The effects of oxygen tension, temperature and food particle concentration at constant pH and salinity on the pumping rate of oysters were determined by accepted methods of measurement.

Simultaneous variation of the effects of these parameters lent itself to computer analysis for defining the optimal pumping rate conditions. The Fortran IV program was devised based upon the equations of J. Coughlan for the estimation of the filtering rate from the clearance of suspensions.

"MSX" — 10 YEARS IN THE LOWER DELAWARE BAY [1]

Susan E. Ford

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N. J. Agricultural Experiment Station
Rutgers University
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More than ten years' data on the "MSX" infection cycle. New infections are acquired dur- epizootic in Delaware Bay planted grounds indicates that the disease has a definite annual ing the summer and intensify as water temperatures cool in the fall. During the winter infections appear to change very little. Higher mortality of infected oysters during late winter and early spring is at least partially responsible for a drop in prevalence at this time. Late spring is a time of increasing prevalence and intensity (presumably, infections which remained subpatent over-winter now develop). Peak prevalence is reached in late May or early June and is followed by a pronounced drop in infections without an accompanying mortality.

Prevalence levels and mortalities were low in 1961 and 1962, but since that time prevalence levels have remained high. Despite the continued presence of the parasite, Delaware Bay oysters no longer suffer the devastating mortalities associated with the onset of the epizootic, and this is interpreted as an expression of resistance. In addition to a lower mortality, resistance is being expressed in control of infections, keeping them localized and at low levels.

Seed bed oysters planted since 1964 show greater resistance than did earlier plants, and oysters from lower Bay stock (under heavy selective pressure since the start of the epizootic) show the highest resistance of any oysters on the planted grounds.

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[1] Supported under PL88-309 contract 3-3-R-3 with the National Marine Fisheries Service.

RECONNAISSANCE AND INVENTORY OF SHELLFISH IN CHESAPEAKE BAY — PRESENT TECHNIQUES AND PROPOSED SYSTEMS — A MANAGEMENT PROBLEM

Frank L. Hamons and Ranford M. Sapp

Fish and Wildlife Administration and Westinghouse Ocean Research and Engineering Center
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Surveys to locate and subsequently classify soft-shell clam beds in Maryland waters of the Chesapeake Bay have been conducted on a full time basis since the fall season of 1968. Efforts before 1968 were sporadic and based largely on the need to mediate conflicts of interest between the oyster and clam industries.

One of the obvious requirements for such a survey, necessary to establish the location and extent of the bar itself, is the additional classification of adjacent bottom, particularly if that bottom produces oysters. Present oyster bar boundaries in Maryland are based on a shellfish survey conducted from 1906-1912, making the resurvey and relocation of oyster bottom a necessary adjunct to the initial charting of soft-shell clam populations. The survey is presently being accomplished using a standard soft-shell clam hydraulic escalator dredge. Certain physical limit-

ations of this gear have proven it to be ineffective in many areas and in some instances detrimental to the bottom being surveyed. These limitations have instigated a search for a system with a wider spectrum of capabilities and less effect upon the environment being scrutinized.

An alternative solution, particularly for depths exceeding those for which the dredge is practical, is a remote sensing system utilizing high resolution side-look sonar to aid in bottom classification. By correlating results of side-look sonar and a recording depth finder, oyster bars and bottom potentially suitable for oysters and clam culture can be identified and mapped at rates or 1,600 acres per day.

A plan to map and classify over 450,000 acres of Chesapeake Bay bottom using a combination of remote sensing and dredging equipment is described and details of proposed sensors are presented.

FILTRATION OF PARTICLES FROM SUSPENSION BY THE AMERICAN OYSTER, *Crassostrea virginica* [1]

Dexter S. Haven and Reinaldo Morales-Alamo

Virginia Institute of Marine Science
Gloucester Point, Virginia

Filtration of particles from suspension by the oyster, *Crassostrea virginica,* was studied in the laboratory. Experiments with naturally occurring particles and with kaolinite gave similar results. Per cent removal of particles in the 1.0-3.0 μ range was about half that of the larger sized particles. The data show that in spite of lower efficiency with which the 1.0-3.0 μ particles were removed, the total volume of these particles constituted the largest single size fraction within the total size range of from 1.0-13.0 μ. The data suggest that particles in the 1.0-3.0 μ range may be important in nutrition of the oyster.

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[1] Supported by U. S. Atomic Energy Commission Grant No. AT-(40-1)-2789.

EXPERIMENTAL APPROACH TO OYSTER-"MSX" INTERACTIONS [1]

Harold H. Haskin and Walter R. Douglass

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Rutgers University
New Brunswick, New Jersey

Previous reports from this Laboratory have indicated an innate resistance to *Minchinia nelsoni* (MSX) in some stocks of *Crassostrea virginica.* Previous data showed that this resistance may not be expressed during periods of low water temperature. The decrease in prevalence and intensity of "MSX" lesions in resistant stocks during spring warming also suggests that the expression of resistance may be temperature controlled.

To explore this, various infected oyster stocks of known history in enzootic waters were removed from the bay in late winter and placed under controlled temperature regimes in the laboratory. Half of each stock was kept at 5°C while the other half was held at room temperature (18.5°C average). Oysters were kept in aerated standing water aquaria with a weekly change of water.

Prevalence and intensity of lesions in oysters held at the higher temperature increased during the first week. Prevalences remained the same in successive samples taken during the first month, while intensities decreased. Prevalence dropped in successive monthly samples while intensities ranged from very light epithelial lesions to heavy general infections. At the lower temperature there was little change in prevalence or intensity over a three-week period. In a second, similar experiment oysters held at 10°C, 15°C and 19-22°C showed an increase in prevalence over two weeks. In two successive weekly samples prevalences remained stationary at 10°C and 15°C, and decreased at 19-22°C. To date these two experiments suggest that resistance to "MSX" may have an optimum at about 20°C or at a somewhat higher temperature.

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[1] Supported under PL88-309 contract 3-3-R-3 with the National Marine Fisheries Service.

RIVER FLOW AND SALINITY ON DELAWARE BAY [1]

Harold H. Haskin, Frederick Krueger and Stewart Tweed

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Rutgers University
New Brunswick, New Jersey

In 1952-3 at the time of the second Delaware Diversion Case, a prediction was made of the probable effects of total diversion by New York City of 800 mgd from the Delaware headwaters on the oyster seed beds of Delaware Bay. This prediction was based on: (1) empirical relation-

ships between gaged river flows at Trenton and observed salinities on selected seed beds over the preceding 25 years, and (2) an estimate of the effect of changing salinity regimes on oyster drill populations.

In the intervening years additional salinity data has been accumulated and changes in distribution of the drills have been observed. New salinity-river flow curves have been constructed, using best-fit techniques for both the original 25-year span of data and the more recent 17-year span. Comparing the older and the more recent periods, there is little difference in the salinity-river flow relationship at lower river flows. At higher river flows (above 15,000 cfs) the curves for the two periods tend to diverge and the divergence increases at the lower bay stations. It appears that at a given, higher river flow there is more salt at a station than in the earlier period. Fortunately for the oyster drill invasion predictions, there has been comparatively little shift in the salinity regimes between the two time periods at the river flows providing critical salinities for the drills at the up-bay limit of their invasion.

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[1] Supported in part by contract with Delaware River Basin Commission

FIELD OYSTER SETTING — COMPONENT SYSTEMS

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A full understanding of oyster setting in the field can perhaps be best approached by considering and investigating each of its component systems. Four systems come to mind; spawning, larval travels during the 2-week larval life span, factors acting to concentrate larvae, and finally factors, if any, stimulating larvae to set. Much research has investigated the first two systems, however, too little emphasis has been placed on the final two.

Set stimulation is described in detail. Implicit in stimulation is an ability of the "mature" larva to "delay" setting and then set in response to environmental stimuli. Field and laboratory evidence is mustered to support such an ability in *Crassostrea virginica*. Thus far, two factors appear to be important in stimulating setting; rapidly increased water temperature, and pheromones from established oyster populations to produce the gregarious setting response.

THE ROLE OF MUCOSUBSTANCES IN THE QUAHOG

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Battelle Memorial Institute
Duxbury, Massachusetts

Based on the results of histochemical studies of mucosubstances in the mantle of the quahog, *Mercenaria mercenaria*, several physiological roles are proposed for the various mucosubstances, particularly the connective tissue mucus. Besides assisting in such expected functions as lubrication, food capture and protection, it is proposed that quahog mucous materials aid in the active transport across cell membranes, especially for the handling of calcium for shell deposition and osmoregulation. It is also suggested that one glycosaminoglycan may serve as a vehicle for sorting certain sulfated compounds and releasing them as needed by the quahog.

CYTOLOGY AND CYTOCHEMISTRY OF LEUCOCYTES OF THE ATLANTIC SURF CLAM, *SPISULA SOLIDISSIMA*

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Surf Clam, *Spisula solidissima*, leucocytes stained with Wright's or Giemsa stains revealed only one cell type: fan shaped that measured an average of 32.5 μ along the long axis and 6.5 μ in width with slender pseudopodia extended 20 μ-50 μ from the main portion of the cell. Compact nuclei stained a blue-purple with Wright's stain and were 5-6 μ in diameter, usually oval but sometimes elongated depending upon the shape of the cell. Fine granular material surrounded the nuclei with 2-5 vacuoles within and on the border of this granular area. The latter material stained a light purple and did not extend into the outer regions of the cytoplasm.

Under phase contrast the leucocytes first appeared spherical but after approximately 5 min began to settle to the glass slide and extended slender pseudopodia. The same fine granular material and vacuoles observed in the Wright's-stained cells were also observed under phase contrast.

Cytochemical technique for acid phosphatase and non-specific esterase revealed active sites for these enzymes concentrated about the nucleus. The enzyme-positive granules exhibited a gradual

decrease toward the periphery of the cell with some granules in the slender pseudopodia. Supravital studies with toluidin blue, bismark brown, janus green B and neutral red were also performed.

FACTORS INFLUENCING OYSTER SETTING IN THE CHESAPEAGE BAY AREA

Allan A. Sollers

Piankatank Oyster Company, Inc.
Williamsburg, Virginia

Man's lack of control over their breeding is cited as being responsible for his inability to maintain sustained yields of oysters. Biological factors, including fouling and crowding, affect more saline populations. Chemical factors, largely unexplored, appear to limit setting in more brackish waters. A program is outlined to explore how these limiting factors are controlled by weather and micro-nutrient levels.

AN ANNOTATED LIST OF PROTOZOAN PARASITES, HYPERPARASITES AND SYMBIONTS OF DECAPOD CRUSTACEA

Victor Sprague and
John Couch

Chesapeake Biological Laboratory
Solomons, Maryland
and
U. S. Department of Commerce
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Biological Laboratory
Oxford, Maryland

This is principally a taxonomic paper, intended to present in a systematic manner a complete list of the protozoa known to be associated with decapods and to deal with a few of the problems in classification and nomenclature. At the same time, such highly relevant matters as host and site of infection, locality and pathogenecity are mentioned.

There is one amoeba in decapods, *Paramoeba perniciosa*, a pathogen of the blue crab along the middle and south Atlantic coast.

Four orders of sporulating protozoa are represented as parasites of decapods. Probably most decapodan species harbor gregarines. About 50 species have been reported. About half of these are members of the Porosporidae, a unique family with alternation of hosts, one host being a decapod and the other a mollusc. Gregarines are not important pathogens. At least eight coccidians occur in decapods. They have no practical significance. The most important sporozoan parasites in decapods are the Microsporida. About 15 species, generally highly pathogenic, have been reported but there are probably many more. At least three species of Haplosporida, one a hyperparsite in trematode metacercariae, are known but we have little evidence on their importance as pathogens of the decapodan host.

At least four orders of ciliates are represented as parasites or commensals of decapods. The following examples are representative of the more interesting decapod crustacean-symbionts or parasites in the major orders of Ciliatea. The holotrichous gymnostome, *Anophrys sarcophaga* Cohn, 1866, occasionally occurs in hemolymph of shore crabs (*Carcinus maenas*) and causes death of its host. The holotrich order Apostomatida contains many species, about 14 of which occur as commensals and parasites on various species of crabs. The apostome, *Synophrya hypertrophica*, found on portunid and carcinid crabs, causes considerable pathogenesis in gills and shell. These ciliates are noteworthy for the synchrony of their life cycles with the molting cycle of their crustacean host. The holotrich order Chonotricha consists of stalked ciliates that are found exclusively on crustaceans with three species occurring only on body surfaces of decapods. Species of chonotrichs on decapods are found in the genera *Chilodochona* and *Kentrochona*. Ciliates of the subclass Peritrichia, more than any other group, have exploited decapod Crustacea as substrates and habitat. Sessile peritrichs, both stalked (13 species) and loricate (22 species), are found on numerous species of crabs, shrimps, lobsters and crayfish. There is little evidence that most of these peritrichs cause any damage to their crustacean hosts. However, certain genera (i.e., *Lagenophrys*) that cover the respiratory appendages or structures of Crustaceans may, in times of environmental stress, interfere with host respiration. Many undescribed species of peritrichs exist on decapod crustacea and little or nothing is known of most crustacean-peritrich relationships. The subclass Suctoria contains at least four different genera and six species that are found on decapod Crustacea. The blue crab, *Callinectes sapidus*, serves as host to an undescribed species of *Acineta* which dwells as a harmless commensal on the crab's gill surfaces.

NSA PACIFIC COAST SECTION

LIVE-CRAB SHIPPING

Harold J. Barnett

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National Marine Fisheries Service
Seattle Technological Laboratory
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Following successful application of the laboratory's technique for air shipping live Dungeness crabs, sone interest developed in the use of truck-trailers for shipping crabs. Working cooperatively with a potential crab shipper, we tested several methods of providing chilled, filtered sea water in a truck-trailer. Good success was obtained when crabs were packed in shallow plastic trays specially fabricated so that a continuous supply of water cascaded from top to bottom in the stacked trays. Utilizing this system a shipper has successfully transported numerous live crab from Washington to California.

THE EFFECT OF TEMPERATURE ON THE LARVAL DEVELOPMENT OF THE HORSE CLAM, *TRESUS* CAPAX, GOULD

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The horse clam, *Tresus capax,* Gould occurs commonly throughout most of the coastal areas of British Columbia. In the southern part of the province it is a late winter or early spring spawner.

In March 1970 adult horse clams were spawned in the laboratory by fluctuating the water temperature. The larvae were raised by standard still water techniques at four temperatures, 5 10, 15 and 20°C. Initial concentration of the larvae was 20/ml which was reduced to 7/ml at the time of settlement. The larvae were fed a mixture of *Isochrysis galbana* and *Chaetoceros calcitrans* at a rate of 8,000 algal cells per larvae per day. The larvae metamorphosed and settled when they attained a shell length between 250-280 μ.

No fertilization and consequently no larval development was obtained when eggs were fertilized at 20°C. At 15°C the larvae had a mean growth increment of 8 μ shell length per day and settled in 23 days. At 10°C the mean increase in shell length was 7 μ per day and they settled in 26 days. At 5°C the mean increase in shell length was 5 μ per day and they settled in 34 days. Growth of the settled clams was faster at 15°C than at 10°C which was faster than 5°C.

HOT WATER AND OYSTERS

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Heated sea water will be a by-product of nuclear power production if ocean water is used for once-through cooling. Should this happen, are there some beneficial uses for this heated effluent? To this end some preliminary studies were conducted to determine how rapidly small Pacific oysters, *Crassostrea gigas,* (2.9 mm diameter) from our hatchery would grow in sea water warmed to 25°C.

Flowing sea water was warmed 13°C above ambient temperature. The unheated water averaged 12.3°C while the heated water averaged 25.1°C. Over a period of 5 months (from 3 March to 4 August 1970) a total of 90 spat were subjected to each of the two temperatures for four-week intervals.

Mortality of the test oysters was 28% in heated water and 18% in unheated water. However, survivors in heated water averaged 14 times larger in surface area at the end of 4 weeks than survivors in unheated water. There were no differences in the food supply.

One possible benefit of heated sea water could be the growing of hatchery produced seed to approximately 3/4 inch diameter. This should insure high survival after planting and could possibly shorten time to harvest.

OBSERVATIONS ON THE DEVELOPING FISHERY FOR CRAYFISH IN WASHINGTON STATE

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A new freshwater commercial fishery for crayfish is on the increase in Washington. Most cray-

fish taken have been exported to Sweden where they are considered a delicacy. This small pot fishery is centered around Bellingham and produces both a canned and a fresh product. Several problems have been encountered, not the least of which is conflict between fishermen and lake or stream-front property owners. The future of this fishery lies in the development of cultural techniques for controlled production of crayfish.

THE PRODUCTION OF EXTRACELLULAR PRODUCTS BY THE MARINE ALGA *MONOCHRYSIS LUTHERI* AS A NUTRIENT SOURCE FOR BACTERIA

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The marine alga, *Monochrysis lutheri*, has been used in laboratory studies of oyster feeding and nutrition. Algal cultures for feeding commercial oysters would use natural sea water containing bacteria. Studies using *M. lutheri* have been made to determine the response of bacteria in algal culture. Four bacteria were cultured on the *M. lutheri* supernatant and were shown to maintain high populations. It was also shown that lower populations of bacteria would occur in algal cultures during log phase than during stationary phase. It is suggested that if algae are cultured on natural sea water, associated bacteria would reach concentrations higher than normal.

THE SHRIMP-FISH SEPARATOR TRAWL: PRELIMINARY OBSERVATIONS ON ITS INTRODUCTION INTO THE OREGON FISHERY

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Since 1967 we have been developing trawls that will separate ocean pink shrimp (*Pandalus jordani*) from fish and debris in the net while it is being towed over the ocean floor. Sorter trawls offer several advantages over conventional shrimp trawls. By allowing unwanted fish and debris to pass through the net, sorter trawls reduce laborious sorting of catches by hand aboard the vessel. They also conserve fish that otherwise would be discarded dead after sorting aboard the vessel and improve the quality of shrimp by eliminating crushing that normally occurs from large quantities of fish in the net when it is lifted aboard.

Knowledge of the behavior of shrimp played a large role in the design of different types of sorter trawls. Behavior of shrimp was studied by underwater TV and a unique Vertical Distribution Sampler which was towed on the ocean floor to determine height of shrimp above bottom under various conditions of light, weather, etc.

Latest version of a separator trawl being evaluated is one converted from a 57-foot Gulf of Mexico semiballoon shrimp trawl. Cost of converting this trawl, which is the one commonly used by Oregon and Washington shrimpers, is less than constructing a new separator trawl. To instruct fishermen in methods of converting their standard trawls, Work Shops were held in Oregon ports in February 1970. These Work Shops were jointly sponsored by Gear Research Base and Oregon State University Cooperative Extension Service.

Preliminary reports from fishermen using sorter trawls after the season opened in March 1970 indicated the trawls effectively sorted fish and debris but that they did not yield as large catches of shrimp as conventional shrimp trawls. Examination of weighed-out-weights and fishing logs, however, showed that boats using sorter trawls produced as much shrimp as vessels with conventional trawls. The true value of sorter trawls became apparent in June and July when large quantities of smelt and other species began contaminating conventional trawls. As a consequence, many additional captains of Pacific Northwest shrimp vessels are now converting their nets to sorter trawls.

OCCURRENCE OF TANNER CRABS (*CHIONOECETES* SP.) IN THE EASTERN BERING SEA WITH CHARACTERISTICS INTERMEDIATE BETWEEN *C. BAIRDI* AND *C. OPILIO*

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Tanner crabs, *Chionoecetes bairdi* Rathbun and

C. opilio (O. fabricius), occur in the southeastern Bering Sea on the shallow shelf (20-100 fm) of outer Bristol Bay. During annual or semiannual trawl surveys since 1968, scientists from the National Marine Fisheries Service Biological Laboratory, Auke Bay, Alaska, have collected data on the distribution and abundance of tanner crabs in Bristol Bay. Crabs with morphological characteristics intermediate between *C. bairdi* and *C. opilio* were first recognized during the spring survey of 1969; data for these crabs were collected separately on surveys in the fall of 1969 and the spring of 1970. The data on relative fecundity, distribution, morphology and size frequency of the 3 forms indicated that hybridization may occur between the 2 species, resulting in crabs with intermediate morphological characteristics, intermediate size, low fecundity and low abundance. The morphological characteristics used to distinguish the 2 species and the intermediate form were documented photographically. Male and female crabs with the intermediate characteristics constituted 2.8 and 1.6% of the tanner crabs captured in the fall of 1969 and 1.0 and 0.4% in the spring of 1970.

SUBTIDAL CLAM EXPLORATIONS IN SOUTHEASTERN ALASKA

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The Exploratory Fishing and Gear Research Base in Seattle modified an east coast hydraulic clam dredge to sample subtidal clam populations along the Pacific coast. During 1969 it was employed in exploratory fishing surveys off the Washington-Oregon coast, where few clams were captured. In December 1969 a survey was conducted in southeastern Alaska to locate areas which could be effectively sampled with the modified dredge. The areas located were dredged during March and April of 1970. Good catches of the Alaskan surf clam (*Spisula alaskana*) were taken near Pleasant Island in Icy Straits, Alaska. The number of clams per 15 min haul ranged from 1/3 bu (38 clams) to 3.7 bu (345 clams). The surf clams had a maximum shell height of 5-1/2 in, and their average was 4-3/4 in.

A PRELIMINARY REPORT ON PACIFIC OYSTER *(CRASSOSTREA GIGAS)* MORTALITY AFTER TRANSFER FROM A NATURAL BED INTO 10°C AND 20°C WATER IN THE LABORATORY

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Pacific oysters (*Crassostrea gigas*), caught as seed in Dabob Bay (summer, 1968), were transferred from Burley Lagoon during April and May, 1970, into 10°C and 20°C water in the laboratory and monitored for mortality rates related to the exposure time at the two temperatures. For a period of 20-25 days, oysters kept at 20°C for 24 hr a day had a 100% cumulative mortality; oysters at 20°C for 16 hr and 10°C for 8 hr a day had a 79% mortality; oysters at 20°C for 8 hr and 10°C for 16 hr a day had a 53% mortality; and oysters at 10°C for 24 hr a day had zero mortality.

A suspected marine molluscan pathogenic bacteria, *Vibrio parahaemolyticus*, was isolated from the saltwater and oysters kept at 10°C and 20°C. Counts of this organism at 20°C gave 440/ml of saltwater and 8,000/gram of oyster. At 10°C the counts were 5/ml of saltwater and <10/gram of oyster. Further experiments have indicated the possibility of this organism being the causative agent responsible for oyster mortalities in this region.

LABORATORY STUDIES ON SETTING OF THE PACIFIC OYSTER

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Several factors were tested for their effect on setting of Pacific oyster (*Crassostrea gigas*) larvae under laboratory conditions. Retention of the larvae on Nitex screening during water changes, constant salinities of 16-34‰ and room lighting of 45-foot-candles did not appear to influence setting. Setting was retarded by rapidly fluctuating salinities, agitation of culture medium by air bubbles, temperatures below 24°C and copper concentrations exceeding 0.1 mg/liter. Poor nutrition during early larval stages and algal cell densities of less than 50,000 cells/ml in the test vessels also reduced setting. Larvae preferred horizontal surfaces to vertical ones but showed little preference for upper or lower horizontal surfaces. Setting was promoted by an oyster

tissue extract on the cultch and by exposure to water that had been pumped by adult *C. gigas*, *Ostrea edulis* or *Mytilus edulis*. There was no species specific response to the exhalant water of the adult bivalves. In the two experiments a more rapid set occurred in Biosea, an artificial seawater, than in filtered sea water.

THE FOOD CONSUMPTION AND GROWTH OF THE LARVAE OF THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*

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Studies are under way at the Oregon State University Marine Science Center, Newport, to determine the feasibility of commercial oyster hatcheries in the Pacific Northwest. This study of the food requirements of Pacific oyster larvae was conducted to help gain a better understanding of some of the factors that will be important to a successful hatchery operation.

Dichromate wet oxidations of samples of Pacific oyster larvae were conducted to determine the relationship between larval shell length and caloric content. These data were used to estimate the total caloric content of test populations of oyster larvae.

The food consumption and growth of Pacific oyster larvae were studied in three experiments that made use of an apparatus that maintained a continuous flow of algae through test chambers containing different numbers of larvae. These constant flow experiments showed that larval growth rate increased as the algal inflow density increased up to 20,000 cells/ml. Subsequent increases in algal density caused larval growth to level off and decline. The results of the constant flow experiments indicate that a continuous feeding system may be of value in a hatchery operation.

Larval food consumption was studied in three additional experiments in which a flow of algae was not used. These standing water experiments provided a means for studying the effects of temperature, larval size and algal density on the food consumption rate of oyster larvae. Larval food consumption was measured as an instantaneous coefficient of food consumption called grazing rate. The standing water experiments showed that larval grazing rate increased with increases in temperature from 10°-24°C. Grazing rate more than doubled with each increase of 5°C. Larval grazing rate was found to increase exponentially with increases in larval shell length and linearly with increases in the caloric content per larva. A third experiment showed that grazing rate is high at low algal densities and declines with increases in the density of the algae.

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HISTOPATHOLOGIC EFFECTS OF COPPER SULFATE ON THE ASIATIC FRESHWATER CLAM, *CORBICULA FLUMINEA*

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Freshwater Asiatic clams, *Corbicula fluminea*, were exposed to various concentrations of copper sulfate and their histopathological and behavioral responses studied. In addition, the tissue recovery potential and starvation effects were analyzed. For low exposure levels (12-50 ppb), the first tissues showing changes were the digestive diverticula, with increased intracellular vacuolization, then the gills, with hemocytic infiltration and increased mucocyte production, and the mantle epithelium, with fragmentations, necrosis, and tissue sloughing. At medium levels of exposure (125-250 ppb), the gills were first affected, followed by the digestive tubules and collecting ducts. For high concentrations (above 250 ppb), the digestive tubules were first affected, followed by the gills and mantle epithelium.

Clams held in concentrations above 250 ppb for more than 14 days showed effects of starvation, and no clams were observed actively siphoning at concentrations of 500 ppb and above. Tissue recovery was complete in clams exposed to 12 ppb, then placed in copper-free water, but was incomplete or only partially complete after exposure to higher concentrations. The mantle epithelium showed the highest recovery potential. Copper was found primarily in the mantle and gill epithelium, the digestive tubules and in the hemocytes.

THE OREGON RED ABALONE RESOURCE, ITS MANAGEMENT POSSIBILITIES

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The natural distribution of red abalone, *Haliotis*

rufescens, in Oregon is from Coos Bay to the California border. Physical barriers have apparently checked the northern advancement of adult animals and southerly near-shore ocean currents during the summer spawning season further reduces the chance of northern distribution while the abalone larvae are in the planktonic stage.

An attempt to extend the distribution of red abalone into favorable habitat north of Coos Bay was initiated in 1967. Juvenile abalone were introduced into Whale Cove near Newport. Adult abalone were also transplanted from California to the northern Oregon coast to provide spawning stock.

A field survey of the abalone population on the southern Oregon coast was begun in 1969. Laboratory spawning techniques are now being developed to rear red abalone larvae to setting size.

Rearing red abalone to setting size and releasing them into the natural environment appears to be an economical and practical method of establishing populations of these highly desirable animals in areas where they do not now exist.

DEVELOPMENT OF A SCALLOP SHUCKER

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Recent activity in harvesting the sea scallop (*Patinopecten caurinus*) from Alaskan waters generated interest in using a mechanical shucker. Local fishermen were not accustomed to the tedious hand-shucking type of operation common on scallop vessels in other areas and began looking around for a mechanized approach. Use of an automated shucker aboard the vessel would significantly reduce labor, cut production costs and relieve the fishermen from the arduous shucking task.

A system of opening and eviscerating scallops was developed and tested. Opening is based on the use of a 5000-6000° F flame, while evisceration is done by water suction. A machine using these concepts was designed. A prototype is being fabricated by a firm that plans to market the unit.

A PRELIMINARY SURVEY INTO THE SETTING AND GROWTH OF THE MANILA CLAM, *VENERUPIS JAPONICA* (DESHAYES)

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Spat collectors containing 7 artificial substrate types were installed in 2 stations in Hood Canal, Washington; Big Beef Harbor and Point Whitney. Replicated Latin square arrays of the treatments were used to determine optimal substrate types in relation to setting and growth of spats. Additional collectors were installed in separated rows and collected at the end of each month from July through November, 1969, and in June, 1970, to secure information on recruitment and growth. Results disclose that setting density at Big Beef Harbor was about tenfold over that at Point Whitney. Differences between treatments in relation to number and size (shell length) were not noticeable. Setting occurred each month during the sampling period July through November; recruitment obscured the rate of growth during this period. At Big Beef Harbor, the mean shell length increased from 2.5 mm in November, 1969, to about 15.0 mm in June, 1970. Collectors left for approximately one year showed bimodal distributions indicating 2 major periods of spawning in the previous year. The possible influence of currents on setting and its implications in this experiment are discussed.

Population surveys were conducted at 3 beaches (the two aforementioned plus Fishermen's Harbor) by means of sampling grids. Attempts at aging clams and obtaining estimates on growth rate were obscured by overlapping year classes.

BACTERIAL CLEARANCE IN THE MARINE GASTROPOD MOLLUSK *APLYSIA CALIFORNICA* (COOPER)

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The clearance rate of bacteria experimentally injected into *Aplysia californica* is variable depending upon the microorganism introduced. One gram-negative marine bacteria (strain Ap5Y) is cleared in 1 or 2 days, depending upon the ambient temperature. A gram-positive marine bacteria (*Micrococcus aquivivus*) is also rapidly

cleared from the hemolymph in 1 day or less, depending upon the temperature. However, the insect pathogen, *Serratia marcescens,* is not cleared from the hemolymph 1 month after injection. The clearance rate of Ap5Y can be expedited with prior injections of live bacteria or heat killed vaccines. An unusual feature of the accelerated clearance rate is that this induced response remains present for at least 1 month after a primary injection of live bacteria. This indicates a primitive anamnestic response is present in *A. californica.* Clearance rates are temperature related, with a quicker elimination occurring at 18°C - 20°C than at 12°C - 14°C.

The serum of *A. californica* possesses a natural bacterial agglutinin against marine bacteria tested and against pig and chicken RBC's. The agglutinin is apparently non-specific, as adsorption with any one of the bacteria completely removes from the serum the ability to agglutinate the other bacterial species and the RBC's. The agglutinin is not related to total serum protein content, but is inactivated completely by heating at 70°C for 20 min and by extraction with either chloroform or phenol. It is a macromolecule as it is not lost upon dialysis against 0.15 M NaCl. It is resistant to freezing, thawing, toluene and xylene. Tentative identification indicates that it is not a lipid or a lipoprotein, but that it is probably a protein. Titers vary between individual animals and with the bacteria used. The agglutinin possibly plays a role in the mollusks defense as an opsonin, because the titer level is depressed after bacterial injection of Ap5Y and it returns to normal as the bacteria are cleared. However, the titer cannot be increased by prior injection, indicating the anamnestic response is probably cellularly mediated. The number of circulating hemocytes is also depressed immediately after bacterial injection, returning to normal as the microorganisms are eliminated, indicating a probable role in phagocytosis and subsequent clearance.

THE USE OF PVC COATINGS IN DUNGENESS CRAB POT CONSTRUCTION

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Experiments were conducted on the utility of polyvinyl chloride (PVC) as a substitute material for preventing or reducing electrolysis of Dungeness crab pots. Six pot frames were coated with PVC in lieu of the traditional rubber wrapping, webbed in the standard manner with stainless steel wire, and grounded to weight bars. Six other pots were constructed of two-by four-inch wire mesh on the standard frame with the entire pot receiving a PVC coating. Three of each type of experimental pot were fished for 3 months by commercial fishermen at Newport, Oregon and Westport, Washington along with conventional gear. The PVC coating held up well to the rigors of commercial fishing, attesting to its duradility. Slickness of the PVC, though, made it difficult to keep pots from sliding on deck. The pots with coated frames and stainless steel mesh fished as well as conventional pots. PVC-coated wire mesh pots fished only about one-half as well as conventional pots. In an effort to understand the apparent reduced catch rates of the PVC-coated wire mesh pots behavior experiments were carried out in a holding pen at Manchester, Washington. In these studies catch rates of PVC-coated pots were 2-3 times greater than conventional pots which is opposite to results from field trials.

TEMPERATURE, SALINITY AND CLAM LARVAE

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Studies were conducted to determine the effects of temperature and salinity on larvae of native littleneck (*Protothaca staminea*), Manila littleneck (*Tapes semidecussata*), and gaper or horseneck clams (*Tresus capax*). Larvae were placed into seawater of various combinations of temperature and salinity at the age of 2 days and reared to setting size. Native littleneck larvae exhibited a narrow range of tolerance to salinity and temperature with optimum conditions at 10-15°C and 27-32 ppt. At 21°C Manila littleneck larvae survived and grew in salinities from 12-32 ppt but optimum growth and survival occurred at 20-28 ppt. Optimum growth and survival of gaper clam larvae occurred at 10-15°C and 21-28 ppt, 20°C was lethal to gaper larvae. Very little growth occurred at 5°C and 28 ppt but when these larvae were placed in 10°C water they grew to metamorphosis.

DEVELOPMENT OF AN *IN SITU* MARINE BIOASSAY WITH CLAMS

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A Pacific oyster embryo *(Crassostrea gigas)* bioassay was developed by the Washington Department of Fisheries to detect and monitor the effects of pollutants, particularly pulp mill wastes, in estuarine waters. In pollution control and abatement proceedings, critics have challenged the applicability of a bioassay which is based on the reproductive success or failure of a species that does not generally reproduce in most areas of Puget Sound.

Therefore, the response relationship between the embryos of Pacific oysters and several species of indigenous bivalves is under investigation. Successful embryo bioassays using laboratory dilutions of sulfite waste liquor have been conducted with the native little-neck (*Protothaca staminea*), horse clams (*Tresus nuttalli* and *T. capax),* and the geoduck (*Panope generosa*).

Bioassays of field water from an area of known pulp mill pollution were conducted in parallel with clam and oyster embryos. These results indicate that the response of horse clams (*T. nuttalli*) and geoduck embryos confirm water quality evaluations made with the Pacific oyster embryos.

SECOND YEAR SURVIVAL AND GROWTH OF PACIFIC OYSTER SEED FROM FIVE SOURCES HELD AT NORTH BAY OYSTER RESERVE

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Oyster seed (1967-catch) from Mangokura and Hojima packing sites in Japan, Pendrell Sound, Canada, and Willapa and Dabob Bays, Washington were placed at the North Bay Oyster Reserve in the spring of 1968 to compare growth and survival of seed from these 5 different sources. Mortality during the second growing year (1969) ranges from a low of 4% (Willapa stock) to 32% (Hojima stock). No relationship was observed between growth and mortality. Growth of all stocks was similar, with size after 2 years directly related to initial size.

THE EFFECT OF DELAYED PLANTING ON PACIFIC OYSTER SEED SURVIVAL AND GROWTH

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Pacific oyster seed (*Crassostrea gigas*) from Mangokura packing site in Japan was planted in successive months from April to August 1969 at the North Bay Oyster Reserve to determine the effect of delayed planting on seed survival and subsequent adult survival. The seed was held in the packing cases at the +5 ft tide level at the Point Whitney lagoon. There was no difference in survival among the April, May, and June plantings, but the July and August plantings underwent heavy mortality, apparently due to holding in the cases. Size of the oysters was related to the month of planting with the earliest planting the largest.

HANDLING MORTALITY ON SOFTSHELL DUNGENESS CRABS

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Previous tagging experiments on Dungeness crab, *Cancer magister,* have shown reduced recoveries of softshells compared to hardshells. Consequently, normal discard handling by crab fishermen has been suspect as a cause of mortality on softshells of unmarketable condition. A preliminary series of experiments, funded under PL 88-309, was carried out in Willapa Bay, Washington, in 1969 to investigate handling mortality on softshell crabs.

Crabs were captured in commercial pots fished overnight, graded and held briefly in running seawater in a compartmented tank, then placed and held in commercial pots at depths of 3-7 fm. In an initial experiment, there was no indication that mortality differed at holding densities of 15, 25, 40 and 60 legal-sized crabs per pot. A sample size of 25 crabs per pot was chosen for subsequent experiments. Since progressive mortality was encountered (approximately 10% in 2 days, 25% in 7 days for softshells), the role of additional handling vs time was investigated on untagged and tagged (Petersen disc) softshells. For comparable holding periods, greater total mortality accompanied additional handling; however, due to variability among replicates the trend was not statistically significant. Mortality

of tagged softshells ranged from 23% (2 days, 1 handling) to 41% (6 days, 3 handlings) and was consistently 6% to 12% higher than of untagged softshells.

Finally, triplicate lots of 25 crabs, untagged and tagged, of 3 grades: I. Hardshell, II. Intermediate, and III. Softshell, were held for 4 days. Total mortality of untagged III's (16%) was significantly higher than I's and II's (4% for both), based on analysis of variance. Mortality of tagged crabs was 9% for I's, 17% for II's, and 23% for III's, and was significantly higher than for untagged crabs. Some tag loss occurred on softshells in the pots.

Initial experiments indicate that discard handling of softshell Dungeness crabs causes a significant mortality. Consequently, crab fishing during periods of softshell abundance would cause a direct loss in total production. The study indicates that past low recovery rates of softshells tagged with the Petersen disc have been due primarily to mortality, but that tag loss was a probable factor.

A CRAB MEAT SEPARATOR

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Using a commercial-size, solid-bowl centrifugal a technique was developed for separating crab meat from shell. The technique involves a cutting or chopping step to reduce the particle size of the feed material to that required by the centrifugal followed by separation in a brine media in the machine.

Working with the shell waste remaining from the Dungeness crab hand-picking operation, we found that as much as 20% by weight of the "waste" could be recovered as edible meat. From blue crab claws we obtained a 31% recovery of meat.

These results created considerable interest from various crab processors throughout the world. Suggested design changes for a centrifugal to handle fishery products, especially crab, were prepared.

OBSERVATIONS ON OYSTER AND SHRIMP CULTURE IN SOUTHERN JAPAN

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During March 1970, the writer made a trip to southern Japan to observe shrimp and oyster culture, and to gather information on the recent oyster mortality problem in the Hiroshima area. During this survey I observed that the Japanese have developed an efficient and profitable culture operation for shrimp. Reasons for the success appear to be:

1. Relatively low labor cost in Japan.
2. Relatively high price for product.
3. A rapidly growing shrimp that reaches 10-15 cm in one year.
4. A government subsidy of larval shrimp at approximately one-half the actual cost.

General observations on oyster culture corroborates those made by previous investigators on methods and efficiency of the operations. Information from Japanese biologists reveals 2 new and rather serious oyster mortality problems in the Hiroshima area:

1. Mortality of 40-80% on the rafts in the outer Hiroshima area caused by a flatworm, *Stylochus ijimai*.
2. Mortality and reduced oyster fatness occurring in the inner Hiroshima area is due to heavy abundance of a polycheatae tube worm on the oyster rafts. Mortality due to this condition is up to 15% oyster; fatness may be reduced by as much as 20%.

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ECONOMIC VALUE OF RESEARCH IN RESOURCES MANAGEMENT; AN EXPOSITION OF DIRECT ECONOMIC BENEFITS TO MANAGEMENT FROM RESEARCH [1]

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INTRODUCTION

For many years the value of scientific research was accepted almost without question. The benefits which had accrued from basic studies accomplished years, decades, and even centuries ago were so obvious to the informed mind that opposition was equated with a return to medieval ignorance. It would be profitable for us to pause and reflect that the physical principles which guided us to the moon were established by Galileo, Copernicus, and Newton, rather than by Robert Goddard or Werner von Braun.

After the launching of the Russian Sputnik, American science enjoyed the flowering of a Golden Age of expansion and liberality. The scientist — especially the physical scientist — became a hero who could almost do no wrong. We reached and surpassed the Russians, and, then, with typical American impatience, came the reaction.

In the past few years the cost accountants, the Budget Bureau, and the executive vice-presidents have demanded from the world of science the same dollar and cents return from research investments as from investments in an automobile assembly line. To scientists, such demands are ridiculous. Scientific research is not an assembly line operation, and while the eventual payoff is inevitable, the results are rarely predictable. Whether through modesty, indifference or timidity, scientists have in general meekly knuckled under to unreasonable fiscal demands and suffered drastic reductions in programs, personnel and financial support. Well, we're not knuckling under. We propose to show how — in one instance at least — scientific research has paid off directly in a commerical fishery.

HISTORY OF EPIZOOTIC

During 1956-57 the State of New Jersey became aware of a disastrous mortality in Delaware Bay oysters. The State of New Jersey sought help from the National Marine Fisheries Service for support of investigations of the causes and methods for the control of the mortalities. The problem was already under initial study at Rutgers University, but the National Marine Fisheries Service had no research capabilities in the area, aside from a small group at Annapolis concerned with oyster predation and ecological studies in Chesapeake Bay. The Service, therefore, awarded financial support for intensified disease studies at Rutgers University and later to the University of Delaware.

In 1957 an unidentified protozoan parasite was consistently found in tissues of dead and moribund Delaware Bay oysters which Dr. John Mackin of Texas A. and M., and Drs. Leslie Stauber and Harold Haskin of Rutgers University nicknamed "MSX" (multinucleate sphere, unknown, subsequently named *Minchinia nelsoni*). The Chesapeake Bay industry and all levels of government became concerned with the hazard posed against their very valuable oyster resources by 1958, and a conference of specialists was convened to consolidate knowledge and focus intelligence on the problem from all possible sources. An informal agreement of the exchange of ideas was instituted on an annual basis during a Shell-

[1] Remarks of Mr. Fred W. Sieling, Fish and Wildlife Admlnistration before meeting of the 61st Joint Annual Convention of the Shellfish Institute of North America and the National Shellfisheries Association at Atlantic City, New Jersey on 22 June 1970.

fish Mortality (Pathology) Conference in 1959. By 1960, extensive mortalities became evident at the mouth of Chesapeake Bay. Scientists at the Virginia Institute of Marine Science (VIMS) searched for MSX organisms and found them along with a similar (but not identical) protozoan in high salinities. Spores of this other organism, called SSO (Seaside Organism), enabled Drs. J. L. Wood and J. D. Andrews of VIMS to classify SSO as a haplosporidan and it was subsequently named *M. costalis*.

The MSX catastrophe emphasized the urgent need for an expanded Federal research facility in Chesapeake Bay to study not only the ecology of the Bay's shellfish resources but disease problems as well; and the Oxford Biological Laboratory (previously located at Annapolis since 1944) was built in 1960. Scientists from Oxford, collaborating with Chesapeake Biological Laboratory and the Fish and Wildlife Administration activated a continuing survey of oyster beds for

TABLE 1. *Annual oyster landings and dockside values Delaware Bay, New Jersey.* [a]

Year	Pounds (In thousands)	Dollars (In thousands)	Averages 1947-1957
1947	9,959	$ 3,613	
1948	8,838	3,473	Ave. lb
1949	9,276	3,649	9,257,000
1950	9,383	3,809	
1951	8,027	3,453	Ave. value
1952	10,246	4,548	$4,237,000
1953	11,626	5,683	
1954	11,669	6,359	Ave. price
1955	8,495	4,206	per lb
1956	7,395	3,807	45.8¢
1957	6,914	4,008	
Total	101,828	$46,408	
1958	3,239	$ 2,393	1958-1969
1959	501	348	
1960	343	280	Ave. lb
1961	1,133	952	1,098,000
1962	1,634	1,483	
1963	556	584	Ave. value
1964	1,142	1,051	$947,000
1965	557	742	
1966	740	832	Ave. price
1967	1,089	957	per lb
1968	1,262	993	86.2¢
1969	982	747	
Total	13,178	$11,362	

[a] The values show the dramatic decrease in Delaware Bay landings by 1959, and the lack of recovery since then. Average annual landings during the period of this epizootic was only 10% of those before the epizootic. Even though average dockside values approximately doubled as oysters became scarcer, losses averaged about $3,000,000 per year. Over the 10 year period dockside losses therefore amounted to $30,000,000. A retail market value of 4-5 times that of the dockside value yields a conservative estimate for losses due to MSX disease in Delaware Bay alone of $120,000,000 over the 10 year period.

TABLE 2. *Annual oyster landings and dockside values Chesapeake Bay, Virginia.* [a]

Year	Pounds (In thousands)	Dollars (In thousands)	Averages 1945-1959
1945	17,536	$ 6,359	
1946	19,765	5,905	Ave. lb
1947	20,654	7,645	19,783,000
1948	21,118	7,025	
1949	18,059	5,788	Ave. value
1950	15,548	5,574	$8,403,000
1951	15,076	5,277	
1952	18,130	7,712	Ave. price
1953	19,511	7,652	per lb
1954	21,225	9,841	42.5¢
1955	21,956	10,016	
1956	21,220	9,901	
1957	20,090	9,847	
1958	25,504	14,126	
1959	21,355	13,374	
Total	296,747	$126,042	
			1960-1969
1960	15,340	$ 10,883	
1961	17,164	13,932	Ave. lb
1962	11,800	9,774	11,526,000
1963	10,518	8,110	
1964	14,150	10,345	Ave. value
1965	12,568	10,291	$8,604,000
1966	9,443	6,494	
1967	9,068	5,959	Ave. price
1968	7,757	5,237	per lb
1969	7,447	5,019	74.7¢
Total	115,255	$ 86,044	

[a] In Virginia, landings decreased approximately 25% by 1960 and have continued to decline thereafter. Average annual production during the 1960-69 epizootic period, is about 1/2 that of the 15 year period before the epizootic. However, dockside prices increased inversely to the decline in landings so that average annual values remain approximately the same before and during the epizootic.

the presence of MSX in the Maryland section of Chesapeake Bay while VIMS personnel continued a similar study in Virginia. The Rutgers group maintained an active research interest in the epizootiology of MSX, although the disease had essentially destroyed Delaware Bay's oyster resources by 1959 (Table 1).

MSX had invaded Chesapeake Bay by 1961-62 to as far as Pocomoke Sound on the Virginia-Maryland line and by 1965 it had seriously affected oyster beds in Dorchester, Wicomico and Somerset Counties (Fig. 1) on Maryland's lower Eastern Shore (Tables 2, 3 and 4). In all cases intrusion of MSX paralleled higher salinities consistently above 15‰ and concomitant with a long period of lower than average rainfall.

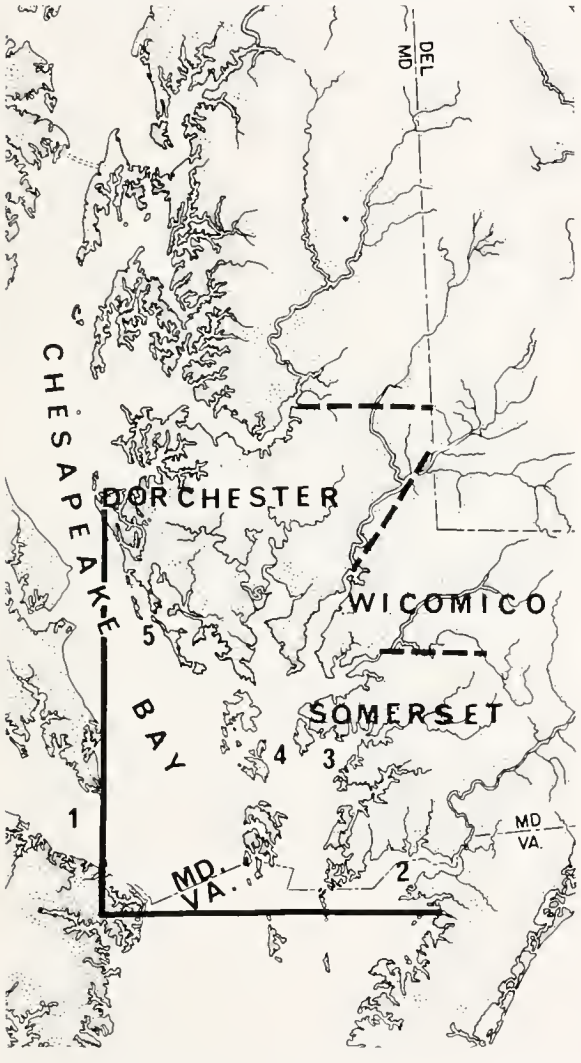

FIG. 1. *Waters within black border are locations of heaviest oyster mortalities and highest MSX prevalence in the Maryland section of Chesapeake Bay. 1. Potomac River; 2. Pocomoke Sound; 3. Manokin River; 4. Tangier Sound; 5. Tar Bay area.*

In the summer of 1965, spores of the MSX organism were found during an intensive study of mortalities in the Manokin River (Figure 1). The finding confirmed the identification of MSX as another haplosporidan and the parasite was named *M. nelsoni* after the eminent biologist Dr. Thurlow R. Nelson, by Drs. Haskin, Stauber and Mackin. Once the nature and ecological requirements of the organism were defined, management could proceed from a well-advised position.

TABLE 3. *Annual oyster landings and dockside values Chesapeake Bay, Maryland.* [a]

Year	Pounds (In thousands)	Dollars (In thousands)	Averages 1945-1959
1945	15,034	$ 5,261	
1946	13,590	4,377	Ave. lb
1947	13,077	4,359	14,865,000
1948	13,285	4,760	
1949	13,718	5,387	Ave. value
1950	14,406	5,521	$6,496,000
1951	14,522	6,692	
1952	16,288	7,165	Ave. price
1953	17,434	7,075	per lb
1954	20,363	9,020	43.7¢
1955	17,272	7,786	
1956	15,844	8,792	
1957	14,144	7,345	
1958	12,027	6,668	
1959	11,966	7,234	
Total	222,970	$97,443	
1960	11,770	$ 8,426	1960-1969
1961	10,337	7,802	Ave. lb
1962	8,138	6,182	11,268,000
1963	7,756	5,618	
1964	7,948	5,461	Ave. value
1965	8,620	6,405	$7,831,000
1966	11,789	8,049	
1967	16,730	11,363	Ave. price
1968	14,874	9,991	per lb
1969	14,715	9,011	69.5¢
Total	112,677	$78,308	

[a] In Maryland, values for the period 1962-66 show a decline in annual landings. Annual landings, statewide, increased to "normal" levels in the following years, even though those in the lower counties remained depressed. (See Table 4) Average annual dockside values increased during 1960-69.

TABLE 4. *Seasonal oyster landings in MSX affected areas of Maryland.*

	Thousands of Bushels						
County	63-64	64-65	65-66	66-67	67-68	68-69	69-70
Dorchester	197	261	55	3	14	24	143
Wicomico	47	45	29	2	33	29	43
Somerset	63	48	41	9	18	7	22
Totals	307	354	125	14	65	60	208

CONTROL RECOMMENDATIONS

Solid information gained by scientists provided the ammunition for making recommendations, which Maryland carefully followed to prevent further spread of the disease, alleviate losses and quicken the rate of recovery in affected areas. Initially, movement of seed oysters into Maryland from MSX areas in other States was prohibited since the mode of disease transmission was not known.

National Marine Fisheries Service's monitoring program was continued and expanded by the Fish and Wildlife Administration of Maryland under the Commercial Fisheries Research and Development Act (P.L. 88-309) to advise on the spread, distribution and prevalence of the parasite in the Maryland section of Chesapeake Bay. This information was useful to management agencies active in seed planting programs to avoid introduction of uninfected seed oysters into MSX-affected areas and to establish quarantines to prevent the transfer of diseased stock to disease-free beds. It was recommended that the shell planting program also be modified to increase spat capture in disease affected areas. It was further recommended that stock surviving several years of infection be left *in situ* and not moved since these resistant survivors might produce MSX-immune progeny. Resistant brood-stock sanctuaries are established in the Manokin River, and scientists in Virginia and New Jersey are also actively developing disease-resistant stock.

MANAGEMENT RESPONSE

A. Seed Planting Program

State biologists conservatively estimated that over 2,000,000 bushels of marketable oysters, valued at over $8,000,000 died in one year on natural oyster bars at the height of the epizootic and approximately 500,000 bushels worth $2,000,000, died on private beds in Maryland. At first Maryland, like other affected states, had little knowledge of methods to mitigate the losses or to conduct its repletion program so that available seed oysters could be used effectively. After the first shock of the loss, which was felt both on many public oyster bars in the southern part of Chesapeake Bay and tributaries, and in the seaside bays by many private planters, considerable thought was given to future seed planting and shell planting programs by the State to increase its production rate in the unaffected areas.

Scientists learned that oysters which had not been exposed to MSX should not be planted in those waters where the disease was enzootic, as these oysters are very likely to be susceptible to the disease. Maryland, therefore, immediately outlawed planting seed oysters into any waters where MSX was known to be enzootic and apt to cause the death of "non-resistant" oysters.

After the distribution pattern of MSX had been determined, emphasis on planting seed oysters was directed from the lower to the upper Bay, above the 15‰ isohaline. Management biologists had the unpleasant duty of visiting watermen in the various counties of Maryland which were seriously affected. The watermen were advised that the State would no longer plant seed oysters in their counties until the disease situation was clarified. Many of these meetings were quite stormy and required much tact and patience. This very serious action caused the dislocation of many watermen's families and great economic losses to the affected areas. However, the watermen and planters cooperated with the State.

The management policy in Maryland has been to distribute seed oysters in proportion to the total number of watermen in each county. Thus each county, even though affected by MSX, would normally be entitled to receive its percentage of whatever seed oysters were moved each year. Scientific evidence advised against the procedure and if it had been followed, large sums of money would have been wasted. Susceptible seed oysters, diverted from MSX areas, amounting to 800,000 bushels, were planted in areas where MSX was absent and were conservatively estimated to yield the same quantity at market size. Oysters marketed from these plantings were of excellent quality and the dockside value was approximately

$4.50 per bushel or worth a total of $3,600,000 to the watermen of the State. We can validly postulate that management, based on scientific findings, has rescued and contributed $14,400,000 directly to the economy of the seafood industry in Maryland during the period 1966 through 1970.[2]

Seed plantings in Maryland waters affected by MSX during the years 1966 through 1970 were reduced to 408,000 bushels, at a cost of $273,000. The majority of the seed oysters came from Tar Bay, located in the MSX affected waters (Fig. 1). It was postulated, however, that they had some resistance and would survive. The expenditure of money during 1966-70 may be compared to 1961-65, when in the same MSX waters 1,208,000 bushels of seed oysters were planted at a cost of $809,000. Thus a direct saving to the State of $536,000 has been effected.

B. Shell Planting Program

The State has concentrated on planting cultch instead of seed oysters in the MSX areas since it is believed that spat surviving the epizootic have a genetic or acquired resistance. The State has based its action on the best information available coupled with practical experience acquired over the years, and production in the MSX areas has increased during the past three years from a low of 14,000 bushels in the 1966-67 season to 208,000 bushels in the 1969-70 season (Table 4).

At present it is speculated that by restricting the introduction of MSX susceptible oysters (from MSX free areas) into enzootic areas, the potential for developing disease-resistant offspring remains greater. However, should the salinity in these areas decline to less than the normal 12-15‰, and MSX is no longer a serious factor in mortalities, the State will consider resuming the previous rate of planting seed oysters.

In the meantime, the State of Maryland, in cooperation with the National Marine Fisheries Service, is conducting an experimental program in the field propagation of an MSX-resistant strain in an area which had been almost completely depleted of oysters by the disease. This experimental work will aid greatly in the decision to resume planting of non-exposed seed oysters and promote an increased rate of repopulation of these areas by disease-resistant oysters.

[2] Calculations based on the economic formula of multiplying the dockside value of oysters by a factor of 4; which represents the retail market value.

SUMMARY

Maryland, fortunately, has large MSX-"clean" areas which have been used to raise our abundant stocks of oysters. Changes in management practices have caused a serious dislocation among many watermen, as well as to the processing and buying role of industry. However, the fact remains that in spite of losing almost one-third of our State production, the industry as a whole has prospered and production has increased to nearly one-third more than what it was before the epizootic of MSX affected Maryland. Therefore, we feel that research from scientific groups is most valuable to management biologists and to the proper utilization of funds appropriated for management of our natural resources.

ACKNOWLEDGMENTS

I should like to acknowledge Dr. Aaron Rosenfield, National Marine Fisheries Service, Oxford, for information and recommendations freely given to the Fish and Wildlife Administration to benefit the Maryland oyster disease rehabilitation program and for his contributions to this report. My thanks to Mr. Haskell S. Tubiash, National Marine Fisheries Service, Oxford, for his assistance in the preparation of this manuscript; to Mr. George L. Ball, Fish and Wildlife Administration, Annapolis, and Mr. William Brey, National Marine Fisheries Service, Easton, Statistical Services, for compilation of some of the statistics used in this paper.

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THE OYSTER PRODUCING POTENTIAL OF PUGET SOUND

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ABSTRACT

An evaluation of the oyster producing potential of Puget Sound reveals that a sustained annual production of up to 6 billion pounds of meat may be possible. Production at this level is based upon using the methods of floating culture developed in Japan in combination with the tremendous fertility and food producing ability of Puget Sound.

Puget Sound is a large estuary located in the north central section of Washington State (Fig. 1), where oyster culture has been carried on for many years. The early oyster industry was based on the Olympia oyster, *Ostrea lurida*, and in later years the imported Japanese or Pacific oyster, *Crassostrea gigas*, has been the primary species. Currently Pacific oyster production in Washington State ranges between 6 and 8 million pounds of meat, with Puget Sound producing between 30 and 40% of the total (Ward, Robison, Nye and Reed, 1968).

Recent attention to utilization of marine resources has prompted an assessment of the possibility for an increase in oyster production in Washington State. Puget Sound, because of its large size, seemed to be a logical area to consider. Evaluation of the oyster producing potential of Puget Sound is a complex task, involving examination of the many widely differing circumstances and conditions which control oyster production. I believe that such an evaluation is both desirable and necessary for three important reasons: 1) a good stewardship of our resources requires a basic understanding of their potential; 2) the current predicted need for additional sources of food makes it necessary to know the capability of our resource; and 3) it is only through full realization of the magnitude of the oyster producing potential of Puget Sound that adequate consideration will be given to this resource in continually increasing competition for use of the water.

Many biological, hydrographic and geographic factors, along with cultural techniques, interrelate to determine the oyster producing potential of Puget Sound. Demand for oysters, economics and competition for use of the water area will determine what portion of this potential will actually be realized. In making this evaluation, I would first like to briefly consider some aspects of demand, and secondly go on to review in some detail the actual producing potential of Puget Sound.

Demand for oysters is governed by price, quality, product desirability, familiarity and availability. Some clues that seem particularly important in evaluating demand are past and present rates of oyster production and the reasons for changes, the increase of numbers of people, and the general availability of food, present and future.

Oyster production in the United States (Engle, 1966) has declined at a fairly constant rate for the past 50 years. These data also indicate that a majority of this decline has occurred on the East Coast of the United States where many areas have gone out of oyster production (for various reasons, including pollution, over-harvest, set failures and oyster diseases). Oyster production on the Pacific Coast, while less than 20% of the nation's total, has remained stable over the past several years.

Changes in human population, both locally and nationally, and the world food supply will also have an important effect on future demand for oysters. There are many different sources of information on the subject of population increase (Anonymous, 1966; Larkin, 1965; Senti, 1967) but

FIG. 1. *Map of Western Washington and Puget Sound.*

there does seem to be some agreement that world population will double by the year 2000, and may go up as much as 10 times the present figure before it levels off. The State of Washington is expected to grow faster than the national rate, and it is anticipated that the population of this state will nearly double by 1985 (Anonymous, 1967).

The available food supply and the potential for increase is another important aspect of this problem. In Anonymous (1966) it is stated that there is now a worldwide shortage of protein food and that about half of the mortality of children between ages 1 and 5 is due to a protein-deficient diet. Agricultural experts (Senti, 1967) indicate real concern about making adequate increases in food production from the land.

It seems clear that because of the nationally decreasing oyster production, the increasing population and the present food shortage, there should be a continuing increase in demand for good oysters. Any area capable of economically competitive production should be able to sell increased quantities of suitable quality oysters.

Next, I would like to consider the oyster producing capabilities of Washington State and particularly of Puget Sound. Some of the factors that are important in such an evaluation are basic fertility of the water, extent of the suitable area available and cultural techniques that fit the environment.

The water must have high fertility (an abundance of oyster food) in order to sustain any major increase in oyster production. Puget Sound has long been recognized by professional oceanographers as a unique body of water. For a number of reasons it is quite high in its supply of chemical nutrients; however, it is only recently that studies have progressed to a point where the tremendous fertility and food producing ability of Puget Sound has been truly recognized. Anderson (1967)[1], studying primary productivity (the basic conversion of chemical nutrients into living plant material) in Puget Sound, comments that primary productivity rates in Puget Sound are among the highest observed in marine waters around the world. Recent work by the Washington State Department of Fisheries (Westley, 1967; Westley, Scholz, Woelke and Tarr, 1967)[2] also confirms the amazing fertility and productivity of Puget Sound.

Primary productivity (or basic plant production) in the sea is no final solution to the problem of producing food for humans. To be of real value to the people this plant production must be converted to animal protein. It is well known that each time conversion to the next higher step in the food chain occurs, there is a major loss of energy. Therefore, the more times the basic plant production has to be converted before the food can be used for humans, the lower the ultimate production will be. Because the oyster feeds directly upon the basic plant material, it is one of the more effective organisms in the marine environment for conversion of plant material to animal protein.

From the foregoing it seems evident that, if the tremendous fertility of Puget Sound could be combined with the efficiency of the oyster in con-

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[1] Anderson, G. C. 1967. Initiation of phytoplankton blooms. Paper presented at Pacific oyster mortality workshop, Seattle, Wash., May, 1967.

[2] Westley, R. E., A. J. Scholz, C. E. Woelke and M. A. Tarr. Pacific oyster mass mortality studies. State of Wash., Dept. of Fish., Summary report, Olympia 45 p.

verting this fertility to animal protein, we would have a highly effective system for large scale production of animal protein.

The next question to consider is the available area for expanding oyster production in Puget Sound. At the present time, many of the more favorable intertidal areas for conventional bottom culture of oysters are in use. While it seems evident that significant increases in oyster production through conventional bottom culture could be made, these increases are definitely limited. There is, however, a different method of oyster culture being practiced in Japan, and to a limited extent here on the Pacific Coast, which uses rafts to float oysters off-bottom (Cahn, 1950; Quayle, 1956). This method would appear to offer tremendous potential for increased oyster production, both because of the greatly increased surface area it would make available, and because it would utilize up to 30 ft of the water depth. Also, by using appropriate modifications of the basic floating method, it would appear that wave action or exposure would not be a limiting factor in any portion of Puget Sound. In addition, floating culture offers advantages of nearly doubling the oyster's growth rate and increasing the fatness. It does have the disadvantages of heavy initial financial outlay (Quayle, 1956).

To determine the total area potentially available in Puget Sound for floating oyster culture, I have utilized data published by the University of Washington Department of Oceanography (McLellan, 1954) on area and volume of greater Puget Sound (Fig 1). Based upon the need for boat access along the shoreline, and the difficulties that would be encountered in anchoring floats in water depths greater than 20 fathoms, the surface areas between the 3 and 20 fathoms depth contours were selected as suitable. There are about 442 square nautical miles of surface area within this depth range in the greater Puget Sound area. If an attempt was made to utilize all of this area, major problems would be encountered because of pollution, water traffic and recreation. However, it seems reasonable to believe that perhaps half of this area could be made available for floating oyster culture if demand and need for food became great enough. This would be an area of about 221 square nautical miles or approximately 187,408 surface acres, or about 28% of the total surface area of Puget Sound.

Various estimates of the yield per acre from floating oyster culture are available. Quayle (1956) reports a figure of up to 8,000 bu per acre per year for Japan. Converting Quayle's figure of 8,000 bu to pounds of meat on the basis of 1 gal/bu, and 8 lb of meat per gallon, we arrive at a figure of 64,000 lb of meat per acre per year. Anonymous (1966) reports 16,000-32,000 lb per acre per year from Japan. Ryther and Bardach (1968) report about 42,000 lb per acre per year from Japan. Thus, the available estimates on annual production of oysters by the floating method range from 16,000 to 64,000 lb per acre. This would seem to clearly demonstrate the tremendous food producing potential of floating oyster culture.

Using 221 square nautical miles for the available area, and 32,000 lb per acre per year (a median figure) as the yield, the calculated potential yield from floating oyster culture would be about 6 billion pounds of meat per year for Puget Sound. This is about equal to the present U. S. fisheries total production of all species.

Many problems would have to be met and overcome to begin oyster production on this scale. First of all, there must be demand; second, this production must be economically competitive; third, an adequate seed oyster supply must exist; fourth, the needed water area would have to be set aside for this use; and fifth, the waters will have to be protected from pollution. Also, since this amount of oyster production would place a tremendous load on the available oyster food supply, careful testing would be necessary to determine the maximum sustained oyster production. However, certain other positive factors indicate the possibility of a change in the outlook for the oyster industry:

1. Population is rapidly increasing and there is a need for new sources of food.
2. Oyster production in Puget Sound would be clearly under United States control and not subject to harvesting by foreign nations. In contrast, any extensive fishery developed on the high seas could be subject to harvesting by foreign nations.
3. Puget Sound is a tremendously rich estuary, capable of enormous food production.
4. The oyster would be one of the more effective methods of converting the food producing potential of Puget Sound into animal protein.
5. Floating oyster culture is a tested method, known to produce enormous quantities of food, and the basic technique could be successfully carried out in Puget Sound.

From this, one fact emerges very clearly, Puget Sound is an extremely valuable resource capable of very substantial food production. I think every effort must be made to protect and preserve this resource so this potential will not be wasted or destroyed.

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CHEMICAL STIMULANTS AFFECTING LARVAL SETTLEMENT IN THE AMERICAN OYSTER [1, 2]

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ABSTRACT

Laboratory experiments confirmed that the presence of spat on shells stimulated spatfall of the American oyster (Crassostrea virginia *Gmelin) and prompted a search for the mechanism of the stimulation. Shell liquor, feces and pseudofeces of the oyster were assayed for presence of natural set stimulants. Highest activity was found in shell liquor which was then subjected to separation techniques including lyophilization and ether extraction to concentrate the set stimulant. The lyophilized residue and an ether extract of oyster liquor as well as a commercial sample of "shellfish glycogen" were found to have a high set stimulant activity.*

INTRODUCTION

The shellfish industry depends on the adequate recruitment of spat to maintain a productive population. Recent management techniques have stressed accurate prediction of spawning coupled with opportune shell plantings (Lindsay, Westley and Sayce, 1958; Nelson, 1952). The development of a consistent method of recruiting natural larvae would be of economic importance to industries in the field of shellfish mariculture.

Knowledge of the response of an oyster larva is necessary to formulate an understanding of its complex setting behavior. A behavior characteristic displayed by the oyster which is relevant to the commercial oysterman is gregarious setting. Properly controlled, the biologist should be able to exploit this natural behavior to obtain desired setting intensities. Cole and Knight-Jones (1949) found that larvae set in greater numbers on shells that already contained 50-100 spat. They suggested that a substance secreted by the new spat may be the effective agent that encourages increased setting.

It appears that Crisp (1967), Bayne (1969) and Hidu (1969) have made the most recent and significant advances in the area of chemical set stimulants. Crisp (1967) reports that oyster larvae may respond to several chemical attractants such as conchiolin, matrix protein, and oyster tissue extract. Bayne (1969) concludes that extracts of oyster tissue containing protein were effective in promoting settlement. Hidu (1969) confirms that oyster larvae display a gregarious setting behavior and states that a water soluble pheromone may be involved in attracting and stimulating larvae to set.

The purpose of this study was to test the gregarious setting response of *Crassostrea virginica*, and to study effects of various natural chemical stimulants on setting. This study provides the basis for isolation, characterization and laboratory synthesis of a set stimulant through the biochemical analysis of shell liquor.

METHODS

Oyster larvae were raised and handled according to procedures discussed by Loosanoff and Davis (1963). All experiments were performed in 4' x 4' setting tanks that hold approximately 75

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gal of sea water. The water was changed daily and filtered through a 5 μ felt bag to remove sediments and larger organisms that might compete with the oyster larvae for food and substrate attachment. One liter of food supplements *Monochrysis* and *Isochrysis* were added daily. For the duration of the experiments water temperatures were maintained at 24-26°C. All shells, plates and tiles used in this experiment were raised off the tank bottom using 3/4" PVC pipe. This procedure assured that the larvae had access to the upper and lower surfaces of cultch. Counts of new spat (upper and lower surfaces combined) were made with a binocular dissecting scope at approximately 1.5 x 10 power.

Presence of spat as stimulant

Experiments 1-2

This portion of the experiment follows the method of Hidu (1969). Spatted shells were placed at random with control shells in a setting tank. The spatted shells contained 40-50, twenty-four hour old spat per shell. Spatted and unspatted shells were selected at random, and spat were counted 48 hr after the larvae were released into the setting tank. This experiment was performed twice. In both experiments approximately 10,000 larvae were used. Results were compared by the t-test (Simpson, Roe and Lewontin, 1960).

Treated cultch as set stimulants

Experiments 3-6

Weathered surf clam shells were steam cleaned and air dried. The shells were divided into four groups, two sets of alternating rows, 20 shells per group. Group A shells were held in seawater for 24 hr and served as a control. Group B and C shells were coated with feces and pseudofeces respectively. The feces and pseudofeces were collected by the method of Haven and Morales-Alamo (1965). Group D shells were swabbed with oyster shell liquor and aged under refrigeration (5°C) with Groups B and C for 24 hr. Refrigeration was needed to prevent the decay of organic substances that might be the stimulation for setting. In this research oyster liquor includes fluid that drains from the shell cavity when valves are pried open as well as juices released when oyster meats were chopped. Experiments 3 and 4 were similar to 1 and 2 except that 55,000 larvae were released in experiment 3 and allowed 48 hr to set and 30,000 larvae were released and allowed 24 hr to set in experiment 4. Results were tested using an analysis of variance test with the F value significant at 95% level of confidence (Simpson *et al.*, 1960).

Experiments 5 and 6 were performed treating asbestos plates with the same three natural substances. All plates were held two weeks in sea water, cleaned and air dried. Experience with artificial cultch has shown us that a period of two weeks is required to leach out substances toxic to settling. The plates were 12 cm² in size. Sixty-four plates were divided into four groups of 16 plates each. All treated plates were held 24 hr under refrigeration prior to placement in the setting tank.

For experiments 5 and 6, approximately 78,000 larvae were released in the setting tank. In experiment 5, eight plates were counted after 48 hr, whereas eight plates were counted after 72 hr in experiment 6. Results of these experiments were not statistically analyzed for reasons to be explained later.

Fractionated Oyster Products

Experiments 7-8

In experiment 7, twenty-two oysters collected in April 1970, weighing 1503 g in the shell, were shucked and 332 g of meat and shell liquor were obtained. The roughly minced meat and juice were vacuum filtered through a coarse sintered glass funnel. The liquor was then refiltered at room temperature through a medium porosity funnel coated with celite. The resulting filtrate was nearly clear and weighed 182 g. It was divided into three portions. The first portion weighed 22 g and was frozen at -70°C and termed oyster liquor. The second portion weighed 85 g and was lyophilized at 40 μ air pressure. Lyophilization is a freeze drying technique in this case designed to preserve and concentrate the solids in oyster liquor. This process yielded 3.112 g of a dry white powder termed lyophilized liquor and 82 g of a colorless liquid termed distillate. Another portion weighing 73 g was continuously extracted with ether and yielded 0.009 g of a transparent oil termed ether extract. The residue from the ether extraction was freed of ether with a stream of nitrogen bubbles and termed raffinate or liquid residue from ether extract. The lyophilized liquor, ether extract and raffinate were stored under nitrogen at 0°C.

In experiment 7, 36 ceramic (Bisque) tiles, 15 cm² were divided into six equal groups. The tiles were treated with solutions of the five products and held 24 hr under refrigeration. All the solutions were prepared in water except the ether extract which was prepared in ethyl alcohol. Solutions were applied to the tiles with small paint brushes. The tiles including controls were arranged in six rows. Approximately 75,000 larvae were released in the tank and allowed 48 hr to set. At this point the experiment was terminated and the spat were counted. Results were analyzed using analysis of variance.

Lyophilized oyster liquor and a commercial glycogen product were used for experiment 8. Seventy-eight oysters weighing 3900 g, collected in June 1970, were shucked to produce 853 g of meat and shell liquor. The contents were filtered twice to yield 447 g of juice. The above procedures were carried out at 0-5°C. The filtrate was frozen at -55°C and lyophilized at 10 μ air pressure. The resulting dry white powder weighed 15.55 g and was divided into two portions. One was briefly exposed to moist air and clumped. A preparation of 5 g of glycogen powder extracted from shellfish was obtained from Mann Research Laboratories, New York and dissolved in 400 ml of water.

In experiment 8, 24 ceramic tiles were held in sea water for two weeks to remove possible toxic materials. The tiles were then scrubbed, air dried and divided into four groups of six tiles each. In addition to control tiles, six each were treated with lyophilized solutions moist and dry, and with the commercial shellfish glycogen respectively. The tiles were held 24 hr under refrigeration, treated again with the respective solutions and held an additional 24 hr under refrigeration. For this experiment tiles were arranged in four rows and approximately 29,000 larvae were released in the tank and allowed 48 hr to set. The spat were counted and the results treated statistically with the analysis of variance test at 95% level of confidence.

RESULTS

Presence of spat as a stimulant

The standard deviations were large in experiments 1 and 2. This may be due to a small number of observations. Nevertheless, the confidence level is significant at 90% for experiment 1. The 2:1 ratio of set on spatted shell compared to unspatted shell in both experiments is suggestive. A review of earlier setting records in our laboratory supports this pattern. We believe that these experiments show the same trends found in the works of Hidu (1969) and Bayne (1969).

Treated cultch as set stimulant

Experiments 3 and 4 indicate that shells treated with oyster liquor yielded the highest spat counts. Experiment 5 agrees with results from 3 and 4 whereas experiment 6 produced a discordant result. Here the control yielded the highest spat count.

Fractionated oyster products

In experiment 7 two fractions, lyophilized liquor and ether extract, showed promise as stimulants. Experiment 8 compared lyophilized liquor with commerical shellfish glycogen and the latter yielded the higher spat counts. Treated tiles had higher spat counts than controls in both experiments. The results of the above studies are summarized in Table 1.

DISCUSSION

Our results support the conclusion of Hidu (1969) that larvae of the American oyster are gregarious. Bayne (1969) demonstrated a similar phenomenon with the European oyster. Field evidence indicates that American oyster larvae are gregarious and the results of Prytherch (1928) may be explained as gregarious setting from the effects of pheromones. Spat are frequently found attached to the shell edges of live, adult oysters. Previous interpreations have stated that these areas are ideal for setting because they are kept clean by the natural pumping of the oysters. Hidu (1969) states that this phenomenon may actually be an indication of larvae reacting to substances released by the adult oyster.

Three naturally emitted substances (feces, pseudofeces and shell liquor) of the oyster were tested as set stimulants. From experiments 3 and 4 we conclude that treated surfaces show significant larval preference over the control shells and that shell liquor induced the highest spatfall.

In experiment 5 and 6 the spat counted at 48 hr show essentially the same pattern with shell liquor being most effective. However, the spat counted at 72 hr completely reverse the earlier trend showing the control with the highest number of spat. This experiment indicates that time is a critical element in these setting studies. Deviation from preferred setting patterns occurred during the course of this study, but only when the cultch was allowed to remain in the tanks longer than 48 hr.

Several explanations are possible for inconsistent results occurring after 48 hr. First, the treated cultch after long periods of time may lose its original value due to hydrolysis or leaching of the set stimulant. Second, treatments of feces, pseudofeces and shell liquor are conducive to the growth of bacteria on the surface of plates. The significance of bacterial slime in setting marine organisms has been documented in fouling studies (Woods Hole Oceanographic Institution, 1952). However, after an extended period of time the concentration of bacteria could increase to the point where they could conceivably become detrimental for the survival of spat. Hidu (personal communication) prefers to conduct these experiments 1-1.5 hr with many replications. He was surprised that any results at all were achieved over such long periods of experimentation. Setting experiments in progress show

TABLE 1. *Numerical data for setting experiments.*

Presence of spat as stimulant						
				spatted	unspatted	t value
Experiment 1						
10 shell count				66	35	1.85
Experiment 2						
9 shell count				327	143	1.71
Treated cultch as set stimulant						
		Control (A)	Feces (B)	Pseudo-feces (C)	Oyster liquor (D)	F value
Experiment 3						
10 shell count		319	1277	994	3300	10.9[a]
Experiment 4						
10 shell count		74	95	95	201	5.0[a]
Experiment 5						
5 plate count		18	51	24	122	No statistical analysis
Experiment 6						
5 plate count		692	364	108	184	"
Fractionated oyster products						
	Control	Oyster liquor	Lyophilized liquor	Distillate	Ether extract	Raffinate	F value
Experiment 7							
6 tile count	291	163	702	69	603	247	2.63[a]
		Control	Shellfish glycogen	Lyophilized liquor-moist	Lyophilized liquor-dry	F value	
Experiment 8							
6 tile count		321	1257	1096	615	8.15[a]	

[a] F value significant at 95% level of confidence.

that the viability of the set stimulant decreases considerably after 24 hr and is closer to Hidu's figures.

Crisp (1967) reports that larvae showed a preference for shell that retained some organic taint of the living oyster. He suggests that larvae are able to detect the protein in the conchiolin matrix of the shell. The larvae also showed a marked affinity for shells soaked in an extract obtained from adult oysters. Crisp (1967) believes that there might be two mechanisms involved with larvae responding both to the proteins from the shell matrix and tissue extracts from adults.

Although not quantified in our experiments, many spat were found clustered around microscopic pieces of oyster tissue present on shells treated with shell liquor (Experiments 3-6). The fact that soaking shells in an extract with the protein matrix intact improved their set stimulant value corroborates that shell liquor may be the strongest natural set stimulant.

We suggest that the pheromones of Hidu's work are a constituent of shell liquor. This appears reasonable for while the oyster is open, there is a constant exchange of shell liquor with the surrounding sea water. The same can be said for feces and pseudofeces except that these two possible stimuli are emitted sporadically and are not circulated the way dissolved substances would be.

Previous work of Hidu (1969) and Crisp (1967) and results of experiments 3 and 4 here indicate that the effective agent may be found by the biochemical analysis of shell liquor and tissue extracts.

Two fractions in experiment 7 showed promise, a lyophilized powder derived from shell liquor-oyster tissue extract and an ether extract derived from the same shell liquor-oyster tissue extract. The high activity observed in the small sample (9 mg)of ether extract suggests that an ether soluble organic compound may be a stimulant.

In experiment 8, a lyophilized powder and a

glycogen preparation were examined as set stimulants. Previous unpublished studies by us have suggested that glycogen is a constituent of oyster liquor. The process of lyophilization would preserve glycogen and water soluble proteins. The work of Bayne (1969) emphatically suggests a protein as an active constituent of spat attractants derived from oyster tissue extracts. He used two standard methods for precipitation of protein. The standard tissue extracts were treated with ammonium sulfate and trichloracetic acid. The precipitates when redissolved in buffered solutions contained a high percentage of water soluble proteins. These two solutions retained the attractant value of the original tissue extract. Bayne (1969) also treated the standard extract with pronase, a proteolytic enzyme, which resulted in the loss of all attractant value. In concurrent work Hidu (personal communication) has isolated a setting stimulant which is a protein. The results of experiment 8 show that both the commercial glycogen preparation and lyophilized powder produced more set than the untreated control tiles. The glycogen attracted the greatest number of spat which indicates it contains a very active constituent. Thus shellfish glycogen may also be a set stimulant.

These preliminary experiments require replication but they are suitable in suggesting trends for the setting preference of oyster larvae. Future experimentation will include tests for specific constituents of shell liquor-oyster tissue extracts. This study has provided new direction for future biochemical isolation of natural pheromones. The results confirm the work of Crisp (1967) and Hidu (1969).

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HOLDING AND SPAWNING DELAWARE BAY OYSTERS (*CRASSOSTREA VIRGINICA*) OUT OF SEASON II. TEMPERATURE REQUIREMENTS FOR MATURATION OF GONADS [1, 2]

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ABSTRACT

Spawning responses are reported for seven lots of Delaware Bay oysters (nearly 400 individuals) held in the laboratory at temperatures ranging from 11.6-29.4°C for periods ranging from 28-248 days. Histological studies of gonadal ripening were conducted on two of the lots of laboratory held oysters and on one lot held at temperatures in the field as a control. Based on the histological examination of oyster gonads and spawning responses as related to the thermal history of the laboratory held oysters, a temperature-time schedule is proposed that should allow one to hold, condition and spawn Delaware Bay oysters in the laboratory or a hatchery on a year-round basis. These oysters required from six to seven times as long to ripen as Long Island oysters at temperatures between 12.0 and 22.0°C. Within this range of temperature, 450 degree days (sum of the daily exposure temperatures above 12.0°C) were required to elicite spawning in at least 50% of the oysters tested.

INTRODUCTION

Success in out-of-season artificial spawning of the American oyster, *Crassostrea virginica* Gmelin, from waters of the southeastern United States has been limited (Loosanoff and Davis, 1963; Maurer and Price, 1968). This study was undertaken to determine the conditions necessary for successfully holding, conditioning and spawning of *C. virginica*, from the Delaware Bay region in the laboratory. Our main objective was to extend the spawning season to produce oyster progeny year-round for research and hatchery purposes. This study provides the basis for a time-temperature schedule that should allow one to condition and spawn Delaware Bay oysters in the laboratory or a hatchery on a year-round basis.

Nelson (1928) and Loosanoff (1942) were among the first to emphasize the importance of temperature and time on gonad development in *C. virginica*. A thorough study of temperature requirements for maturation of Long Island Sound oysters was carried out by Loosanoff and Davis (1952). Although the work by Loosanoff and Davis (1952) offers a relatively well defined temperature-time schedule for the ripening of oysters from Long Island Sound, other studies show the existence of physiological races with respect to temperature requirements in reproduction (Stauber, 1950). For example, the temperature requirements for gonad development and spawning of the northern populations are definitely lower than those of the southern groups (Loosanoff and Nomejko, 1951; Loosanoff, 1958; and Loosanoff, 1969).

Our results indicate that it is possible to predict the temperature exposure necessary for laboratory maturation of Delaware Bay oyster

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gonads. However, additional work should be done to confirm and refine the temperature-time schedule offered here under more closely controlled environmental conditions especially in regard to the nutrition of the oyster via supplemental feeding.

METHODS

Approximately 400 oysters (two to five years old) were held in the University of Delaware Shellfisheries Laboratory on the Broadkill River adjacent to Roosevelt Inlet in Lewes for periods ranging from 28-248 days by techniques reported by Maurer and Price (1968). These oysters represent native stocks collected from wild populations from Delaware Bay and its tributaries. These oysters were divided into lots for laboratory temperature exposures (Table 1).

Lots I through IV were placed in the laboratory on 11 May 1967 before ambient water temperatures exceeded 12.0°C. Lot V was placed in the laboratory on 8 March 1968 before ambient water temperatures had risen above 3.0°C while Lots VI through VIII were collected from the Mispillion River on 31 January 1969, when the temperature of the river water was 3.0°C.

Oysters in Lots I through IV were immediately subjected to the laboratory temperatures indicated. In Lot V the laboratory temperature was increased gradually over a 24 hr period. Oysters in Lots VI and VII were acclimated to experimental temperatures by increasing the temperature approximately 3.0°C per day.

Lot VIII was used as a field control and was placed in a plastic-coated wire basket (Marlboro Wire Goods Co., Marlboro, Mass.) and suspended from an oyster raft moored adjacent to the Shellfisheries Laboratory so that it hung about 0.3 m under the surface of the Broadkill River. These oysters experienced ambient water temperatures which were taken with a continuously recording thermistor probe. Acclimation of oysters in Lots VI through VIII was achieved by 10 February 1970 which for the purposes of that experiment is considered time zero.

Water temperatures of laboratory holding tanks were measured daily with a maximum-minimum recording thermometer. The maximum daily range of temperature experienced by oysters in the laboratory was approximately 4°C, but generally the water temperature fluctuated less than 2°C.

The holding temperatures for Lots I through IV were selected in an effort to retard spawning until fall and winter. Lot V was exposed to 29.4°C water in order to accelerate spawning. The temperature exposures of Lots VI through VIII were selected to demonstrate the effects of temperature on gonadal maturation.

Groups of 2-15 oysters (usually ten) were removed from the holding tanks after conditioning at the regimes indicated (Table 1) and subjected to spawning techniques of Loosanoff and Davis

TABLE 1. *Periods and temperatures for conditioning oysters to spawn in laboratory experiments.*

Lot No.	Period 1	Period 2	Period 3	Time of experimental spawning
I	118-147 days @ 16.1°C	—	—	September-October 1967
II	151 days @ 16.1°C	8-15 days @ 18.0°C	—	October-November 1967
III	152 days @ 11.6°C	47 days @ 15.0°C	42-49 days @ 15.0°C	January 1968
IV	152 days @ 11.6°C	47 days @ 15.0°C	36-49 days @ 19.4°C	January 1968
V	28 days @ 29.4°C	—	—	April 1968
VI	99 days @ 14.7°C	—	—	May 1969
VII	71 days @ 21.8°C	—	—	April 1969
VIII	128-130 days @ 0.5-21.2°C	—	—	June 1969 [a]

[a] Held in trays on a raft in the Broadkill River adjacent to the Shellfisheries Laboratory.

TABLE 2. *Temperature exposures and spawning responses of seven lots of Delaware Bay oysters* (Crassostrea virginica).

Group	Time of Exposure (days)	No. of oysters per lot	Temperature Exposure[a] in degree days minimum	maximum	Per cent of oysters Spawned	Mean spawning response time[b]
III	241-248	60	267	288	20%	83 hr 24 min
VI	99	9	297	—	44%	—
IV	235-248	47	407	503	60%	41 hr 36 min
I	118-147	182	484	619	—	32 hr 48 min
II	159-166	49	667	709	59%	18 hr 42 min
VII	71	12	710	—	83%	5 hr 50 min
VIII[c]	128-130	29	263	282	66%	4 hr 15 min

[a] Calculated using the formula $T_e = T_x - X\ (12.0°C)$ where T_x = sum of the daily exposure temperatures above 12.0°C and X is the number of days when T was 12.0°C or above.

[b] Expressed as the delay between the initial application of a spawning stimulus (thermal, chemical, or both) and the first sighting of sperm or eggs.

[c] This group was held in the Broadkill River on a raft and exposed to natural water temperatures.

(1963) with slight modifications by Maurer and Price (1968). Spawning manipulations involved transferring oysters from conditioning (holding) tanks to battery jars or aquaria where they were then immersed in Broadkill River water that had been heated to 30.0-31.0°C. If the thermal stimulus was ineffective, a sperm or egg suspension was added to the water to provide an additional spawning stimulus.

Oysters that spawned were sexed and isolated to permit calculations of the time required for response and the percentage that released sexual products (except for Lot I). Spawning sometimes occurred when the investigators were not in the laboratory. In such cases an estimate of the time of the spawning was made from the stage of development of the larvae. The percentage of individuals spawned was determined by direct observation.

Histological sections were made by fixing ten oysters in each lot (VI through VIII) at two week intervals following the methods of Loosanoff (1969). In addition animals were dissected and rough estimates of gonad condition and glycogen content were made.

The relationship between temperature regimes (Table 1) and gonadal development, as judged by spawning responses (Table 2), was calculated with the following assumptions: 1) the temperature below which gonad development is arrested for Delaware Bay oysters is 12.0°C (Loosanoff 1958), 2) in calculating degree-days exposure (Te), we used the formula, $T_e = T_x - x\ (12.0°C)$ where Tx is the sum of the mean daily exposure temperatures in degrees centigrade where the daily exposures are above 12.0°C and x is the number of days when the daily exposures were above 12.0°C. When average daily exposures are less than 12.0°C they are omitted from the calculation, e.g., the initial exposures of 11.6°C for 152 days in Lots III and IV are omitted, and 3) gonadal development is directly related to the total degree-day exposure above 12.0°C in these Delaware Bay oysters.

RESULTS

General spawning observations

Data in Table 2 demonstrate a direct relationship between temperature exposure and percent of oysters spawned under laboratory conditions and an inverse relationship between temperature exposure and the spawning response time.

Spawning occurred at a minimal level (20%) in laboratory held oysters when the temperature exposure was as low as approximately 270 degree days (corrected to 12.0°C). Loosanoff and Davis (1963) employed the 50% level of spawning as one criterion of ripeness in oysters. Using this criterion, successful laboratory spawning was achieved in Delaware Bay oysters after a minimum of 450 degree days (Fig. 1). In contrast, successful ripening to spawning occurred under the relatively natural field conditions on the raft at approximately 270 degree days (Lot VIII).

Crassostrea virginica from Delaware Bay were not ripened successfully at high temperatures under the conditions described. Lot V was exposed to a temperature of 29.4°C (range 28.7-30.4). Al-

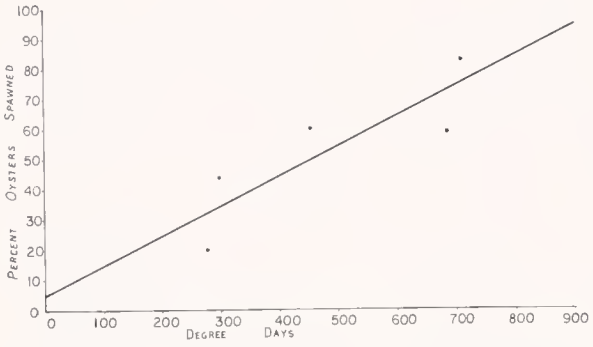

FIG. 1. *The relationship of spawning success (measured as percent oysters spawned) and degree day exposure (above 12°C) in five lots of oysters conditioned in the laboratory.*

though gonads ripened until one male was induced to spawn after 27 days, the brood stock condition deteriorated rapidly thereafter and subsequent spawning efforts were unsuccessful. A *Bucephalus* infection was suspected as a causative agent to brood stock deterioration.

Histological Studies and Associated Spawning Responses

Controls at field temperatures (Lot VIII)

Histological sections of Mispillion River oysters were examined to determine their pre-experimental condition (10 February 1969). Gonads were in typical winter stages with thin undifferentiated gonadal layers. After 71 days at field temperatures (ranging from 0.5-14.0°C) the gonads showed evidence of ripening. However, no oysters in this lot spawned when subjected to thermal and chemical stimulation at that time. The tissues of these oysters had small follicles containing undifferentiated cells characteristic of winter stages. A few sections showed some indications of early stages of gametogenesis with either spermatogonia or some ovogonia. On 3 June, 113 days after the start of the experiment when field temperatures had reached 19.9°C, dissections and gross observation indicated that gonadal tissue was slightly swollen. Attempts to stimulate spawning were unsuccessful. Sections revealed that follicles were scattered but well defined with females containing growing ovocytes and males containing spermatids and some spermatozoa. Nineteen of 29 oysters were successfully spawned 128-130 days after the initiation of the experiment when water temperature in the field had reached 21.0°C (Table 2).

Observations at 15°C (Lot VI)

The initial gonadal condition (10 February) was as described for Lot VIII. After 28 days of conditioning, scattered follicles in early stages of spermatogenesis were observed in histological sections. By day 50, gross observations and dissections indicated that these oysters might be induced to spawn but attempts were unsuccessful. Tissues showed that proliferating follicles contained ovocytes and ripe spermatozoa but gonads were definitely not in the ripe condition.

Dissections and gross observations on day 71 indicated that gonads had ripened substantially since the last examination period. Upon stimulation of a test sample of ten oysters, a single male spawned. Histological sections revealed that the follicles were full of spermatozoa. After 99 days at 15°C, two females and two males were induced to spawn (Table 2). Histological examinations of males and females showed ripe spermatozoa and eggs respectively.

Observations at 22°C (Lot VII)

The gonads of oysters taken from the field (10 February 1969) were undifferentiated. After 28 days follicles contained mainly spermatogonia or early spermatocytes. By day 56, mature sperm were present. Ovocytes were beginning to develop by 28 days and by 56 days nearly mature eggs were present. By day 70, the gonads were full of mature sperm or eggs. Spawning of ten oysters was attempted on day 50 without success. On day 70, however, ten (nine females and one male) of 12 animals tested were spawned successfully.

DISCUSSION

The most important points supported by this work are: 1) Delaware Bay oysters require approximately six to seven times as long to ripen at temperatures between 12.0 and 22.0°C as do Long Island oysters, 2) the cumulative temperature exposure is more significant in ripening Delaware Bay oysters to spawning than exposure to "high" temperatures per se, and 3) at temperatures of approximately 12.0°C or less ripening does not occur in Delaware Bay oysters.

Our ultimate goal in this research is to produce a formula from which one may generate predictions for ripening oysters throughout the year. Although our data should be refined through further efforts for more reliable predictability, we feel that they do support the hypothesis that temperature-time spawning schedules can be developed for Delaware Bay oysters.

The data presented here support the conclusion that a minimum temperature exposure of 450 degree days is required for satisfactory ripening

(50% level) of these oysters (Fig. 1). Spawning percentages and response times improved progressively as temperature exposures increased from 400-700 degree days (Table 2 and Fig. 1). For research or hatchery work where short spawning response times and a high incidence of spawning are desired, 700 degree days would be a reasonable standard to use. Based on that standard, the following simple formulae were derived by the authors to generate temperature-time spawning schedules for Delaware Bay oysters:

$$(1) \quad D = \frac{700}{T - 12}$$

$$(2) \quad T = \frac{700}{D} + 12$$

where D is exposure time in days and T is the daily mean exposure temperature within the approximate range of 12.0-22.0°C, using Delaware Bay oysters removed from the field in winter and spring before ambient water temperatures have risen above 12.0°C.

Predictions derived from the formulae above are consistent with findings published by Loosanoff (1958, 1969) that, New Jersey (Delaware Bay) oysters held at 12°C exhibited little or no gamete development even though held for 78 days, and 2) New Jersey (Delaware Bay) oysters held at 21°C exhibited a successful level of spawning after 78 days.

Although our estimate of the temperature level below which no gonadal activity occurs was partially based on Loosanoff's statement (1958), this figure was arrived at independently by us in an empirical manner. For each lot of oysters, total degree day exposure times were calculated for the minimum, mean and maximum number of days that each lot was held. These totals were corrected for possible zero gonadal activity levels ranging from 5 to 13°C. We found that a zero level of 12°C provided corrected degree-day exposure figures that were most consistent with the relative spawning successes of these oysters (Table 2). That is to say, when the relationship of spawning success and degree day exposure is derived, the y intercept of the regression line is nearest zero when 12°C is used as the zero level of gonadal activity (Fig. 1).

Although this study will provide a guide for others in the southeastern United States for conditioning and spawning oysters in the laboratory, several comments can be offered: 1) a more definitive study should be undertaken to elucidate the effect of nutrition and other environmental factors on gonadal ripening rates for Delaware Bay oysters as is evidenced by comparing field held oysters (Lot VIII) with laboratory held oysters (Lots I-VII), and 2) those to the south of Delaware Bay may find it necessary to condition *C. virginica* for longer periods than reported here.

Galtsoff (1964) reported that a single oyster may pump from several to 30 liters of water per hour depending on its size and environmental conditions. In this study, approximately two liters of water per hour per oyster were provided at all temperatures which may have retarded ripening to some degree at higher temperatures. Unfortunately, our facilities were not equipped for greater water flows or supplemental feeding at the time of these experiments.

However, the average time required for 50% of oysters to produce ripe gametes as predicted by Loosanoff and Davis (1952) for Long Island oysters at 15.0°C is 26.5 days, at 20.0°C is 7.9 days and at 25.0°C is 5.4 days. The average time required for 50% of the oysters in our experiments to ripen to spawning (450 degree days) at those same temperatures (15.0°C is 150 days, 20.0°C is 56 days, and 25.0°C is 35 days) is a rather constant ratio of 6-7 times longer. Presumably, if we had been starving our oysters at higher temperatures the ratio of ripening times would increase significantly with increase in temperature.

There is little information in the literature concerning ripening times of oysters south of Long Island Sound. Hidu, Drobeck, Dunnington, Roosenburg and Beckett (1969) were able to ripen about 25% of their Chesapeake Bay oysters from winter condition to spawn by an exposure to temperatures averaging 23-24°C for six weeks. Our formula indicates that at those temperatures Delaware oysters would require from five to ten weeks to ripen to a 60-80% level using exposures of 450-700 degree days respectively (Table 2). However, it is clearly speculative to attempt to compare spawning responses of oysters from different regions unless they have been handled in a similar manner resulting in data intended for spawning predictions.

ACKNOWLEDGMENTS

The dedication and expertise of Mr. Earl Greenhaugh, Resident Manager of the Shellfish Laboratory, and George Reinsfelder, Research Assistant, greatly facilitated this study. Thanks are due to Mr. Richard Keck, Research Fellow, for capably preparing the histological materials used in this study. We also wish to thank Drs. Jonathan E. Taylor and Marenes Tripp who critically reviewed the manuscript.

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SETTING OF THE AMERICAN OYSTER RELATED TO ENVIRONMENTAL FACTORS AND LARVAL BEHAVIOR

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ABSTRACT

Factors were defined which may be important in producing the consistently heavy oyster, Crassostrea virginica *(Gmelin), setting on the Delaware Bay tidal flats along the west coast of the Cape May Peninsula. A transect of 12 stations extending 4/5 mile offshore measured setting over two seasons with cement-board panels placed throughout the water column. Oysters set heavily at the very inshore intertidal area and also 1/2 mile offshore at a slope which bounds the flats from deeper water. The slope setting area coincided with heavy concentration of mature larvae. At the inshore area, similar setting rates were associated with only a tenth of the slope larval concentration indicating existence of factors promoting stimulaiton of set. The offshore slope was the area of steepest gradient in current velocity. The most inshore area was characterized by relatively great salinity and temperature increase with flood tides.*

Laboratory-reared oyster larvae, when subjected to salinity and temperature increases as experienced inshore, were stimulated to set in response to the temprature, but not the salinity factor. A gregarious tendeney in setting, however, reduced the significance of these findings. Individual cultch shells were not differentially "preferable" in oyster setting. However, the presence of spat on cultch shells attracted and stimulated setting.

The offshore slope area may be important in the primary concentration of larvae. Inshore setting probably is promoted by factors which may stimulate setting, such as temperature increases and the presence of oyster populations which may promote a gregarious response.

INTRODUCTION

This paper describes and seeks to explain the heavy intertidal oyster set (*Crassostrea virginica*) which is regularly received (Nelson, 1959) in Delaware Bay on the western coast of Cape May, New Jersey. By so doing, it hopes to bring to light factors which may be important in producing oyster set in other areas.

The Cape May tidal flats (Fig. 1) comprise an extensive intertidal area, averaging about a half mile in width, bordering almost the entire western shore of the Cape May Peninsula. Offshore this area is defined by a relatively sharp slope area (Fig. 2) which then grades into the deeper areas of the bay. Delaware Bay is a relatively shallow drowned river mouth estuary with from five to six feet of tidal range. Prevalent wind mixing prevents formation of seasonal salinity or temperature stratification. Oyster setting on the Cape May flats usually is intermittently heavy from June to October, depending on prevailing bay temperatures which, in turn, affect adult oyster conditioning and spawning and, apparently, larval survival. In scoring the consistency of setting here, Nelson (1934) stated that 1933 produced no oyster set on the Cape Shore Flats and that a failure of this sort happened only once or twice in 20 years.

This study utilized a combined field and laboratory approach to describe setting patterns and to

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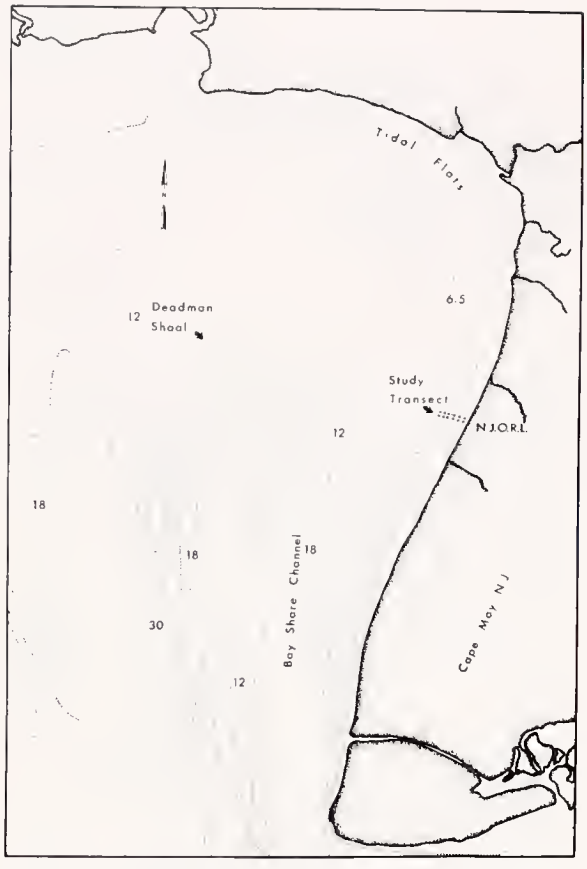

FIG 1. *The Delaware Bay, Cape May area of New Jersey showing study transect. NJORL = New Jersey Oyster Research Laboratory. Depths in feet at mean low water.*

learn of possible interactions between oyster larval behavior and environmental factors. The area was characterized biologically and hydrographically over a field transect of 12 stations which extended the width of the tidal flats plus a similar distance offshore. Laboratory-reared oyster larvae were then utilized to determine the importance of hydrographic and biological factors which may have stimulated oyster setting in the field.

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MATERIALS AND METHODS

Field Studies

Field studies were conducted for two years, 1965 and 1966, over a series of 12 stake stations which extended over the tidal flats to approximately 4,000 ft offshore (Figs. 1-3). Stations were arranged in two parallel lines (north and south) which were approximately 200 ft apart; the distance between stations within each line was approximately 800 ft. The following biological and hydrographic information was collected over the transect:

1. *Oyster set*

A series of 6-inch asbestos board panels were suspended throughout the water column from the bottom to high tide levels during the summers of 1965 and 1966 (Figs. 2, 3). Distance between the panels at the inshore stations (1, 2 and 3) was 2 ft and 4 ft at offshore stations 4, 5 and 6. Panels were notched and held in a horizontal position by a taut nylon line which was equipped with knotted line halters (Fig. 3). The line was anchored to the bottom by a solid cement block and at the top by a metal rod extending horizontally about 1 ft from the stake. "Bottom" panels were secured to the top of the cement blocks and were raised from the true bottom by no more than 4 inches. Stations were paired with regard to distance from shore and depth of panels.

Sets of panels were harvested after being immersed for week-long periods throughout the 1965 and 1966 summer seasons. Weekly measurement periods extended from 17 June to 8 September 1965 and from 25 June to 30 August 1966. Harvested panels were placed immediately in slotted wooden boxes to permit drying without disturbance of surfaces.

Oyster set on the upper and lower surfaces of dried panels was counted with a binocular microscope. The "bottom" panels were counted on the upper surface only. Four replicate sets of panels were used interchangeably throughout the two seasons. After counting, panels were made ready for reuse by scraping with a stiff brush in hot water. This treatment effectively removed all organisms from the panels.

Cross sectional diagrams showing lines of equal set were constructed for each weekly measurement period over the two seasons. North and south lines were averaged in the calculation, with set being expressed as total per panel (0.5 ft^2) per measurement week. Appropriate correction factors adjusted set figures for the bottom panels.

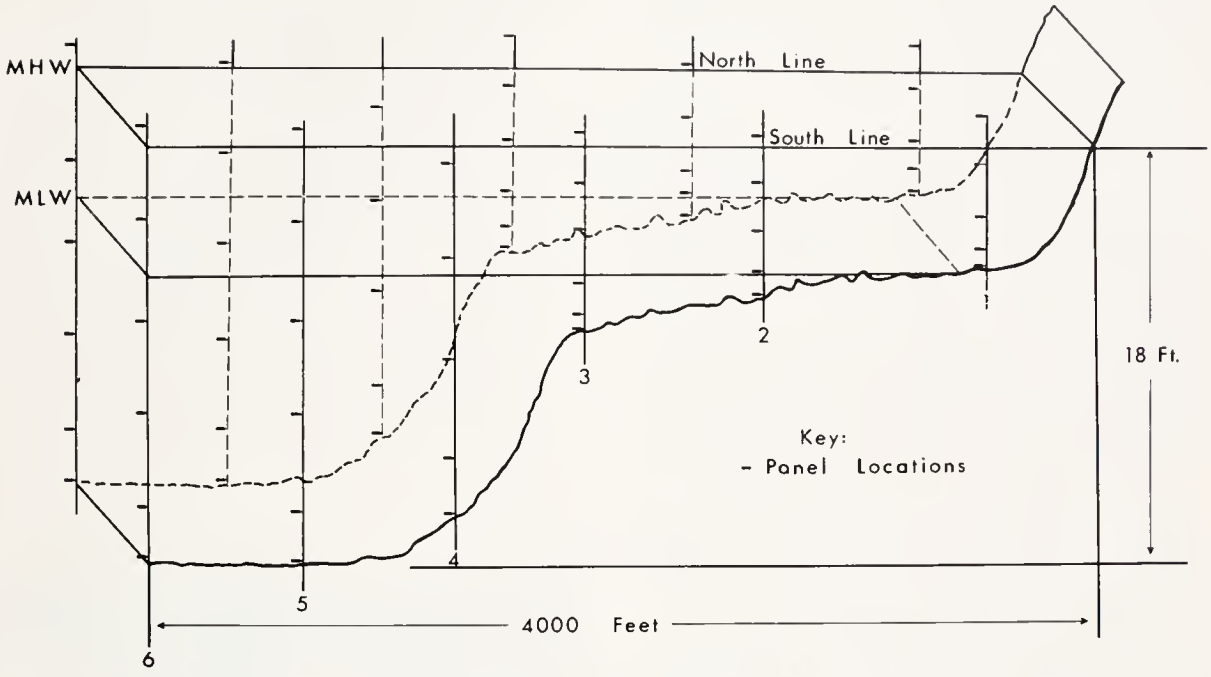

FIG. 2. *A three-dimensional view of Cape May field transect stations. Stations were arranged in two parallel rows which extend from the most inshore flats (Sta. 1) to deeper water approximately 4,000 ft offshore (Sta. 6).*

2. *Correlations of oyster larval abundance and occurrence of set*

Measurements were made on the south transect on 2 July and 14 July 1966 as follows:

Occurrence of "mature" and "eyed" larvae over tide cycles: Stations 1, 2, 4 and occasionally Station 6 (Fig. 2) of the south line were sampled at three levels (bottom, middle and surface) at 8 times spaced over a 12-hour period which coincided with a complete flood and ebb tide. Samples of 200 liter volume were procured in three minutes with a portable gasoline driven pump. Oyster larvae were concentrated with a 160 μ mesh plankton net and later counted under a binocular scope. The mesh size allowed capture of only the larger, more mature oyster larvae, those commonly called "mature" and "eyed". Earlier stage larvae, "straight-hinge", "early" and "late umbo" were not captured with the 160 μ mesh net.

Intensity of oyster set over the transect at the time of larval density measurements: Shellbags with 10 cleaned oyster shells were placed at the bottom and 4 feet above at Stations 1 and 2 and at the bottom plus 5 and 10 foot levels at offshore Station 4 (Fig. 2). Bags were placed and pulled twice during the day's run, with exposure times closely coinciding with periods of ebb and flood tide. Shells were counted for oyster set on inner shellfaces only.

Salinities and temperatures over the transect at 3-foot depth intervals at the times of plankton measurement: A Beckman Model RS5-3 portable electrodeless induction salinometer was used.

3. *Hydrographic Studies*

Field salinities and temperatures were measured on the south transect on eight sampling days in 1965 and 1966 (21 July, 10, 21, 27 August 1965; 2, 14 July, 9, 23 August 1966). The six transect stations were visited approximately hourly during 12 hours, with salinities and temperatures being measured at 3-foot vertical intervals with the salinometer.

Current velocities were measured in a similar manner over the same transect on 9 and 23 August 1966. Twelve-hour periods included six visits to each station at regularly spaced intervals. A Gurley Current Meter measured current velocities at 3-foot intervals throughout the water column.

From the salinity, temperature and current velocity data, estimates were made of prevailing hydrographic conditions and vertical and horizontal gradients as affected by tide stage.

Laboratory Studies

Laboratory studies included tests of the effects of salinity and temperature increase on oyster setting and an investigation of the gregarious setting response of oysters. Methods of larval culture have been described elsewhere (Haskin, 1964, 1965, 1966[2]; Hidu, Drobeck, Dunnington, Roosenburg and Beckett 1969).

1. *Salinity and Temperature Factors*

The effect of a salinity fall and rise and a temperature rise on oyster setting was determined in three replicate experiments in a factorial design suitable for the analysis of variance.

Setting-stage, laboratory-reared oyster larvae were aliquoted in equal numbers (approximately 10,000) in each of four 15-liter plastic containers. For 11 hr larvae were subjected to salinity and temperature changes as follows:

Culture 1. "Constant Conditions"
Salinities and temperatures were held relatively constant over an 11-hour period, with 10 oyster shells exposed to oyster set during time periods 0-1, 1-3, 3-5, 5-7, 7-9 and 9-11 hr. During each of these periods, a new batch of ten oyster shells was immersed. Temperatures in the three repetitions of the experiment ranged from 22-25°C; the within- experiment ranges were ± 5°C. Harvested shells were counted on both faces to determine total set. Salinities were constant within experiments; salinities ranged between 28 and 29‰ between experiments.

Culture 2. "Temperature Rise"
Salinity was held constant throughout. Temperature was held constant during the first 5 hr after which temperatures were raised 4-6 degrees within a 2-hour period and held near the higher levels for 4 additional hours. Culture water was heated by holding the experimental containers inside a larger vessel which was filled with hot tap water. Uniform heating inside the experimental container was maintained by air bubble mixers. All other cultures, including the control, contained the air bubblers. Shell measurement periods were as in Culture 1.

Culture 3. "Salinity Fall and Rise"
Temperatures were held constant. Salinities during the second through fifth hours were dropped approximately 3 ‰. During hours 5-11, salinities were gradually raised approximately 3 ‰. Salinities were lowered and raised by dripping in lower and higher salinities from vessels above. A constant water level in the experimental vessel was maintained by an automatic siphon which was screened to prevent escape of larvae. Samples from the siphon effluent were titrated with silver nitrate to determine salinities.

Culture 4. "Salinity Fall Followed by Salinity and Temperature Rise"
Salinity in this culture was manipulated as

FIG. 3. *Intertidal field transect station at low tide showing panel placement. The halter line is secured at the bottom by a solid cement block and at the top by a metal rod extending perpendicularly from the stake.*

[2] Haskin, H. H. 1964, 1965, 1966. Unpublished mimeo reports, Annual reports to U. S. Bur. Comm. Fish. Contract 14-17-0007-226 of PL 87-580 and sub-projects 3-3-R of PL 88-309, Rutgers, The State University, New Brunswick, N. J.

in Culture 3; temperature was manipulated as in Culture 2. This treatment measured a possible interaction of salinity rise and a temperature rise in affecting oyster set.

The three replicate experiments were analyzed by a factorial model analysis of variance (Simpson, Roe and Lewontin, 1960). The first 5 hr (the period before the salinity and temperature rises, Fig. 9) were considered in a separate analysis with groups (1-4) and time 0-1, 1-3, 3-5 hours) considered as fixed factors, and replications (3) and shells within groups (10) considered as random factors.

The second part of the experiments (including the salinity and temperature increases) was subjected to a separate factorial model analysis of variance. In this case, fixed factors were considered to be salinity rise (0 and 3 ‰), temperature rise (0 and 4°C), and time (5-7, 7-9, and 9-11 hr). Random factors were replicates (3) and shells within groups (10).

Although there was some amount of variation between replicates in absolute salinities and temperatures and exact timing and degree of rise of these factors, the separate runs were thought to be sufficiently uniform to be considered replicates in the analysis.

2. Gregarious Setting Response

Erratic results (heavy setting in some cultures in the control period) in the salinity-temperature experiments prompted an investigation of the gregarious setting response in the American oyster. These results have been published elsewhere (Hidu, 1969); however, the hypotheses tested and results will be briefly summarized here since they have bearing in explaining setting observed in the field. Hypotheses tested in the laboratory were as follows:

A. Individual cultch shells differentially attract setting oysters.
B. Cultch position in the culture container affects amount of set accumulated.
C. Presence of spat on cultch attracts more set than unspatted cultch in the same water mass.
D. Presence of spat stimulates setting of larvae in greater numbers per unit of time than occurs if spat are absent.

RESULTS

Field Oyster Set Patterns — 1965 and 1966

Cape Shore set patterns are presented as cross-sectional diagrams expressing weekly patterns for both seasons as lines of equal set intensity (Figs. 4A-4H). Data from north and south lines were combined in the calculation since patterns on each were very similar. In case of a light set, occasionally several weeks' data were combined to form a single emergent pattern.

The oyster set in 1965 was very light at the Cape Shore, with only 751 oysters setting on the panels during the entire season. The week of 26 August-2 September dominated the season with approximately 60% of the measured set. Setting here (Fig. 4A) was largely confined to the subtidal areas of offshore Stations 5 and 6. Setting occurred from mean low water to 8 feet below, with a very light accumulation below this level. The light set of the rest of the summer is presented as a composite pattern (Fig. 4B). The slope area separating flats from deeper water had greatest set. Set on the bottom in 1965 was practically nonexistent. No set would have been seen if panels had not been present throughout the water column.

In contrast, 1966 was a good setting season, with over 100,000 oysters being counted on panels from 25 June-1 September. Good weekly sets occurred between 25 June-25 July, followed by light and spotty sets in late summer. The 1966 set is thus presented as weekly patterns to 25 July, with the remainder of the season as a composite (Figs. 4C-4H).

The 1966 set may be divided into two distinct types. First, the early season patterns (25 June-12 July, Figs. 4C-4E) were characterized by relatively heavy setting at the slope area which divided the flats from deeper water. A second concentration of set was measured at the very inshore stations, with a relatively light set at the mid-flat stations. The offshore slope set was most intense at panels 2-4 feet off the bottom, whereas the inshore set was heaviest at the bottom and graded off very rapidly above 2 feet from the bottom.

Later 1966 patterns (after 12 July, Figs. 4F-4H) retained the slope setting; however, the inshore patterns were lost.

Correlations of Larval Abundance and Occurrence of Set

The simultaneous transect sampling of oyster larval abundance and occurrence of set revealed interesting differences between the close inshore and the slope setting areas. Larval samples, taken over two days, revealed high numbers of mature and eyed larvae concentrated at the offshore slope area (Table 1, Figs. 5A, 5B). Numbers of larvae ran as high as 24 per liter; a density of mature larvae much in excess of anything yet sampled on Delaware Bay. Conversely, inshore larval densities were lighter, reaching maximums of slightly more than 1 per liter.

Set on shellbags exposed for flood and ebb

tides during the larval sample periods revealed a different picture (Table 1). Heavy sets were recorded at the slope area, coinciding closely with the heavy larval concentrations and the heavy set areas recorded in the panel study. Similar heavy setting, however, was recorded at the close inshore area. The persistent scarcity of larvae in this area indicated that factors may be stimulating the inshore mature larvae to set.

The degree of stimulation is shown by the

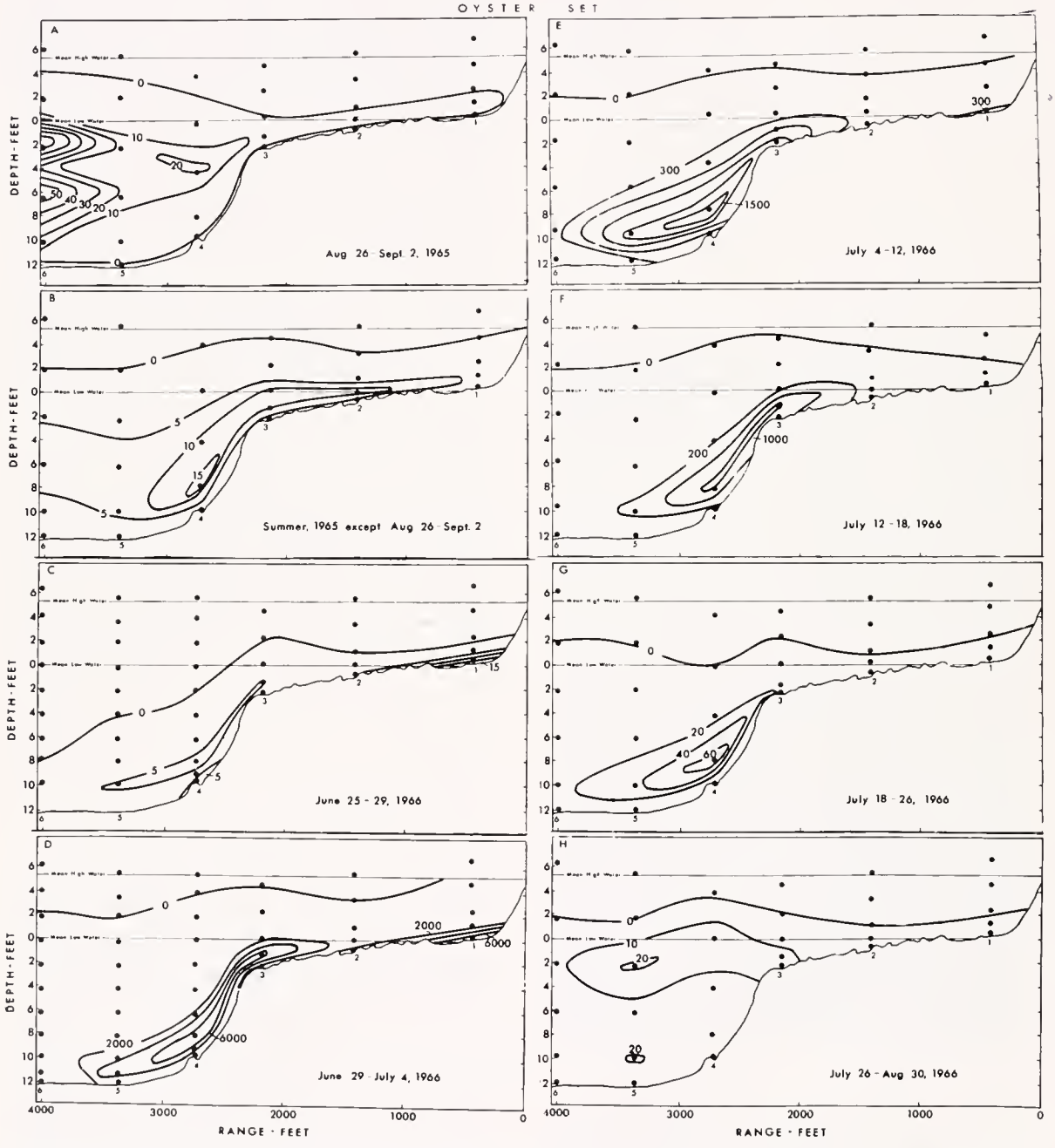

FIG. 4. *Oyster set at Cape May transect during 1965 and 1966 expressed as set per panel (0.5 sq. ft.) for the weekly measurement periods. Dots indicate panel location.*

TABLE 1. *Cape Shore combined field transect measurement of oyster larval density and shellbag set intensity with calculated index of "stimulation to set." Plankton sample and shellbag locations are depicted in Figures 5 and 6.*

Station and level	Date (1966) tide	Shellbag No. set	Shellbag Time of immersal (EST)	Larval samples larvae/200 liters Time	Larval samples larvae/200 liters liters	Larval exposure to shellbags in hours	Set per shellbag per hours of larval exposure
1 [a]	2 July	283	0527-	1025	6	48	5.9 (omit)
Bottom +1'	ebb		1300				
"	flood	1212	1615-	1615	258	1257	.96
			2315	1811	148		
				1900	257		
				2127	109		
"	14 July		0520-	0645	10	23	.22
	ebb	5	1000	0735	1		
"	flood	28	1200-	1300	2	74	.38
			1755	1436	5		
				1617	15		
				1740	45		
							$\bar{x}$ = .52
1	2 July	0	0527-	—	—	—	—
Bottom +4'	ebb		0800				
"		40	1745-	1817	77	513	.078
	flood		2315	1900	87		
				2133	102		
"	14 July	0	0520-	0645	1	1	0
	ebb		0700				
"		1	1500-	1617	2	14	.036
	flood		1755	1740	10		
							$\bar{x}$ = .038
2	2 July	106	0538-	1040	0	358	.28
Bottom +1'	ebb		1300	1220	102		
"	2 July	10	1555-	1555	301		
	flood		2310	1747	252	1273	.0078
				1933	90		
				2155	150		
"	14 July	12	0525-	0714	719	2181	.0055
	ebb		1000	0920	13		
"	14 July	38	1200-	1200	20	529	.072
	flood		1845	1400	136		
				1640	41		
				1845	366		
							$\bar{x}$ = .091
2	2 July	15	0538-	—	—	—	—
Bottom +4'	ebb		0800				
"	2 July	2	1745-	1753	178	467	.0043
	flood		2310	1944	104		
				2202	36		
"	14 July	2	0525-	0714	323	646	.0031
	ebb		0713				
"	14 July	0	1500-	1640	4	30	0
	flood		1845	1845	19		
							$\bar{x}$ = .002>

TABLE 1. *(cont.)*

4	2 July	1116	0550-	0653	74	6435	.17
Bottom +1'	ebb		1445	1105	209		
				1300	1185		
				1420	2556		
"	2 July	34	1445-	1519	1439	3781	.009
	flood		2300	1715	427		
				2000	29		
				2225	265		
"	14 July	100	0533-	0555	5	6332	.016
	ebb		1130	0801	45		
				0940	4786		
				1105	195		
"	14 July	23	1130-	1335	88	429	.054
	flood		1810	1700	36		
				1807	24		
							$\bar{x} = .063$
4	2 July	72	0550-	1300	18	2787	.026
Bottom +5'	ebb		1445	1426	2643		
"	2 July	6	1445-	1525	492	2324	.0026
	flood		2300	1718	206		
"	14 July	13	0533-	0615	28	944	.0138
	ebb		1130	0801	35		
				0940	659		
				1105	8		
"	14 July	14	1130-	1340	94	516	.029
	flood		1810	1700	67		
				1807	212		
							$\bar{x} = .018$
4	2 July	1	0550-	0714	320	1263	.00079
Bottom +10'	ebb		1445	1105	61		
				1300	58		
				1433	531		
"	2 July	2	1445-	1532	298	903	.0022
	flood		2300	1721	25		
				2013	64		
				2232	106		
"	14 July	1	0533-	0622	5	112	.0067
"	ebb		1130	0801	2		
				0940	80		
				1105	1		
"	14 July	2	1130-	1345	36	151	.0165
			1810	1700	3		
				1807	2		
							$\bar{x} = .0065$

[a] Bottom +1' indicates shellbag placed 1 foot above bottom.

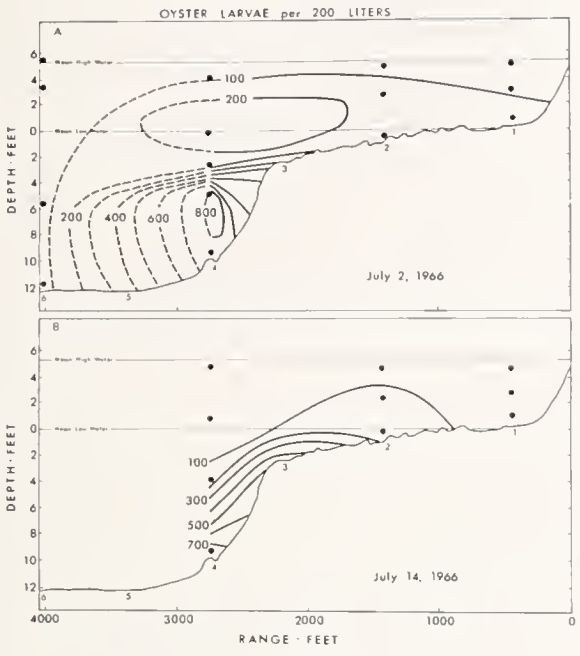

FIG. 5. *Mean densities of "mature" and "eyed" oyster larvae per 200 liters of sample taken over two 12-hour tide cycles. Mean densities were calculated from several samples taken throughout the day at the indicated locations. Dotted contour lines indicate tenuous conclusions based on only five samples from Station 6.*

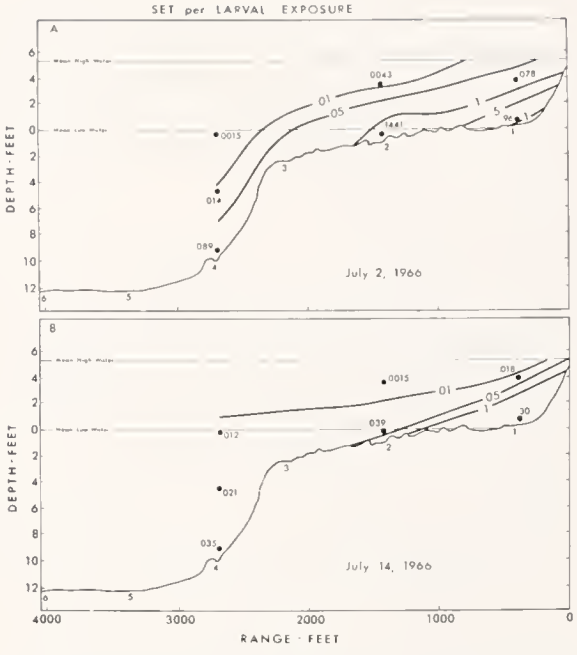

FIG. 6. *Oyster set at Cape May as a function of larval exposure. Contour and sample location figures express set per shell bag per day per "larval hours exposure". For example, a figure of 1.00 means that one set was received for one mature larva present in 200 liters for one hour adjacent to the shell bag. Larval densities with time were interpolated from successive plankton samples.*

calculated figure of set per shellbag per "larval hours" of exposure (Table 1, Figs. 6A, 6B). This figure, using reasonable assumptions, calculates set per shellbag related to larval exposure by figuring duration of exposure to the measured larval densities. The calculated figures on two measurement days at Inshore Station 1 at the bottom were approximately 10 times greater than calculated figures for the slope area (Figs. 6A, 6B). This may indicate that a mature larva at the inshore area has over 10 times more inclination to set than a larva in offshore areas. Similar reductions in "inclination to set" were recorded at higher levels in the water column at each station. Although there was considerable larval exposure at the higher shellbags, there was very little corresponding set.

It must be noted that the correlations between larval abundance and setting were established in only two days of sampling. The inshore-offshore differences, however, were striking. Additional information is needed to draw firm conclusions about set stimulation in the field.

Hydrographics

1. Current velocities

Measurements of 9 and 23 August are presented as cross sectional diagrams with iso-velocity contours (Figs. 7A, 7B). All data of 12-hour runs are combined in average velocity calculations.

Characteristically, currents run nearly parallel to the shoreline [3]. Relatively high velocities are associated with the offshore area with a great slackening of currents as the flats are encountered. On 9 August velocities at the deep water stations averaged between .50 and 1.50 ft/sec with greatest average velocity being recorded above the mean low water mark. The lowest velocities were associated with the bottom in all cases. On 23 August, with more tidal range, average offshore velocities ranged between 1.0

[3] Van Winkle, W. Unpublished rhodamine dye study. 1965.

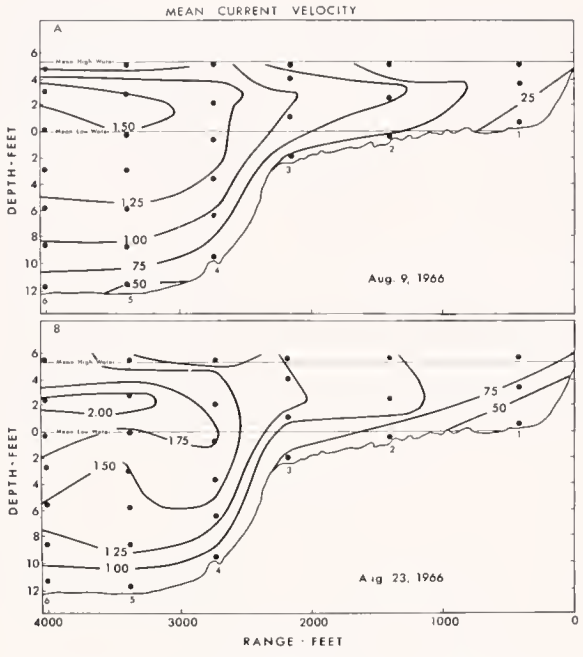

FIG. 7. *Mean current velocities (ft/sec) measured on 9 and 23 August 1966 expressed as iso-velocity contour lines.*

and 2.0 ft/sec. At the inshore stations, average velocities were considerably lower, ranging between 0.25 and 1.25 ft/sec.

The slope, separating flats from deeper water, was an area of great current velocity change as evidenced by considerable convergence of velocity contour lines. This may have considerable bearing on the concentration of larvae as discussed later.

2. Temperatures

Cape Shore summer water temperatures were very weakly stratified, vertically (Table 2). Average vertical gradients at all stations (6-18 foot depth) for six measurement days ranged only between 0.46 and 1.00°C. The shallowness of the bay, plus characteristic wind mixing, appears to prevent summer temperature stratification.

Water temperatures at the inshore intertidal stations varied widely, whereas the deepwater stations had more uniform temperatures (Fig. 8, Table 2). The inshore water temperatures, especially Station 1, responded greatly to prevailing air temperatures and solar heating. Temperature increases were most marked during periods of early flood tides, when apparently heat was absorbed from sun-baked tidal flats. The data of 10 August (Fig. 8) are typical in this regard. Offshore Stations 4-6 temperatures ranged about 25-27°C throughout the tide cycle. In contrast, the most inshore intertidal station had morning temperatures of 25-27°C during the ebb tide. With the flood tide in late afternoon, the most inshore temperatures were initially 33°C, six degrees above temperatures recorded here at the last of the ebb tide at 10:00 am. The high temperatures remained at Station 1 until at least high slack water. Similar, but less pronounced, increases were measured at the mid and outer flat Stations 2 and 3. Other measurement days confirmed that the very high temperatures at Station 1 persisted well past high slack water in mid-afternoon and evening.

3. Salinities

Cape Shore salinity ranges followed a pattern which was inverse to the temperatures. The most offshore stations with greater currents had the greatest salinity ranges. The inshore stations with a lower prevailing current velocity had considerably less salinity range.

Several salinity averages compiled on six tide-cycle runs are summarized on Table 3. Salinity ranges throughout tide cycles at all stations were between 0.23 and 4.09 ‰. Vertical salinity gradi-

TABLE 2. *Summary of mean water column temperatures and maximum vertical temperature gradients measured over tide cycles (12 hr) on six days at stations 1S-6S.*

Station	Mean temperature C°			Maximum vertical temperature gradient		
	Max. day	Min. day	Med. day	Max. day	Min. day	Med. day
1	30.09	25.67	26.80	1.31	.05	.78
2	27.67	24.95	26.11	1.10	.39	.46
3	27.11	24.72	26.19	1.19	.30	.60
4	26.90	24.60	25.88	1.36	.51	1.00
5	26.71	24.62	25.75	1.87	.36	.90
6	26.67	24.67	25.55	1.62	.40	.62

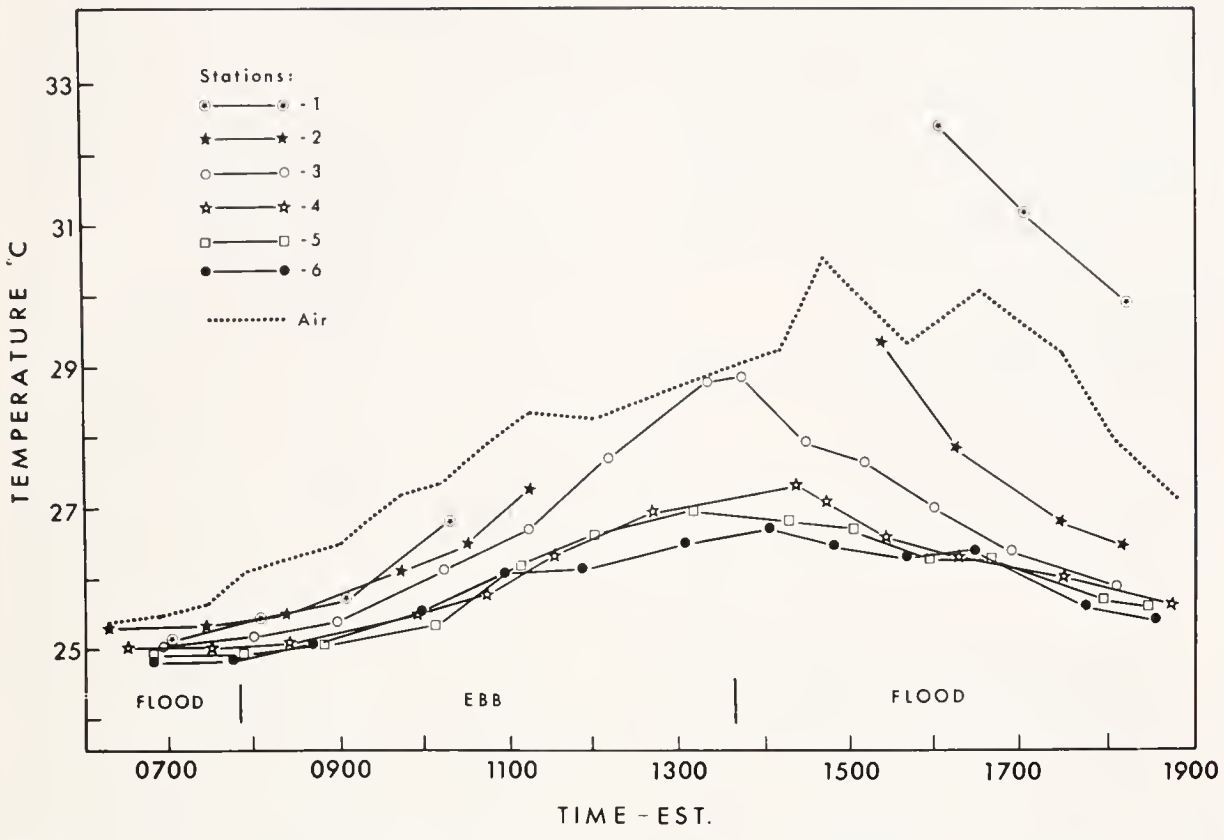

FIG. 8. *Mean temperatures taken over a 12-hour tide cycle over stations 1-6 on 10 August 1965. Mean values were calculated from measurements taken at 3-foot intervals throughout the water column.*

ents, as with temperatures, were very weakly defined, with a mean gradient at all stations at 0.64 ‰. Bottom salinity ranges over tide cycles were very similar to the overall ranges and varied between 0.19 and 3.78 ‰. Absolute salinities ranged between 21.11 and 28.68 ‰, with an average of 24.65 ‰.

On occasion, unusually high salinities were encountered at the most inshore Station 1 at early flood tide. A comparison of flood tide salinities

TABLE 3. *Summary of several tide cycle (12 hr) salinity ranges (‰) measured over stations 1S-6S on six days. Measurements were with induction salinomcter with vertical intervals at three feet. Days were chosen at random with respect to previous wind and precipitation experience.*

Station	Max. salinity range (12 hr) all levels			Max. vert. sal. gradient at single meas.			Bottom sal. range (12 hr)			Total absolute salinity range on all days	Mean salinity on all days
	Max. day	Min. day	Med. day	Max. day	Min. day	Med. day	Max. day	Min. day	Med. day		
1	1.18	.23	.42	.35	.15	.18	1.15	.19	.38	23.18-28.29	24.69
2	1.79	.38	.86	.85	.09	.42	1.79	.35	.86	21.11-27.20	24.52
3	2.90	.44	2.29	1.10	.06	.52	2.76	.39	1.80	21.66-28.09	24.63
4	3.95	.63	1.80	1.35	.15	.77	3.72	.46	1.81	21.63-28.65	24.67
5	4.03	.65	2.00	1.58	.18	.90	3.78	.59	1.95	21.79-28.61	24.66
6	4.09	.74	2.32	1.24	.16	.97	3.78	.74	2.27	22.00-28.68	24.71
All stations	4.09	.23	1.90	1.58	.06	.64	3.78	.19	1.80	21.11-28.68	24.65

taken nearly simultaneously at Stations 1 and 2, during the first three hours of the flood tide, revealed about a 1 ‰ difference on eight measurement days. Apparently the high salinity water originated from tidal flat pools formed by drainage from extensive salt marshes nearby. With early flood tide the high salinity, warmed water probably was lifted to the very inshore areas. The possible importance of this factor in stimulating larvae to set was investigated in the laboratory.

Laboratory Studies

Effects of salinity and temperature changes on setting

Results of three trials of salinity and temperature change experiments are shown in Figure 9

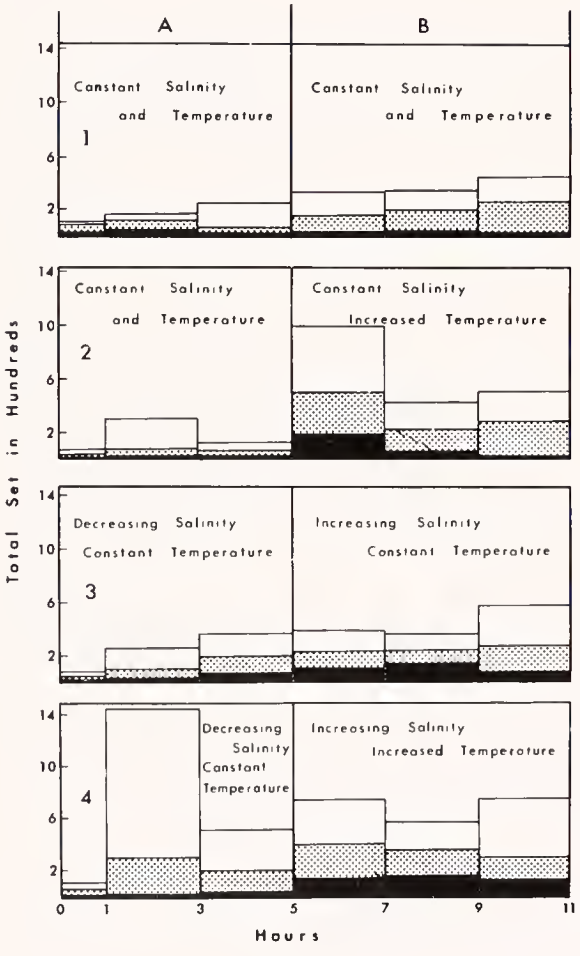

FIG. 9. *Effects of laboratory salinity and temperature changes on oyster setting. Blocks show total set per 10 cultch shells exposed for 2-hour periods. Three replicates of the experiment are shown with block shading.*

as the total set per treatment per measurement period. Two analyses of variance were calculated for the entire experiment. The first analyzed components of variation in the initial five-hour period (Part A, Fig. 9) before the increases in temperature and salinity. The second analysis assessed the importance of components of variation in the period of increase in salinity and temperature during the last six hours of the experiments (Part B, Fig. 9).

Of the fixed factors in Part B, only the temperature increase significantly increased setting rates, and this to the 95 per cent confidence level (Table 4) [4]. Neither time, salinity rise, or the interactions between these and other factors affected setting rates. Of the random factors, there were significant differences between replicates of the experiment; different overall setting rates were experienced between the three experiments. It is difficult to place similar numbers of larvae between experiments which are physiologically able to set during the experimental period. Only these larvae will effectively enter the experiment.

The analysis of the initial 5-hour control period (Part A) revealed considerable variability which weakened the case for the significant effect of the temperature increase of Part B (Table 4). Here, there was found again a significant difference between replicates (C) as might be expected, but in addition, 99 per cent level significance was attributed to the effect of an interaction of groups (A) and replication (C). Significance was also attributed to effects of an interaction of time (B) and replicates and the triple interaction of groups (A), time (B) and replicates (C). There was no difference in setting intensity between groups (A) and thus it may be concluded that the lowering of salinity in Groups 3 and 4 did not affect setting rates.

The heavy sets received only in culture 4 in preliminary control period (Fig. 9) were puzzling. Nearly all of the set was concentrated on one or two of many exposed cultch shells. It appeared that particular shells in some manner stimulated the setting of oysters. This occurrence prompted an inspection of other frequency distributions of set on cultch shells and the described patterns were indeed quite typical in nearly all laboratory cultures. An investigation then determined the cause of such sporadic setting and unusual set

[4] Later experiments tested only the temperature factor in set stimulation and again found increased temperature to cause significantly increased setting rates (Lutz, Hidu and Drobeck, 1970).

TABLE 4. *Analysis of variance of* Crassostrea virginica *setting in salinity and temperature change experiments.*

Part A. Preliminary control period

Factor	Levels
Groups (A)	1, 2, 3 and 4
Time (B)	0-1 hour, 1-3 3-5
Replication (C)	3
Shells (D)	10 per cell

Source of variation	d.f.	Sum of squares	Mean squares	F
Total		2170.39		
A	3	106.13	35.38	1.77
B	2	148.65	74.32	6.10
AB	6	60.66	10.11	.92
C	2	245.83	122.92	30.70**
AC	6	119.53	19.92	4.94**
BC	4	48.59	12.5	3.01*
ABC	12	132.11	11.00	2.72*
ABCD	324	1308.90	4.04	

Part B. effects of salinity and temperature rise

Factor	Levels
Salinity (A)	No change, + 4 ppt
Temperature (B)	no change, + 4°C.
Time (C)	0-2, 2-4, 4-6 hr
Replication (D)	3
Shells (E)	10 per cell

Source of variation	d.f.	Sum of squares	Mean squares	F
Total	359	2171.18		
A	1	.83	.83	.09
B	1	78.64	78.64	81.80*
AB	1	.98	.98	.68
C	2	19.71	9.86	1.27
AC	2	4.29	2.14	.70
BC	2	25.64	12.82	3.60
ABC	2	10.93	5.46	5.94
D	2	169.31	84.66	15.45**
AD	2	17.95	8.98	1.64
BD	2	1.91	.96	.17
ABD	2	2.87	1.44	.26
CD	4	31.13	7.78	1.42
ACD	4	12.23	3.06	.56
BCD	4	14.28	3.57	.65
ABCD	4	3.70	.92	.18
ABCDE	324	1776.77	5.48	

distribution. The results of this work have been published elsewhere (Hidu, 1969) but the conclusions are listed here since they have relevance in interpreting this field study:

1. Individual cultch shells are not differentially attractive to setting larvae.
2. Cultch position has a significant effect in set accumulation, i. e., bottommost layers of cultch in laboratory cultures attract significantly more set than higher layers.
3. New spat on cultch shells attracts set to that substrate and also stimulates setting.
4. The effective agent appears to be a waterborne pheromone emitted from metamorphosed oysters. Two-month-old spat located inside larval-proof plankton mesh bags stimulated a set on cultch shells

located outside the bags. More recent information (Hidu, Veitch and O'Brien, 1970) also indicated a waterborne agent, since sea water in which adult oysters had recently pumped stimulated setting and modified the distribution of set on cultch shell in the laboratory.

5. This information indicates that the American oyster sets gregariously, thus the presence of established populations in the field may attract and stimulate setting from physiologically mature larvae.

DISCUSSION

Oyster setting at Cape May, or indeed any other area, may be a function of two types of factors. First are the hydrographic regimes that determine and modify the travels of the larva from spawning until it has the ability to set. Second are factors which may stimulate the mature larva to set. This study gave indications that both types of factors may act in quite a systematic fashion to provide a good setting area. It builds on other information that is available on larval occurrence and setting in Delaware Bay and elsewhere.

There has been much discussion of the movement of larvae during their 2-week life span ending in the arrival of the mature oyster larvae on various setting grounds in Delaware Bay. First, it has been observed that early stage larvae, "straight-hinge" and "early umbo", tend to be randomly distributed in the water column throughout the tidal cycle; however, later stages, "mature" and "eyed" larvae, are more benthic in distribution (Kunkle, 1957). At early and maximum flood tides, the older stages are more uniformly distributed vertically. During times of ebb and slack water, the late stage larvae apparently congregate near the bottom. Haskin (1964) confirmed Kunkle's observations and in addition found in the laboratory that the older stage larvae responded to salinity changes. This possibly explained their differential distribution with tide stage. Thus, in Delaware Bay, with a net down-bay movement of surface waters and net up-bay movement of bottom waters and an over-all counterclockwise circulation pattern in the lower bay, it has been postulated (H. H. Haskin, personal communication) that oyster larvae which may originate in the upper bay planted grounds and seed beds are carried down bay. As the larvae become older and are more benthically distributed, they are carried up the channels, i. e. Bay Shore Channel (Fig. 1), reaching the Cape May area as mature larvae.

Factors which may act to concentrate setting larvae within specific, more well defined areas may be as important as the processes which modify larval movement during their 2-week life span. In this study, there were great concentrations of late-stage larvae near the bottom at the sharp slope area which separates the flats from the deeper water channel areas. Changes in current pattern in this area — a sharp transition from higher velocities offshore to lower velocities over the flats — may be responsible for the concentration of larvae. Possibly the larvae are concentrated, dropped from suspension like inanimate particles, by the eddies and slack water produced at the edge of the flats. Such concentrating effects have been noted elsewhere. Nelson (1921) and Roughley (1933) stated that larvae tended by some means to be concentrated in eddies and slack water, thus providing for areas of great setting. Roughley contended that the best spatting grounds are those over which water is caused to eddy by the contour of the land and tidal flow. The importance of current patterns within specific areas thus should be studied more closely and their importance to larval concentration determined. The inshore setting of larvae at Cape May may be aided by the concentrating effects of current patterns at the slope area.

Factors which may stimulate setting in mature larvae also appear to be important. In this study, similar sets were obtained at the close inshore intertidal areas with only 1/10 of the larval exposure as at the slope area indicating the existence of such factors. Two factors may have been important in stimulating the very heavy inshore sets. Laboratory experiments indicated that temperature increases, such as were encountered at very inshore areas on midday flood tides, stimulated setting in oysters. Similar effects have been noted at the National Marine Fisheries Service Biological Laboratory at Milford, Connecticut (Warren Landers, personal communication), where heaviest sets in outdoor rearing pools occur in the midmorning hours, during time of most rapid temperature rise. Thorson (1964) in a review paper concluded that temperature increases may be important in setting. He viewed the setting response of marine invertebrate larvae as part of a more general response to light. Studies indicated that most larvae, including *C. virginica*, are "photopositive" throughout the larval life span but such factors as salinity changes and increased heat may act as a stimulus for a photonegative response and that this in turn is associated with setting. Thorson indicated that such a response mechanism would allow an organism some "selection" of an area of setting; for example, set differentially in the intertidal zone. It might be noted that *C. virginica*, south of

the Chesapeake area, is most prominent in the intertidal zone. However, such populations in northern areas are practically nonexistent because of winter losses.

Gregarious setting may be a second factor producing the very heavy inshore sets at Cape May. In the laboratory portions of this study, the gregarious response was evident, apparently mediated by pheromones emitted by metamorphosed oysters. In the field at Cape May, although the importance of gregarious setting was not determined, it was noted that associated with the inshore area was a large yearling oyster population. Station 1S which was in close proximity to the oyster bed had over twice the setting intensity over two years as did the adjacent Station 1N. Of course there has been much other work in defining the importance of gregarious setting. Cole and Knight-Jones (1949) originally observed that oyster larvae, *Ostrea edulis*, in outdoor rearing tanks, were attracted to shells which already contained spat. Later, Knight-Jones (1952) found that *O. edulis* probably sets gregariously in the field. Cultch shells on well-stocked oyster grounds in British rivers contained significantly more spat than cultch on immediately adjacent areas which were not stocked with oysters. Knight-Jones emphasized the probable importance of gregarious setting by eliminating other possible reasons for the heavier set: "It is most unlikely that the heavy falls of spat on the Shop Laying during 1948 and 1949 were due to that ground being especially favorable for setting through some intrinsic quality such as cleanliness or exposure to current". Recently, Cranfield (1968) noted in New Zealand that the oyster, *O. lutaria*, set most heavily immediately on an established oyster bar and very much lighter in adjacent areas. Cranfield surmised that gregarious setting may be the mechanism.

To conclude, evidence is accumulating which indicates that the establishment of oyster populations is the result of an orderly interaction between environment and the larva during its 2-week life span and especially during time of setting. Factors regulating bay-wide movement of larvae, such as the salinity response and vertical position (Pritchard, 1953) appear to have importance in producing set areas. Factors which produce concentrations of setting larvae may be important. The role of currents and eddies should be investigated more intensively.

Finally, environmental stimuli producing a set from mature larval populations may also be important in population establishment. Here increased temperatures and a gregarious setting response were important in the laboratory. Both may have been important at inshore areas in the field which appeared to be stimulatory to set. More intensive work is needed to determine the relative importance of these and other factors in set stimulation in the field. Such findings will ultimately allow us a better understanding of how oyster populations are established, permitting us to discover new potentially important setting areas and to manipulate natural conditions to produce new setting areas.

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SETTING, GROWTH AND MORTALITY OF *CRASSOSTREA VIRGINICA* IN A NATURAL MARSH AND A MARSH ALTERED BY A HOUSING DEVELOPMENT [1]

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ABSTRACT

In February 1969, asbestos plates for collecting oyster spat and partitioned trays containing eight size groups of juvenile oysters were placed in (1) a dead-end canal in a housing development created by dredging, bulkheading, and filling part of a coastal marsh; and (2) a dead-end bayou in an unaltered part of the same marsh in West Bay, Texas. The plates and juvenile oysters were then monitored periodically for the next 12 months.

Temperature, salinity, dissolved oxygen, total phosphorus, inorganic phosphate-phosphorus, Kjeldahl nitrogen, nitrite, turbidity, pH, CO_2, total alkalinity and carbonate alkalinity were monitored in the water at both locations. Eight of the above variables were compared to the growth and mortality of the juvenile oysters.

Spat set from late May until October 1969, with the greatest settlement in September. Although never heavy, setting was 14 times greater in the marsh than in the altered area.

Juvenile oysters grew faster in the marsh than in the altered area. Within each site the individual size groups increased in mean length at similar rates. The average increase in length was 52 mm/year in the natural marsh and 33 mm/year in the altered area. The average increase in weight was 136 g/year in the natural area and 86 g/year in the altered area, and was greatest in the largest size group in both areas.

Mortality rates of juveniles were similar among all size groups in the development and greatest among the largest size groups in the marsh. The annual rate of mortality averaged 91% in the altered area and 52% in the natural area. Dermocystidium marinum *was not detected in three spot checks during June, July and August.*

Greatest differences in spatfall, growth and mortality between areas were in the summer when dissolved oxygen was lower in the narrow dead-end canals in the altered area. Also several plankton blooms followed by very low oxygen and then fish kills occurred in the canal during the summer.

INTRODUCTION

One and three-tenths million kilograms of oyster meats valued at $1.3 million were harvested from the Galveston Bay system in 1968, totalling 86% of the Texas oyster harvest (Farley, 1969). This oyster harvest from this bay system was taken in less than half of the total water area, however, because of harvesting restrictions relating to fecal pollution (Fig. 1). If the amount of sewage discharge into the system increases, more of the bay area will be closed to commercial harvest, and alternate methods and areas will be needed to supply the demand for oysters.

[1] Contribution No. 312 from the National Marine Fisheries Service Biological Laboratory, Galveston, Texas.

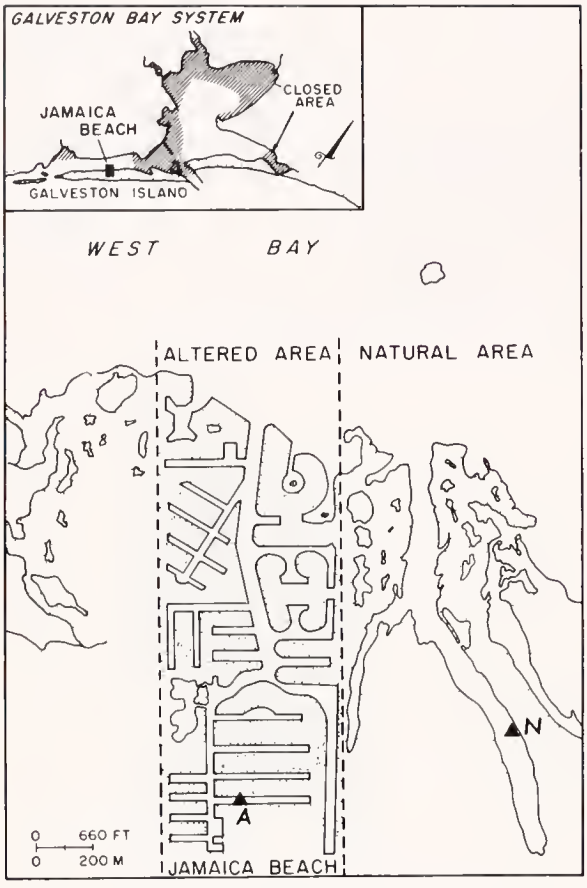

FIG. 1 *Areas of the Galveston Bay system closed to shellfish harvest in 1969-1970 because of pollution (Texas State Department of Health, 1969), and locations of sampling stations in West Bay, Texas.*

Many coastal areas such as West Bay, Texas (Fig. 1), though meeting public health standards for oyster harvest, do not produce large quantities of oysters because of environmental limitations. Production of oysters in the open part of West Bay is low, probably because high salinities provide excellent habitat for the oyster drill, *Thais haemastoma,* and the fungus parasite, *Dermocystidium marinum.* Salinity is lower in many of the bayous adjacent to the bay, however, and they would probably support sizable populations of oysters if sufficient substrate were available.

Large areas of shallow bay and marsh are being developed for housing sites along the Gulf coast. This type of alteration involves dredging, filling and bulkheading. Bulkheads in the developments provide substrate for attachment of oysters and are potentially useful for intensive oyster culture because (1) the water is deeper than adjacent natural areas, (2) culture techniques could be applied without seriously interfering with other uses, (3) the waters should remain unpolluted in the new developments because of requirements for treatment of sewage and (4) the areas are easily accessible by boat or automobile.

The general objective of this study was to determine the feasibility of utilizing bulkheaded canal areas for economic oyster production. Specific objectives were (1) to compare the setting rates of oyster spat and the growth and survival of juvenile oysters in a marsh area altered by channelization and bulkheading with those in an undisturbed marsh and (2) to determine how juvenile growth and mortality rates vary in relation to the environmental parameters affected by the habitat modification.

STUDY AREA AND METHODS

Jamaica Beach, located on the Galveston Island shoreline adjacent to West Bay (Fig. 1), was selected for study because it is one of the largest housing developments on the Island, has the most complex canal system of any of the developments, is bordered by natural marsh areas and is located in an area of West Bay approved for oyster harvesting by the Texas State Department of Health (1969).

Sampling station "A" was established in a dead-end canal adjacent to a bulkhead in the development and station "N" was established in a natural bayou east of the development (Fig. 1). Water depth (mean low tide) was 1.1 m at A and 0.8 m at N. Many adult oysters were attached to the bulkheads at station A, and a small reef was located within 50 m of station N.

Twelve hydrological variables were monitored at each station during alternate weeks from the oyster mortality and spatfall checks, from 20 March to 15 November, and monthly from December to February by Pullen, Proctor and Trent (MS.)[2]. Water temperature was monitored continuously by a recording thermograph at each station and salinity was determined whenever the oysters were checked in addition to the determinations made during the hydrological study.

Three pairs of asbestos plates were suspended in the water at each station to determine the rate of oyster spat set. Each plate was 12.7 cm^2 and 0.3 cm thick and had one smooth surface. The plates

[2] Pullen, E. J., R. R. Proctor and L. Trent. (MS.). Environmental differences between a natural estuarine area and a similar area altered by a land-fill housing development.

were seasoned in sea water for 2 weeks and scraped clean before initial use. The individual plates of a pair were placed horizontally, with smooth surfaces facing each other, 2.5 cm apart on a wooden rack suspended vertically from a tripod field structure. Each pair of plates was located 0.3 m below the previous pair. The frame rack was suspended at each station so that the upper pair of plates was located at mean low water. The plates were replaced with clean ones at 2-week intervals. Spat attached on the central 100 cm^2 area of the smooth side of each plate were counted with the aid of a binocular microscope; this count was used as the unit of observation.

The growth rates of juvenile oysters were determined using unattached oysters placed in two trays near the spat collectors in each area. The trays were standard 81 x 46 x 10 cm polyvinyl-coated (laboratory cart) baskets similar to baskets used in oyster mortality studies by Hofstetter, Heffernan and King III (1965). Each tray was lined with 1.3-cm mesh galvanized wire screen and was partitioned with strips of screen into 18 squares (13 x 13 cm). Juvenile oysters used were dredged in February 1969, from an oyster reef near the middle of Galveston Bay and were from a late summer spawn in 1968 (R. P. Hofstetter, personal communication). The oysters were separated from the dead shell, cleaned of major fouling and sorted into groups having shell length ranges of 5 mm (25-29, 30-34, 35-39 mm, etc.). The oysters were placed in running sea water for 2 weeks so that most of the mortality resulting from handling occurred prior to initial measurement and placement in the field.

Before placing in the trays, total length to the nearest millimeter and the weight to the nearest 0.1 g of each oyster were recorded. Seventy-two oysters were placed in each tray. Four oysters in the following size ranges were placed in the number of compartments indicated in parentheses: 25-29 mm (1); 30-34 mm (2); 35-39 mm (3); 40-44 mm (3); 45-49 mm (3); 50-54 mm (3); 55-59 mm (2); 60-64 mm (1). Within each tray, the 18 groups of four oysters each were placed randomly in the compartments.

The trays were placed 2 ft apart on frames positioned 0.3 m below mean low water at stations A and N. Total length was recorded for each oyster once per month and weight was recorded every 2 months. The oysters were lightly washed prior to measuring and major attached fouling organisms were carefully removed prior to weighing. Whenever the oysters were removed for measuring, the trays and partitions were cleared of major fouling and algae. Excessive algae was also removed during each mortality check.

The oysters were examined every 2 weeks to monitor mortality. Dead oysters were replaced with live oysters having about the same shell length and a similar shell configuration until the supply of reserve oysters held in trays at each station was exhausted. We assumed that the bias resulting from a lack of growth of each dead oyster for a period of, at most, 2 weeks would not appreciably affect our results, and that the variability in weight was random between the replaced oyster prior to its death and the reserve oyster used to replace it. All dead oysters were replaced until 20 August in the altered area and 24 September in the natural area. Not all of the dead oysters were replaced subsequently. The number of oysters remaining in the trays, therefore, diminished after these dates. The original 144 oysters and their substitutes in each area had diminished to 119 and 45 at the natural and altered sites, respectively, by the end of the study on 18 February 1970. Annual mortality rates were computed by converting all the 2-week rates to instantaneous rates, then totalling and converting the sum to annual rates.

The possibility of infection of the juveniles with *D. marinum* was checked by thioglycollate culture of the flesh (Ray, 1966) of dying oysters which were recovered while still relatively undecomposed.

HYDROLOGY

Eight of the 12 hydrological variables monitored showed apparent seasonal or areal differences (Fig. 2). Temperatures were similar between areas but followed the typical seasonal pattern. Salinity was low in the spring, rose rapidly in June, and remained above 23‰ for the rest of the period

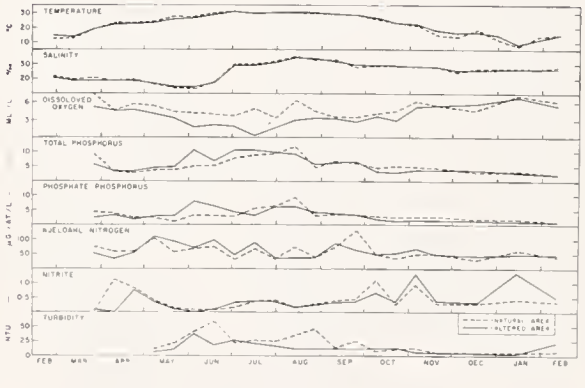

FIG. 2. *Comparisons, between the canal and bayou, of the values of eight hydrological variables by date and area.*

in both areas. Dissolved oxygen was considerably lower in the canal than in the marsh during the late spring and early summer. Total phosphorus and phosphate-phosphorus values rose in late spring in both areas and maintained higher levels in the altered area than in the natural area during this period. Kjeldahl nitrogen levels fluctuated erratically and averages were highest during spring and summer in both areas. Nitrite levels were lowest during late spring and summer in both areas. The waters were more turbid during spring and summer, and turbidity was higher in the natural area. Total and carbonate alkalinity, pH and CO_2 values were similar between dates and areas.

SPATFALL

Only 197 spat attached to the central 100 cm^2 of the smooth surfaces on the suspended plates during the study. Over three-fourths of these spat set on the plates with the smooth surfaces facing down. Setting occurred from 28 May to 1 October in the natural area and from 6 August to 1 October in the altered area, with the peak spatfall in both areas being in late September (Fig. 3). In a previous study in West Bay, Hopkins (1931) observed the first spat settlement in the middle of May. He noted that setting was irregular and took place during short time periods. More recent studies in Galveston Bay showed that spatfall started in late May or early June in 3 of 4 years and in April the other year (Hofstetter, 1960, 1963). During 1969, in Galveston Bay, peak setting

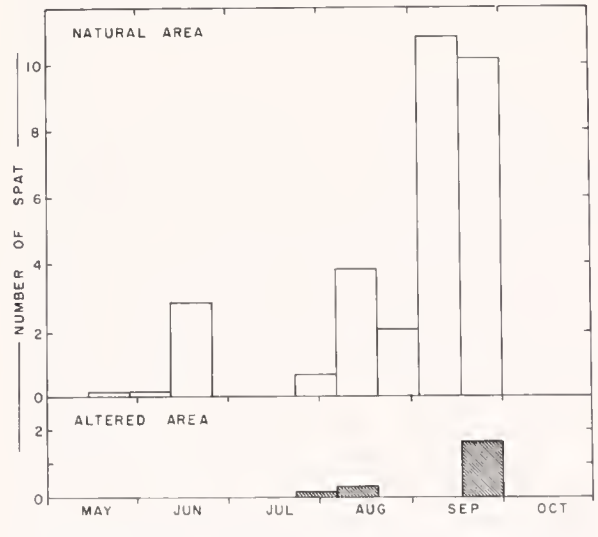

FIG. 3. *Mean number of spat per plate by date and area.*

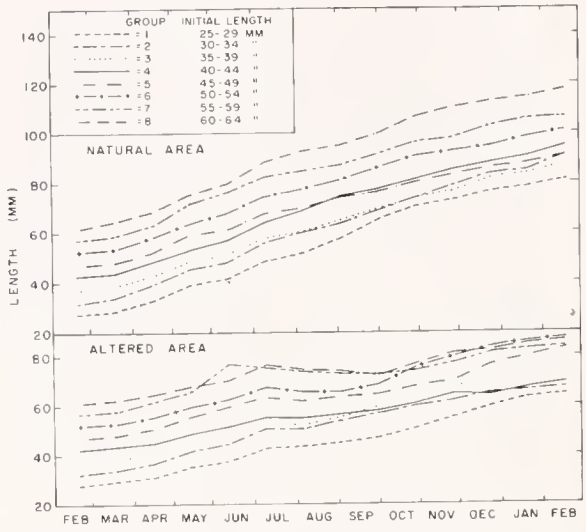

FIG. 4. *Mean lengths of juvenile oysters by size group, date and area.*

of oyster spat was observed in July, with a second setting period observed in September-October (R. P. Hofstetter, personal communication). Our peak spatfall occurred during this second setting period observed in Galveston Bay.

Spatfall in the natural area was about 14 times greater than in the development. The peak spatfall in the natural area, however, was lower than Hofstetter (1959) observed during a light spatfall year, 1958, on the commercial oyster reefs in Galveston Bay. Spatfall greater than a trace occurred over a time period so short that attempts to establish correlations between spatfall and hydrological variables would be meaningless. All setting did occur, however, when temperatures averaged above 25°C. (Fig. 2) and this result was similar to previous data reported by Hopkins (1931) in another area of West Bay.

GROWTH

Growth in length was compared among eight size groups of juvenile oysters for each area (Fig. 4) and between areas with size groups combined (Fig. 5). Growth in the natural area was almost linear and was similar among size groups. In contrast, growth was depressed from July through September in the altered area, especially in the the large size groups. The average increase in length (sizes combined) for the 12 months was 55 mm in the natural area and 32 mm in the altered area, or 72% greater in the natural area. The oysters at the end of the study were about 18 months old and averaged 87 mm. Growth in

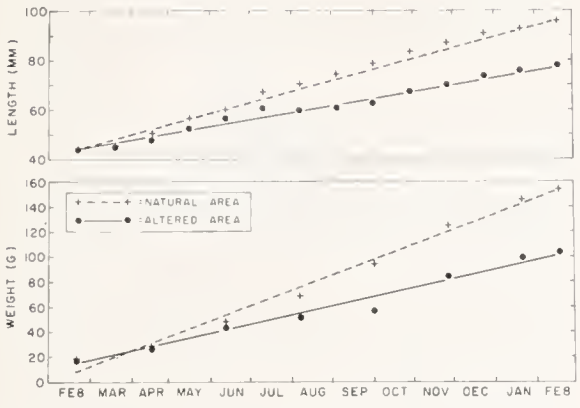

FIG. 5. *Mean lengths and weights of juvenile oysters (size groups combined) by date and area.*

length of juvenile oysters was significantly and positively correlated to temperature in the natural area and to dissolved oxygen in the altered area (Table 1).

The average increment in length observed in the natural area was greater than the maximum reported by Hofstetter (1963) for oysters from commercial reefs in Galveston Bay. In studies of oysters in trays, however, he observed growth rates similar to ours (R. P. Hofstetter, personal communication). Menzel (1955) reported that oysters averaged 75 mm 10 months after setting at Aransas Pass, Texas.

Growth in weight was compared among the size groups by area (Fig. 6) and between areas (Fig. 5). Unlike length, weight increased at a greater rate in the larger oysters in both areas. Similar to length increments, the rate of weight gain was depressed during June-September in the altered area. This depression was most pronounced in the largest oysters. The average increase in weight (sizes combined) was 136 g in the natural area and 86 g in the altered area, or 58% greater in the natural area.

MORTALITY

Annual mortality rates of juvenile oysters were similar among size groups in the altered area, ranging from 82.6-94.0%, whereas in the natural area there was much variation among size groups, ranging from 31.6-77.9% (Fig. 7). Mortality was greatest during the warmest months in

TABLE 1. *Correlation coefficients (r) between hydrological variables and length increments and 2-week mortality rates of juvenile oysters in the natural and altered areas in West Bay, Texas.*

Hydrological variable	Area	Length increment		Mortality rate	
		df	r	df	r
Temperature	Natural	11	0.59[a]	24	0.61[b]
	Altered	11	-0.09	23	0.69[b]
Salinity	Natural	11	-0.06	24	0.76[b]
	Altered	11	-0.40	24	0.70[b]
Dissolved oxygen	Natural	11	-0.41	19	-0.37
	Altered	11	0.56[a]	19	-0.51[a]
Total phosphorus	Natural	11	0.11	19	0.65[b]
	Altered	11	-0.39	19	0.41
Phosphate-phosphorus	Natural	11	0.07	19	0.63[b]
	Altered	11	-0.35	19	0.29
Kjeldahl nitrogen	Natural	11	0.33	19	0.03
	Altered	11	0.26	19	-0.01
Nitrite	Natural	11	0.06	19	-0.08
	Altered	11	0.06	19	-0.13
Turbidity	Natural	11	0.32	19	0.23
	Altered	11	0.13	19	0.05

[a] 95% confidence

[b] 99% confidence

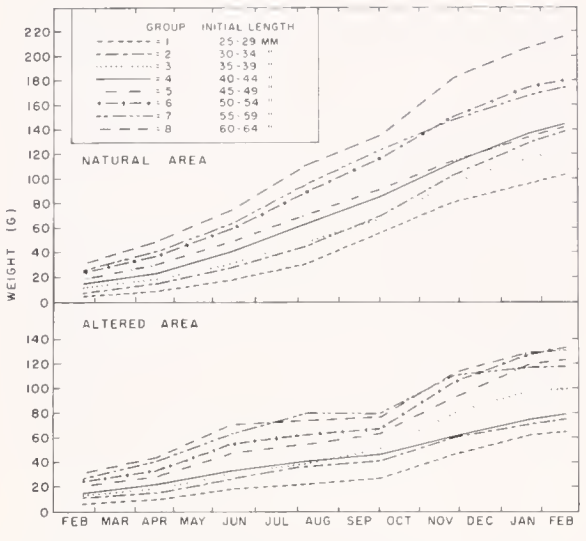

FIG. 6. *Mean weights of juvenile oysters by size group, date and area.*

both areas (Fig. 8). In the altered area, mortalities were above 15% during each 2-week period from 25 June to 15 October. During the same period, mortalities in the natural area ranged between 4 and 11%. The period of peak mortalities did not coincide in the two areas. The months of greatest mortalities were similar to those reported by Hofstetter (1967) on the Galveston Bay commercial reefs but his greatest monthly rate of 10% (in June 1967) was less than we observed from July to October in the altered area.

The average annual mortality rate of oysters (sizes combined) was 52.2% in the natural bayou and 91.2% in the altered canal and was greater

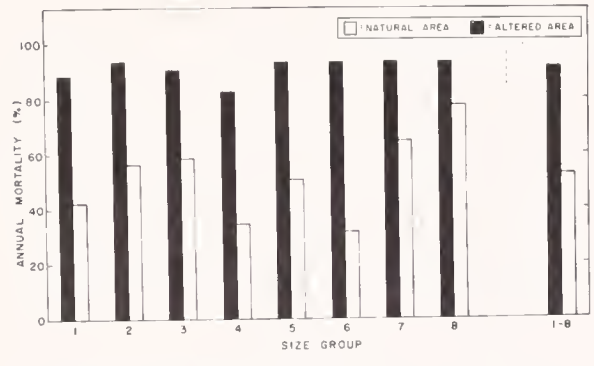

FIG. 7. *Annual mortality rates of juvenile oysters by size group and area, and for size groups combined.*

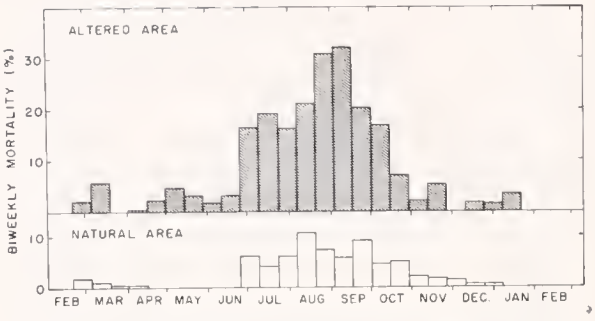

FIG. 8. *Biweekly mortality rates of juvenile oysters by date and area.*

in the canal for every size class. Mortalities on two commercial reefs in Galveston Bay during spring, summer and fall ranged from 35 to 52% during 1963-65 and from 24 to 30% in 1966, a low salinity year (Hofstetter, 1966). A tray study conducted during the same seasons on the same reefs in 1965 had mortalities of 25% for 9 months. Because only the winter, the season of lowest expected mortality, was excluded, the 9-month mortalities were probably not much below the annual rates. Assuming this, the mortalities we experienced, even in the natural bayou, were generally above those in Galveston Bay. In Louisiana, however, Mackin (1961) noted that tray studies by various authors showed that the usual annual mortality of oysters one year old or older is between 50-70% and may be as low as 30% or as high as 90%. Using these criteria, mortality in the natural area of our study was slightly below average whereas, in the altered area mortality was slightly above the high extreme observed in Louisiana.

Juvenile mortality rates were significantly and positively correlated to water temperature and salinity in both areas and to total and inorganic phosphate-phosphorus in the natural area (Table 1). Correlations to temperature and salinity were probably indirect relations. Mackin and Wray (1949, 1950) noted in Louisiana that though excessive mortalities occurred only when there were both high salinity and high temperature, high mortality did not always occur when those conditions prevailed. In Galveston Bay, when Hofstetter (1967) noted the rise and fall of mortality rates with temperature and salinity during 1967, he also reported a high incidence of *D. marinum*. The total phosphate and inorganic phosphate-phosphorus relation with mortality may be the result of over-nutrification similar to that observed on Long Island Sound in 1950-55 where a high concentration of phosphates, resulting from discharge of duck-farm wastes, occurred and

caused heavy algal blooms (Wallace, 1966). In addition to high mortality, the oysters became poorer in quality and size and could no longer be marketed.

Dissolved oxygen was inversely correlated to mortality in both natural and altered areas (Table 1). Low dissolved oxygen was probably not, however, the direct cause of most of the mortality. Mackin and Wray (1949, 1950) observed that, except as part of the disease syndrome, oxygen depletion may be ruled out as a contributing factor of mortalities. In laboratory experiments, Sparks, Boswell and Mackin (1958) showed that oysters survive for several days in less than 1.0 ppm O_2; one survived 120 hr after the O_2 was reduced below 1.0 ppm. It is not clear, however, how other possible stresses (phytoplankton blooms, fish kills and fouling of the water by dead fish) associated with the low dissolved oxygen affected mortality rates.

Other factors possibly causing a higher mortality in the altered area were examined. Dying oysters were collected on 2 July, 30 July and 13 August and cutured for *D. marinum*, with negative results. This was unexpected because infections are usually prevalent each summer in Galveston Bay (Heffernan and Hofstetter, 1960; Hofstetter, 1967). Mudworm, *Polydora websteri*, blisters and boring sponges, *Cliona* sp., occurred at about the same frequency in both areas. Mussels, barnacles and bryozoans on the oysters were about equally abundant at removal time (every 2 months) between the two areas. Boring clams, *Diplothyra smithii*, and oyster drills, *T. haemastoma*, were never observed at either study site.

DISCUSSION

Setting, growth and survival rates observed during this study were much more favorable for oyster production in the natural bayou than in the small dead-end canal located in the housing development. Our study was not representative of either the entire natural marsh or altered area since we intentionally chose study locations in the upper reaches of each area, furthest from the bay. We located away from the open bay to monitor more completely the differing effects the natural marsh with its bayous and the development with its greatly altered habitat may have on the oysters. Also within the development the canals furthest from the bay were often the narrowest and were usually located in areas where most of the houses had already been built. We therefore believed that whatever impact the housing development might have on the oyster environment would be greatest at the site selected.

The standing crop of oysters in the natural bayou was low probably because of lack of suitable substrate for attachment. Therefore, production could be increased, at least during some years, by providing more substrate.

The standing crop of oysters on the bulkheads in many areas of the development was and is high. In the spring of 1969, adult oysters were more abundant than now in the development canal where station A was located. A majority of those attached above the mean low tide level died during the summer of 1969. This heavy mortality did not occur in the canals located close to the bay (Fig. 1). Indications are that environmental conditions for oyster production in the canal at station A were not as unfavorable in one or both of the previous 2 years as in 1969 because spatfall and subsequent survival were apparently more successful in at least one of the immediately preceding years.

We believe that poor setting and growth, and high mortality in the canal were caused directly or indirectly by heavy plankton blooms that occurred during the summer. During these blooms, the oxygen level decreased drastically at night, resulting in at least four fish kills from May to August. Either high concentrations of plankton, or low dissolved oxygen, or "foul" water resulting from the decaying fish, or all three, probably depressed setting, growth and survival. Previous experiments have shown that oyster feeding may severely diminish or cease altogether in either high plankton (Loosanoff and Engle, 1947) or low oxygen (Sparks *et al*, 1958) concentrations, and that some species of algae produce toxic metabolites which can kill oyster larvae (Davis, 1969). Also, the need for "nonfouled" water for good oyster production has been indicated (Galtsoff, 1964; Wallace, 1966).

Possible causes of heavy plankton blooms and resulting oxygen depletions in the upper end of the development in 1969 included poor water circulation, inadequate water exchange and high nutrient levels. Wind-driven circulation responsible for reaeration of the waters in the development is less than in the natural area because of houses blocking and diverting prevailing winds and because many of the canals are narrow and are perpendicular to the direction of prevailing summer winds. Water depths at mean low tide in the development averaged about 1.5 m but were often much greater, sometimes over 3 m, whereas depths in the natural area averaged about 0.6 m but were always less than 1 m. With the average tide level change of 0.3 m, this means that only about one-fifth of the volume of water in the development exchanges with the bay during a normal tidal cycle, whereas about one-half exchanges per cycle in the natural area. Nutrient

levels were about the same (nitrogen) or slightly higher (phosphates) in the canal than in the bayou. It is possible, however, that because of reduced water exchange, nutrient levels in parts of the development are too high to maintain a balanced ecological system.

The feasibility of utilizing bulkheaded canal areas for economic oyster production appears doubtful in developments unless the canal systems are designed to insure good water circulation. Though these areas may have enough years of good oyster production to maintain a sizable standing crop, it is unlikely that the production would be consistent enough to support intensive commercial utilization.

ACKNOWLEDGMENTS

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A PARASITE AND DISEASE SURVEY OF CONNECTICUT OYSTERS

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ABSTRACT

Histologic sections of 1,337 oysters, Crassostrea virginica, *from New Haven Harbor, Connecticut, were examined for the presence of parasites and disease. Seven parasites were found, including types resembling plasmodia of* Minchinia costalis *and* M. nelsoni. *One oyster was found with a neoplasm and seven others with a disorder of the ova of unknown etiology. There was no evidence of any disease epizootic in the New Haven area, although one moribund oyster was found to be heavily infected with plasmodia of the* M. costalis *type.*

INTRODUCTION

Widespread damage to the oyster *(Crassostrea virginica)* resources of Delaware, Chincoteague and Chesapeake Bays has been caused by protozoan parasites (Haskin, Canzonier and Myhre, 1965; Andrews, 1967; Couch and Rosenfield, 1968). Oystermen in other areas of the Atlantic seaboard are concered about the possibility of similar disease-caused mortalities in their local waters.

The New York State Conservation Department in 1965 requested the National Marine Fishery Service Biological Laboratory, Oxford, Maryland, to examine oysters taken from Long Island waters. A small percentage of these oysters, all but one of which were from Great South Bay, were infected with plasmodia of *Minchinia nelsoni* [1].

METHODS

Both native New Haven oysters and oysters introduced to New Haven waters from Norwalk, Connecticut, were sampled each month in 1966 and 1967. No mortalities had been noted in Norwalk oysters, and it was thought that these animals might not have had previous experience with *Minchinia,* and therefore be more susceptible to the disease. Introductions of Norwalk oysters were made in November 1965, and in February, May and August 1966. The Norwalk oysters were suspended in wire trays above the New Haven oyster beds. Monthly samples of 15 oysters were taken from the beds and from the trays of introduced oysters.

The oysters were scrubbed, opened and 5-7 mm-thick transverse slices were taken from the palps through the visceral mass. These tissues were fixed in Davidson's, dehydrated in alcohol and embedded in paraffin. Six micron sections were stained with hematoxylin and eosin or Azure-eosin and examined for parasites and pathology.

RESULTS AND DISCUSSION

Seven types of protozoan parasites were found in the oysters. In order of decreasing prevalence, they were: a small ciliate found on the gill epithelium, a larger ciliate found on the gills, an amoeboid organism in the gut epithelium, plasmodia of sporozoan parasites resembling *M. nelsoni* and *M. costalis,* a ciliate in the tubules of the digestive diverticula and spores of a gregarine, *Nematopsis ostrearum.* A neoplastic condition was found in one oyster and abnormal ova

[1] (Unpublished report, BCF Biological Laboratory, Oxford, Md., to Division of Marine Fisheries, New York State Conservation Department, 5 May 1966).

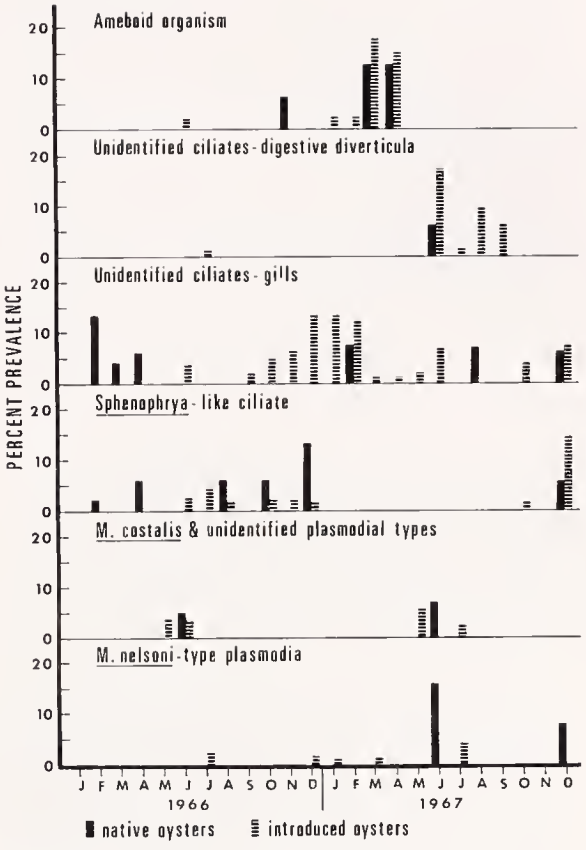

FIG. 1. *Prevalence of protozoan parasites.*

in seven oysters. The monthly prevalence of each parasite was followed for two consecutive years (Fig. 1). The low prevalences of the neoplasm and the diseased ova have not been graphically presented. The specific identification of the ciliates found in histologic sections was not determined since neither living specimens nor whole mounts were available for study. The specific identification of the haplosporidans was based solely on plasmodial morphology because the definitive stages (spores) were absent from the study material.

Small, gill-infesting ciliate (Fig. 2)

These small ciliates varied in size from 4.5 x 7.0 μ to 6.5 x 10.0 μ and had an elliptical macronucleus approximately 1.0 μ in diameter. The organisms were found on, or slightly embedded in, the host's gill and resembled small thigmotrichs of the family Ancistrocomidae which are known parasites of lamellibranch gills (Kozloff, 1946a, 1946b, 1946c). There were seldom more than two of these organisms per oil immersion field. Maximum prevalence of 13% was reached in the winter months, and both the native and introduced oysters were almost equally infected.

Sphenophrya-like ciliate (Figs. 3, 4)

This organism exhibited a variable morphology, reaching maximum size of 15.0 x 23.0 μ. The maximum size of the elliptical macronucleus was 6.5 x 12.0 μ and the micronucleus was approximately 2.5 μ in diameter. Cilia were not seen. The organism appeared to be attached to the gill epithelium, and frequently had the semicircular or crescent-shape characteristic of the genus *Sphenophrya*. Infestations seldom involved more than one or two gill filaments, although the filaments were often heavily infested. This parasite occurred in a small percentage of oysters throughout the year, was slightly more abundant in fall and winter and was more prevalent in 1966 than in 1967. Again, both the native and introduced oysters were about equally affected. At the densities encountered in this study, neither this ciliate nor the preceding organism caused any observable pathology.

Amoeboid organism (Figs. 5, 6, 7)

The gut epithelium of oysters infected by this organism had a vacuolated and disorganized appearance (Fig. 5). The parasite occurred in a round form and in an elongate, apparently motile form (Fig. 6). The round form reached a maximum of 10.5μ in diameter with a nuclear diameter of approximately 2.5 μ. A centrally located nucleolus was sometimes visible, but was usually obscured by the densely stained nucleoplasm. Cytoplasm was granular and often had a vesicular appearance. The round form appeared to be a cyst within a host cell, often containing two organisms (Fig. 7). Whether the host's cells were phagocytes or epithelial cells could not be determined. Elongated forms were often seen extracellulary. Possibly they were migrating from the epithelium toward the lumen

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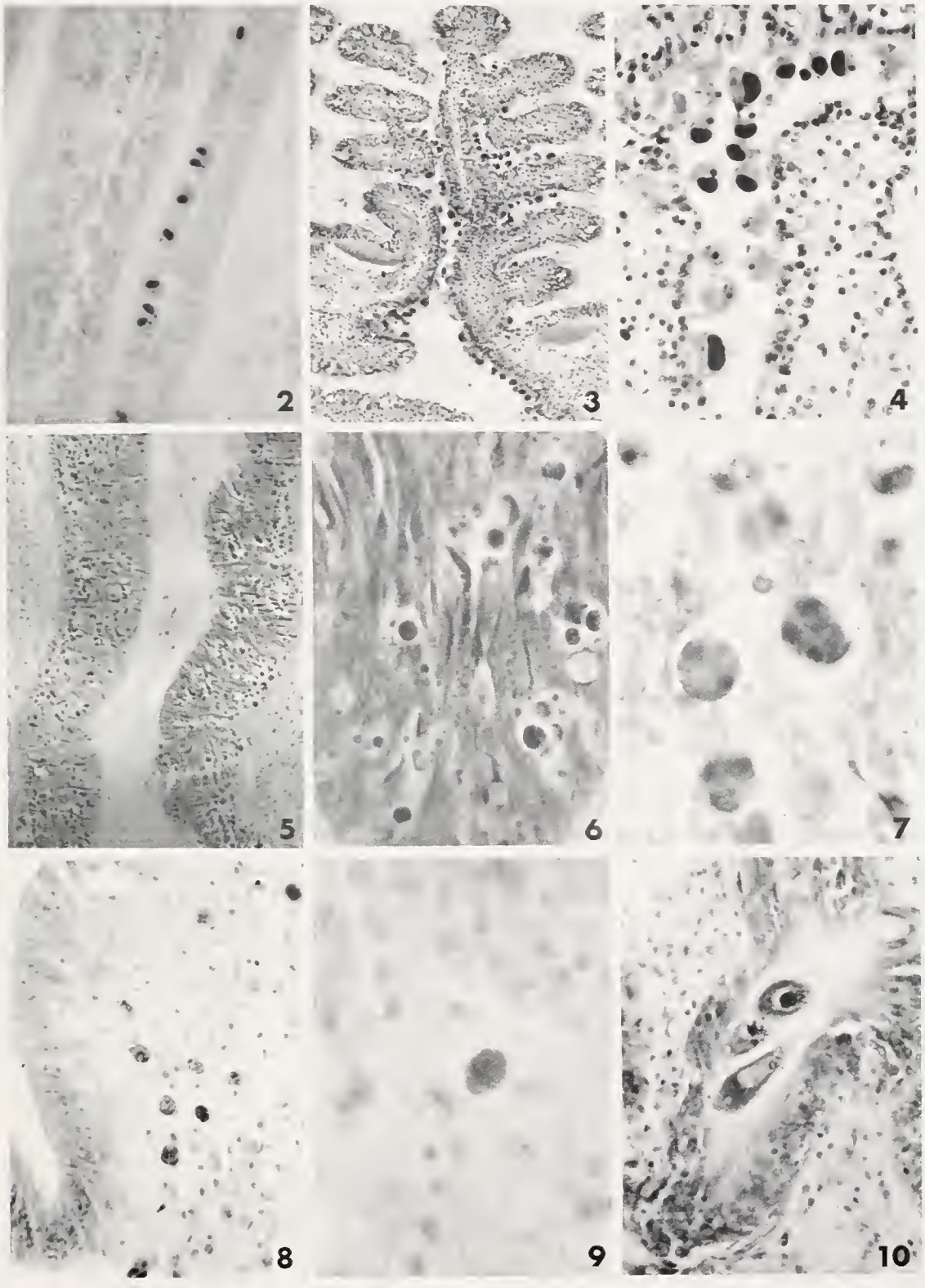
FIGS. 2-10. *Protozoan parasites seen in histologic sections of Connecticut oysters. Azure-eosin stain.* FIG. 2. *Small ciliate infecting gills, X700;* FIG. 3. *Large ciliate infecting gills, X175;* FIG. 4. *Large ciliate infecting gills, X700;* FIG. 5. *Ameboid organism in intestinal epithelium, X175;* FIG. 6. *Ameboid organism in intestinal epithelium showing encysted and elongated forms, X700;* FIG. 7. *Ameboid organism in intestinal epithelium showing cysts containing two organisms, X1750;* FIG. 8. *Plasmodia in mantle, X700;* FIG. 9. M. costalis *type plasmodium in mantle, X1750;* FIG. 10. *Ciliate in digestive diverticula, X700.*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of the gut. In the lumen these forms were often seen within phagocytes. The parasite seemed to be restricted to the gut epithelium, usually of the intestine and stomach, but in one heavy infection it also occurred in the digestive diverticula. It was never seen in other tissues, nor did it ever penetrate the basement membrane of the epithelium. There was no evident hemocytic response in the tissues, but in advanced infections the gut lumen was filled with phagocytes.

Twelve of the 14 infections were found in March and April, and 11 of the 14 occurred in the introduced oysters. All but one infection occurred in 1967.

Haplosporidans (Figs. 8, 9)

Small plasmodia were seen in various tissues and were most common in the vesicular connective tissue of the gill and palp regions. In size and structure they resembled the plasmodial stages of *Minchinia* sp. described from oysters in Delaware, Chesapeake and Chincoteague Bays (Wood and Andrews, 1962; Haskin, Stauber and Mackin, 1966). Only one oyster was heavily parasitized; most infections were of a light or possibly remissive (Farley, 1968) nature. The heavily parasitized oyster was moribund and had an *M. costalis* type infection. Increased numbers of hyaline hemocytes (Farley, 1968) were seen, and many of the infected oysters contained abundant pigment in the connective tissue.

Twenty-five oysters contained these organisms. In 10 of these the plasmodia were morphologically similar to those described for *M. nelsoni*, while five others resembled *M. costalis*. Ten other infections were considered to be remissive stages. It must be emphasized that no spores were found and identifications must be considered tentative.

Prevalence of these organisms was highest in mid-summer samples. The percentage of infected oysters was greater for the native population, and was slightly greater in 1967 than in 1966.

Katkansky and Warner (1970) found *M. costalis* infections in *C. virginica* in Tomales Bay, California, during July 1967 and 1968. Spores were found in one oyster. These oysters were originally imported to California from New Haven, Connecticut, in October 1966.

Ciliates in the digestive gland (Fig. 10)

The ciliates found in the lumina of the digestive diverticula appeared to belong to the family Ancistrocomidae. Ciliates similar to these have been reported from oysters in Chesapeake Bay (Burton, 1963) and in Louisiana (Mackin, 1962). No host response was evident, and infestations in the Connecticut oysters were always light.

This parasite was found in 12 oysters. It was present only during the summer months, was more abundant in introduced oysters than in bed oysters and was more abundant in 1967 than in 1966.

Nematopsis ostrearum

A few spores of this gregarine were found in the gill and mantle of one native oyster from the June 1967 sample. *Nematopsis ostrearum* was first described by Prytherch (1940). Further investigation by Sprague and Orr (1955) failed to demonstrate pathogenicity in the molluscan host. Landau and Galtsoff (1951) found the parasite in 30% of 3-year-old oysters from Great South Bay, Long Island, in 1942. These oysters had been transplanted from New Haven in 1939. The infection was primarily confined to the adductor muscle. The absence of this tissue in sections examined during the present study might account for our single observation. Landau and Galtsoff (1951) speculate that *Nematopsis* may have been introduced to the Connecticut area with seed oysters brought from Delaware Bay.

Diseased Ova

This disorder was not noted during the initial examination of the Connecticut material. It was recently discovered in oysters from the Atlantic, Gulf and Pacific coasts of the United States (Farley and Buchanan, personal communication). Reexamination of the native New Haven oysters revealed the presence of this disease in seven specimens from April and July collections of 1966 and 1967. Introduced oysters were not reexamined. The disease is characterized by nuclear and cytoplasmic hypertrophy. Abnormal DNA replication in the nucleus is revealed by fuelgen staining. The disease appears to affect only a few eggs in any given oyster.

Neoplastic disease

One introduced oyster from the June 1966 sample had a proliferative cell disease of apparent mesenchymal origin. The primary lesion was in the gill, but the atypical cells characterizing this disease were found scattered throughout the blood sinuses and vesicular connective tissue of the oyster. Studies of this and similar conditions in other mollusks are continuing at our laboratory.

DISCUSSION

The sampling procedures used in this study were designed to investigate the epizootiology of *Minchinia* disease, similar to an earlier study by Couch and Rosenfield (1968). Although there appear to have been some differences between 1966 and 1967 parasite prevalences, the numbers of

oysters contained in any one sample were too small to provide statistically significant data. The parasite prevalence data would have been more meaningful had larger samples been taken from the native population.

The study did indicate that at least seven protozoans (three ciliates, two or more haplosporidans, an amoeboid organism, and *Nematopsis)* parasitize oysters in the New Haven area. Because of their low prevalence and the apparent lack of host response to all but the haplosporidans, these organisms did not appear to have been epizootic or to have been causing heavy mortalities in oysters at the time of this study. The neoplastic disease seemed to be an isolated occurrence. The importance of the diseased ova condition could not be evaluated. Studies on oysters from other areas indicate that this may be an ubiquitous condition normally occurring in low prevalences and low intensities.

ACKNOWLEDGMENTS

The author is indebted to Dr. Anthony Calabrese, National Marine Fishery Service Biological Laboratory, Milford, Connecticut, for collection of the samples and Mr. C. A. Farley, National Marine Fishery Service Biological Laboratory, Oxford, Maryland, for aid in parasite identification, and interpretation of the results.

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THE HISTOPATHOLOGICAL EFFECTS OF VARIOUS DOSES OF IONIZING RADIATION ON THE GONAD OF THE OYSTER, *CRASSOSTREA GIGAS* [1, 2]

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ABSTRACT

Early developing female oysters (Crassostrea gigas) *were irradiated with various doses of gamma radiation. Three degenerative syndromes were observed in the gonads of oysters irradiated with sublethal doses. Although some residual (germinal) cells and ova were killed directly by the radiation, the ultimate manifestation of gonad degeneration — denudation — was caused by radiation effects on the cell renewal system of this tissue. Mitotic inhibition of the residual cells occurred and, with the possible exception of 1-Krad oysters, was permanent. Thus, the cell renewal system of the gonad is destroyed and eventually the gonad becomes lined by squamous cells. Gonad degeneration was rapid in lethally irradiated oysters but was not related to effects on the cell renewal system. Rather, the ova, ovocytes and residual cells in these oysters were killed directly by the radiation.*

INTRODUCTION

Recent renewed interest in invertebrate pathology has stimulated many studies on basic cellular phenomenon in invertebrates such as reaction to injury and wound repair, inflammation, the ability of blood cells to replace lost or injured cells, reactions to tissue implants and the existence or non-existence of immune mechanisms and tumors. These studies have provided the impetus for the revival of interest in comparative pathology.

To our knowledge, no one has conducted an extensive study on the histopathological effects of ionizing radiation on an invertebrate. We feel the oyster represents an ideal invertebrate for such a study as more is known about cellular and histopathological phenomena of this animal than any other invertebrate, excluding insects.

The fact that ionizing radiation affects the ability of a cell to divide was one of the earliest discoveries of radiation biologists. Bergonie and Tribondeau (1906) described the selective action of X-rays on epithelial tumors and tumors caused by beta-irradiation. As a result of their experiments, they formulated the following "law": "X-rays are more effective on cells which have a greater reproductive activity; the effectiveness is greater on those cells which have a longer dividing future ahead, on those cells the morphology and the function of which are least fixed." In other words, the sensitivity of cells to irradiation is in direct proportion to their reproductive activity and inversely proportional to their degree

[1] This study was made possible through a grant, UI-1039-03, (Shellfish Sanitation Training Grant) from the U. S. Public Health Service.

[2] Part of a thesis in partial fulfillment of the requirements for a Doctor of Philosophy degree in Fisheries, University of Washington, by the senior author.

of differentiation (Bacq and Alexander, 1961).

Studies on the cellular or histopathological effects of ionizing radiation on invertebrates are generally restricted to protozoans and insects. There is a relatively voluminous literature on protozoans that encompasses all areas of radiation biology. Many of the studies of insects are confined to the effects on mature sex cells. Radiation has been used to elucidate regenerative or repair mechanisms in planarians (Dubois, 1949; Mix and Sparks, 1969), and histopathological effects of irradiation were also described by Mix and Sparks (1969). Studies of other invertebrates, except for LD_{50} determinations and the effects of irradiation on mature sperm and ova are limited. Mix (1970)[3] reviewed the available information on cellular and histopathological effects of ionizing radiation on invertebrates.

Mix and Sparks (1970) described the histopathological degeneration of the digestive tissues of the oyster, *Crassostrea gigas*, after various doses of gamma irradiation. These authors also described regeneration of digestive tubules damaged by irradiation (Mix and Sparks, 1971). With the exception of these studies, virtually no work has been done on histopathological syndromes and, except for some insects, few results have been interpreted with regard to effects on cell renewal systems. The present study describes degeneration of the germinal elements of the gonad and its relation to the cell renewal system of that organ.

MATERIALS AND METHODS

Only a brief description of materials and methods will be included since they were described in an earlier paper (Mix and Sparks, 1970). Five hundred second-year oysters (in their third year of life) were divided into nine groups of 55 animals each and color-coded with Fuller Speedtec enamel. The eight experimental groups of oysters were lowered separately into the irradiator in sealed boxes and irradiated with gamma radiation from a cobalt-60 food irradiator (M. K. II. Serial No. 2 designed by Brookhaven National Laboratory). Control oysters were simply lowered into the unit. Doses given to the eight groups were: 1, 5, 10, 20, 50, 100, 200 and 400 Krads at a dose rate of 6 Krads/min. Dose rate was determined by the Fricke method (Hine and Brownell, 1956) and represents absorbed dose.

After irradiation, the oysters were moved to the U. S. Public Health Service, Northwest Marine Health Sciences Research Laboratory located on Burley Lagoon, Purdy, Washington, and maintained in experimental flats. The flats were wooden troughs 102 cm wide, 203 cm long, 25 cm deep, and contained 180 liters of seawater. The usual flow rate in the flats was 12 liters/min. To accommodate the relatively large number of oysters initially present, it was necessary to use two of the flats for two days. After removing the first two days' samples, the remainder were moved to one flat.

Three oysters from each dosage group were sampled at 0, 1, 2, 4, 8, 12, 24, 48 and 96 hr and two oysters from each group at 6, 8, 12, 16, 24, 30, 43, 59, 76 and 90 days. Each oyster was immediately shucked and fixed individually for 24 hr in 16 oz squat jars containing 300 ml of Davidson's fixative. They were then dehydrated through a graded series of ethanol solutions and xylene for 24 hr, embedded in Paraplast, sectioned at approximately 7μ and stained with Harris' hematoxylin and eosin.

RESULTS

Normal development and histology

The genus *Crassostrea* exhibits protandric hermaphroditism. The majority of individuals begin sexual development as males and later change to females -- usually during the third year for *C. gigas* (Katkansky and Sparks, 1966).

Loosanoff (1942) described the annual sexual cycle of *C. virginica* on the Northern Atlantic Coast. Katkansky and Sparks (1966) partially confirmed this description for *C. gigas* and we (Sparks, Mix, Weitkamp, DesVoigne and Jones, unpublished research), have further substantiated these finding for the latter species. The gonad is in a state of quiesence during the cold season. Proliferation of gametes occurs in the spring and *C. gigas* generally matures by late summer. If spawning does not occur (it frequently does not on the West Coast of the United States), resorption of gametes by leucocytes begins during the fall and continues into the winter and following spring.

The histological structure of the gonad thus varies, depending on the age of the oyster, degree of maturation, season, temperature and the amount of food present. In adult oysters in which ova or sperm have been resorbed, the germinal epithelium consists of undifferentiated germ cells. As gametogenesis progresses, maturing gametes eventually fill the lumen of the follicle. Since the

[3] Mix, M. C. 1970. The histopathological effects of ionizing radiation on the Pacific oyster, *Crassostrea gigas*. An examination of degenerative syndromes, cellular reparative mechanisms, and their relation to normal cell renewal systems. Ph.D., Thesis. Univ. of Washington. 168 p.

transformation of germinal cells into ova is a gradual process which does not involve all cells of the germinal epithelium at the same time, numerous undifferentiated (residual) cells are usually found along the inner periphery of the follicle (Fig. 1) (Galtsoff, 1964). After maturation, the gonads contain mature ova or sperm as well as undifferentiated cells. Phagocytosis becomes pronounced as large numbers of leucocytes invade the gonoducts. Following resorption, the oyster is in an undifferentiated stage and sex cannot be accurately determined. Ovocytes, when sufficiently developed, may be distinguished from spermatocytes by their large nuclei and granular cytoplasm (Galtsoff, 1964).

Histopathology of irradiated oysters

Four histopathological syndromes will be described: one acute, lethal radiation syndrome of oysters receiving 200 or 400 Krads and three acute, sublethal syndromes of oysters receiving 1, 5, 10, 20, 50 or 100 Krads.

1. Lethal syndrome

24 hr (after irradiation)

The only cells affected were a small number of early developing ovocytes, which were karyolytic or pycnotic. Residual (generative or germinal) cells remained normal and mitotic activity was common in these cells. Light leucocytic infiltration was observed in some gonoducts.

48 hr

Leucocytic infiltration increased in most oysters. Residual cells often possessed nuclei with clumped chromatin or were karyolytic. Larger ova possessed vaculated cytoplasm and some pycnotic ova had been sloughed into the lumen of the gonoduct.

96 hr

Degeneration, manifested by nuclei with clumped chromatin, vacuolated cytoplasm, pycnotic and sloughed ova and leucocytic infiltration, continued. The germinal epithelium was pale, basophilic staining and large areas were necrotic.

144 hr

By 144 hr, many fibroblasts had infiltrated the gonoducts, germinal epithelium and surrounding areas. Intraluminal leucocytes were necrotic and liquified. Ciliated epithelium of the peripheral ducts was necrotic and many residual cells, ovocytes and ova had become pycnotic and were being sloughed.

2. Sublethal Syndromes

Three patterns of degeneration are described. The first consists of oysters irradiated with 1 Krad, the second of oysters irradiated with 5, 10, or 20 Krads and the third of oysters irradiated with 50 or 100 Krads.

a. 1-Krad oysters

24-96 hr

The peripheral gonoducts were invaded by leucocytes by 24 hr. The number of blood cells in the gonoducts increased through 48 hr. No pycnotic ova or residual cells were observed.

96-144 hr

Massive number of blood cells and fibroblasts had invaded the gonoducts and surrounding tissue (Fig. 2). Few degenerating ova or residual cells were found.

6-12 days

The inflammatory response continued, but became less intense after 8 days. No changes were seen in the residual cells, but some developing ova became pycnotic and had been sloughed.

12-16 days

Fibroblasts became the predominant cell type seen in and around the gonads, with heaviest concentrations found in the peripheral ducts. Pycnotic and sloughed ova were commonly observed and the germinal surfaces were becoming denuded by 16 days.

16-76 days

The germinal epithelium was generally denuded by 30 days, but by 50-60 days some maturing ova were observed and by 76 days, a few normal-appearing ova were seen in the remaining portion of the gonad (Fig. 3).

FIG. 1 *The gonad of a nonirradiated female oyster. 128X.*

FIG. 2. *The gonad of a female oyster 144 hours after irradiation with 1 Krad. Note the invasion of leucocytes throughout the gonad. 128X.*

FIG. 3. *The gonad of a female oyster 76 days after irradiation with 1 Krad. Note the scattered normal-appearing ova. 128X.*

FIG. 4. *The gonad of a female oyster 8 days after irradiation with 20 Krads. Note the pycnotic ova among the normal residual cells. 320X.*

FIG. 5. *The gonad of a female oyster 16 days after irradiation with 20 Krads. Note the sloughed pycnotic ova. 320X.*

FIG. 6. *The completely denuded gonad of an oyster 43 days after irradiation with 50 Krads. 128X.*

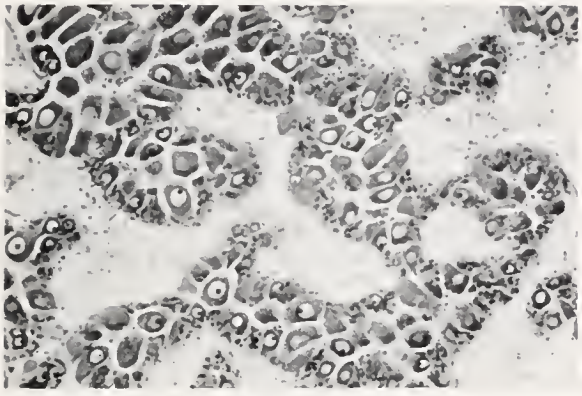

FIG. 1

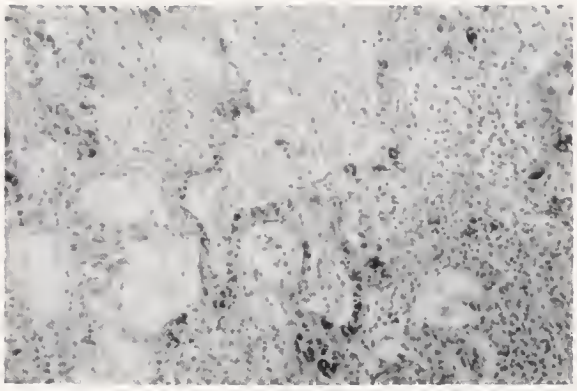

FIG. 2

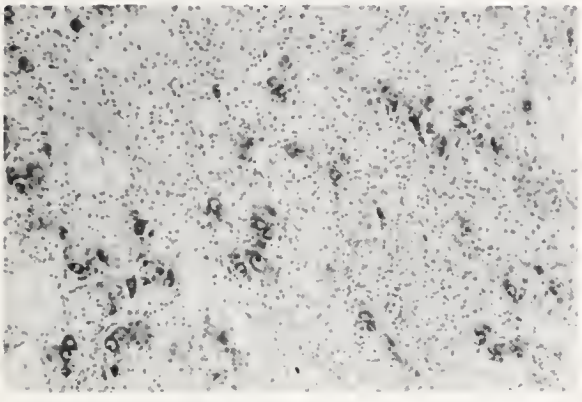

FIG. 3

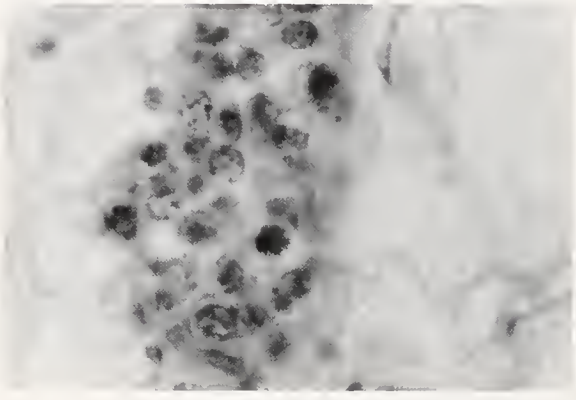

FIG. 4

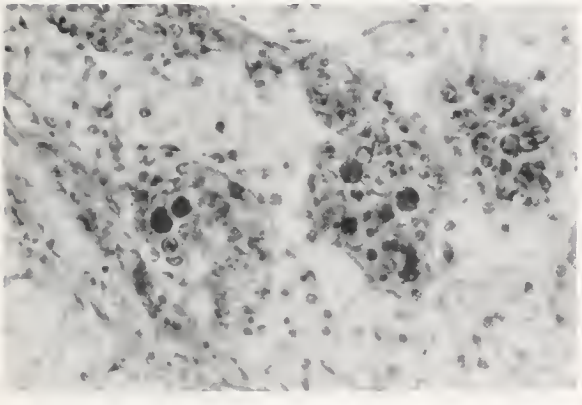

FIG. 5

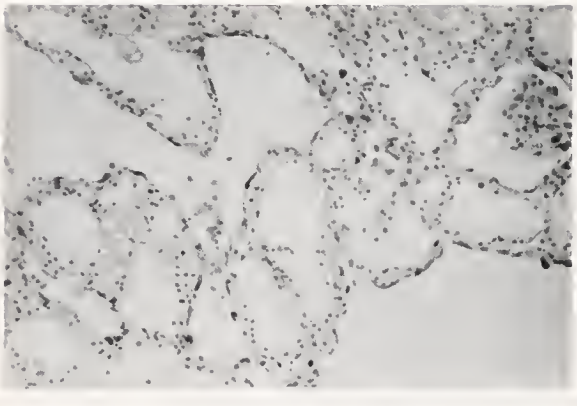

FIG. 6

b. 5-, 10- and 20-Krad oysters

24-96 hr

Light leucocytic infiltration was observed in most areas of the gonad through 96 hr. Pycnotic ova appeared at 96 hr, earlier than in 1-Krad oyster.

96hr-8 days

The number of pycnotic ova increased dramatically by 144 hr. Inflammation of the gonad continued as adjacent blood vessels became filled with blood cells, followed by pavementing of the vessel wall and subsequent migration into the gonadal tissues. Leucocytes (primarily granular) and fibroblasts were commonly observed in the cellular infiltrate. By 8 days many developing ova were pycnotic and were frequently sloughed into the lumen of the gonoduct (Fig. 4). Residual cells typically possessed clumped chromatin or were pycnotic.

8-16 days

Moderate to heavy leucocyte and fibroblast infiltration continued. The germinal epithelium was populated by necrotic ova and residual cells interspersed with normal cells and cells in early stages of degeneration (Fig. 5). Clumped chromatin and vacuolated cytoplasm were seen in larger ova.

16-30 days

Few further changes were observed. By 30 days most of the germinal epithelium was necrotic. Gonoducts were filled predominately with phagocytes.

30-43 days

By 43 days scattered residual cells and some pycnotic ova were all that remained of the germinal epithelium.

43-76 days

Complete denudation of the germinal surface had occurred by 59 days and most ducts were lined by abnormal squamous cells (Fig. 6).

c. 50- and 100-Krad oysters

The degenerative syndrome for these oysters followed basically the same sequence described previously, differing only in time of appearance (see Table 1 which summarizes the degenerative syndromes). The basic difference was in the magnitude and time of appearance of the inflammatory response. In general, leucocytic infiltration occurred later and was initially less intense than in oysters irradiated at lower doses, but increased sharply at 20-30 days — much later than in lower dose groups.

DISCUSSION

Age, sex and maintenance temperatures are all factors that affect the radiation response of mammals (Rugh, 1953). We felt that irradiation of second year oysters, in February, would reduce the variability of these factors, and would thus minimize their effect on radiation-induced pathological effects. Pacific oysters at this time of year have generally completed resorption of unspawned, or residual, gonadal products from the previous summer and, therefore, the large numbers of leucocytes associated with resorption are not present to confuse or alter the radiation response. The transition from the male phase to the female phase in these protandric hermaphrodites typically occurs during the winter and early spring of their second year on the bed (Sparks, Mix, Weitkamp, and DesVoigne, unpublished research). Thus, oysters used in this study were all generally in the same stage of sexual development — early developing female.

The "Law of Bergonie and Tribondeau" is usually accepted as a generalization concerning the greater effectiveness of ionizing radiation on tissues with rapidly dividing cell populations than on tissues in which little or no cell division occurs (Haber and Rothstein, 1969). Considerable progress has been made in recent years in under-

TABLE 1. *Time sequence of degeneration in gonads of oysters exposed to various doses of radiation.*

Pathological condition	1 Krad	5 Krads	10-20 Krads	50 Krads	100 Krads
Aggregation of leucocytes and fibroblasts in blood spaces	4-12 days	4-16 days	6-16 days	6-16 days	6-16 days
Earliest period of infiltration into germinal epithelium	24 hr	48 hr	48 hr	96 hr	96 hr
Time of heaviest inflammation	144 hr	144 hr	8 days	12-30 days	12-30 days
Time germinal epithelium denuded	—	43 days	30-43 days	30-43 days	30-43 days
Proliferation following denudation	yes(?)	no	no	no	no

standing normal cell population kinetics in mammals, thus providing background information for more precise interpretation of radiation effects (Stohlman, 1959). Tissues with rapid cell turnover depend for their integrity on continued cell production, and perturbations of the cell renewal systems can be brought about, perhaps most dramatically, by exposure to ionizing radiation (Patt and Quastler, 1963).

The effects of irradiation on most cell renewal systems can be reduced to a simple basic scheme; initially there is impaired cell production with little change in rate of cell decay, which leads to cell depletion; the degree of depletion depends on the degree to which production is impaired. Thus, radiation causes aplastic cytopenia, or, if a cell population vanishes completely, acytosis (Patt and Quastler, 1963). More or less complete restoration of the system is possible if the organism survives until the proliferation of stem cells can be successfully resumed. The reactions to various oyster tissues and cells may be explained within this scheme, although it is difficult to make a precise analysis because of the paucity of knowledge regarding cell renewal systems in this organism.

The gonads of the oyster contain the most active cell population with regard to proliferation or mitotic cell division. Normally, microscopic examination reveals many mitoses in the germinal epithelium of this tissue. Following all levels of irradiation, there is impaired cell production as evidenced by: the absence of mitotic figures by approximately 144 hr after irradiation; the appearance of pycnotic residual cells indicating probable abortive mitosis shortly after irradiation; and finally, the eventual denudation of the gonad after irradiation with doses greater than 1 Krad. Thus, two mechanisms of aplastic cytopenia occur in germinal cells of the gonad. Initially, necrosis occurs and is characterized by a morphological sequence consisting of chromatin clumping, pycnosis and karyolysis. The term "interphase death" has been used to describe this syndrome. However, this characterization is not entirely appropriate; "interphase" should be restricted to cells between mitoses (Patt and Quastler, 1963), which would exclude ovocytes and maturing ova. The second mechanism of aplastic cytopenia involves post-irradiation mitotic inhibition and, ultimately, death of the cell.

Following mitotic inhibition, complete repair of radiation damage apparently does not occur in the remaining residual cells since repopulation of denuded germinal epithelium was not observed in any irradiated group, with the possible exception of oysters irradiated with 1 Krad. There are several potential sources of ova observed in 1-Krad oysters 76 days after irradiation. These include: small ovocytes that are not damaged by irradiation and subsequently mature; ovocytes that may repair (subcellular repair) the initial radiation damage and then mature; they may arise from uninjured or repaired (subcellular repair) residual cells or; they may come from differentiated leucocytes.

To recapitulate: the germinal epithelium of the gonad (proliferating residual cells and small ovocytes) is destroyed by all doses of irradiation used in this study (1-400 Krads). Acute degeneration consists of rapid cell death caused directly by irradiation and is manifested by chromatin clumping, karyolysis, pycnosis and subsequent cell sloughing soon after irradiation. Mitotic inhibition of the remaining residual cells also occurs and, with the possible exception of 1-Krad oysters, is permanent. Thus, the cell renewal system of the gonad is destroyed and eventually the gonad becomes lined by squamous cells and is denude of any normal cell type.

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CHRONIC EXPOSURE OF OYSTERS TO DDT, TOXAPHENE AND PARATHION [1]

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ABSTRACT

Oysters, Crassostrea virginica, *were reared from juveniles (27 mm mean height) to sexual maturity in flowing sea water chronically polluted with low levels (3.0 ppb, μg/l, or less) of DDT, toxaphene and parathion. The study was performed in two phases over a period of 2 years, the insecticides being administered as a mixture during the first phase and separately in the second phase of the experiment. The weights of oysters grown in a mixture (1.0 ppb each of DDT, toxaphene and parathion) of the three insecticides were significantly less ($\alpha = 0.05$) than control oysters after 9 months. This loss of weight was about 10% of the total body weight (including shell) of the oysters. Weights and heights of separate groups of oysters reared in sea water containing about 1.0 ppb of either DDT, toxaphene or parathion were not statistically different from controls.*

Oysters reared in the pesticide mixture had tissue changes associated with kidney, visceral ganglion, gills, digestive tubules and tissues beneath the gut. A mycelial fungus was also present, indicating a breakdown in the oyster's natural defense against this parasite. Eggs and spermatozoa removed from the oysters developed into 24-hour trochophore larvae.

The oysters accumulated relatively high levels of DDT and toxaphene but eliminated them during a 3-month depuration period.

INTRODUCTION

The insecticides DDT, toxaphene and parathion are acutely toxic to oysters at concentrations of 1.0 ppm (mg/l) or less (Butler, 1963), but our study demonstrates that young oysters will grow to sexual maturity in sea water containing approximately 1.0 ppb of one or all three of these insecticides.

Low concentrations of several insecticides have been found in a number of streams throughout the United States (Manigold and Schulze, 1969). That insecticides reach estuarine waters is shown by the fact that more than 50% of the oyster samples and many fish and shrimp samples taken from inshore waters of the Gulf of Mexico for the BCF Estuarine Monitoring Program contained DDT (Butler, 1969). We have collected samples of estuarine water in Northwest Florida that contained up to 0.3 ppb DDT or 1.7 ppb methoxychlor immediately following the spraying of adjacent beaches for control of stable flies, *Stomoxys calcitrans*. Undoubtedly, the pesticide content of estuarine waters fluctuates with rainfall, disposal of pesticide wastes, and the direct applications of insecticides to marshes and beaches for arthropod control.

Experiments at this and other laboratories demonstrated that oysters can tolerate limited amounts of certain pesticides for various lengths of exposure, but these experiments were not of sufficient duration to delineate effects on oysters grown in estuarine waters chronically polluted with low concentrations of one or more pesticides. The purpose of this study was to determine some of these effects by rearing oysters in flowing sea

[1] Contribution No. 116.

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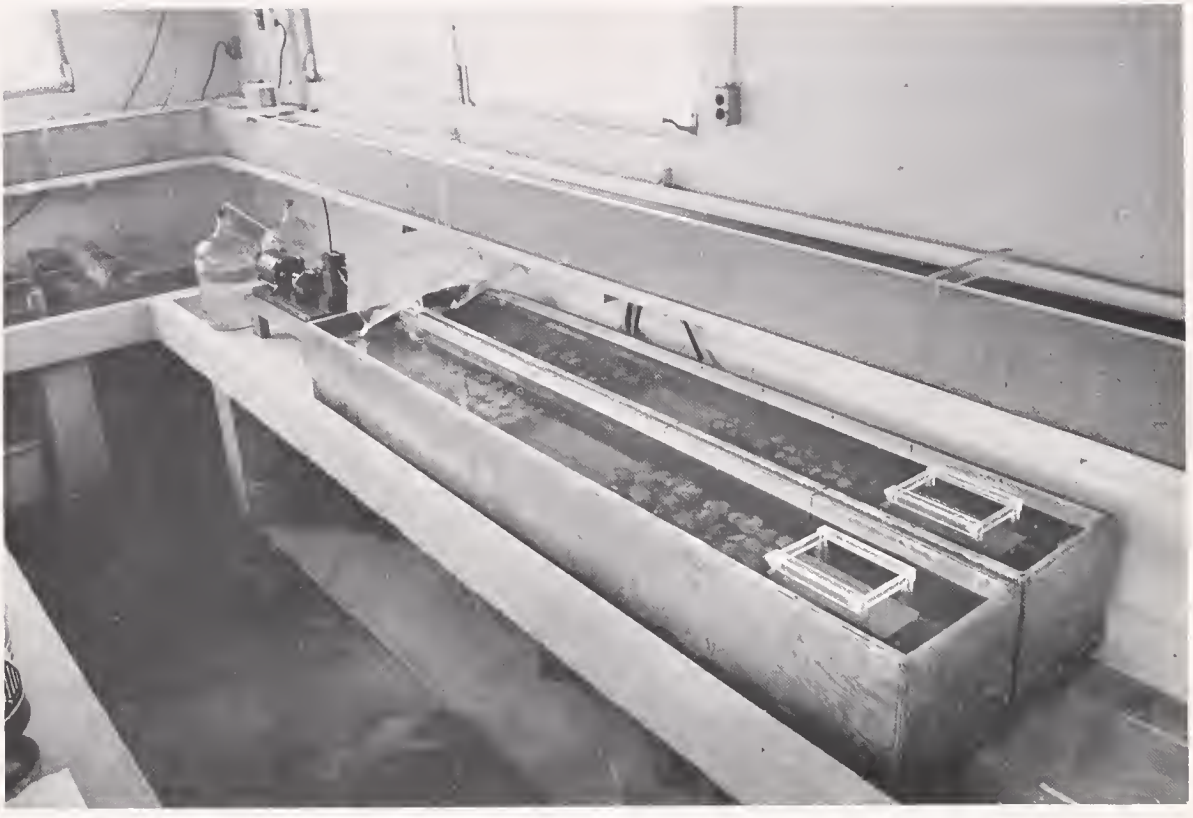

FIG. 1. *Flow-through system for rearing oysters in pesticide-polluted sea water.*

water containing DDT, toxaphene and parathion. These insecticides are widely used in the United States and are often applied as mixtures to agricultural crops.

This study was made in two phases at Gulf Breeze, Florida. From September 1967 to September 1968 oysters were reared in sea water containing a mixture of DDT, toxaphene and parathion (Experiment I). From September 1968 to September 1969, separate populations of oysters were reared in sea water containing either DDT, toxaphene or parathion (Experiment II). Each year, the experimental oysters grew in the pesticide-polluted water for the first 9 months and then in "clean" water for the last 3 months.

EXPERIMENT I. CHRONIC EXPOSURE OF OYSTERS TO A MIXTURE OF DDT, TOXAPHENE AND PARATHION

MATERIALS AND METHODS

Holding facilities

One hundred two-month old oysters were placed in each of two fiberglass-lined, wooden tanks of about 180 liters capacity and 244 cm long, 30 cm wide and 25 cm deep (Fig. 1). Positioning the oysters on compartmented, plastic racks facilitated handling and identification of individual animals. Calibrated plastic tubes siphoned 380 liters of unfiltered sea water per hour from a constant-level trough into each of the oyster tanks. The oysters obtained all of their food from this constant flow of sea water which was pumped directly from Santa Rosa Sound into the "wet" laboratory. Since the flow of sea water through each tank remained constant during the experiment, the same amount of planktonic food was available to each oyster.

Test solutions

A dual channel Sigmamotor pump equipped with a Zero-Max speed changer continuously metered 120 ml/hr of solvent (20% acetone, 80% distilled water) containing DDT[3], toxaphene and

[3] p,p' DDT [1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane], 99.3% pure, Geigy Chemical Corporation; parathion (0,0-Diethyl-O-*P*-nitrophenyl phosphorothioate), 99.6% pure, American Cyanamid Company; toxaphene ($C_{10}H_{10}Cl_8$), technical grade containing 67-69% chlorine, Hercules Powder Company.

parathion into the experimental tank and the same amount (120 ml/hr) of solvent without pesticide into the control tank. Mixing troughs with baffles facilitated mixing of the pesticides and solvent with the sea water flowing into the tanks. The desired concentration of pesticide in the experimental tank was 1.0 ppb of each of the three insecticides. Analyses of the water each month verified the concentration of each insecticide. Flow rate of the metering pump was checked daily. Average and range of concentration (quantitated with gas chromatography) measured during the 36-week exposure were 0.6 (0.3-0.8) ppb DDT, 0.8 (0.6-1.0) ppb parathion and 0.8 (0.6-1.0) ppb toxaphene.

The concentration (1.0 ppb) of each insecticide used in these experiments was selected on the basis of results obtained from acute toxicity tests and from residues found in estuarine water following field application of certain pesticides. In 96-hour exposures, we found that DDT inhibited shell growth of juvenile oysters at 10 ppb and above, toxaphene at 100 ppb and above, and parathion at 1000 ppb and above. A mixture of equal parts of the three insecticides inhibited shell growth at 100 ppb (96 hr). We detected 0.3 ppb DDT in the waters (15 m from shore) of Santa Rosa Sound approximately eight hours after windrows of dead marine grasses on the adjacent beaches were sprayed for stable fly control. When the same area was treated with methoxychlor the following year, we measured 1.7 ppb of this compound in the water.

Measurement of growth

The oysters were carefully cleaned and weighed individually each week for 48 weeks and the weights of the two groups compared statistically using Student's t-test (Snedecor and Cochran, 1967). We used the in-water weighing technique described by Andrews (1961) to obtain the weight of each live oyster. The weight of each oyster was estimated to the nearest 0.01 g on a top-loading balance with suspension attachments (Fig. 2). The in-water weight of an oyster is approximately half the weight in air and is mostly shell weight, since the specific gravity of oyster meats is close to that of sea water. This technique proved to be an objective method for measuring changes in weight that occurred from week to week.

Sampling for residue analyses and histopathological examination

The object of the residue analyses was to determine the accumulation of pesticides by the oysters and the depuration rate after exposure. Five oysters were removed from each group for residue analysis after 12, 24 and 36 weeks (ten oysters) of exposure and at 4-week intervals thereafter. Tissues of each five-oyster sample were homogenized and an aliquot analyzed by electron capture gas chromatography, using the method described by Duke, Lowe and Wilson (1970). Calculated residues were based on wet tissue weight.

FIG. 2. *Suspension-weighing of oysters in sea water.*

Consulting pathologists examined individual oysters after 24 and 36 weeks of pesticide exposure and after 48 weeks when the oysters had been in "clean" water for 12 weeks. A table of random numbers was used to avoid bias each time oysters were selected for residue analyses or pathological examination.

RESULTS

Effects on growth

During the 48-week experiment, control oysters grew from a mean weight of 1.67 g to 37.92 g. In the same period, experimental oysters grew from a mean weight of 1.67 g to 34.89 g. The mean weights of both groups showed a gradual change in weight in relation to age (Table 1). The mean weight of control oysters was consistently greater than that of the experimentals after 6 weeks of exposure but did not show a

TABLE 1. *Mean in-water weight of control and insecticide-exposed (1.0 ppb each of DDT, toxaphene and parathion) oysters for 48 consecutive weekly weighings.*

Week	Seawater[a]		Control oysters		Experimental oysters		Difference
	Mean temp. (°C)	Mean salinity (‰)	N	Mean weight (g)	N	Mean weight (g)	Mean weight (g)
0 (15 Sept. 1967)	25	27	100	1.67	100	1.67	0
1			100	2.01	100	1.99	0
2			100	2.43	100	2.43	0
3			100	2.78	100	2.77	0
4			100	3.22	100	3.23	0
5			100	3.76	100	3.76	0
6			100	4.30	100	4.28	0.02
7			100	4.88	100	4.84	0.04
8			100	5.42	100	5.38	0.04
9			100	5.99	99	5.94	0.05
10			100	6.70	99	6.65	0.05
11			100	7.44	99	7.39	0.05
12 (6 Dec. 1967)	16	29	100	8.12	99	8.05	0.07
13			95	8.95	94	8.78	0.17
14			95	9.78	94	9.52	0.26
15			95	10.48	94	10.15	0.33
16			95	11.11	94	10.70	0.41
17			95	11.79	94	11.28	0.51
18			95	12.37	94	11.74	0.63
19			95	13.11	94	12.35	0.76
20			95	13.71	94	12.84	0.87
21			95	14.45	94	13.44	1.01
22			95	15.03	94	13.85	1.18[c]
23			95	15.58	94	14.29	1.29[c]
24 (28 Feb. 1968)	9	28	95	16.14	94	14.72	1.42[c]
25			85	16.59	84	15.20	1.39[c]
26			85	17.33	84	15.77	1.56[c]
27			85	17.98	84	16.28	1.70[c]
28			85	18.79	84	16.94	1.85[c]
29			85	19.71	84	17.70	2.01[c]
30			85	20.80	84	18.62	2.18[c]
31			85	22.01	84	19.59	2.42[c]
32			85	23.21	84	20.59	2.62[c]
33			85	24.39	84	21.60	2.79[c]
34			85	25.40	84	22.53	2.87[c]
35			85	26.49	84	23.55	2.94[c]
36[b] (21 May 1968)	25	29	85	27.50	84	24.70	2.80[c]
37			65	28.89	64	26.34	2.55
38			65	29.70	64	27.37	2.33
39			65	30.51	64	28.29	2.22
40			65	31.19	64	29.27	1.92
41			60	31.79	59	29.57	2.22
42			60	32.70	59	30.63	2.07
43			60	33.52	58	31.23	2.29
44			60	34.09	58	31.92	2.17
45			55	35.63	53	32.76	2.87
46			55	36.27	53	33.38	2.89
47			55	36.87	53	34.05	2.82
48 (13 Aug. 1968)	29	30	54	37.92	53	34.89	3.03

[a] Mean temperature and salinity of seawater during a 7-day period.

[b] End of pesticide exposure.

[c] Statistically different at the $\alpha = 0.05$ significance level where the absolute value of the differenece between means of the two groups were compared for the case where the variability of the two groups was unknown but assumed to be approximately equal.

statistically significant difference ($\alpha = 0.05$) until the 22nd week. This difference continued to exist in weekly weighings until the pesticide exposure stopped at the end of 36 weeks. At this time, the mean weight of 85 control oysters exceeded that of 84 experimental oysters by 2.8 g ($t = 2.28$; d.f. = 167). This difference represented about 10% of the total body weight (including shell) of the exposed oysters. After the oysters had been in uncontaminated water for an additional 12 weeks, the difference in mean weight was approximately the same but was no longer statistically different ($\alpha = 0.05$; $t = 1.55$, d.f. = 105) with the reduced sample size. Only two experimental oysters died during the experiment — one in the 8th week and one in the 43rd week. One control oyster died in the 48th week.

Accumulation and retention of pesticides

Experimental oysters accumulated relatively high levels of the two chlorinated hydrocarbons, DDT and toxaphene, but very little parathion, an organophosphorus compound (Table 2). Parathion was either metabolized very rapidly or not accumulated by the oysters. Each value in Table 2 represents the pesticide residue (ppm, mg/kg) in five oysters, except in week 36 (end of exposure) when we analyzed ten oysters from both control and experimental groups. Pesticide residues reached maximum levels at week 24 when the oysters contained 91.0 ppm of DDT and metabolites, 30.0 ppm toxaphene and 0.36 ppm parathion. We cannot fully explain the drop in pesticide levels at week 36, but believe that some of the pesticide was lost through spawning. Butler (1966) found that the gonad of oysters stored about twice as much DDT as the digestive tract and associated organs. He also demonstrated the localization of DDT and its metabolites DDE and DDD in the gametes of sexually mature oysters.

After 12 weeks in clean water, the remaining oysters contained no toxaphene or parathion and only background levels (same as controls) of DDE.

Histopathology — 24 weeks

Mr. Gilbert Pauley, consulting pathologist, examined five oysters taken from each group after 24 weeks of exposure. Oysters exposed to the pesticides exhibited apparent pathology associated with the kidney, visceral ganglion, tissues beneath the gut, possibly the gills, and most frequently, the digestive tubules. The epithelial cells of the digestive tubules were necrotic in four of the experimental oysters. This necrosis was similar to that found in oysters injected with turpentine (Pauley and Sparks, 1965). An unknown fungus was present in two of the experimental oysters. The gonads of both control and experimental oysters were immature and sexually undifferentiated — a normal condition for their age and time of sampling.

Histopathology — 36 weeks

Mr. Pauley examined five control oysters and ten pesticide-exposed oysters after 36 weeks (end of pesticide exposure). The ten experimental oysters (6 females, 3 males, 1 hermaphrodite) were infected to various degrees with a mycelial fungus that invaded and caused lysis of the mantle, gut, gonads, gills, visceral ganglion and kidney tubules. Some tissues showed an intense inflammatory reaction and leucocytic infiltration. The control oysters were normal in most respects.

Mr. Pauley summarized his findings as follows:

> I think it is significant that the fungus was present in *all* of the experimental oysters in this group (half were infected in the first group) and that it had not been present in

TABLE 2. *Accumulation of pesticides by oysters exposed to a mixture (1.0 ppb each) of DDT, toxaphene and parathion.*

Week	Experimental oysters					Control oysters				
	DDE	DDD	DDT	Toxaphene[a]	Parathion	DDE	DDD	DDT	Toxaphene[a]	Parathion
	(ppm of wet tissue weight)					(ppm of wet tissue weight)				
12	5.8	<0.5	18.0	1.0	0.12	0.023	ND	0.029	ND	ND
24	21.0	0.98	69.0	30.0	0.36	0.032	0.050	0.037	ND	ND
36[b]	13.0	0.20	29.0	9.0	0.07	0.027	0.026	0.015	ND	ND
40	7.6	0.065	9.8	3.0	ND	0.028	0.015	ND	ND	ND
44	1.4	0.011	0.44	ND	ND	ND	ND	ND	ND	ND
48	0.017	ND	ND	ND	ND	0.015	ND	ND	ND	ND

[a] Values approximated.

[b] End of pesticide exposure.

ND = Not detectable, less than 0.010 ppm.

any of the control animals. It does show that there was something in one of the pesticides (or the synergistic effect of the three) that caused a break down in the oyster's natural defense against this parasite. The lack of a cellular response is somewhat unusual, but not in the case of this particular fungus, according to Mackin (1962), who stated that this mycelial fungus in the host produced little cellular response. I was able to find very little difference in the two sets of male oysters, but there was a significant histological difference in the females. Those female oysters exposed to the pesticides were not as mature as the controls, as exhibited by the smaller follicles, less mature eggs, more numerous immature ova, and in many places, a leucocytic infiltration or hyperplasia of the germinal epithelium. Leucocytic infiltration was also noticed among the sperm of experimental male oysters.

Almost always there was a slight edema beneath the gut of the animal, and this was sometimes accompanied by an unusual leucocytic infiltration.

The reaction in the ganglion, kidneys and gills was not as distinct and spectacular as in the other organs, but nevertheless it was present.

The dilation effect caused by the reduction in the height of the epithelium of the digestive tubules is similar to the effects in oysters that were injected with turpentine (Pauley and Sparks, 1965) and in the kidney tubules of fish subjected to Mirex (Van Valin, Andrews and Eller, 1968). It is interesting to note that in oysters the pesticide is selective for the smaller unciliated digestive tubules, and does not affect the larger digestive ducts that are ciliated.

Eggs and spermatozoa removed from both control and experimental oysters before tissue fixation developed into 24-hour trochophore larvae. We did not have the necessary food and facilities to attempt rearing of the larvae.

Histopathology — 48 weeks

Mr. Pauley and Dr. Ronald Taylor, consulting pathologists, examined ten oysters taken from each group after 48 weeks. At this time the experimental oysters had been in clean water for 12 weeks following the 36-week exposure.

In a summary report, Dr. Taylor stated that the experimental oysters looked much healthier than those examined at the end of 36 weeks. Control oysters, however, showed an increased amount of pathology relative to those examined earlier. This was probably due to increasing age and laboratory holding conditions. The oysters subjected to pesticides were generally in an earlier stage of gonadal development than the controls.

In the opinion of Dr. Taylor, oysters subjected to pesticides as described in this experiment are capable of returning to a more normal condition after being returned to nonpolluted water.

EXPERIMENT II. CHRONIC EXPOSURE OF OYSTERS TO SEPARATE CONCENTRATIONS OF DDT, TOXAPHENE AND PARATHION

In Experiment II separate groups of oysters were reared in sea water containing about 1.0 ppb of either DDT, toxaphene or parathion. We wished to determine the effects of each insecticide separately. The test conditions were essentially the same as in the first experiment, except that fewer oysters and smaller aquaria were used for each group.

MATERIALS AND METHODS

Holding facilities

Fifty two-month old oysters were placed in each of four plastic aquaria (75 cm long, 30 cm wide and 18 cm deep) of about 25 liters capacity. These oysters were maintained in the "wet" laboratory described in Experiment I. A fifth group of 50 oysters was placed in a wire basket and suspended in open water from an outside dock. The position of the four indoor aquaria and oysters in each group were randomly assigned. Young oysters were selected at random from a homogeneous population which set and grew on cultch suspended from a dock. As in Experiment I, the four indoor groups were positioned on compartmented plastic racks for ease in handling and identifying individual oysters. Three of the four groups were designated as experimental oysters to receive pesticide-polluted sea water, the fourth group being control oysters. The fifth group of oysters suspended from the dock was maintained as a "field" control for comparison with oysters grown in the laboratory. Each of the four laboratory groups of oysters received 154 liters of unfiltered sea water per hour. These flows remained constant throughout the experiment. More water was available to each oyster as specimens were removed for residue analyses and pathology.

Test solution

Separate stock solutions of DDT, toxaphene and parathion were metered into three of the aquaria and the same amount of solvent into the control. This was done exactly as in Experiment

I, except that the insecticides were administered separately, rather than as a mixture. The desired concentration of insecticide was 1.0 ppb. Water samples taken from the three experimental aquaria each month contained an average of 0.6 (0.3-1.1) ppb DDT, 0.8 (0.6-1.0) ppb parathion and 0.7 (0.2-0.9) ppb toxaphene.

Measurement of growth

Oysters in each of the five groups were cleaned, weighed and measured individually at 2-week intervals (as opposed to 1-week intervals in Experiment I) for 48 weeks. Oysters were weighed to the nearest 0.01 g (in-water weight) and height measured to the nearest millimeter. Weights and measurements from the five groups were compared statistically, using a single factor analysis of variance.

Sampling for residue analyses and histopathological examination

Five oysters were removed from each of the five groups for residue analyses of tissues after 12, 24 and 36 weeks of exposure and after 48 weeks when all oysters had been in "clean" water for 12 weeks.

A consulting pathologist examined five oysters from each group after 24 and 36 weeks of pesticide exposure and after 48 weeks when the oysters had been in "clean" water for 12 weeks.

RESULTS

Effects on growth

Statistical analyses of biweekly weights and measurements of all groups of oysters showed no effects of the individual insecticides on growth. After 12 weeks of exposure, the mean weight of control oysters was consistently greater than that of oysters reared in DDT or toxaphene polluted water, but these differences were never statistically different ($\alpha = 0.05$) at any time during the experiment. Heights (measured from umbo to distal end) of oysters in the four laboratory groups were not statistically different ($\alpha = 0.05$) during the pesticide exposure. The fifth group of oysters, suspended from the dock and maintained as a field control, developed into deeper "cup-shaped" oysters with shorter shells than those reared in the laboratory. Field control oysters were not considered a part of the actual experiment, but were used to evaluate laboratory holding conditions and possible pathology of test oysters. Mean weight and height of all groups of oysters at 12-week intervals during the experiment are shown in Table 3. The decrease in population size at each 12-week interval is due to removal of oysters for residue analysis or pathological examination. Mortality was negligible in all groups.

No differences in general appearance of meats of oysters reared in the laboratory were detected. The small samples were inadequate for determining a true condition index.

Accumulation and retention of pesticides

The oysters accumulated relatively high levels of the DDT and toxaphene but little parathion (Table 4). Experimental oysters were analyzed only for compounds to which they were exposed. Levels of DDT and metabolites in both groups of control oysters were determined at each sampling period. The drop in pesticide residue level at week 36 was probably due to spawning.

The oysters exposed to toxaphene and parathion for 36 weeks purged themselves of accumulated residues when held in "clean" water for 12 weeks. The oysters exposed to DDT contained less than one percent of the accumulated DDT and metabolites after the same "flushing" period.

Histopathology — 24 weeks

Dr. Ronald Taylor examined five oysters from each group for pathology or other abnormality. All oysters were immature and in the earliest stages of sexual development. Oysters reared

TABLE 3. *Mean in-water weight and height of control and insecticide-exposed oysters at 12-week intervals.*

(Week)	Seawater[a]		Mean in-water weight (g) and height (mm) of 5 groups of oysters														
	Temp	Salinity	Control (lab)			Control (field)			DDT (1.0 ppb)			Toxaphene (1.0 ppb)			Parathion (1.0 ppb)		
	(°C)	(‰)	N	Weight	Height	N	Weight	Height	N	Weight	Height	N	Weight	Height	N	Weight	Height
0 (Sept. 23, 1968)	26	31	50	1.69	29	50	1.86	28	50	1.77	29	50	1.73	29	50	1.59	27
12 (Dec. 17, 1968)	10	31	48	8.41	41	47	7.68	41	48	8.06	41	49	7.84	42	50	8.49	43
24 (Mar. 11, 1969)	10	29	43	14.19	48	42	12.80	45	43	13.69	49	43	13.17	50	45	14.47	50
36 (June 3, 1969)[b]	25	27	32	23.25	58	31	21.00	52	28	20.93	55	30	20.09	56	31	21.38	56
48 (Aug. 22, 1969)	27	27	19	29.38	63	20	28.41	58	18	25.23	60	20	28.69	65	20	29.69	64

[a] Mean temperature and salinity of seawater during a 7-day period.
[b] End of pesticide exposure.

TABLE 4. *Accumulation of pesticides by oysters exposed to 1.0 ppb DDT, toxaphene or parathion.*

		Pesticide residue (ppm of wet tissue weight)				
Week	Treatment	DDE	DDD	DDT	Toxaphene[a]	Parathion
12	p,p'-DDT	12.	0.79	63.		
	Toxaphene				20.	
	Parathion					0.24
	Control (lab)	0.026	0.019	0.019		
	Control (field)	0.028	0.029	0.040		
24	p,p'-DDT	31.	0.28	45.		
	Toxaphene				23.	
	Parathion					0.24
	Control (lab)	0.037	0.034	0.035		
	Control (field)	0.037	0.031	0.029		
36[b]	p,p'-DDT	16.	0.94	43.		
	Toxaphene				8.0	
	Parathion					0.082
	Control (lab)	0.029	0.024			
	Control (field)	0.026	0.034			
48	p,p'-DDT	0.14		0.020		
	Toxaphene					
	Parathion					
	Control (lab)	<0.010		<0.010		
	Control (field)	<0.010		0.017		

[a] Values approximated.

[b] End of pesticide exposure.

outside (field controls) appeared to be slightly more developed than the other oysters.

In Dr. Taylor's opinion, the three insecticides had no significant observable effect on the oysters.

Histopathology — 36 weeks

After 36 weeks of exposure, Dr. Taylor examined five oysters from each of the five groups. All oysters examined were sexually mature, both sexes being represented in each group.

Dr. Taylor summarized his findings as follows:

The inside and outside controls differ as follows:

1. The outside (field) controls show a much higher incidence of leucocytic infiltration among the Leydig cells.
2. The inside (laboratory) controls demonstrate more edema in various parts of the body. In the following analysis, the experimentals are compared to the inside controls only.
   a. The single most pathological animal is in the DDT-treated group.
   b. If we disregard the single most pathological animal from each group, the parathion-treated animals emerge as the most pathological group followed closely by the inside controls — the DDT and toxaphene treated groups, the least pathological.
   c. Edema in various parts of the body is most evident among the parathion-treated animals, especially beneath the gut.
   d. The digestive tubules appear more pathological among the parathion-treated oysters than among the others.
   e. The toxaphene-treated animals were the only ones to show any lysed Leydig cells, increased incidence of pigmented cells, or pathology of visceral ganglion or adductor muscle.
   f. There appears to be no significance to the incidence of parasites (cestodes, gregarines, copepods) among the various groups.
   g. The results of this experiment are not clear cut. I could not say unequivocally that any of the experimental groups were clearly more pathological than the controls. However, there is the *suggestion* that parathion and toxa-

phene are causing some pathology. I would want to repeat the experiment using more animals and to analyze the results statistically before coming to any conclusion.

Histopathology — 48 weeks

Five oysters from each group were examined by Dr. Taylor at the end of 48 weeks. All oysters had been in unpolluted water for 12 weeks at this time. The laboratory controls had more pathology than the field controls, but in comparing the laboratory controls and the three experimental groups, Dr. Taylor reported, "in summary, no one group appears significantly more pathological than any other group."

CONCLUSIONS

Growth was affected and pathology produced in oysters reared in sea water containing a mixture of about 1.0 ppb each of DDT, toxaphene and parathion. These changes were not evident in separate groups of oysters reared in sea water containing about 1.0 ppb of either DDT, toxaphene or parathion alone. We do not know whether the differences in results of the two experiments were due to the greater total amount of insecticide, synergism among the three toxicants or to both.

This study provided some insight on effects of these insecticides on oysters grown in estuarine waters chronically polluted with low levels of one or more of the toxicants.

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REARING THE BAY SCALLOP, *AEQUIPECTEN IRRADIANS* [1]

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ABSTRACT

Bay scallops, Aequipecten irradians, *collected from various bays along the Eastern Shore of Virginia and from Bogue Sound, North Carolina, were conditioned and spawned in the laboratory, out of their normal spawning period. A thermal stimulus of 21-27°C was used to stimulate spawning, and larvae set in 10-19 days using cultured algae as food.*

Juvenile scallops were held in plastic trays in the laboratory for one week, then moved to outdoor tanks with flowing, unfiltered seawater. They remained there until they were about 2 mm in width, then moved to plastic screened wooden floats in the field where they reached an average minimum market size (50 mm) in 12-13 months.

Mortality of larvae, early post-set scallops and adults is described.

The bay scallop appears to be amenable to mariculture. The biological feasibility of rearing bay scallops from egg to market size has been established.

INTRODUCTION

The Bay scallop, *Aequipecten irradians,* contributed $11,962,000 to the United States fishery between 1960-67 (Lyles, 1969), ranking in value among bivalve mollusks behind oysters, clams and sea scallops.

The bay scallop was the source of a small fishery along the seaside of the Eastern Shore of Virginia before the disappearance of the eelgrass in the early 1930's. Lyles (1969) indicates an average of 888,333 lb was taken from Virginia waters (not necessarily limited to Eastern Shore waters) between 1920-1932 contributing a yearly average of $102,666 to the state's fishery.

In spite of its commercial value, little attention has been given to rearing the bay scallop to market size. Belding (1910), in discussing its artificial propagation in Massachusetts waters stated, "It would be impossible to raise the young embryos in sufficient numbers for commercial hatching." He further stated, "There is but one way now known of artificially aiding the scallop industry, i.e., by transplanting in the fall the abundant set from exposed places to the deeper water before the 'seed' is killed by the winter."

Since Beldings work with the bay scallop, Wells (1927) was able to spawn and raise the larvae past the setting stage thus providing the initial step for its culture. Loosanoff and Davis (1963) described the methods to condition, spawn and raise the larvae to metamorphosis. Turner and Hanks (1960) and Sastry (1966) substantiated the feasibility of conditioning the bay scallop out of season.

At the Eastern Shore Laboratory of the Virginia Institute of Marine Science, investigations into rearing bay scallops began in 1968. This species was believed suitable for mariculture for a number of reasons:

1. Most important, this species has a high market value necessary to support a mariculture operation.
2. Markets and consumer acceptability were established.
3. Natural scallop populations fluctuate due to year class failures (Belding, 1910). Culture techniques could stabilize the supply and make it possible to develop new markets.
4. Hatchery techniques of conditioning, spawning and rearing bay scallop larvae

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had been successfully demonstrated.

5. Rapid growth to market size is characteristic of this species (12-17 months in Massachusetts waters, Belding, 1910; 10 months in more favorable North Carolina waters, Gutsell, 1928), and growth rate could probably be increased by selection of brood stock (Loosanoff and Davis, 1963).
6. Automatic shucking devices used for the calico scallop, *Argopecten gibbus,* could, with little or no modifications, be adapted for the bay scallop, alleviating labor and other problems inherent to hand shucking operations.

MATERIALS AND METHODS

Description of Area and Procurement of Brood Stock

All scallops were reared at the Wachapreague Laboratory, with the exception of those used in a preliminary study to determine the feasibility of using pens for holding scallops to market size. Floats were held in Finney Creek near the laboratory. Tidal amplitude is 1.2-1.5 m. Temperatures ranged from −1.1-29.8°C and salinities from 21.4-32.5‰ from November 1969 to October 1970. Surface current data from August to October 1970 averaged 19.3 cm/sec. Scallops in floats were covered with 7.5-13 cm of water.

The pen was set up on a tidal flat in Assateague Channel. Temperature, salinity and current were about the same as in Finney Creek. Tidal amplitude is about 1.2 m. The scallops were always covered with at least 30.5 cm of water.

Initial brood stock consisted of 66 adult scallops collected from Metomkin, Burton, Swash and Hog Island bays along the Eastern Shore peninsula of Virginia, and from Bogue Sound, North Carolina, from October through December 1967. Scallops for the study of the feasibility of using pens were reared by a hatchery on Long Island and sent to Virginia on 9 July 1970.

Conditioning Procedures

Conditioning was accomplished by holding 6-10 scallops in fiberglass boxes (60 x 45 x 13 cm) with approximately 20 liters of raw, standing seawater. Water was changed three times per week, and scallops were fed one liter of a mixture of algal solution per day. After about a week at 18°C, temperatures were raised to 20-22°C for 3-8 weeks. Conditioning was carried out in December and January.

Gonadal condition was checked in live animals by grasping a gaping scallop with thumb and forefinger, the flesh of the fingers acting as wedges between the valves. With the valves slightly opened, the gonad could be observed as a slightly bulbous, triangular structure lying anterior to and partially encircling the adductor muscle. The bay scallop is a functional hermaphrodite (Belding, 1910; Gutsell, 1930) with the testis comprising the anterior border of the gonad running from near the ventral tip to the dorsal base where it becomes slightly enlarged. The ovary occupies the more posterior portion.

When ripe, the ovarian portion becomes reddish-orange, and the testis cream-colored, although a black pigmented epithelium sometimes obscures the initial color change of the former.

Spawning Procedures

When gonads appeared ripe, spawning procedures were begun using methods described by Loosanoff and Davis (1963). Spawners were placed in 2 liter glass finger bowls or 1.5 liter pyrex dishes and stimulated to spawn by raising the water temperature from ambient to 27-30°C. Occasionally a sperm suspension was needed to stimulate spawning.

Fertilization sometimes occurred simultaneously with spawning, when an individual scallop released both sperm and eggs. More often, only one sex product was released by an individual scallop. As soon as spawning occurred, adults were removed from the spawning dishes and the ova in each dish were fertilized with approximately 2 ml of sperm suspension. Care was taken to introduce only a small amount of sperm suspension since high densities of spermatozoans were suspected of causing a high percent of deformed larvae.

Larval Rearing Procedures

Fertilized eggs were passed through a 153 μ nylon screen to remove clumps of fecal and tissue matter and collected in calibrated containers. They were then counted using methods described by Loosanoff and Davis (1963) and placed in 20 gal polyethylene garbage cans at a density of 1-2 million per 60 liters of seawater (17-34 per ml). Temperatures of larval cultures ranged from 20-28°C during the larval period.

The water was changed three times a week by siphoning water and larvae through nylon screens. These were constructed by fusing nylon screen to a 10 cm section of a 30 cm diameter plexiglass tube with 1, 2 dichloroethane. The mesh size of the screen was increased as the larvae grew. At each water change the larvae were concentrated on screens and washed into calibrated containers for subsampling and counting. This procedure allowed observation of growth, condition, mortality, setting, bacterial activity and effects, if any, of competitors, predators or saprophytes. Measurements were made on the first 10 larvae found in

a randomly chosen microscopic field, using an ocular micrometer. Decisions were made at that time on the disposition of the larvae (how many per container or tray), and the larvae were redistributed to containers.

As larvae grew, the number per can was decreased until there were approximately 200,000 per can (4/ml). At this density the larvae measured 148-216 μ (all measurements of larvae and scallops refer to the height or distance from umbo to ventral edge) and were starting to set.

Seawater used during the conditioning, spawning and immediate postset period was pumped from Finney Creek, cleared of particulate matter by a Westfalia Clarifier, model KDD 605 (Centrico, Inc., Englewood, N. J.) and treated with ultra-violet light. Unicellular cultures of *Monochrysis lutheri*, *Phaeodactylum tricornutum* or *Dunaliella tertiolecta* were fed to the conditioning scallops and the larvae throughout their 10-19 day larval period, at the rate of 1 liter per day. A mixture of two or three species was generally used. Occasionally, as a supplement to the above food, seawater filtered through a 15 μ dacron bag filter was added at a rate of 16 liters per 60 liter water change.

Nursery Procedures

Once the larvae had set, they were moved to plastic photographic trays (55 x 67 x 10 cm) at a density of approximately 200,000 per tray. They were left for about a week to allow them to grow before being moved to wooden outdoor tanks (2.5 x 0.61 x 0.1 m) with unfiltered flowing

FIG. 1. *Floats used to hold scallops until market size.*

seawater. These tanks were constructed of three-quarter inch (1.9 cm) plywood and painted with an epoxy coating. A maximum of 500,000 scallops could be placed in each tank.

Initially, when 2 mm in height (after about 2-3 weeks during the warmer months), scallops were moved to wooden, rectangular floats 210 cm long, 61 cm wide, and 15 cm deep (Fig. 1). These were constructed of three-quarter inch (1.9 cm) pine boards and covered top and bottom with fiberglass window screen (16 mesh per inch) or plastic netting. Subsequent observations indicated that it was possible to hold them in the tanks until 10 mm in height before being moved to floats. This eliminated using floats covered with the two smaller mesh screens and reduced the unit effort.

In approximately 12-13 weeks the scallops measured about 25 mm in height and had been moved from floats with window screen to ones with large plastic netting (mesh size, 16 x 22 mm). They remained here until market size (50-65 mm).

The pen, used in the preliminary study mentioned above, was constructed of hardware cloth (12.5 mm mesh) tacked to poles that had been pumped into the bottom to give an area 10' x 10' x 6'. The scallops rested on the relatively hard mud-sand bottom. They averaged 16.1 mm on 9 July 1970, the start of the experiment. They were placed in a quarter inch hardware cloth cage until 14 August when they were large enough (24 mm in height) to be released into the 1/2 inch mesh 10' x 10' x 6' pen. On 24 November 1970, they were collected using dip nets.

RESULTS AND DISCUSSION

Utilizing 66 adult scallops collected in 1967, three filial generations have been produced. The progeny have been used as brood stock and for other studies related to the mariculture of the bay scallop. The initial group of 66 adults was successfully conditioned and stimulated to spawn as early as February. Despite bi-weekly efforts, spawning during February, March and early April was infrequent; however, by mid-April when the gonads appeared more fully developed, spawning was stimulated quite easily and as frequently as twice a week.

Although self-fertilization is believed to be uncommon in nature (Belding, 1910; Gutsell, 1930), it was a common occurrence in the laboratory. The larvae obtained by self-fertilization in the laboratory appeared normal, and, in fact, one isolated scallop spawned hermaphroditically for nine weeks during the late summer and early fall of 1968 producing nine groups of larvae that displayed normal growth, setting and survival. By either method, fertilization was quite successful, leaving less than an estimated 1% of each group of eggs unfertilized.

The fertilized eggs reached the straight hinge stage in 18-28 hr at temperatures between 20-28°C (with faster development occurring at higher temperatures). The larvae averaged 73.27 μ in height at straight hinge stage, and usually doubled in size in 5 days with temperatures over 20°C and with adequate food. The percent of fertilized eggs that reached setting ranged from 2.4-7.8% (average 5%). This represents, at best, a rough estimate due to the difficulty in counting set larvae. The losses were probably due to several factors; disease (Tubiash and Chanley, 1963; Loosanoff and Davis, 1963) and zooplankton predators or competitors which passed through the filter system. The authors suspect a large number of pelecypod larvae would never reach the setting stage due to generic deficiency. Larvae normally began setting in 10-19 days, with most occurring in 10-14 days. One group set in six days. Soon after setting, the scallops formed a firm byssal attachment.

During the early post-setting period to 2 mm in height, mortalities often reduced the number of live scallops by an estimated 50-80%. This high post-set mortality often occurs with pelecypods (Loosanoff and Davis, 1963). During metamorphosis the nutritional needs of the scallops may change, requiring a different food than the type available; this could directly or indirectly contribute to this mortality. Smothering may also contribute to the mortalities. Matthiessen and Toner (1966), culturing bay scallops in Massachusetts waters, found little mortality associated with metamorphosis. They estimated that of the 14-28% mortality occurring at this time approximately 5% could be attributed to mechanical loss due to the cleaning of trays.

Scallops from 2 mm in height to market size suffered an estimated 50% mortality. The sides and screened bottoms of the floats frequently became fouled with hydroids, algae, tunicates and mud. This fouling undoubtedly reduced circulation in the floats and probably had an adverse affect on growth and survival. Also, in the area where the floats were being held, a strong tidal flow, possibly combined with boat traffic, often caused the scallops to become washed to one side or end of the float, which, along with the fouling, caused the float to tip in the water. If the float was not righted many scallops died, apparently from smothering.

It is believed that improved handling techniques could reduce mortalities occurring between 2 mm and market size. Putting stabilizing wings on the floats (Fig. 1) has helped to alleviate the tipping.

Moving the floats to an area with less current and less boat traffic, using a deeper float so scallops are below the surface, submerging floats, chemically treating floats to prevent fouling, or using a different method for holding scallops may improve survival during this period.

Growth and Mortality of Scallops Held in Floats

F_1 and F_2 generation scallops (total of 10,652) were placed in floats in November 1969 and monitored for growth and mortality until November 1970. Data collected from one group of F_2 generation scallops (3 months old in November 1969), held at a density of 44/ft², is typical for scallops held in surface floats in the Wachapreague area (Figs. 2 and 3).

Growth from December 1969 to April 1970 was negligible, while the average growth rate from May 1970 through August 1970 (Fig. 2) was 7.0 mm/month.

Growth and temperature data (Fig. 2) indicate that maximum growth occurs above 10°C. Observations, however, have indicated that growth (measured as the height of the scallop) decreases as the scallop approaches 50 mm. This is shown in Fig. 2 where, between August and November 1970, the growth rate has decreased even though temperatures were above 10°C. Density of scallops in the float may also have had an affect on growth of the larger scallops.

Assuming minimum market size to be 50 mm (Belding, 1910), a few F_2 scallops were marketable in approximately 11 1/ 2 months. However, a mean size of 50 mm was not reached until about the 13 month (Fig. 2) mainly due to a lack of growth during the winter months (1969-1970).

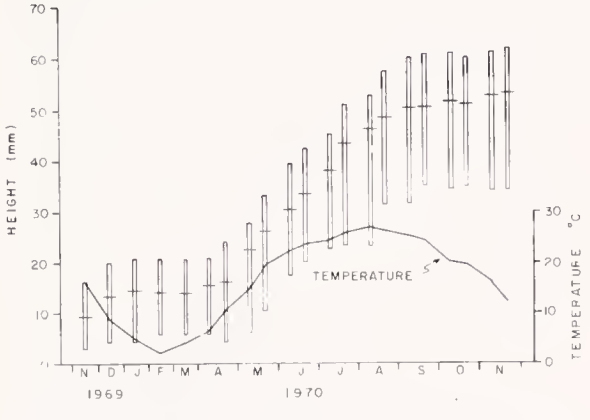

FIG. 2. *Average monthly growth and range of F_2 scallops reared in floats from November 1969 through November 1970. Scallops spawned August 1969.*

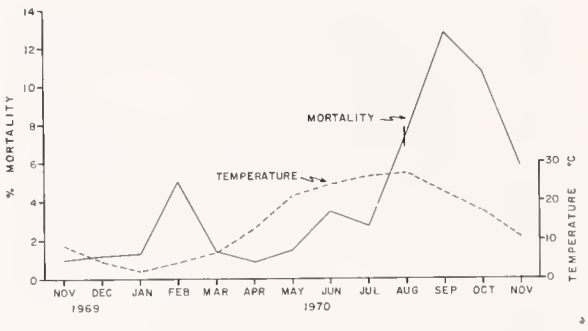

FIG. 3. *Average monthly mortality for F_2 scallops reared in floats from November 1969 through November 1970. Scallops spawned August 1969.*

This suggests that if in the experimental area scallops were spawned in January, February, and March and moved to natural waters by the end of April and averaged 10.0-15.0 mm by the end of May, they would be market size in October (8-10 months). Improved handling techniques and/or genetic improvement may reduce even more the time it takes to reach market size. Adjusting the spawning schedule will allow marketable scallops to be available at most any time of the year. However, methods for holding scallops may have to be adjusted during the winter months due to the danger of ice. Surface floats during the winter are not adequate where ice is common.

Growth of scallops held in the pen from July to November 1970 averaged 8.3 mm/month. Scallops grew from 16.1 mm to 57.4 mm, going from egg to market size in 6 months.

Average monthly mortalities (Fig. 3) generally remained below 7% from November 1969 to November 1970. The increase in mortality noted in February is attributed to sub-zero temperatures and ice in January. The increase from June through September is believed due to two factors: a general physical decline of the animals and overcrowding. Disease, parasites, and factors mentioned earlier may have influenced mortality. However, senescence, referred to by Belding (1910), was probably the more important factor. Belding mentions that this period of physical decline begins at 18 months and nearly eliminates each year class by the 26 month. Mortality of F_2 scallops began to increase at the tenth month (Fig. 3). Confinement in floats and high summer temperatures possibly weakened them so the period of physical decline began earlier. Gutsell (1930) also noted that only a few scallops reached 2-years of age, but stated that "development of sexual products in preparation for a second spawning began and continued normally until

death intervened." This, he states, "suggests not death from old age but from some pathologic factor." Some scallops, when spawning in the laboratory, discharge pieces of gill tissue, suggesting that spawning itself could be responsible directly or indirectly for some deaths and poor condition. Sastry (1966) mentions that of all the scallops examined throughout the reproductive period in North Carolina waters, those in spawning condition were least tolerant to all test temperatures (10, 20, 25 and 30°C). The decrease in mortality in October and November 1970 (Fig. 3) is believed due mainly to decreasing temperature resulting in a decrease in metabolic activity.

Although much work remains, mainly the determination of optimum densities and optimum depth for holding scallops in floats, the biological feasibility of rearing the bay scallop from egg to market size has been established. Optimization of all procedures is necessary, as well as further study of methods for holding scallops from about 10 mm to market size.

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ASSOCIATION BETWEEN POST-JUVENILE RED HAKE AND SEA SCALLOPS

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ABSTRACT

First evidence of a special association between post-juvenile red hake and sea scallops was obtained from a photograph of these species in their natural habitat on the sea bottom 200 km east of Cape Cod, Massachusetts.

Post-larval and juvenile red hake, *Urophycis chuss* (Walbaum) customarily inhabit the mantle cavity of sea scallops, *Placopecten magellanicus* Gmelin. This association was first reported by Goode (1884) and later by Welsh (1915), Bigelow and Schroeder (1953), Edwards, Livingstone and Hamer (1962), and most recently by Musick (1969). It frequently has been observed by scallop fishermen and by biologists engaged in studies of the sea scallop. Musick (1969) refers to this relationship as inquilinism, a form of commensalism, usually where one organism lives within another.

Small hake swimming into and out from the scallop's mantle cavity cause little or no interruption in the scallop's pumping rhythm, particularly when the hake are small. When hake reach a length of 10 or 12 cm they are generally too large to be accommodated by most scallops. It was generally assumed that when hake reached approximately this size it was the termination of their association. However, we have found evidence of post-juvenile association between these two species in which a half-grown red hake is snuggled against a live sea scallop (Fig. 1).

The relationship between red hake and sea scallops continues after the hake have grown too large to enter the scallop's mantle cavity. The photograph shows both animals lying in the center of a shallow, basin-like depression in the sediment. (Previous photographs revealed that sea scallops commonly dwell in shallow pocket-like depressions in sandy sediments.) Total length of the hake is about 30 cm and it is probably mature. Height (length) of the scallop is 15 cm. The ventral surface of each animal is resting on the sediment and the hake's tail and left thoracic region are pressed against the scallop. The distal end of the hake's left pelvic fin is angled upward, indicating contact with the scallop. The right pelvic fin lies back horizontally and the right pectoral fin is angled obliquely forward. The scallop's valves are not closed, as would be the case if it were disturbed or irritated. Rather, the valves are held in the normal feeding attitude with the tentacles and velar folds slightly protruding, notwithstanding the fact that the hake is touching the scallop's sensory tentacles and blocking light to numerous pallial eyes. The scallop obviously is not disturbed by the hake's presence.

Sea scallops in the aquarium at the NMFS Biological Laboratory, Woods Hole, when disturbed by contact with fish, crabs, starfish, or other sea scallops, respond by snapping the valves shut. Moreover, if the contact is strong, the scallop will swim away from the intruder. In the situation shown in the photograph, neither of these responses resulted from contact with the red hake.

Considering that red hake 15-18 cm were observed curled around empty surf clam (*Spisula solidissima*) shells and rocks off New Jersey and New York (Wicklund, 1966), post-juvenile hake do not appear to specifically require a scallop for shelter or thigmotactic security. Sea scallops, which presumably have been conditioned to the

FIG. 1. *A half-grown red hake, about 30 cm long, partially encircling a live sea scallop on the sea bottom of Georges Bank. Scale bar at right represents 10 cm.*

presence of post-larval and juvenile red hake will accept external contact of post-juvenile red hake without antagonistic response. This late-stage, external association between these two species may be much more complex than that indicated by the few observations reported.

The photograph was taken by the authors 22 August 1965, on the southeastern part of Georges Bank, 200 km east of Cape Cod, Massachusetts, at 41° 14' N. Lat.; 67° 04' W. Long.; water depth 64 m. It was obtained by means of a sled-mounted underwater camera towed from the R/V *ALBATROSS IV* (cruise 65-11, station 44) during an investigation of benthic invertebrate communities in the Georges Bank region.

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PERCENTAGE OF SOLIDS AND LENGTH-WEIGHT RELATIONSHIP OF THE OCEAN QUAHOG

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ABSTRACT

Percentage of solids of ocean quahogs, Arctica islandica, *were determined for samples taken from offshore Long Island, New York. Solids averaged 18.5% for clams between 30 and 129 mm in shell length. Dry meat weights increased most rapidly between clam lengths of 92-113 mm. Calculated yields of bushel quantities are given for two areas off Long Island and New Jersey where clams are plentiful.*

INTRODUCTION

Ocean quahogs *(Arctica islandica)* are an underutilized source of clam meats from the Continental Shelf bordering the Middle and North Atlantic States (Merrill, Chamberlin and Ropes, 1969; Parker and McRae, 1970). The average annual landing from 1960-69 of 150,000 pounds of shucked meats is a poor indication of the potential catch from this abundant natural resource. Estimated landings of 473,000 pounds in 1969 and 1,743,370 pounds in 1970 (W. E. Brey, personal communication) and patents on techniques to process the clam (Wendt, 1969) indicate a positive industry attitude to utilize the resource. Greater expansion of the ocean quahog fishery could supplement other clam products in the United States. The surf clam *(Spisula solidissima)* industry presently supplies better than half of the demand for clam products in the United States but demand for clam meats is constantly increasing. Research on the density, distribution and total solids of surf clams in the Middle Atlantic Bight included the ocean quahog, because populations of the latter were found mixed with, or nearby, surf clam populations and also at greater depths (Merrill and Ropes, 1969a).

This paper reports on total solids and length-weight relationship for ocean quahogs. The quantity of dry meat actually recovered and its percentage relative to the wet weight of the clam meat is expressed as total solids. Several factors, however, have been found to influence the total solids in bivalves, such as the oyster and surf clam (Engle, 1958; Shaw, Tubiash and Barker, 1967; Barker and Merrill, 1967). I shall present measurements of the dry meat weights for ocean quahogs of various sizes, relate these measurements to bushel quantities, and discuss biological factors that may have affected the values.

Sample collection and treatment

Samples of ocean quahogs were taken from beds off Long Island, New York, on 20 June through 2 July 1969, during a cruise of the research vessel ALBATROSS IV. A sample consisted of the shucked, intact meats and shell liquor from as many as 25 clams having shell lengths within a 10-mm size interval. In all, 10 groups of clams were collected and frozen immediately after shucking.

The methods given by Shaw, Tubiash and Barker (1967) were used to determine the percentage of solids. Each sample was thawed, the meats drained for 5 min, weighed to the nearest centigram, and then homogenized in a blender. About a 20-gm aliquot of the homogenate was placed into a pre-weighed evaporating dish and weighed. The dish was set into the freeze-dryer unit and processed to a constant weight. The average percentage of solids per clam was determined using the following formula:

$$\text{Percentage of solids} = \frac{\text{dry weight of meat X100}}{\text{wet weight of meat}}$$

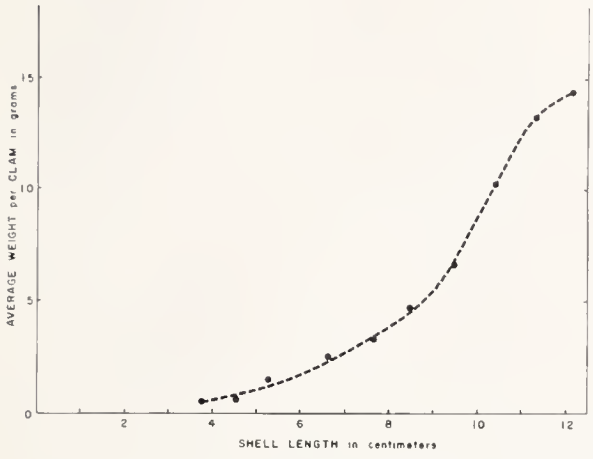

FIG. 1. *Dry weight of meats (in grams) of ocean quahogs,* Arctica islandica, *relative to shell length from samples taken during late June and early July 1969 off Long Island, New York.*

Meat weights relative to clam size

An average dry meat weight of 1 gm per clam was found for those 49 mm long and subsequently an even gram of weight was gained for every 6-8 mm increase in shell length to 92 mm (Fig. 1). At 92 mm the average dry weight of meat was 6 gm. Thereafter, a gram was added for every 2-3 mm increase in shell length to 113 mm. The rate of addition of dry weight of meat decreased for clams greater than 113 mm.

Values for wet weight of meats of ocean quahogs relative to shell size closely paralleled dry weight values with one exception: wet weights of ocean quahogs larger than 113 mm steadily increased in contrast to the dry total solids. Thus, the largest clams tended to be more watery than the smaller ones.

Meat weights relative to bushel quantities

The fishing industry measures wet meat weight for bushel quantities. Wet meat weights of a bushel of ocean quahogs were calculated for two general areas where this species was found in abundance during cruises of the research vessel UNDAUNTED in 1965 (Merrill and Ropes, 1969b; Ropes and Merrill, 1969). Numerous shell-length measurements provided data on the sizes most likely to be taken by a fishery. Off Long Island, New York, ocean quahogs averaged 88 mm long, and plus or minus one standard deviation from the mean included clams 75-101 mm long; off New Jersey, ocean quahogs averaged 93 mm long, and plus or minus one standard deviation from the mean included clams 82-105 mm long (Table 1). The calculated meat weights per bushel of New Jersey clams at all three length measurements were greater than the comparable values for Long Island clams. For example, based on the average shell length, a bushel of New Jersey clams was 1,015 gm (2 1/4 lb) heavier than clams from Long Island. From a production standpoint, high densities of clams off Long Island, however, may compensate for the lower meat yields.

Meat yields of ocean quahogs have been reported by other investigators. In each case important information was omitted that would permit a critical comparison with my results. Arcisz and Sandholzer (1947) gave a value of "about 12 pounds" of meats per 90-pound bushel for ocean quahogs from beds off Rhode Island. No description of the procedures used to determine meat yields and the sizes of the clams sampled was presented. A 10-pound per bushel meat yield was reported by Mendelsohn, Parker, McRae, King and Joyce (1970), but no other information was included. DeWolf and Loosanoff (1945) reported a meat yield of 1.2 oz (34 gm) per clam for ocean quahogs averaging 83 mm long. Their value was 9 gm higher than ours for the same size clam and, although no information was given on the procedure to obtain the value, a bushel containing 145 clams of that weight would amount to 4,930 gm (10.9 lb). All of the reported values are within those given in Table 1. Yet, it might have been possible to identify the source of variation in the values if the location of the sample, the sizes of clams used and method of determining meat yields had been included in all of the studies.

The percentage of solids value (18.5%) for

TABLE 1. *Calculated wet meat weights per bushel of* Arctica islandica *in two Middle Atlantic sample areas.*

Sample area	Shell length (mm)	Wet meat weight in gm and (lb) per bu [d]
Long Island	75[a]	2755 (5.94)
	88[b]	4133 (9.11)
	101[c]	7540 (16.62)
New Jersey	82[a]	3408 (7.51)
	93[b]	5148 (11.35)
	105[c]	8700 (19.18)

[a] One standard deviation below the average.

[b] Average.

[c] One standard deviation above the average.

[d] A standard U. S. bushel contained an average of 145 clams.

ocean quahogs compares favorably with those found for other bivalves, even though different techniques were used. Engle (1958) reported an annual mean value for oysters *(Crassostrea virginica)* of 17.0%, Harriman (1954) reported a value of 18.4% for soft-shell clams *(Mya arenaria)*, and Barker and Merrill (1967) reported a value of 21.4% for surf clams (*S. solidissima*). In all of these studies a decrease in percentage of solids coincided with spawning. Loosanoff (1953) identified July and August as the spawning period for ocean quahogs off Point Judith, Rhode Island. It is possible that the percentage of solids reported here for ocean quahogs is slightly high because the samples were taken just before spawning. Samples taken at intervals during the reproductive cycle would be necessary to clearly identify variation in percentage of solids due to gonad condition.

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PHOTOGRAPHIC METHOD FOR SURVEYING CLAM POPULATIONS [1]

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ABSTRACT

A photographic method for surveying softshell clam, Mya arenaria *beds is described and evaluated. The equipment, which includes an underwater camera affixed to an aluminum frame, is diver-operated and positioned during sampling. Some preliminary results concerning spacial arrangements in juvenile and adult beds are discussed.*

INTRODUCTION

Although a variety of adequate survey methods for estimating the density of clam populations are available, many possess specific disadvantages. The Maryland hydraulic dredge (Manning, 1959) has been successfully employed at our laboratory (Pfitzenmeyer, 1960; Pfitzenmeyer and Drobeck, 1963) as the primary sampling tool, but it, too, has limitations for certain types of research. Because of operational necessities (hydraulic jetting), *in situ* relationships can not be determined; and all sizes of clams are not equally collectable, due to selectivity of belt mesh size.

SCUBA or diving have been commonly employed (Forster, 1954; Morgans, 1959) as benthic survey tools as have photography (Owen, 1951; Veevers, 1952) and TV (Barnes, 1963). Carriker (1967 pg. 445) termed diving, underwater TV and other direct observational methods one of the ". . . most promising innovations in the study of benthos.", however, they have been infrequently applied in a quantitative manner. In order to study certain parameters of spacial arrangement in *Mya* beds, underwater photography, as a sampling tool was investigated. The present report gives construction and operational details of a photographic survey method.

METHODS

A Nikonos "Amphibious" 35 mm camera was affixed by a utility clamp (E. H. Sargent & Co. S-19140) to an adjustable floodlight stand (Fuller and d'Albert Inc., "Picstand #122") that was, in turn, mounted on an aluminum frame (Fig. 1). The frame was fitted with a clamp cradle for the flash attachment (Fig. 1,a) and a wooden carrier for flashbulbs (b). Positional adjustments of the camera could be made in both the horizontal and vertical planes by loosening wingbolts (c). A metric ruler, which could be extended into the photographic field was attached to the aluminum frame (d).

Even though an underwater close-up lens system for the camera was available, the land lens (Nikkor 1:2.5, f-35 mm) proved satisfactory for all operations. Camera settings were routinely held at the following: distance to subject = 2.75 feet, f =22, speed = 1/250, measured distance to subject = 0.5 meter. Three types of film were used in preliminary trials (Kodak Tri-X pan, Plus-X, and Panatomic-X) all with excellent results. Special negative developers, that increase ASA ratings, were unsuccessfully employed and Kodak "Microdol" was used thereafter. The latter, with slow development times, permitted sharp resolution of detail. Survey sample shots were taken both with and without flashbulbs (#6), depending on water clarity. Flash lighting occasionally produced "washed" negatives indicating that stoboscopic or diffuse lighting should be examined as a possible alternative. Low contrast

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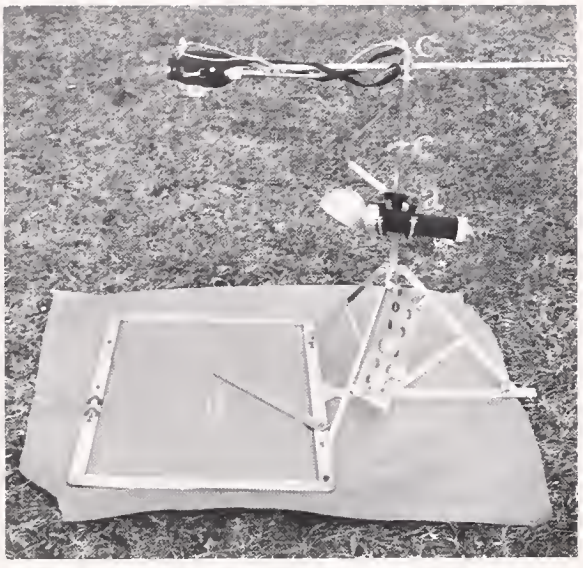

FIG. 1. *Photograph of the camera and stand used in surveying clam beds. Legend: (a) flash and cradle attachment, (b) flash bulb holder, (c) wing bolts and clamps used for adjustment of camera, (d) ruler shown extended into photographic field. Also shown (unlettered) are line levels used to adjust the camera in the horizontal plane. Photo credits: E. Dunnington and M. Reber.*

paper was not required with most sample negatives; it proved necessary when turbid conditions prevailed.

In sampling, the camera and stand were manually transported and hand operated. Depending upon water depth, the operator used either a snorkel or SCUBA. Care was required in positioning the stand as slight movements caused localized turbidity that prevented clear photographs. With practice, however, it was possible to photographically survey (20-40 shots) a large area in less than an hour. The area included in a sample was approximately 120 cm^2 and photographic enlargement (8 x 11 in.) was essentially 1:1.

RESULTS

Typical photographic results show the siphons of young-of-the-year *Mya* (1967 year class) protruding from the substrate (Fig. 2). Young clams were extremely numerous in this sample. The two large spherical objects (one near center, one near left edge) are tunicates, *Mogula manhattensis*. The one near the center borders a typical adult *Mya* siphon hole and others may also be seen in the right half of the photograph. Adult siphon holes are characteristically non-circular in appearance. This may be due to substrate characteristics or to continual extension and retraction of the siphon with erosion of the edge. Regardless of the cause, it does not appear practicable to employ the siphon hole as an estimator of clam size as was initially hoped. Large clams retract their siphons at the slightest mechanical or visual disturbance. Merely occluding the ambient light caused retraction. Siphons of retracted clams could still be seen by the diver if the line of sight was directly over the siphon-hole. However, the siphons were usually re-extended (enough to be photographed) after a brief time period.

Although no actual measurements of clam shell length were made in the present study, approximate sizes are known from previous collections made at this station on a quarterly basis over a five year period. Examination of these collection data suggest that the juvenile and adult clams categories, used in the present study, represent shell lengths of approximately 25 and 80 mm respectively.

An extreme density of young clams has been emphasized by an enlargment of the photographs (Figs. 2, 3). Notice the abundance of these clams and the fact that many siphons are contiguous to each other. This pattern is in contrast to that characterizing adult beds in this area. Adult clams are more regularly spaced and the distance between clams (measured in a direct line from a given individual to the nearest neighbor) is about twice that of young clams. The average, between-clam distance for adults was approximately 20 mm while that for juveniles was 9 mm in a test series of 45 measurements.

Clam densities for both juvenile and adult beds were estimated by griding the photographic enlargement and counting the number per cell. The number of cells per sample counted varied from approximately 10-100. These estimates were expressed to the common base of a square meter sample. Density estimates obtained by different readers varied slightly, but there was fair agreement between estimates from different operators (5.6 vs 7.5 x 10^3 clams per M^2) for the densest samples which were the most difficult to quantify. In addition, the variances for these counts were small suggesting that highly accurate counts can be obtained and that the counts are reproducible, as long as samples are read by the same person. A concurrent hydraulic and photographic sampling has not been performed and this should be done for comparative purposes.

Clark and Evans (1954) suggested a technique by which spacial relationships between animals of the same species may be measured. This measure depends upon between-animal distance

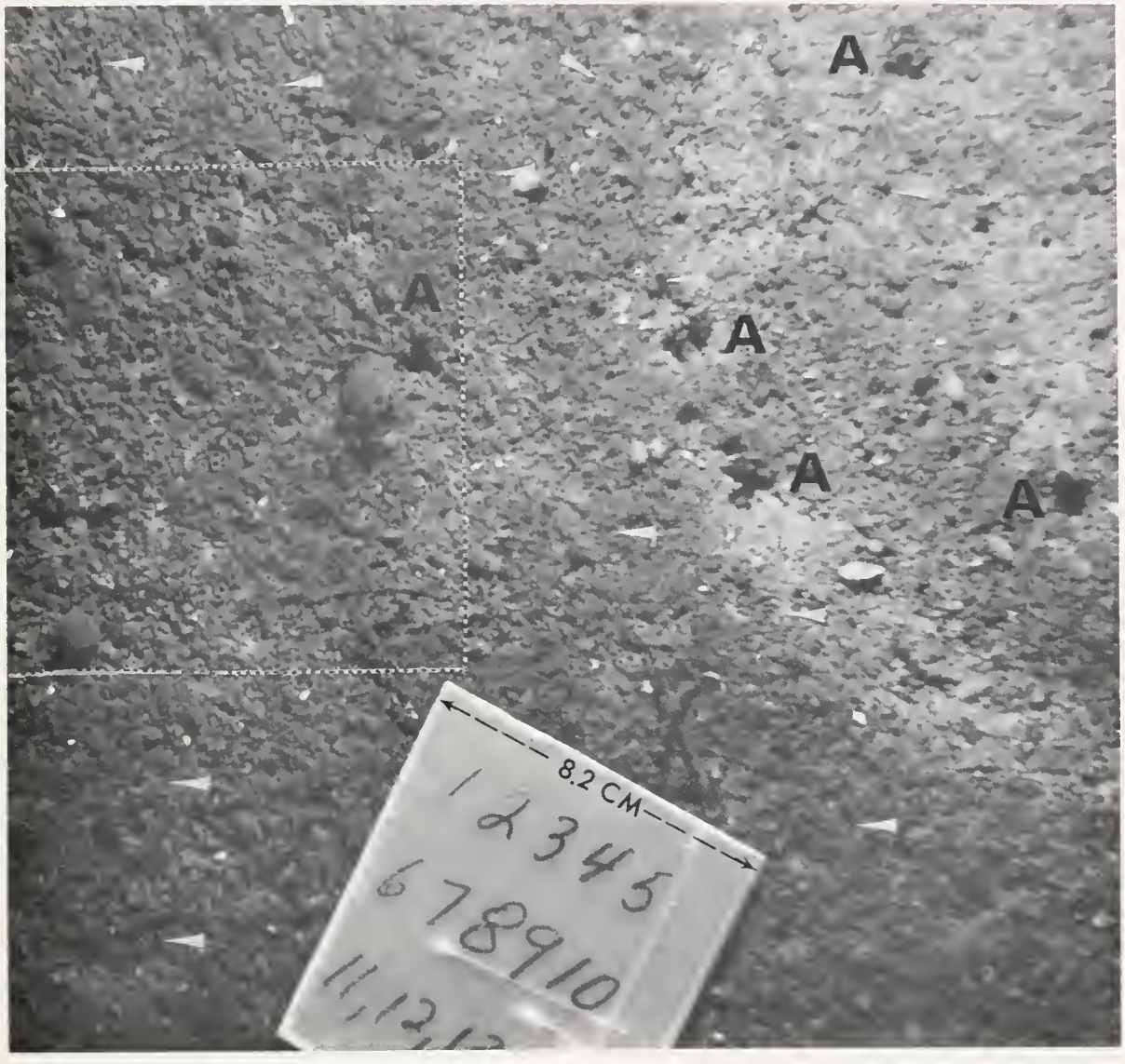

FIG. 2. *An example of a bottom photograph from Jack Bay Station. "A" denotes siphon holes of adult clams. The siphons have been retracted and are not visible. At bottom, center is a plastic plate used for numbering the survey series. A metric scale appears on the plate. Arrows mark some of the more obvious siphons of juvenile clams. Inset area is shown, enlarged, in Fig. 3.*

and density per unit area and is expressed as R, a number which ranges from 0 to slightly greater than 2. The maximum value occurs with a regular geometric lattice wherein all individuals are equidistant from each other. Values less than 1 suggest clumped distributions. Values close to 1 suggest a random distribution and those above 1 to the upper value indicate regularly spaced distributions. This measure was applied to both juvenile and adult photographic samples. Data for juveniles gave R values of slightly less than 1-1.5 and suggest that the spacial arrangement for this life history stage may be clumped or random with respect to its neighbors. In contrast, adult samples gave R values of about 1.8, indicative of a highly spaced condition i.e., clams are more uniformly positioned in relationship to each other.

By extension, this may mean that an individual adult clam inhibits or exerts some influence over its proximal neighbors. Also, since the juvenile pattern is close to random, as judged from the R value, it is possible that spacing of *Mya* is age-determined and the maturational pattern is from

FIG. 3. Enlargement of Inset area from Figure 2. Some extended siphons of juveniles are marked by arrows, but many more appear that have not been indicated. Siphons have the appearance of a figure "8" with darkened centers. Distance between the tunicates (labelled "m") is 8.9 cm.

nearly random to regularly spaced. These interpretations are speculative but may have application in stimulating research or in developing a theory of population control for this species.

DISCUSSION

These preliminary results indicate that the photographic method is useful as a survey tool. Many random samples in a particular locality may be taken in the time period required to take a single dredge sample. Additionally, since the photographic method does not disturb the animal or the substrate, it would be possible to monitor exact sites through time with high precision.

Preliminary measurements of between-clam distances (Clark and Evans, 1954) for both adults and juveniles suggest that the former are more regularly spaced. Whereas the juveniles are clumped or randomly placed in reference to each other, adults are more nearly geometrically arranged. Thus it may be that larger clams inhibit setting or continued growth in close proximity to themselves.

A possible disadvantage to the photographic method is its dependence upon moderately good water clarity. This is not true of the hydraulic dredge and most other benthic samplers.

ACKNOWLEDGMENTS

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SEASONAL FACTORS RELEVANT TO COLIFORM LEVELS IN THE NORTHERN QUAHAUG [1]

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ABSTRACT

Few coliform organisms can be recovered from the northern quahaug, Mercenaria mercenaria, *when the water temperature falls below 10°C for considerable periods of time, even though they are growing in heavily polluted waters. This could be due to a more marked effect of low temperatures on microbial uptake as contrasted to elimination, resulting in a gradual decrease in the coliform levels in the animals. A second possibility is death of the organisms within the animal. In the work reported, the effect of seasonal changes on the presence of fecal coliforms in animals from a polluted area is documented. The greater inhibitory action by temperatures of 10°C and lower and by turbidities above 10 Jackson Turbidity Units on uptake as compared to elimination of* Escherichia coli *by the quahaug is demonstrated. Data on the effect of temperature and turbidity changes on the experimental uptake and elimination of* E. coli *by the animal are presented. Possible bases for these differences are discussed.*

INTRODUCTION

Very few coliform organisms can be recovered from the northern quahaug, *Mercenaria mercenaria,* during the winter at latitudes where the water temperature remains below 10°C for considerable periods of time even though the animals are taken from highly polluted waters. Although the "hibernation" of the animal with a corresponding marked reduction in feeding activity under the influence of low temperatures and other seasonal factors is inherent to any explanation for these observations, the death of the organisms already present and their physical elimination must be considered. The physical elimination of the organisms requires that the processes of uptake and elimination of coliform organisms are not equally affected by environmental parameters, especially temperature, which operate under these conditions. In previous publications (Cabelli and Heffernan, 1970; Heffernan and Cabelli, 1970), the influence of various environmental factors on both the uptake and elimination of *Escherichia coli* by the quahaug was considered. The work presented documents the effect of season on the levels of coliforms in animals growing in polluted waters. The influence of temperature and turbidity on experimental elimination of *E. coli* relative to its uptake is also considered, and data on the seasonal effects impinging on both these processes are presented.

MATERIALS AND METHODS

The procedures used to prepare *E. coli* suspensions for experimental contamination of the quahaugs, the source of the animals, and the experimental systems and methods to examine uptake and elimination of the bacteria by the quahaugs have been described previously (Cabelli and Heffernan, 1970; Heffernan and Cabelli, 1970). The water in the flow through system during both uptake and elimination was maintained at a flow rate of 13 ml/min/animal and at a salinity of 30-31‰. A pour plate procedure on a modified MacConkey agar medium incubated at

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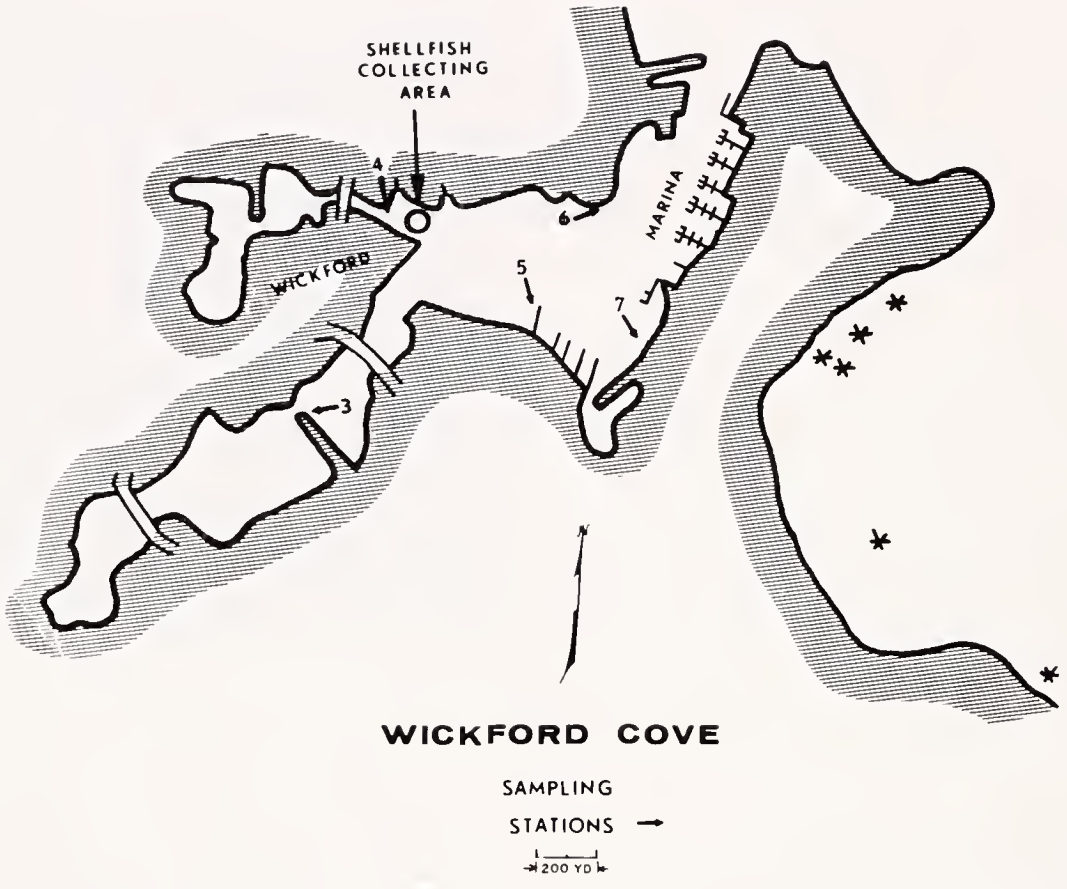

FIG. 1 *Location of sampling stations in Wickford Cove, Rhode Island. The numbered stations corresponed to those given in Table 1. Stippled areas indicate intertidal zone.*

45.5°C in an air incubator (Cabelli and Heffernan, 1970) was used for enumerating *E. coli* in the artificially contaminated animals and a portion of the coliform population — those capable of growing on this medium at the specified temperatures (ET coliforms) — in naturally contaminated quahaugs.

Accumulation indices were obtained from experiments in which individual animals were assayed for *E. coli* after a 24 hr exposure to *E. coli* inoculated sea water. Indices were obtained by dividing the median *E. coli* level per 100 gm of tissue in the accumulating animals by the level in the water per 100 ml (Cabelli and Heffernan, 1970).

The influence of seasonal factors on the levels of ET coliforms in animals taken from polluted waters was studied by periodically collecting animals from a sampling station in Wickford Cove, Rhode Island (Fig. 1) and assaying pools of at least six animals each for their ET coliform content. Water samples from this station and several adjacent to it (Fig. 1) also were assayed for coliforms and fecal coliforms using a 12-tube MPN test (American Public Health Association, 1962) as well as the plating procedure. Fecal coliform levels were found to be comparable to ET coliform levels with seawater and shellfish samples collected from Wickford Cove.

Temperature acclimatization of animals, removed during the fall and winter from waters at falling and low ambient temperatures, was attempted by maintaining the quahaugs for specified periods of time in flowing seawater heated to 20°C.

The turbidity of environmental seawater in the experimental system was increased artificially by continuous addition of a 1% bentonite suspension to the mixing boxes at a rate which maintained the desired turbidity in the holding tanks. The median particle size of bentonite particles in the suspension was 2 μ.

RESULTS

The levels of coliforms, fecal coliforms and ET coliforms in the water at the sampling stations are shown in Table 1. From Figure 2, it can be seen that the numbers of ET coliforms in the animals decreased during the fall to an undetectable level during the winter and increased again during the spring. The organisms reappeared in the quahaugs during the week of 17 April when the water temperature had risen to 10°C, and the levels in the animals increased rapidly during the subsequent four weeks. The level of ET coliforms in the quahaugs remained high during the summer, gradually decreased during the fall and fell below the sensitivity of the assay method during the winter. These observations were in keeping

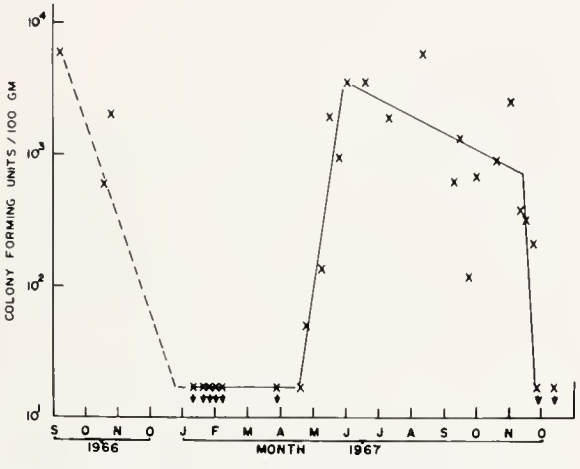

FIG. 2. *Seasonal fluctuations in ET coliform levels in quahaugs taken from a single polluted area. ET coliform levels are expressed as colony forming units (CFU) per 100 gm of animal tissue (includes liquid in the shell). A broken line was used to show the decline in levels during the fall of 1966 because of the paucity of data.*

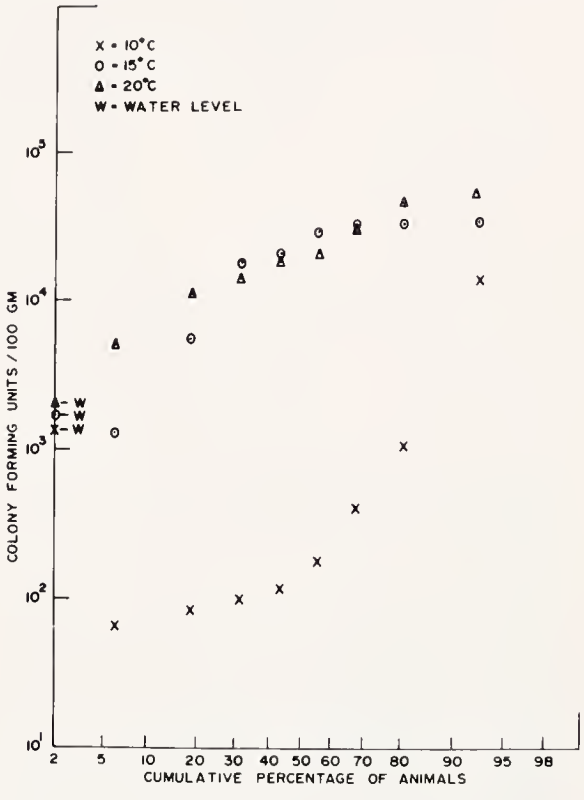

FIG. 3 *Experimental accumulation of* E. coli *by the Northern Quahaug as a function of water temperature. Each point indicates the level of* E. coli *in an individual animal. The water level (W) is the number of* E. coli *expressed in CFU/100 ml of the water. The accumulation interval was 24 hr, salinity and flow rate of the seawater were 30-31‰ and 13 ml/min/animal, respectively.*

TABLE 1. *Coliform, fecal coliform and ET coliform levels of water from sampling stations adjacent to the shellfish collection area.*

	Coliform MPN/100 ml			Fecal Coliform MPN/100 ml			ET Coliform Count CFU/100 ml		
Station	Fall	Winter	Spring	Fall	Winter	Spring	Fall	Winter	Spring
4	>2400	>2400	4090	>2400	1070	590	2470	1210	468
	>2400	>2400	1070	>2400	1300	170	4734	2305	93
5	>2400	142	570	1800	19	86	2573	12	56
	570	290	360	280	120	142	257	112	88
6	93	1100	720	76	160	64	48	165	202
	76	460	760	16	280	31	13	232	56
7	320	62	1500	93	6	106	100	17	63
	145	460	260	16	67	32	15	65	32

TABLE 2. *Accumulation of* E. coli *at 20°C as a function of environmental conditions of the animals preceding uptake.*

Ambient water temp [b]	Uptake period (hr)	Accumulation factor [a] for animals acclimatized at 20°C for weeks			
		0	1	3-4	9-10
15°C	24	2.2	3.6	2.9	5.7
	48	2.7	2.7	9.4	7.9
<12°C	24	3.5	—	1.3	2.7
		4.4			
		1.4			
	48	3.5	—	1.9	—
		2.5			

[a] Accumulation factor — median level of "steady state" animals/level in water.

[b] Temperature of water at time animals removed for test or acclimatization.

with those made in the laboratory concerning the influence of the water temperature on the experimental accumulation of *E. coli* by the quahaug.

At 10°C, most of the animals did not concentrate the organisms from the water (Fig. 3). Even when the quahaugs were allowed to accumulate *E. coli* at the optimal temperature of 20°C, if they were collected during the fall and winter from waters whose ambient temperatures had dropped to 15°C or less, the accumulation indices (Table 2) were lower than those seen with summer animals (about 7; Cabelli and Heffernan, 1970). Acclimatization for 3-10 weeks at 20°C of a group of animals removed from 15°C water on 10 October did result in accumulation indices similar to those obtained with summer animals. This was not achieved with animals removed three weeks later from waters whose ambient temperatures had fallen below 12°C (Table 2). The elimination rates of *E. coli* however, did not differ appreciably between temperatures of 10 and 20°C (Fig. 4).

Similary, the effect of turbidity was more pronounced on the uptake than on the elimination of *E. coli* by the quahaugs. While a turbidity of 25 JTU, achieved by adding bentonite to the water to a final concentration of 25 mg/liter, markedly reduced accumulation (Fig. 5), the same concentration of bentonite, if anything, may have increased the elimination of the organisms from the animals (Fig. 6).

Data suggest that the death of ET coliforms in the living animal also may be important in accounting for the absence of these organisms in the quahaug during the winter (Fig. 7). In those experiments, the numbers of ET coliforms in naturally polluted animals subjected to dry storage at temperatures between 6 and 18°C were determined over a period of two weeks.

During the middle of April when the ambient water temperature had risen to 10°C and fecal coliforms first were detected in the naturally contaminated animals (Fig. 2), quahaugs placed in 20°C water appeared to be hyperactive in their ability to concentrate *E. coli* from the water (Table 3). This hyperactivity reached a peak the

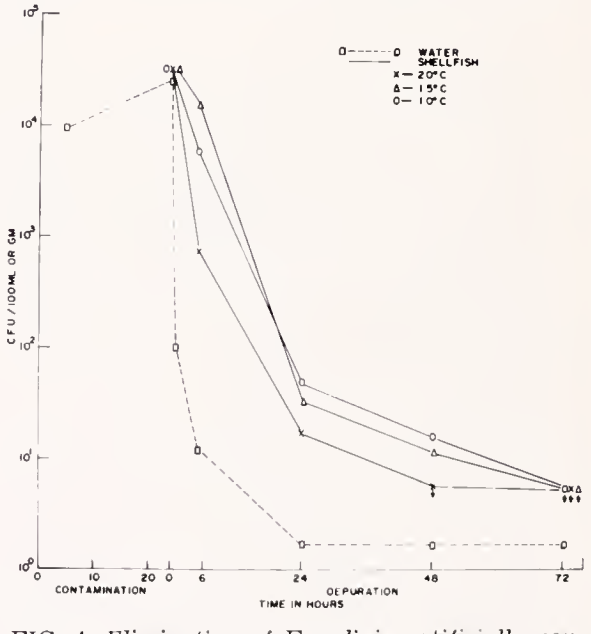

FIG. 4. *Elimination of* E. coli *in artificially contaminated quahaugs as a function of water temperature. The* E. coli *levels as obtained from pools of six animals each are expressed as a function of the elimination interval. The broken line shows the* E. coli *level in the water. Salinity was 30-31‰ and the flow rate was 13 ml/min/animal.*

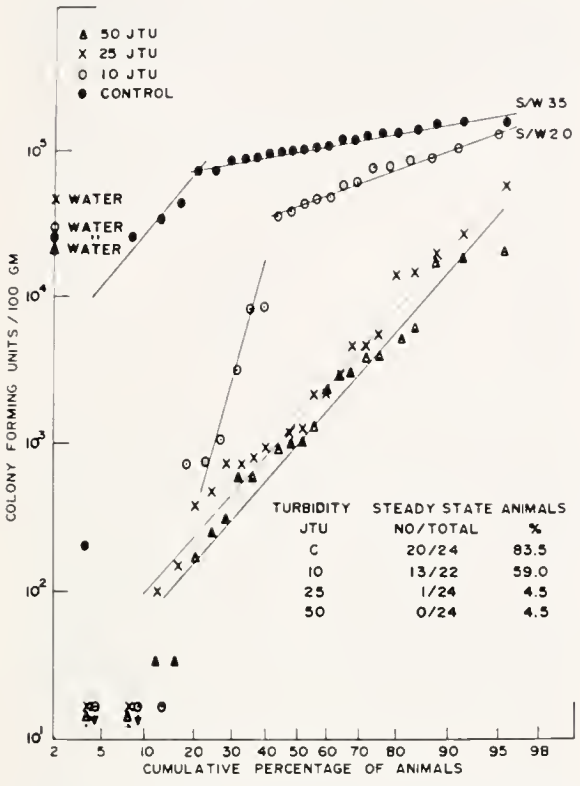

FIG. 5. *The experimental uptake of* E. coli *by the quahaug in waters of varying turbidities achieved by the addition of bentonite to the water. JTU — Jackson Turbidity Units. The points represent the levels in individual animals. "Steady state" animals are defined as those animals whose* E. coli *levels were in that portion of the bimodel distribution curve in which the levels in the animals exceeded that in the water. The median* E. coli *level of the "steady state" animals divided by the level in the water was used to calculate the accumulation index (S/W). Temperature, 20°C; salinity 30-31‰; flow rate, 13 ml/min/animal.*

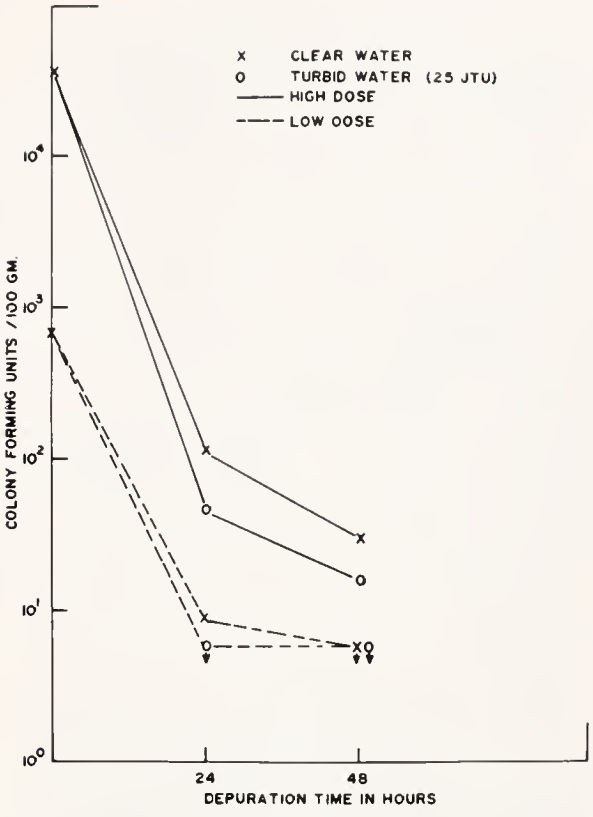

FIG. 6. *The elimination of* E. coli *from artificially contaminated quahaugs as a function of the turbidity of the water. The turbidity of the water was increased by the addition of bentonite to the environmental water. The* E. coli *levels in four pools of six animals each are expressed as a function of the elimination interval. "High dose" and "low dose" indicate the initial levels of* E. coli *in the animals following the accumulation phase of the experiment. Temperature, 20°C; salinity, 30-31‰; flow rate, 13 ml/min/animal.*

following week and then declined. Accumulation at ambient temperatures followed a similar pattern but was delayed by approximately one week (Table 3). This effect of "thermal shock" also was observed at approximately the same time during the previous year.

The accumulating ability of the quahaug appeared to be extremely sensitive to rather small changes in temperature — or some other seasonal factor — during the spring and the fall when the animals were going into or coming out of so-called "hibernation". Thus, as noted above, a rise in temperature of 1-2°C during April, when the ambient temperature had reached 10°C, markedly increased accumulation. Conversely, during late September a drop in the ambient temperature to 18-19°C decreased the accumulation index to a value of 2.1.

DISCUSSION

If, as suggested by the data, environmental influences can affect the uptake and elimination of bacteria disproportionately, an explanation must be sought in the underlying anatomical and physiological mechanisms. Since most of the organisms are concentrated within the digestive system of the animal (Cabelli and Heffernan, 1970), the mechanisms underlying uptake prob-

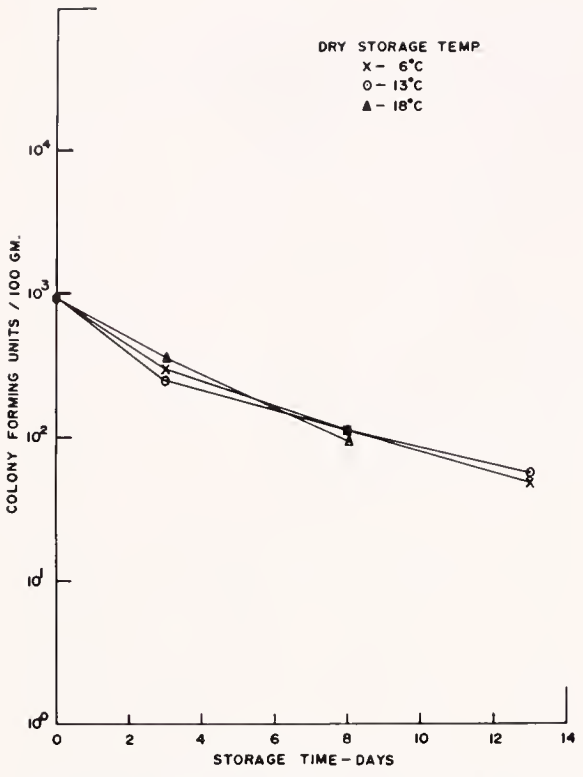

FIG. 7. *Changes in the ET coliform levels in naturally polluted shellfish during "dry storage at various temperatures. The mean of the levels in two pools of six animals each was used to define each point.*

ably are those involved in the ciliary action controlling transport of water across the gills and suspended particulates over the gills and palps to the mouth. The effect of environmental factors such as temperature, salinity, etc., on these functions have been examined by several investigators (Vernberg, Schlieper and Schneider, 1963; Rice and Smith, 1958; Coughlin and Ansell, 1964).

Elimination of *E. coli* already within the digestive system requires, in addition, transport of the organisms through this system and ejection to the outside environment as fecal material. The influences of environmental conditions on these mechanisms have received little attention. That elimination can take place in the absence of pumping is known from the observation of voided deposits alongside animals maintained in "dry storage". It may be related to the "dribbling" of foreign material in the absence of water transport which Hillman (1963) associates with a fourth fold in the mantle. The effect of temperature on ciliary activity of the epithelium lining areas of the digestive tract has not been studied as it has with gill tissue.

TABLE 3. *Accumulation indices for the experimental uptake of* E. coli *by quahaugs at ambient and 20°C temperatures collected during rising water temperatures in the spring.*

Date	Ambient temp.°C	Accumulation index at ambient	20°C
1/16	5	—	2.5
3/17	7	—	3.1
4/17	10	<1	14.7
5/1	11	13.9	27.8
5/23	12	21.4	7.4
5/31	15.5	16.5	11.8

If, as shown by the data, bacterial accumulation, but not elimination, is highly sensitive to small change in temperature during the fall, the decrease in coliform levels in the animals during this time and their absence during winter "hibernation" can be explained as a consequence of the unbalanced effect of falling water temperatures on the two processes. The above not withstanding, from data on growth rates (Pratt and Campbell, 1956), animal activity (Feng and Haskin, 1963) and the uptake and elimination of *E. coli*, 10°C appears to be a minimum for physiologic activity in the northern quahaug.

Feng and Haskin (1963) have demonstrated that effects of temperature on several characteristics associated with the feeding activity of the northern quahaug, as determined in the laboratory, are modified by ambient environmental conditions. The data from the present study demonstrating hyperactive accumulation of the bacteria as the animals come out of "hibernation" during April and May in Rhode Island substantially agree with Feng and Haskin's findings in animals harvested during March in Delaware Bay. In both instances, hyperactivity was observed with temperatures from 10-20°C; and, around 10°C, a sharp increase in activity was obtained as a consequence of a small rise in temperature.

A similar sensitivity to small changes in temperature was observed at this laboratory with animals harvested in the fall during falling temperatures. No similar stimulation on the elimination of *E. coli* has been observed. To the contrary, animals contaminated artificially with *E. coli* during the period of hyperactivity in the spring did not eliminate the organisms as readily as those contaminated during the summer (Heffernan and Cabelli, 1970). This may be related to the observation that the time required for the clam to eliminate the organisms is dependent on the initial contamination level. Thus, when the *E. coli*

density in the environmental water is high or if the density is low but the animals are hyperactive, there could be an increase in the absolute numbers of organisms which lodge in the quahaugs at locations or in niches less accessible to cleansing mechanisms. Cytologic techniques will be required to clarify this point.

The quantity of suspended material required to inhibit uptake of bacteria by *M. mercenaria* appears to be comparable to or somewhat less than that required to influence water transport in *Crassostrea virginica*. In the present study, the addition of 10 mg/liter (10 JTU) of bentonite only slightly affected uptake, while 25 mg/liter (25 JTU) completely inhibited accumulation. Lund (1957) found that 10 mg/liter of Fuller's earth did not measurably influence water transport in *C. virginica;* Loosanoff and Tommers (1948) observed a 50% decrease in the rate of water transport when 100 mg/liter of Fuller's earth was added to the water.

It would appear that both death of the fecal coliforms in the animal undergoing "hibernation" and a more pronounced inhibition of uptake relative to elimination could be responsible for low levels of coliforms observed in quahaugs taken from polluted waters during the winter at temperatures less than 10°C. Evidence has been presented (Rindge, Clem, Linkner and Sherman, 1965) implicating quahaugs taken from grossly polluted waters in Greenwich, Connecticut, between December and April as vehicles for the transmission of infectious hepatitis. Therefore, it becomes important to determine whether or not viruses do not die in the animal as rapidly as the indicator or if the processes underlying elimination of the virus are more sensitive to the effects of temperature than those governing removal of the bacteria. Data obtained in identical experimental systems indicate that the elimination of Poliovirus I (Liu, Seraichekas and Murphy, 1967) is more sensitive to the effect of temperatures between 10-15°C than the elimination of *E. coli* (Heffernan and Cabelli, 1970).

It is significant, however, that microbiological standards governing shellfish harvest for human consumption quite correctly are based upon coliform levels in the overlying waters of the growing areas rather than those in the animals themselves. Thus, difficulties which could arise because of this situation (U. S. Department of Health, Education and Welfare, 1965) are eliminated.

ACKNOWLEDGMENT

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THE ELIMINATION OF BACTERIA BY THE NORTHERN QUAHAUG: VARIABILITY IN THE RESPONSE OF INDIVIDUAL ANIMALS AND THE DEVELOPMENT OF CRITERIA[1]

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ABSTRACT

Trials were performed to determine the distributions of residual ET coliform levels in individual quahaugs following depuration. The distributions were dependent on the initial level of contamination in the animals and on the manner by which they were polluted, i.e., artificially with cultures of Escherichia coli *as opposed to naturally in polluted waters. From the data obtained with the naturally polluted animals, a monitoring system for the depuration process and some tentative target residual levels were developed for further evaluation of the process with "moderately polluted" animals. This was accomplished in 15 trials which showed that, following depuration of moderately polluted quahaugs, a product could be produced which, in terms of these coliform organisms, was as good or better than that currently accepted from approved areas.*

INTRODUCTION

It has been known for some time that bivalve molluscs taken from polluted waters can reduce their content of enteric micro-organisms when placed in an environment free of these organisms. The commercial application of this principle to the purification of shellfish to be consumed as food (depuration) has been used in Europe with oysters and mussels and in the United States with soft-shell clams.

In previous studies, significant environmental parameters relevant to the uptake and elimination processes in the quahaug, *Mercenaria mercenaria*, were examined (Heffernan and Cabelli, 1970; Cabelli and Heffernan, 1970; Cabelli and Heffernan, 1971). *Escherichia coli* and/or ET coliforms — those coliforms capable of colony formation on a modified MacConkey agar pour plate at an air incubation temperature of 45.5°C — were used as models for the behavior of enteropathogenic bacteria and possibly viruses and as logical organisms for monitoring the depuration process. Factors such as the temperature, salinity, turbidity and flow rate of the environmental water, the spacial arrangement of animals in the basket and the initial level of contamination in the animals were examined. Preliminary data on the variability in animal response during depuration were presented.

In the work described, individual animals were examined for their residual ET coliform levels following depuration to study the variability in animal response. This information then was used to examine the feasibility of the depuration process for the elimination of bacteria, possible criteria for measuring the sanitary quality of the final product and a means of monitoring the process. Finally, the attainability of the target residual levels was examined in a series of trials using the proposed monitoring process.

MATERIALS AND METHODS

The experimental systems and conditions used during artificial contamination and subsequent

[1] Contribution No. 36: Northeastern Water Hygiene Laboratory, Narragansett, Rhode Island 02882. A part of the Environmental Protection Agency, Cincinnati, Ohio. 45213.

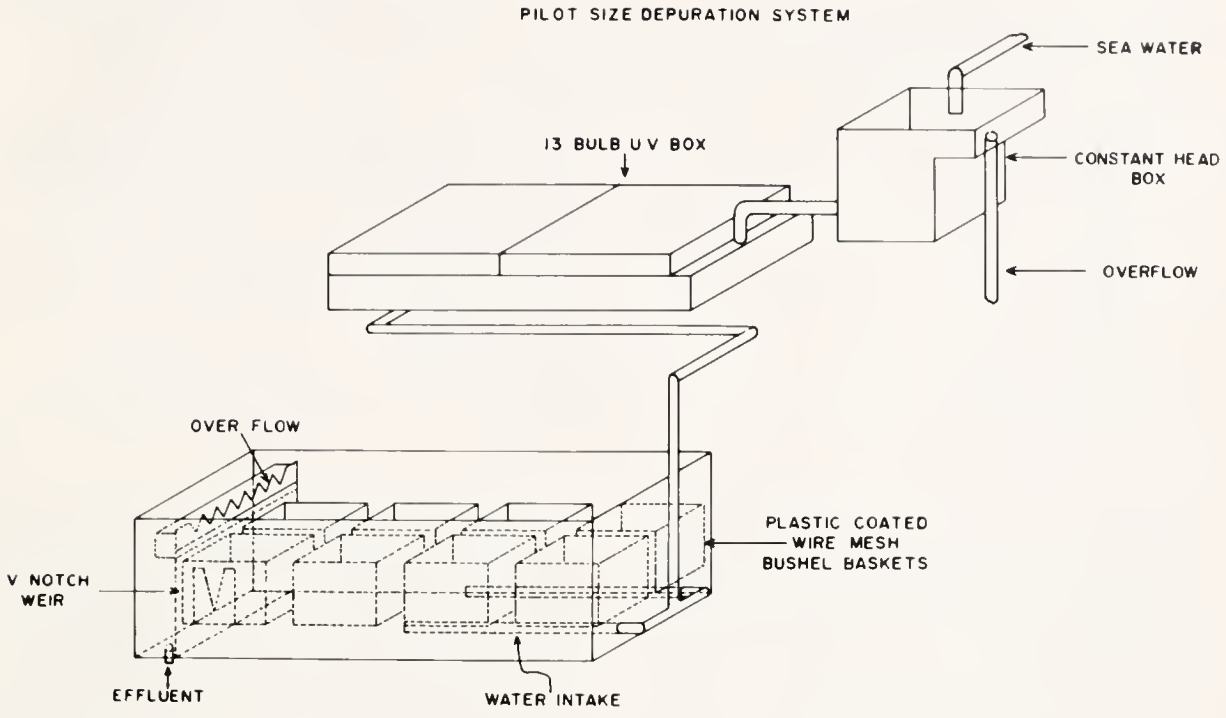

FIG. 1. *"Pilot plant" depuration system.*

depuration are those described in previous reports (Cabelli and Heffernan, 1970; Heffernan and Cabelli, 1970). The "pilot plant" system (Fig. 1) was used exclusively for those trials with naturally polluted animals. The environmental conditions maintained in the depuration system were as follows; temperature, 20°C; salinity, 30-31‰; water flow rate, 13 ml/animal/min; depth of animals in the basket, 3 in.

Naturally polluted quahaugs *(M. mercenaria)* were obtained during the late spring, the summer and the early fall from polluted areas in Narragansett Bay, Rhode Island. In the second series of trials, the quahaugs were harvested from sites likely to yield only moderately polluted animals with initial ET coliform levels of 100-1000 Colony Forming Units (CFU) per 100 gm. Depuration was initiated upon receipt of the

TABLE 1. *Residual levels following depuration of quahaugs artificially contaminated with* E. coli.

Initial level CFU/100 gm	Depuration Time (hr)	Number of animals	Median	Mean [a]	Residual levels, CFU/100 gm distribution
2.2×10^5	48	24	33	61	<25(5 [b],25(5),33(2),50(6),75(2),100(2),275,375
4.3×10^4	48	24	<17	<17	<17(22),17,33
3.0×10^4	48	24	17	32	<17(12),17(6),33(2),50,83,100,282
3.0×10^4	48	24	<17	18	<17(15),17(5),33(2),66,83
4.6×10^3	48	24	<17	<17	<17(24)
4.0×10^3	24	24	<17	<17	<17(24)
2.7×10^3	48	24	<17	<17	<17(24)
3.3×10^2	24	24	<17	<17	<17(23),17
3.0×10^2	24	24	<17	<17	<17(19),17(4),33
3.0×10^2	24	24	<17	<17	<17(24)

[a] Arithmetic mean.

[b] ( ) - number of animals in parenthesis.

TABLE 2. *Residual ET coliform levels following depuration of naturally polluted animals.*

Initial level CFU/100 gm	Depuration Time (hr)	Number of animals	Median	Mean [a]	Residual levels, CFU/100 gm distribution
$3.1x10^3$	48	50	<17	396	<17(40),17(2),100,150,618,1100,1750,2070,5840,7860
$3.1x10^3$	48	24	58	149	<17(5),17,50(3),66(3),116(2),150,166(2),199,216,250 (2),300,365,880
$2.0x10^3$	48	24	<17	<17	<17(24)
$1.9x10^3$	48	24	<17	82	<17(21),66,630,1100
$9.5x10^2$	48	50	<17	55	<17(39),17(6),33(2),50,979,1280
$5.0x10^2$	48	24	<17	<17	<17(24)
$4.2x10^2$	48	24	<17	<17	<17(23),166
$3.4x10^2$	48	24	<17	<17	<17(23),133
$1.4x10^2$	48	24	<17	<17	<17(23),17

[a] Arithmetic mean.

animals in the laboratory. Following a depuration period, usually of 48 hr duration, animals were removed and assayed for ET coliform levels individually in pools of six animals each (Cabelli and Heffernan, 1970).

RESULTS

Several trials were performed to compare residual ET coliform levels following depuration in naturally polluted quahaugs and in animals artificially contaminated with *E. coli*. Distributions of levels obtained in the trials with animals artificially contaminated with *E. coli* are shown in Table 1 and those with naturally polluted animals in Table 2. The trials were grouped arbitrarily according to the inital contamination density. Residual levels of the indivldual animals in each of the groups were ranked and plotted on log-probability paper so that predictions of 95 and 99 percentile levels could be obtalned. These plots are shown in Figure 2. With low initlal contamination densities, few animals had detectable residual levels; and, hence, the slopes of the lines are poorly defined. The median, mean and 95 and 99 percentile levels for each of the groups are shown in Table 3.

To compare the elimination of ET coliforms in naturally polluted animals to that of *E. coli* in artificially contaminated quahaugs, the mean, 95 percentile and 99 percentile residual levels for each group were plotted against the mean initial dose; extrapolations made to a common initial density of 580 organisms/100 gm are shown in Table 3. Higher residual levels were obtained with naturally polluted animals than with those artificially contaminated with *E. coli* (Table 3).

The next step in the development and evaluation of an operational bacterial depuration process for the quahaug required the establishment of a target residual level for the product and a monitoring system to determine if it could be achieved. Previous data provided several useful insights into this problem: (1) the evaluation would have to be performed with naturally polluted animals; (2) only animals with initial ET coliform levels less than about 950 CFU/100 gm

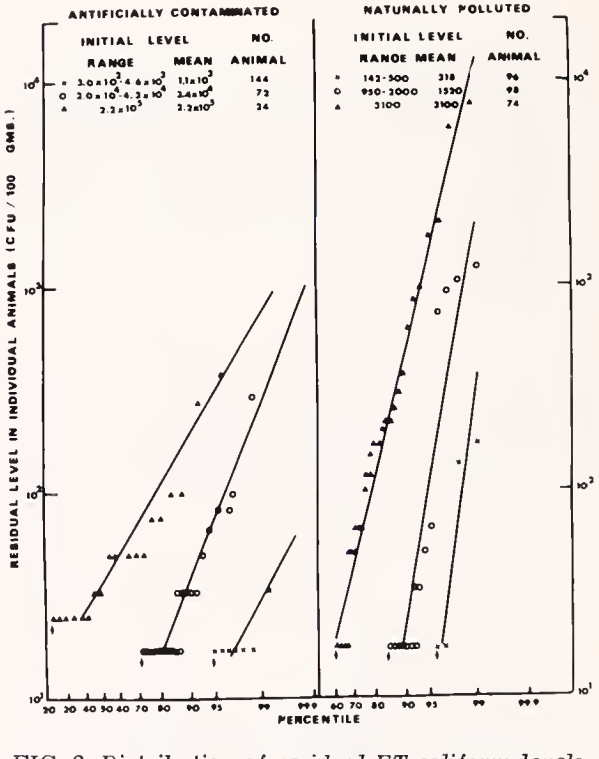

FIG. 2. *Distribution of residual ET coliform levels in individual animals grouped according to the united level of contamination.*

TABLE 3. *Comparison of* E. coli *and ET coliform residual levels following depuration of artificially and naturally polluted quahaugs.*

Initial levels Range	Mean	No. of trials	Contamination of Method animals	Residual levels in individual animals median	Mean [a]	Percentile 95	99
3.0×10^2-4.6×10^3	1.1×10^3	6	144 Artif.	<17	8.5	<17	30
3.0×10^4-4.3×10^4	3.4×10^4	3	72 Artif.	<17	25.0	74	270
2.2×10^5	2.2×10^5	1	224 Artif.	66	65.0	298	710
Extrapolation	580		Artif.	<17	6.0	5.0	22
1.4×10^2-5.0×10^2	3.2×10^2	4	96 Natur.	<17	11.0	<17	320
9.5×10^2-2.0×10^3	1.5×10^3	3	98 Natur.	<17	50.0	110	1800
3.1×10^3	3.1×10^3	2	75 Natur.	<17	310.0	1400	12000
Extrapolation	580		Natur.	<17	17.0	16.0	470

[a] Arithmetic mean. Values of <17/100 gm (sensitivity of assay method) set at 8.0/100 gms.

could be expected, with reasonable regularity, to cleanse themselves to very low levels in 48 hr; (3) in a successful trial at least 90% of the animals could be expected to cleanse themselves below the level of sensitivity of the assay procedure; and (4) the ET coliform residual level based upon comparibility to animals taken from approved areas should be less than 50 CFU/100 gm[2].

A sampling system, in which four pools of six animals each were examined, was selected as a compromise between the need to determine the composition of ET coliform residual levels in a representative number of individual animals and logistic limitations. A tentative target was selected wherein one of the limitations was that the ET coliform levels in the four pools did not exceed 25 CFU/100 gm. This was expected to define a product whose residual ET coliform levels was considerably less than the median from approved areas (Table 3). An additional limitation was that the levels in at least two of the pools be less than the sensitivity of the assay method, 17 CFU/100 gm, and that only one of the two remaining pools could exceed that level. Therefore, the large majority of the individual animals would be expected to have residual ET coliform levels below the sensitivity of the assay procedure. The combination of acceptable levels in the four pools as defined by the target criteria is shown in Table 4.

Fifteen trials were performed in the "pilot plant" system with naturally polluted animals at mean initial ET coliform densities between 140-2100 CFU/100 gm. After a 48-hr treatment interval, they were assayed as previously indicated. The criteria for residual levels were exceeded in only two of the trials; and in one of the two instances, the initial level (1.2×10^3 CFU/100 gm)

[2] This is based upon a maximum ET coliform value for the animals of 50 CFU/100. gm. The value of 50 was derived from the water coliform MPN of 70 as noted previously, 70/1.4 = 50.

TABLE 4. *Acceptable ET coliform residual levels following depuration.*

Pool	Residual levels per pool of six animals in CFU/100 gm									
1	<17	<17	<17	<17	<17	<17	<17	<17	<17	<17
2	<17	<17	<17	<17	<17	<17	<17	<17	<17	<17
3	<17	<17	<17	<17	<17	<17	17(1)	17(1)	17(1)	17(1)
4	<17	17(1)[a]	33(2)	50(3)	66(4)	83(5)	17(1)	33(2)	50(3)	66(4)
Total[b]	<4.2	4.2	8.3	12.5	16.7	20.2	8.3	12.5	16.7	20.2

[a] Number of colonies (N) per 6 gm of homogenate assayed from pool (P).

[b] Total CFU/100 gm = $\dfrac{N_{p1} + N_{p2} + N_{p3} + N_{p4}}{4 \times 6} \times 100$

TABLE 5. *Depuration of naturally polluted quahaugs using the prescribed monitoring system.* [a]

Initial level	Number of pools with a residual level of				
	<17	17	33	50	150
1.4x10[b]	4				
1.5	3	1			
1.6	3	1			
1.9	4				
3.0[b]	2	1			1
3.5	2	1	1		
4.0	4				
5.7	3	1			
5.9	2	2			
7.5	4				
8.8	3			1	
1.2x10[c]	4				
1.2	2	1			1
1.4	3	1			
2.1	2	1	1		

[a] Temp., 20°C; salinity, 30-31‰; flow rate, 1 gal/bushel /min.

[b] Rejected as unacceptable.

[c] Median initial levels — 570.

exceeded an acceptable initial level ($9.5x10^2$) In both instances, the mean level of 42 CFU/100 gm was attributable to a high value (150 CFU/100 gm) for only one of the three pools (Table 5).

The frequency with which the different possible distributions occurred is shown in Table 6. Also included in Table 6 is a projected distribution of the ET coliform densities in the individual animals if it is assumed that the level in any given pool of six quahaugs is attributable to a

TABLE 6. *Frequency of acceptable combinations of ET residual coliform levels in pooled samples* [a]

Residual ET coliform levels as CFU/100 gm		
In pools of 6 animals	In individual animals	Frequency [b]
<17 (4)[c]	<100 (24) [d]	5
<17 (3), 17 (1)	<100 (23), 100 (1)	4
<17 (3), 33 (1)	<100 (23), 200 (1)	
<17 (3), 50 (1)	<100 (23), 300 (1)	1
<17 (3), 66 (1)	<100 (23), 400 (1)	
<17 (3), 83 (1)	<100 (23), 500 (1)	
<17 (2), 17 (2)	<100 (22), 100 (2)	1
<17 (2), 17 (1), 33 (1)	<100 (22), 100 (1), 200 (1)	2
<17 (2), 17 (1), 50 (1)	<100 (22), 100 (1), 300 (1)	
<17 (2), 17 (1), 66 (1)	<100 (22), 100 (1), 400 (1)	

[a] Assumes that levels in a pool of six animals are contributed by a single quahaug.

[b] As observed from experimental data.

[c] Number of pools in parenthesis.

[d] Number of animals in parenthesis.

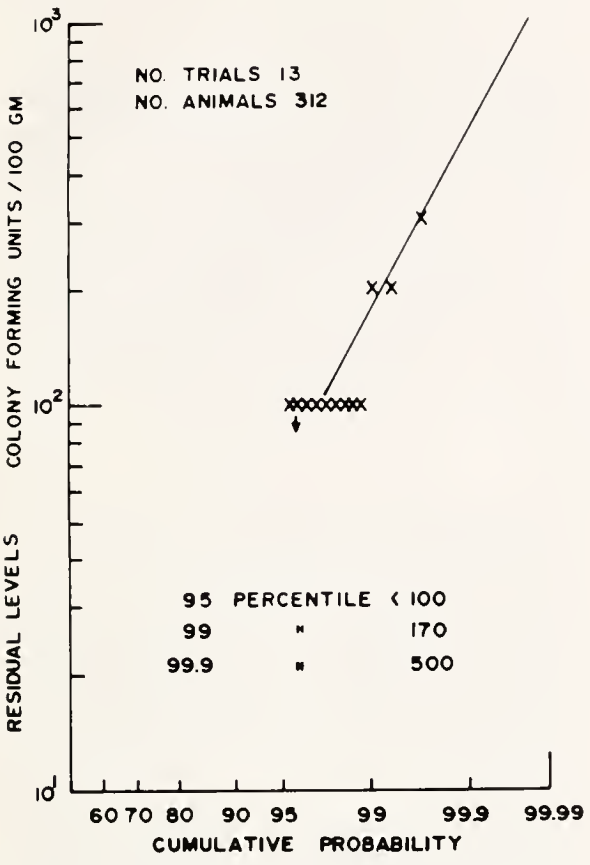

FIG. 3. *Residual levels of ET coliforms in individual animals extrapolated from data with pooled samples. Data taken from Tables 5 and 6. Mean initial level of contamination for naturally polluted quahaugs used in each trial varied between 1.2 X 10^2 — 2.1 X 10^3 CFU/100 gm.*

single animal. The probability plot (Fig. 3) of the projected levels in individual animals provides a prediction of the 95, 99 and 99.9 percentile residual levels following depuration of a population of similarly polluted quahaugs.

DISCUSSION

The marked difference observed in the depuration of naturally polluted quahaugs as opposed to those artificially contaminated with *E. coli* raises the question as to the adequacy of data obtained with animals artificially contaminated with either viruses or bacteria when these data are used to determine the feasibility of depuration. Beyond this rather obvious point, the basis for the difference is of interest. It is possible that the mechanics of harvesting animals from their natural environment is more detrimental to this aspect of the animal's physiology than their transfer from basketed storage in the laboratory. Furthermore, with depuration of artificially contaminated quahaugs, any stress imposed by manipulation would be more likely to manifest itself in decreased activity during the uptake rather than the subsequent elimination phase of the trial.

A second possibility for the difference is the manner in which organisms are presented to the animal. During artificial contamination, the organisms taken from a pure culture, appropriately diluted and mixed into the water, are presented to animals in small particles containing one to a few cells. Under natural conditions, animals burrowed into the bottom sediments may ingest organisms contained in larger particles which are derived from sewage effluents and which have settled to the bottom. These particles could, by virtue of their size, be carried to a location(s) within the animal from which their elimination during depuration is more difficult. Thus, the nature of the pollution source as well as the proximity of the animal to it could be as important to depuration as the level of pollution in the animals. Further experimentation will be needed to establish the extent to which each of the possibilities accounts for the differences observed. The data showing that elimination was obtained following contamination for 1-14 days (Heffernan and Cabelli, 1970) would argue against the duration of the contamination period as a major factor.

The observation that the elimination of coliforms in naturally polluted quahaugs is more protracted than in animals artificially contaminated with *E. coli* may have a significance beyond that of questioning the validity of data on residual levels obtained with artificially contaminated animals. It is possible, that during accumulation (1) the nature of the particle containing the coliform organisms, (naturally vs artificially contaminated animals), (2) thermal shock (fall, winter and spring animals which are artificially contaminated at 20°C; Cabelli and Heffernan, 1971) and (3) high levels of coliforms in the environmental water (Heffernan and Cabelli, 1970) may result in increasing the absolute number of organisms which are deposited at some site in the quahaug which is less accessible than the digestive gland to the elimination of the organisms via the normal feeding — cleansing mechanisms. This possibility should be investigated since it could provide insights not only into a means of improving the depuration process but also into the validity of a bacterial

system as an indicator of viral depuration.

The data obtained from the examination of the residual ET coliform levels in the individual animals clearly demonstrates that the prime problem in the application of the normal feeding — cleansing mechanism of the quahaug to the elimination of bacterial pollutants is the variability in the response of the individual animals. In fact, about one percent of the quahaugs cannot be expected to appreciably reduce their level of ET coliforms. However, by restricting depuration to "moderately polluted" animals and by the use of the proposed rejection criteria, both the number of inactive or minimally functioning animals in the final product and their influence on the overall sanitary quality of the depurated product can be reduced. Obviously, this by no means implies that the problem of variability in the response of individual animals has been overcome. Rather, its impact has been decreased by the rejection of instances in which there are excessive numbers of minimally functioning quahaugs. A partial solution to the problem of animal variability lies in the stimulation of the feeding-cleansing activities of the animals by chemical or physical means. This approach also could decrease the required treatment period and minimize seasonal influences on the process.

It can be seen that the target residual level used to monitor the process, namely an average residual ET coliform value of 25 CFU/100 gm for the four pools of six animals each, would result in a product whose ET coliform levels are about half that of animals taken from the upper limit of an approved area. The data show that, with the methods used, elimination to this residual level is readily attainable. Since only 24 animals are sampled and none can have a residual ET coliform level in excess of 500 CFU/100 gm, 4.2 % of the animals from an acceptable run could contain residual levels in excess of this value. The data from the thirteen acceptable trials when projected would suggest that only 0.12 % of the animals would be expected to have residual levels in excess of 500 CFU/100 gm (Fig. 3).

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ASSOCIATION AFFAIRS

ANNUAL CONVENTION

The National Shellfisheries Association's 1970 Convention was held jointly with the Shellfish Institute of North America and the Oyster Growers and Dealers Association of North America, Inc. from 21-24 June 1970 at Atlantic City, New Jersey.

Officers and Executive Committee members elected for the term 1970-1971 were:

President	Frederick W. Sieling
Vice-President	Roy E. Drinnan
Vice-President	Kenneth K. Chew
Secretary-Treasurer	Mrs. Helen J. Haynie
Members-at-Large	R. Winston Menzel
	Michael Castagna
	Theodore B. Ford
Editor, NSA Proceedings	William N. Shaw

John W. Ropes of the National Marine Fisheries Service Biological Laboratory, Oxford, Maryland is Custodian of back issues of the NSA Proceedings.

Mr. James B. Engle was elected to honorary membership in the National Shellfisheries Association. A plaque was presented to Mr. Harry C. Davis, former Assistant Laboratory Director, National Marine Fisheries Service Biological Laboratory, Milford, Connecticut for his contributions to the shellfish industry.

It was recommended that a shrimp symposium be held at the 1971 annual meeting. Dr. Kenneth K. Chew and Mr. Daniel W. Gotshall were appointed to serve as a committee to prepare the symposium for the meeting.

The membership has steadily grown with the membership now totalling 338 general members, 8 Honorary members and 3 Life members, with 86 active library subscribers and 4 abstract companies.

The Pacific Coast Section of the NSA met jointly with the Pacific Coast Oyster Growers Association on 21-22 August 1970 at the Double Tree Inn, Seattle, Washington. Officers of the Section elected for the term 1970-1971 were:

Chairman	Ronald E. Westley
Vice-Chairman	Robert B. Herrmann
Secretary-Treasurer	Walter Jakubowski

There will be a 1971 joint meeting of the National Shellfisheries Association and the Shellfish Institute of North America in Seattle, Washington.

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

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Original papers given at the Annual Association Convention and other papers on shellfish biology or related subjects will be considered for publication. Manuscripts will be judged by the Editorial Committee or by other competent reviewers on the basis of originality, contents, clarity of presentation and interpretations. Each paper should be carefully prepared in the style followed in the 1965 PROCEEDING (Volume 56) before submission to the Editorial Committee. Papers published or to be published in other journals are not acceptable.

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